Supercritical CO_2 Extraction of β-Carotene and Lycopene from Tomato Paste Waste

T. Baysal,[†] S. Ersus,[†] and D. A. J. Starmans*[,‡]

Food Engineering Department, Engineering Faculty, Aegean University, 35100 Bornova, Izmir, Turkey, and Institute of Agricultural and Environmental Engineering B.V., P.O. Box 43, NL-6700 AA Wageningen, The Netherlands

Lycopene and β-carotene were extracted from tomato paste waste using supercritical carbon dioxide (SC-CO_2). To optimize supercritical fluid extraction (SFE) results for the isolation of lycopene and β-carotene, a factorial designed experiment was conducted. The factors assessed were the temperature of the extractor (35, 45, 55, and 65 °C), the pressure of the extraction fluid (200, 250, and 300 bar), addition of cosolvent (5, 10, and 15% ethanol), extraction time (1, 2, and 3 h), and CO_2 flow rate (2, 4, and 8 kg/h). The total amounts of lycopene and β-carotene in the tomato paste waste, extracts, and residues were determined by HPLC. A maximum of 53.93% of lycopene was extracted by SC-CO_2 in 2 h (CO_2 flow rate = 4 kg/h) at 55 °C and 300 bar, with the addition of 5% ethanol as a cosolvent. Half of the initially present β-carotene was extracted in 2 h (flow rate = 4 kg/h), at 65 °C and 300 bar, also with the addition of 5% ethanol.

Keywords: *Lycopene; carotene; tomato paste waste; extraction; supercritical CO_2; cosolvent*

INTRODUCTION

The carotenoids are natural pigments that provide the natural yellow, orange, and red colors of fruits, vegetables, plants, birds, and marine animals. These colors are a result of the presence of conjugated double bonds that also provide the carotenoids with antioxidant properties by the successful delocalization of captured free radical species. According to epidemiological studies by Siemensma (1996), carotenoids play an important role in the prevention of cancer, cataracts, and aging diseases such as heart disease. There also exists an inverse relationship between the consumption of foods containing carotenoids and the risk of lung, intestinal, skin, and bladder cancer as respectively described by Burton and Ingold (1984), Krinsky (1988), Conett et al. (1989), Stich and Anders (1989), Block et al. (1992), Blot et al. (1993), Marchand et al. (1993), and Kornhauser et al. (1994). In addition to this it was shown by studies of Bureau and Bushway (1986) that retinol and several carotenoids (β-carotene, β-cryptoxanthin, zeaxanthin, lutein, capsorubin, capsanthin, lycopene, and capsanthol) were involved in the cytoprotective injury of gastric mucosa. Besides some applications of carotenoids for their provitamin A activity, they are widely used as colorants in food. Carotenoids are added directly to many food products such as butter, popcorn, salad dressings, and beverages or indirectly via animal uptake in, for example, chicken and fish as described by Britton (1992), Birtigh et al. (1995), and Vega et al. (1996).

Hitherto, artificial dyes were commonly used in the food industry, but increasing interest in the use of natural products has led to the production of natural food colorants. Lycopene is the principal compound responsible for the characteristic red color of tomatoes. As determined by Gross (1987), the total lycopene content in tomatoes varies between 90 and 190 μg (g fresh wt)$^{-1}$. Sharma and Maguer (1996) reported the occurrence of lycopene in different fractions of tomato fruit such as tomato skin, the water insoluble fraction, and the fibrous fraction including fiber and soluble solids. Their results indicated that 72−92% of the lycopene was associated with the water insoluble fraction and the skin. Tomato extracts and especially skin extracts contain high amounts of lycopene. As already indicated by Sadler et al. (1990), the widespread use of tomato paste as a colorant makes lycopene a commercially interesting natural pigment.

From the figures above it is clear that tomato paste waste is a natural and very economical source for coloring material. The waste during tomato processing is mainly obtained in the form of seeds and skin residues. Al-Wandawi et al. (1985) showed that the seeds were a rich source of edible oil, whereas the seed flakes were found to be a good protein source. Because of their nutritive value, these wastes are normally used as animal feed.

The results of the analysis of tomato waste indicated that the hydrocarbon carotenoid "lycopene" is located mainly in the tomato skin with concentrations of up to 120 μg/g of wet sample. Additionally, β-carotene was found to be present in a concentration of 3 μg/g of wet sample. Compared to the concentrations of lycopene (167.9 μg/g of wet sample) and β-carotene (10.6 μg/g of wet sample) in tomato paste end-product, the values found in the waste material are considerable. It can clearly be stated that a large quantity of the natural color of tomatoes is lost as waste in tomato processing.

Compared to traditional techniques, new isolation techniques should provide better processing in terms of solvent use to improve product quality. Most extraction

* Corresponding author (telephone 31-317-476592; fax 31-317-425670; e-mail d.a.j.starmans@imag.wag-ur.nl).

[†] Aegean University.

[‡] Institute of Agricultural and Environmental Engineering B.V.

10.1021/jf000311t CCC: $19.00
Published on Web 10/14/2000

methods of carotenoids use organic solvents such as hexane, ethanol, acetone, methanol, tetrahydrofuran, and petroleum ether. These solvents were used in the research performed by Chandler and Schwartz (1987), Tan (1988), Heinonen et al. (1989), Sadler et al. (1990), Khachik et al. (1992), Hakala and Heinonen (1994), and Tonucci et al. (1995). As indicated by Barth et al. (1995), traditional solvent extraction of carotenoids is time-consuming, requires multiple steps, and consumes large amounts of organic solvents. The amount and the price of organic solvents directly influence the total cost of producing an acceptable extract/product. Moreover, when the final product is used as a food ingredient, it is absolutely necessary to remove all potentially toxic solvents.

Carotenoids are a group of pigments that can easily degrade when exposed to heat, light, and/or oxygen. In the traditional methods of isolation by solvent extraction described by Favati et al. (1988) and Chao et al. (1991), the risk of thermal degradation and/or oxidation of the extracts should not be underestimated. Because either pathway will lead to a distinctly worse extraction product, an improved carotenoid extraction method should be considered.

Supercritical fluid extraction (SFE) has already proven itself as an attractive technique for selectively removing compounds from food matrices. Specifically, SFE offers the possibility of mild extraction conditions combined with low energy requirements for solvent recovery. The high selectivity of the extraction process and the reduced potential for oxidation of the extracted materials make this technique especially suitable for extractive isolation of natural pigments such as carotenoids. Although capital costs necessary for the extraction setup are high, SFE may be an attractive alternative for the extraction of natural pigments as described by Chao et al. (1991) and Barth et al. (1995).

In this study, the process conditions during the extraction of coloring materials from tomato paste waste with supercritical carbon dioxide extraction have been optimized with respect to pressure, temperature, carbon dioxide flow, extraction time, and cosolvent addition in order to selectively obtain lycopene and β-carotene.

MATERIALS AND METHODS

Materials. Tomato paste waste was obtained from Tukas A.S., a local producer of tomato paste and other food products in Turkey.

Sample Preparation. For preservation purposes, tomato paste waste [dry matter content = 24% (±2%)] was dipped in a 5% sodium metabisulfite solution and dried in a greenhouse (20 kg/m^2) for 5 days. The pooled dry material (dry matter content = 94 ± 2%) was subsequently packed into polyethylene pouches (containing 4.5 kg each), which were stored and then subsequently shipped to The Netherlands in secured cardboard boxes.

Prior to each SFE extraction, 60 g of dried tomato paste waste was taken from a pouch and successively ground using a cutmill (type 5M1, Retsch GmbH, Haan, Germany) equipped with a 3 mm sieve.

Supercritical Fluid Extraction. Extraction of tomato paste waste was performed in duplicate using a randomized block design. The extraction was carried out in a polyvalent pilot plant extraction setup (Sitec Sieber Engineering A.G., Zurich, Switzerland), which is schematically shown in Figure 1. Liquid carbon dioxide entering the apparatus is cooled in condensor C before it is pressurized and passed into the system. The desired flow rate was adjusted manually before and during the experiment. A PI 4-20 flow meter (Micro Motion, Boulder, CO) was used for the continuous measurement of the carbon dioxide flow rate. The temperatures of the extractor, carbon dioxide, and separators 1 and 2 were automatically regulated through the recirculation of thermostated water from three individually regulated water baths.

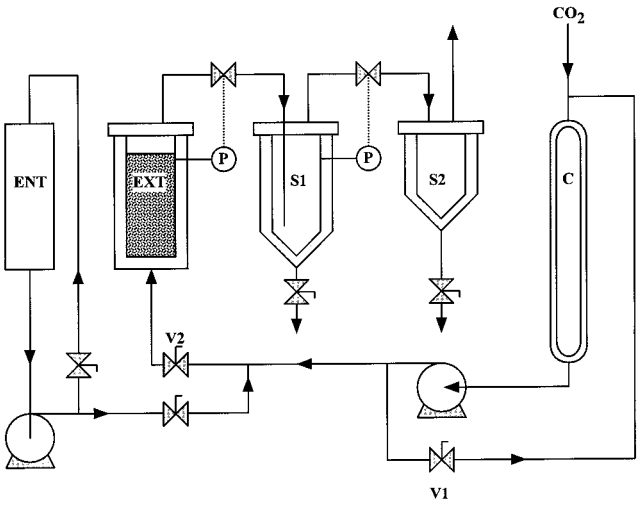

Figure 1. Schematic presentation of the setup for SFE.

The material to be extracted was loaded into a cylindrical container that was equipped with steel mesh filters on both ends, thus enabling carbon dioxide to pass the cylinder without transport of solids to the exterior. After prepressurization of the total system and the regulation of the carbon dioxide flow rate, the extractor (EXT) was depressurized and the cylinder was subsequently placed inside the extractor, after which the complete carbon dioxide flow was redirected toward the extractor using valves VI and V2. The temperature/pressure combination of the extractor and the temperature/pressure combinations of both separator vessels S1 and S2 were controlled individually. The extraction was stopped by redirecting the carbon dioxide flow again to recirculation over the condensor. The solid residue was removed from the extractor after stepwise depressurization of the entire system. Subsequently, both separator vessels were rinsed with hexane, and both extracts were collected in brown bottles to prevent UV-activated degradation of the extract.

Analysis Methods. *Total Solids.* Total solids of tomato paste waste before and after extraction with supercritical carbon dioxide was determined gravimetrically. Approximately 2 ± 0.1 g of tomato paste waste was preweighed and subsequently dried in a vacuum oven set at 100 mmHg and 75 °C in triplicate until a constant weight was reached. Total solids were calculated as a percentage of the initial weight.

Carotenoid Extraction. The determination of the total amount of β-carotene and lycopene in tomato paste waste and residue material was performed according to the AOAC methods used by Williams (1984). The carotenoids were extracted from 2 g of either control or residual material using a hexane/acetone/absolute alcohol/toluene mixture as solvent. The obtained extracts were put into brown bottles that were subsequently flushed with nitrogen and stored at −22 °C until HPLC analysis. The lycopene and β-carotene yields in both the extracts and the solid residue are expressed as the percentage of carotenes extracted from cryogenically milled starting material (100%). All extracts were filtered through a 0.22 μm filter paper (Syrfil-MF, 25 mm syringe filter, Costar Corp., Cambridge, MA) before direct injection onto the HPLC column.

Carotenoid Determination. The total amount of β-carotene and lycopene in the extracts was determined using the HPLC procedure of Sadler et al. (1990). The extracts were diluted with HPLC grade hexane to different volumes in such a way that the concentration fell inside the range of the calibration curve. The calibration curve was determined by injecting 20 μL samples of 2.5, 5.0, 7.5, and 10 ppm of pure β-carotene [type

Table 1. Process Conditions during SFE of Tomato Paste Waste

expt	variables					constants[a]
temp	T_{ext}	= 35	45	55	65 °C	P_{ext} = 300 bar flow rate = 4 kg/h
pressure	P_{ext}	= 200	250	300	bar	T_{ext} = 65 °C flow rate = 4 kg/h
time	time	= 1	2	3	h	T_{ext} = 65 °C flow rate = 4 kg/h
	rate	= 2	4	8	kg/h	P_{ext} = 300 bar flow rate = 4 kg/h
flow rate	time	= 4	2	1	h	T_{ext} = 65 °C P_{ext} = 300 bar total CO_2 = 8 kg
cosolvent	eth	= 5	10	15	%	T_{ext} = 65 °C P_{ext} = 300 bar flow rate = 4 kg/h

[a] In all experiments $T_{sep1} = T_{sep2} = 35$ °C, $P_{sep1} = 80$ bar, and $P_{sep2} = 1$ bar.

2, synthetic minimum 95% (HPLC grade, Sigma Chemical Co.)] and lycopene (from tomato, 90−95%, Sigma Chemical Co.).

A Waters 2690 (Alliance) HPLC with a 996 photodiode detector (Waters Associates,, Milford, MA) was used for lycopene and β-carotene determination. Isocratic separation of unknown samples (20 μL injection volume) was achieved on a Waters Symmetry C18 (5 μm) (3.9 × 150 mm) HPLC cartridge column. The mobile phase methanol/THF/water (67:27:6) was perfused at a flow rate of 1.5 mL/min.

Statistical Analysis. One-way analysis of variance (ANOVA) was performed to interpret the extraction yield for both lycopene and β-carotene. The statistical significance of the effect of temperature, pressure, extraction time, flow rate, and cosolvent was determined at the 0.05 level.

Experimental Design. *Influence of Extraction Temperature.* Milled samples (53 g each) were extracted using the described method. It was hypothesized that the extraction temperature had a twofold influence on the obtained extract. On the one hand, elevation of the temperature would increase the total amount of extracted material as found by Vega et al. (1996), whereas, on the other hand, the quality of the extract would suffer from thermal degradation during the extraction procedure. To investigate the influence of temperature on the obtained extract, experiments were carried out at extractor temperatures of 35, 45, 55, and 65 °C. Both the extraction time and the carbon dioxide flow rate were kept constant during these experiments at 2 h and 4 kg/h, respectively. The pressure inside the extractor and separators 1 and 2 during these experiments was kept constant at values of, respectively, 300, 80, and 1 bar. The operating temperatures of separators 1 and 2 were set to 35 °C during these series (Table 1). The collected extracts were analyzed by means of the described HPLC procedure.

Influence of Extractor Pressure. Another important extraction parameter is the pressure inside the extractor. To obtain more insight into the influence of this parameter on the extraction yield, experiments were carried out at the most promising temperature (found in the previous experiment). The extractions were performed using three different extraction pressures (200, 250, and 300 bar).

Influence of the Carbon Dioxide Flow Rate. When a non-equilibrium semi-industrial extraction is performed, the carbon dioxide flow rate may have an influence on the extraction efficiency. At optimum temperature and pressure, experiments were performed in which the carbon dioxide flow rate was varied. To compare the results, the experiments were conducted in such a way that in each experiment a total of 8 kg of CO_2 was used. In this way, it is hypothesized that only the contact time and the intensity of mixing between the CO_2 phase and the sample is varied without changing the separation characteristics ($T_{extractor} = 65$ °C, $T_{separator1} = 35$ °C, $P_{extractor} = 300$ bar, $P_{separator1} = 80$ bar).

Table 2. Effect of Temperature on SFE Efficiency of Carotenes

extractor temp (°C)	isolation of lycopene[a] (%)	isolation of β-carotene[a] (%)
35	7.58 c	32.58 b
45	17.17 b	39.75 a
55	17.83 b	38.77 a
65	21.86 a	43.02 a

[a] Different letters within columns indicate significantly different values ($P < 0.05$).

Influence of the Addition of Cosolvent. The extraction of compounds from plant material is greatly influenced by the hydrophobicity/hydrophilicity of the extraction fluid. The lack of a dipole moment in CO_2 molecules decreases the extractive properties of supercritical carbon dioxide for somewhat polar compounds. In fact, the chemical composition of extracts in experiments using SC-CO_2 resembles that of experiments in which hexane is used. To facilitate the isolation of less hydrophobic compounds, polar solvents can be added to the supercritical carbon dioxide mixture.

The influence of a cosolvent on the extraction properties of supercritical carbon dioxide was studied by performing extraction experiments in which ethanol was added to the supercritical carbon dioxide flow at four distinct levels of 0, 5, 10, and 15 wt %. The obtained extracts were pretreated and analyzed as described before.

RESULTS AND DISCUSSION

The initial total lycopene and β-carotene contents in dried tomato paste waste were found to be 309.6 ± 15 and 29.6 ± 3 μg/g (dry basis), respectively. For determining the influence of particle size on the extraction, experiments were done with and without milling. Before the parameters were optimized, for the same supercritical conditions without milling application, the amounts of extracted lycopene and β-carotene were 5 ± 0.4 and 2.5 ± 0.3 μg/g, respectively. When the 3 mm diameter milled samples were used for supercritical extraction, 24 ± 2.4 μg/g of lycopene and 9.5 ± 1.2 μg/g of β-carotene could be extracted. Results show that the extraction efficiency increased ∼5 times for lycopene ∼4 times for β-carotene when using milled tomato paste waste (sieve hole diameter = 3 mm). This indicates that an increased surface area enhances carotene extractability dramatically. During the final experiments particle size and flow rate of CO_2 were kept constant.

Effect of Temperature. The influence of temperature on the supercritical extraction yields of lycopene and β-carotene from tomato paste waste is shown in Table 2. Increasing temperature increases the solubility of the carotenoids, which results in higher yields. The highest temperature used for the extraction (65 °C) gave the maximal extraction yield.

The total lycopene recovery is only 30−45% of the initially present material, whereas nearly all of the β-carotene is accounted for. A possible explanation for this phenomenon could be the decomposition of lycopene during the extraction procedure. Comparison of the recovery values indicates that ∼40% of the β-carotene is extracted, whereas only 20% of the lycopene is found in the extract. Furthermore, an increase in temperature has a positive influence on the extraction yield of both carotenoids. The results show that the temperature dependence of the lycopene extraction yield at constant pressure (300 bar) was higher than that for β-carotene.

The effect of temperature on extraction was more difficult to assess than the effect of pressure. An increase in extraction temperature results in an in-

Table 3. Effect of Pressure on the SFE of Carotenes

pressure of extractor (bar)	recovery of lycopene[a] (%)	recovery of β-carotene[a] (%)
200	18.88 b	20.19 b
250	16.62 b	26.94 b
300	21.86 a	43.02 a

[a] Different letters within columns indicate significantly different values ($P < 0.05$).

Table 4. Effect of Time on the SFE of Carotenes

extraction time (h)	recovery of lycopene[a] (%)	recovery of β-carotene[a] (%)
1	13.92 b	24.15 b
2	21.86 b	43.02 a
3	13.15 b	21.86 a

[a] Different letters within columns indicate significantly different values ($P < 0.05$).

creased solute vapor pressure. The density of SC-CO_2, at constant pressure, decreases as temperature increases, but the magnitude of such a density change becomes smaller at elevated pressures. Marentis (1988) and Spanos et al. (1993) also pointed out that an increase of temperature had no apparent effect on the β-carotene extraction at intermediate pressures (27.6 MPa).

Effect of Pressure. The density of supercritical carbon dioxide increases from 0.85 to 0.95 kg/m^3 with increasing pressure from 200 to 300 bar. The results shown in Table 3 show no significant difference in lycopene and β-carotene extraction yields if the pressure is changed in this range. This indicates an increase in extractability of carotenes at increasing SC-CO_2 density. It was technically not possible to work at higher extractions pressures with the available apparatus. These results and the result found in the literature by Vega et al. (1996) indicate that at an extraction pressure of 400 bar the carotene yield will increase.

Studies by Sakaki (1992) showed that an increase of temperature had a stronger effect on the solubility than an increase of pressure. Spanos et al. (1993) found that solubility may be controlled by a balance between SC-CO_2 density and solute (carotene) vapor pressure changes as the temperature increases. Both lycopene and β-carotene extracted at 300 bar had highest recovery values.

Effect of Extraction Time and Flow Rate. The extraction time was taken as a constant during the experiments for the optimization of temperature and pressure. To further minimize the extraction time and thereby also minimize the costs related to the extraction procedure, additional experiments were performed during which the extraction time and flow rate of supercritical CO_2 were varied.

For optimization of the extraction time, 1, 2, and 3 h extraction periods were studied with the optimized parameters. The influence of the extraction time on the yield of lycopene and β-carotene is given in Table 4.

The highest carotene yield was obtained with an extraction time of 2 h. It is possible that 1 h is not enough for extracting carotenes and that at 3 h increased degradation occurs.

To determine the effect of flow rate during the extraction on the carotenoid yield, the duration of individual extraction experiments was adapted in such a way that for all cases exactly 8 kg of CO_2 was used. The results of these experiments are given in Table 5.

Because the total amount of CO_2 was constant throughout the experiments, the differences found were

Table 5. Effect of Flow Rate on the SFE of Carotenes

flow rate (kg/h)	extraction time (h)	recovery of lycopene[a] (%)	recovery of β-carotene[a] (%)
2	4	13.92 b	30.42 b
4	2	21.86 a	43.02 a
8	1	19.46 a	33.54 b

[a] Different letters within columns indicate significantly different values ($P < 0.05$).

Table 6. Effect of the Addition of Cosolvent to the SFE Fluid on the Extraction of Lycopene

	recovery of lycopene		
temp (°C)	ethanol[a] (5%)	ethanol[a] (10%)	ethanol[a] (15%)
35	22.86 c	13.38 e	14.10 e
45	34.34 b	17.42 cde	16.80 de
55	53.93 a	17.55 cde	20.87 cd
65	51.05 a	29.54 b	18.86 cd

[a] Different letters within columns indicate significantly different values ($P < 0.05$).

Table 7. Effect of Addition of Cosolvent to the SFE Fluid on the Extraction of β-Carotene

	recovery of β-carotene		
temp (°C)	ethanol[a] (5%)	ethanol[a] (10%)	ethanol[a] (15%)
35	37.07 cd	37.31 cd	38.96 cd
45	42.74 bc	39.88 cd	37.54 cd
55	47.02 ab	42.14 bc	33.95 d
65	49.95 a	46.99 a	33.87 d

[a] Different letters within columns indicate significantly different values ($P < 0.05$).

a result of mixing conditions in the extractor. Optimum extraction yields were obtained at a flow rate at 4 kg/h for both lycopene and β-carotene. For lycopene the recovery value at a flow rate of 8 kg/h was not found to be significantly different from the value obtained at a 4 kg/h CO_2 flow rate. β-Carotene isolation was less than optimal at a flow rate of 8 kg/h.

Effect of Cosolvent Addition. With the optimized parameters (pressure = 300 bar; extraction time = 2 h; flow rate = 4 kg/h), both the cosolvent (ethanol) addition effect on the extraction yield of lycopene and β-carotene and the effect of temperature on the cosolvent effectiveness were investigated. It was found that effects of cosolvent addition on the yield of the supercritical extraction of lycopene and β-carotene varied with the temperature during the extraction. It appears that when too much ethanol is added, the extraction is hindered due to the decreased homogeneity of the extraction mixture.

The recoveries of lycopene and 3-carotene are shown in Tables 6 and 7, respectively. The highest lycopene and β-carotene yields were obtained with 55 °C temperature and 5% ethanol and 65 °C and 5% ethanol, respectively.

Brunner and Peter (1982) showed that the solubility of materials with low volatility is increased when using ethanol as entrainer instead of supercritical gas alone. According to Vega et al. (1996), the two main effects of ethanol are solubility enhancement and temperature dependence of such solubility enhancement.

Other research concerned with the isolation of carotenoids is found for the extraction of carrots by Barth et al. (1995). At a pressure of 400 bar and a temperature of 40 °C, 5% cosolvent ethanol yielded better extracts than when 10% is added to supercritical carbon dioxide at 300 bar. Also, Vega et al. (1996) extracted carotenes from carrot pulp using SC-CO_2 and ethanol as an entrainer and obtained recoveries of 50−65% with 5 and 10% ethanol.

Table 8. Optimal Extraction Conditions

	pressure (bar)	temp (°C)
extractor	300	65
separator 1	80	35
separator 2	1	35
CO_2 flow (kg/h)	4	
extraction time (h)	2	
cosolvent addition (EtOH %)	5	

As expected, higher recoveries were obtained by the addition of ethanol. Extractions without ethanol resulted in ≈25% recovery for lycopene and 45% recovery for β-carotene, whereas extractions with 5% ethanol resulted in 55% lycopene and 50% β-carotene recoveries.

CONCLUSIONS

Total or 100% recovery of supercritical extracted carotenes was not possible because of degradation of the product. Extractions in the absence of ethanol resulted in 20 and 40% recoveries of lycopene and β-carotene, respectively, whereas addition of 5% ethanol resulted in recoveries of both species of 50%.

It is necessary to mill the tomato paste waste to get a better extraction efficiency. When using the described apparatus for the extraction, it is not recommended to mill the waste to particles <2 mm. Such particles will block the steel mesh filters and the piping between extractor and separators.

The highest carotene yield of 3 mm milled tomato paste waste was obtained under the conditions given in Table 8.

It can be expected that temperatures >65 °C will give even higher extraction yields. On the other hand, this might cause an increase in carotene degradation. Carotenes have a high molecular weight and are difficult to extract with SC-CO_2 of relatively low density. The results presented here and the values found in the literature indicate that extraction pressures up to 400 bar can give higher yields.

LITERATURE CITED

Al-Wandawi, H.; Abdul-Rahman, M.; Al-Shaikhly, K. Tomato processing wastes as essential raw materials source. *J. Agric. Food Chem.* **1985**, *33*, 804−807.

Barth, M. M.; Zhou, C.; Kute, K. M.; Rosenthal, G. A. Determination of optimum conditions for supercritical fluid extraction of carotenoids from carrot (*Daucus carota* L.) tissue. *J. Agric. Food Chem.* **1995**, *43*, 2876−2878.

Birtigh, A.; Johannsen, M.; Brunner, G. Supercritical-fluid extraction of oil-palm components. *J. Supercrit. Fluids* **1995**, *8*, 46−50.

Block, G.; Patterson, B.; Subar, A. Fruit vegetables and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1−29.

Blot, W. J.; Li, J. Y.; Taylor, P. R. Nutrition intervention trials in Linxian, China: Supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J. Natl. Cancer Inst.* **1993**, *85*, 1483−1492.

Britton, G. Carotenoids. In *Natural Food Colorants*; Hendry, G. A. F., Houghton, J. D., Eds.; Blackie: Glasgow, U.K., 1992; p 280.

Brunner, G.; Peter, S. On the solubility of glycerides and fatty acids in compressed gases in the presence of an entrainer. *Separation Sci. Technol.* **1982**, *17* (1), 199−214.

Bureau, J. L.; Bushway, R. J. HPLC determination of carotenoids in fruits and vegetables in the United States. *J. Food Sci.* **1986**, *51* (1), 128−130.

Burton, G. W.; Ingold, K. U. β-carotene and unusual type of lipid antioxidant. *Science* **1984**, *224*, 569−573.

Chandler, L. A.; Schwartz, S. J. HPLC separation of cis−trans carotene isomers in fresh and processed fruits and vegetables. *J. Food Sci.* **1987**, *52* (3), 669−672.

Chao, R. R.; Mulvaney, S. J.; Sanson, D. R.; Hsieh, F.; Tempesta, M. S. Supercritical CO_2 extraction of Annatto (*Bixa orellana*) pigments and some characteristics of the color extracts. *J. Food Sci.* **1991**, *56* (1), 80−83.

Conett, J. E.; Kuller, L. H.; Kjelsberg, M. O.; Polk, B. F.; Collins, G.; Rider, A.; Hulley, S. B. Relationship between carotenoids and cancer. *Cancer* **1989**, *64*, 126−134.

Favati, F.; King, J. W.; Friedrich, J. P.; Eskins, K. Supercritical CO_2 extraction of carotene and lutein from leaf protein concentrates. *J. Food Sci.* **1988**, *53*, 1532−1536.

Gross, J. *Pigments in Fruits*; Academic Press: London, U.K., 1987.

Hakala, S. H.; Heinonen, I. M. Chromatographic purification of natural lycopene. *J. Agric. Food Chem.* **1994**, *42*, 1314−1316.

Heinonen, M. I.; Ollilainen, V.; Linkola, E. K.; Varo, P. T.; Koivistoinen, P. E. Carotenoids in Finnish foods: Vegetables, fruits, and berries. *J. Agric. Food Chem.* **1989**, *37*, 655−659.

Khachik, F.; Goli, M. B.; Beecher, G. R.; Holden, J.; Lusby, W. R.; Tenorio, M. D.; Barrera, M. R. Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *J. Agric. Food Chem.* **1992**, *40*, 390−398.

Kornhauser, A.; Wamer, W. G.; Lambert, L. A.; Wei, R. R. β-carotene inhibition of chemically induced toxicity in vivo and in vitro. *Food Chem. Toxicol.* **1994**, *32* (2), 149−154.

Krinsky, N. I. The evidence for the role of carotenes in preventative health. *Clin. Nutr.* **1988**, *7*, 107−112.

Marchand, L. L.; Hankin, J. H.; Kolonel, L. N.; Beecher, G. R.; Wilkens, L. R.; Zhao, L. P. Intake of specific carotenoids and lung cancer risk. *Cancer Epidemiol. Biomarkers Prevent.* **1993**, *2*, 183−187.

Marentis, R. T. Steps to developing a commercial supercritical carbon dioxide processing plant. In *Supercritical Fluid Extraction and Chromatography*; Carpentier, B. A., Sevenants, M. R., Eds.; ACS Symposium Series 366; American Chemical Society: Washington, DC, 1988; pp 127−143.

Sadler, G.; Davis, J.; Dezman, D. Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. *J. Food Sci.* **1990**, *55*, 1460−1461.

Sakaki, K. Solubility of β-carotene in dense carbondioxide from 308 to 323K and from 9.6 to 30 MPa. *J. Chem. Eng. Data* **1992**, *37*, 249−251.

Sharma, S. K.; Maguer, M. L. Lycopene in tomatoes and tomato pulp fractions. *Ital. J. Food Sci.* **1996**, *2*, 107−113.

Siemensma, A. D. *Natural Mixed Carotenoids and Supplementation of Foods*; Quest International: Bussum, The Netherlands, 1996.

Spanos, G. A.; Chen, H.; Schwartz, S. J. Supercritical CO_2 extraction of β-carotene from sweet potatoes. *J. Food Sci.* **1993**, *58*, 817−820.

Stich, H. F.; Anders, F. The involvement of reactive oxygen species in oral cancers of betel quid/tobacco chewers. *Mutat. Res.* **1989**, *214*, 47−61.

Tan, B. Analytical and preparative chromatography of tomato paste carotenoids. *J. Food Sci.* **1988**, *53* (3), 954−959.

Tonucci, L. H.; Holden, J. M.; Beecher, G. R.; Khachik, F.; Davis, C. S.; Mulokozi, G. Carotenoid content of thermally processed tomato-based food products. *J. Agric. Food Chem.* **1995**, *43*, 579−586.

Vega, P. J.; Balaban, M. O.; O'Keefe, S. F.; Cornell, J. A. Supercritical carbondioxide extraction efficiency for carotenes from carrots by RSM. *J. Food Sci.* **1996**, *61*, 757−765.

Williams, S. *Official Methods of Analysis of the Association of Official Analytical Chemists*; AOAC: Arlington, VA, 1984.

Received for review March 8, 2000. Revised manuscript received July 25, 2000. Accepted August 14, 2000.

JF000311T