LWT - Food Science and Technology 43 (2010) 39–44

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effects of supercritical CO_2 fluid parameters on chemical composition and yield of carotenoids extracted from pumpkin

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ARTICLE INFO

Article history:
Received 14 January 2009
Received in revised form
30 June 2009
Accepted 6 July 2009

Keywords:
Supercritical fluid extraction
Carotenoid
Composition
Pumpkin

ABSTRACT

Pumpkin is a traditional food that is grown extensively worldwide and is believed to be beneficial to human health due to its high contents of carotenoids. The carotenoids in pumpkin were extracted by organic solvents and by supercritical carbon dioxide (SC-CO_2), and then they were identified, quantified, and compared. β-carotene (31 to 40 g per 100 g of total carotenoids) was the predominate carotenoid in pumpkin. Lutein and lycopene contents were much higher in SC-CO_2 extracts than those in organic solvent extract. *Cis*-β-carotene increased by more than two times in the SC-CO_2 extracts, even at a relatively low temperature of 40 °C, over those in the solvent extracts, indicating both enhanced solubility and isomerization from *trans*- to *cis*-β-carotene. The influences of modifier (10 mL/100 mL), temperature (40–70 °C), and pressure (25–35 MPa) of SC-CO_2 extraction on the change of carotenoid yields were also investigated. The highest yield (109.6 μg/g) was obtained at 70 °C and 35 MPa, with a 73.7% recovery. Selective extraction could be achieved by adjusting the temperature and pressure. Higher proportions of all-*trans*-β-carotene extracts were achieved at 40 °C under both 25 MPa and 35 MPa conditions. In order to extract more *cis*-isomers, a higher temperature of 70 °C was preferred.

1. Introduction

Carotenoids are currently the most widespread pigments used in the food industry as functional food ingredients or colorants (Krinsky, 1998). Epidemiological studies have shown that people with high carotenoid intake and high plasma levels of carotenoids have a significantly reduced risk of cancer, cardiovascular diseases, cataracts, and heart disease (Lidebjer, Leanderson, Ernerudh, & Jonasson, 2007).

Pumpkin is a gourd-like fruit of the genus *Cucurbita* and the family *Cucurbitaceae*. It is grown all around the world for food, animal feed, or ornamental sales. It became an important food source of carotenoids worldwide when the presence of relatively high contents of provitamin A carotenoids were observed (principally β-carotene, α-carotene, and sometimes β-cryptoxanthin) (Speek, Speek-Saichua, & Schreurs, 1988). Different cultivars and species of pumpkin contained different carotenoids (Arima & Rodriguez-Amaya, 1988; Azevedo-Meleiro, & Rodriguez-Amaya, 2007; Kurz, Carle, & Schieber, 2008). Murkovic, Mülleder, and Neunteufl (2002) found that a wide range of varieties of pumpkins contained common carotenoids: β-carotene (0.06–7.4 mg/100 g), α-carotene (0–7.5 mg/100 g), and lutein (0–17 mg/100 g). Hence, there is considerable industrial interest in extracting carotenoids from pumpkins to develop functional food ingredients.

Supercritical carbon dioxide extraction (SC-CO_2) of carotenoids is an alternative to the traditional, low selectivity, and toxic organic solvent extraction method because it provides a non-toxic and faster extraction process. SC-CO_2 extraction of carotenoids has been widely applied to various materials, such as tomato, chili pepper, sweet potato, and carrot (Perva-Uzunalic, Skerget, Weinreich, & Knez, 2004; Spanos, Chen, & Schwartz, 1993; Sun & Temelli, 2006; Vasapollo, Longo, Rescio, & Ciurlia, 2004). Due to distinct solubilities of different kinds of carotenoids, selective extraction could be achieved by adjusting the temperature and pressure of SC-CO_2.. Montero et al. (2005) selectively extracted β-carotene from *Synechococcus* sp. by properly adjusting the operating conditions of SC-CO_2, achieving a purity higher than 95%.

Supercritical fluid extraction of carotenoids from pumpkin has only been reported by Seo, Burri, Quan, and Neidlinger (2005). However, the carotenoid profile reported in SC-CO_2 extract is not as conclusive as that reported by others using organic solvent extraction. The supercritical conditions were tested by the

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0023-6438/$ – see front matter
doi:10.1016/j.lwt.2009.07.003

adjustment of one variable at one time, which is time consuming and neglects the interaction of variables. Moreover, the tested temperatures were as high as 110 °C, which may result in the degradation of carotenoids. The present study was designed to analyze the carotenoid profiles of pumpkin flesh, to investigate the influence of modifiers in the SC-CO_2 extraction on the extraction yield, and to explore the influence and interaction of two independent experimental variables, namely temperature and pressure, on the yield of carotenoids from pumpkin.

2. Material and methods

2.1. Pumpkin samples and chemicals

Samples of fresh and fully ripe pumpkin (*Curcurbita moschata,* orange) from the local market were peeled and seeded, and then the fleshes were chopped into small pieces. Approximately 1 kg raw material was freeze-dried until reaching a final moisture content of about 10 g/100 g (dry weight) and ground with a grinder (SmartGrind Deluxe, Black & Decker, Mirama, FL, USA) to an average particle size about 1 mm (corresponding to 18 mesh).

Standards of mixed carotene isomers from carrots (≥ 95%, Approx. β: α = 2:1), β-carotene (≥95%), lutein (≈90%), and lycopene (≥95%) were purchased from Sigma-Aldrich Co.(St. Louis, MO, USA). The HPLC grade solvents were all from Caledon Laboratories LTD.(Georgetown, Ont., Canada). Carbon dioxide for supercritical fluid extraction was purchased from Praxair Product Inc.(Kitchener, Ontario, Canada).

2.2. Supercritical fluid extraction

An automated Speed SFE NP model 7100 instrument (Applied Separation Inc., Allentown, PA, USA), equipped with a pump (Module 7100) and a 10-mL thick-walled stainless cylindrical extractor vessel, was used for carotenoid extraction. CO_2 was cooled by a refrigerated bath (RB-5, Techne Cambridge Ltd., UK) before pressurizing and pumping into the system. The extractor was filled with defatted cotton on both ends, thus preventing CO_2 from transporting solids out. A freeze-dried sample of 0.4 g was weighed and used for each extraction. Constant weight of extraction was achieved after 40 min. The flow rate of the CO_2 at the exit of the system was controlled and kept constant at 1.5 mL/min (±0.05, sensitivity or accuracy of control on the equipment) for during the experiments. The pressures and temperatures were varied to determine the effects of them on the extraction yield according the experimental design in present study. All samples were collected in 25 mL brown sample vials to minimize UV-activated degradation and stored at −20 °C prior to quantitative analysis. The dry extract was washed from the vial with 4 mL hexane, and then dried under a stream of nitrogen. After re-dissolving in 1 mL hexane, the extract was stored at −20 °C until HPLC analysis.

2.3. Solvent extraction

Freeze-dried pumpkin samples (0.1 g) were mixed with 2 mL of ethanol (95 mL/100 mL), vortexed for 1 min, and then mixed with an equal volume of hexane. The hexane layer was removed and collected, and the extraction was repeated until the color of the final residue became very light. The extracts were dried under nitrogen and then re-dissolved in 1 mL hexane. To calculate the recovery, it was assumed that the organic solvent extraction extracted 100% of the carotenoids from the freeze-dried sample.

2.4. HPLC analysis

Carotenoids were determined according to the method of Kurz et al. (2008) with slight modifications. Separation was performed on an Agilent 1100 HPLC apparatus (Agilent Technologies, Waldbronn, Germany) equipped with a reverse phase analytical polymeric C_{30} column (250 mm l. × 4.6 mm i.d., 5 μm particle size) (YMC, Inc. Wilmington, NC, USA) and a UV diode array detector (monitored at 450 nm). The mobile phase consisted of methanol/methyl *tert*-butyl ether (MTBE)/water (81:15:4, v/v/v; A), and methanol/ MTBE/water (4:92:4, v/v/v; B). Gradient chromatography was run at 0.42 mL/min as follows: 0–60.0 min, solvent B increasing linearly from 0% to 80%; 60.0–65.0 min, solvent B increase to 100%; 65.0–70.0 min, solvent B decrease to 0%; 70.0–80.0 min, isocratic with 0% B. The injection volume was 20 μL.

Standard solutions were injected and the concentrations were adjusted accordingly. Peaks were identified according to the retention time (t_R) and UV absorption patterns of the standards. Peaks without standards were tentatively identified according to references (Kurz et al., 2008, Tsao, Yang, Young, Zhu, & Manolis, 2004). Considering that no *cis*-isomer standards were available, the quantification of β-carotene isomers was done by using the same response factor as all-*trans*-β-carotene. Similarly, the response factor for lutein was used for the quantification of lutein esters.

2.5. Experimental design and statistical analysis

The effects of temperature (40–70 °C) and pressure (25–35 MPa) with the presence of ethanol as modifier on total carotenoid yields with SC-CO_2 extraction from pumpkin were investigated using a Central Composite Design (CCD). Table 1 gives the levels of variables in coded and actual units. All the experimental units (run) were replicated three times. A full second-order polynomial model of the design was used to evaluate the yield (response variable, Y) as a function of independent variables (x) and their interactions (Eq. (1))

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_j x_j + \sum \beta_{ii} x_i^2 + \sum \beta_{jj} x_j^2 + \sum \beta_{ij} x_i x_j \qquad (1)$$

where Y is the response, β_0 is the constant coefficient, β_i, β_j are the linear coefficients, β_{ii}, β_{jj} are the quadratic coefficients, β_{ij} is the linear-by-linear interaction coefficient, and x_i, x_j are the coded values of independent variables.

Experimental design, data analysis, and quadratic model building were conducted using the software Design Expert (Version 7.1.4 trial, Stat-Ease Inc., Minneapolis, MN, USA). Five replicates at the center of the design were used to allow for the estimation of a pure error sum of squares. Differences between variables were

Table 1
Central composite design experimental design for SC-CO_2 extraction of carotenoids from pumpkin and results obtained with the presence of ethanol as modifier at ratio of 90:10 (dry weight of pumpkin sample: volume of ethanol).

Runs	Factor values		Response	
	Temperature (°C)	Pressure (MPa)	Total carotenoids (μg/g)	Recovery (%)
1	40.0 (−1)	25.0 (−1)	29.1 ± 5.3	19.5%
2	70.0 (+1)	25.0 (1)	97.6 ± 12.2	65.6%
3	40.0 (−1)	35.0 (+1)	62.3 ± 6.2	41.8%
4	70.0 (+1)	35.0 (+1)	109.6 ± 14.4	73.7%
5	33.8 (−1.4)	30.0 (0)	39.6 ± 6.4	26.6%
6	76.2 (+1.4)	30.0 (0)	88.6 ± 12.0	59.5%
7	55.0 (0)	22.9 (−1.4)	61.6 ± 4.1	41.4%
8	55.0 (0)	37.1 (+1.4)	99.7 ± 6.0	67.0%
9	55.0 (0)	30.0 (0)	76.5 ± 4.3	51.4%