JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Biosynthesis of ^{14}C-Phytoene from Tomato Cell Suspension Cultures (*Lycopersicon esculentum*) for Utilization in Prostate Cancer Cell Culture Studies

JESSICA K. CAMPBELL,[†] RANDY B. ROGERS,[‡] MARY ANN LILA,[†,‡] AND JOHN W. ERDMAN, JR.*[,†]

Division of Nutritional Sciences, University of Illinois at Urbana−Champaign, 905 South Goodwin Avenue, and Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana−Champaign, 1201 South Dorner Drive, Urbana, Illinois 61801

This work describes the development and utilization of a plant cell culture production approach to biosynthesize and radiolabel phytoene and phytofluene for prostate cancer cell culture studies. The herbicide norflurazon was added to established cell suspension cultures of tomato (*Lycopersicon esculentum* cv. VFNT cherry), to induce the biosynthesis and accumulation of the lycopene precursors, phytoene and phytofluene, in their natural isomeric forms (15-*cis*-phytoene and two *cis*-phytofluene isomers). Norflurazon concentrations, solvent carrier type and concentration, and duration of culture exposure to norflurazon were screened to optimize phytoene and phytofluene synthesis. Maximum yields of both phytoene and phytofluene were achieved after 7 days of treatment with 0.03 mg norflurazon/40 mL fresh medium, provided in 0.07% solvent carrier. Introduction of ^{14}C-sucrose to the tomato cell culture medium enabled the production of ^{14}C-labeled phytoene for subsequent prostate tumor cell uptake studies. In DU 145 prostate tumor cells, it was determined that 15-*cis*-phytoene and an oxidized product of phytoene were taken up and partially metabolized by the cells. The ability to biosynthesize, radiolabel, and isolate these carotenoids from tomato cell cultures is a novel, valuable methodology for further in vitro and in vivo investigations into the roles of phytoene and phytofluene in cancer chemoprevention.

KEYWORDS: Phytoene; phytofluene; carotenoids; tomato; *Lycopersicon esculentum*; prostate cancer; norflurazon

INTRODUCTION

An abundance of epidemiological evidence suggests that a diet rich in fruits and vegetables is associated with a reduced risk of several forms of cancer (*1*−*3*). Phytochemicals present in fruits and vegetables have been proposed to provide protection from carcinogenesis through various biological actions (*4*). With respect to tomatoes, increased consumption of tomatoes and tomato products has been significantly associated with a reduced risk of prostate cancer in several epidemiological studies (*5*). Carotenoids are natural yellow, orange, and red pigments present in tomatoes and possess a wide range of proposed biological functions, including antioxidant and anticarcinogen properties and immunoprotection (*6*). Lycopene, the most abundant tomato carotenoid (*7*), has been the primary focus of both in vitro and in vivo studies examining the relationship between increased intake of tomatoes and reduced risk of prostate cancer. However, recent studies suggest that other tomato phytochemicals may also modulate prostate cancer. We demonstrated that in an N-methyl-N-nitrosurea−androgen-induced rat carcinogenesis model, a diet containing whole tomato powder significantly inhibited the development of prostate cancer as compared to a control diet, whereas a lycopene-supplemented diet was not as effective (*8*). Small, short-term clinical studies with prostate cancer patients have found therapeutic benefits with tomato supplementation, including a reduction in prostate and leukocyte DNA damage, reduced prostate specific antigen levels, positively modulated volume and grade of prostate intraepithelial neoplasia, and altered biomarkers of prostate cell growth and differentiation (*9*, *10*). These studies indicate that tomato phytochemicals, in addition to lycopene, may also modify prostate carcinogenesis.

Phytoene and phytofluene, biosynthetic carotenoid precursors of lycopene, are also present in mature tomatoes, although approximately 5- and 11-fold less than that of lycopene, respectively (*7*). The predominant isomer of phytoene in tomatoes is 15-*cis*-phytoene, whereas several *cis*-phytofluene isomers are present in smaller quantities (*11*−*14*). Significant quantities of both phytoene and phytofluene have been detected in human serum and tissues including liver, lung, breast, skin,

* To whom correspondence should be addressed. Tel: 217-333-2527. Fax: 217-333-9368. E-mail: jwerdman@uiuc.edu.
[†] Division of Nutritional Sciences.
[‡] Department of Natural Resources and Environmental Sciences.

10.1021/jf0581269 CCC: $33.50
Published on Web 01/11/2006

colon, and prostate (*15*, *16*). With a 4 week dietary supplementation of tomato juice in healthy volunteers, mean changes from baseline plasma concentrations of phytoene and phytofluene were greater than that of lycopene, even though tomato juice concentrations of phytoene and phytofluene were less than that of lycopene (*15*). Furthermore, in rats fed a tomato carotenoid extract for 10 weeks, phytoene and phytofluene uptake by the liver were much greater than expected, based on the relatively low percent of these carotenoids in the tomato extract (*17*). Overall, these studies suggest preferential uptake of phytoene and phytofluene, as compared to lycopene. Additionally, both phytoene and phytofluene have demonstrated anticarcinogen and antioxidant properties in vitro (*18*−*20*). Because of the considerable quantities of phytoene and phytofluene in tomatoes, their relative bioavailability, and potential bioactivity, it is essential to begin elucidating the potential health benefits of these carotenoids. However, studies examining the roles of phytoene and phytofluene in prostate cancer prevention have been limited, primarily because these carotenoids are not commercially available, especially in their natural isomeric conformations, for in vitro or in vivo experiments.

To discern the biological roles of phytoene and phytofluene in prostate cancer inhibition, the pure compounds must be administered in the same natural isomeric forms found in tomatoes and tomato products. Through utilization of radiolabeled carotenoids, in vitro or in vivo metabolism studies can be conducted to quantify the extent to which carotenoids and their metabolites accumulate in the prostate (*21*, *22*). The overall goals of the current work were to develop a novel tomato cell culture system to produce and radiolabel 15-*cis*-phytoene and subsequently determine if 15-*cis*-phytoene could be taken up and metabolized by a human prostate cancer cell line, DU 145. Our first objective was to modify the biosynthesis of carotenoids in a tomato cell suspension culture system with the utilization of a phytoene desaturase inhibitor, norflurazon, to consequently accumulate 15-*cis*-phytoene and *cis*-isomers of phytofluene in vitro. Furthermore, it was our second objective to biosynthesize radiolabeled 15-*cis*-phytoene, by providing uniformly labeled ^{14}C-sucrose as a carbon source to the tomato cell suspension cultures, for subsequent isolation through reverse phase preparatory high-pressure liquid chromatography (HPLC) analysis. Finally, ^{14}C-15-*cis*-phytoene was utilized to evaluate the uptake and metabolism of 15-*cis*-phytoene by human prostate DU 145 cancer cells.

MATERIALS AND METHODS

Tomato Cell Suspension Culture and Norflurazon Treatment. Tomato cell suspension cultures were established using a method previously described (*23*). Tomato callus was induced from sepal explants of greenhouse-grown tomato plants, *Lycopersicon esculentum* cv. VFNT Cherry, on agar-solidified medium. The medium used for callus induction contained Murashige and Skoog basal salts (*24*), Nitsch's vitamins (*25*), myo-inositol (100 mg L^{-1}), and 3% sucrose, supplemented with plant growth regulators 2,4-dichlorophenoxyacetic acid (2 mg L^{-1}) and 6-benzylaminopurine (0.1 mg L^{-1}). Callus was subcultured by transferring 1 cm^3 portions to fresh media every 2 weeks. Once friable callus was obtained, approximately 2.0 g of callus was transferred to 40 mL of liquid suspension induction media, which was identical to callus induction media, contained in 125 mL Erlenmeyer flasks, and placed on a rotary shaker at 150 rpm. The resulting cell suspension cultures were maintained by transferring 6.0 mL aliquots of established culture (2.0 mL settled cell volume) to fresh media at 2 week intervals.

To induce carotenoid accumulation, cells were transferred to a production medium identical to liquid suspension induction medium but replacing the plant growth regulators with indole-3-acetic acid (5 mg L^{-1}) and all-*trans*-zeatin (2 mg L^{-1}). Cultures were maintained by subculturing at 2 week intervals to fresh liquid production media for the duration of the experiments. All phases of cell culture growth occurred at 24 °C with light exclusion to protect the carotenoids from photodegradation.

Norflurazon (a gift from Syngenta, Greensboro, NC) stock solutions were prepared in either ethanol or dimethyl sulfoxide (DMSO), filter sterilized, and aseptically added to liquid suspension cultures at final culture concentrations of 0.3, 0.03, or 0.003 mg/40 mL fresh media. The duration of culture exposure to norflurazon was for 3, 5, 7, or 9 days.

^{14}C-Labeling of Tomato Cell Suspension Cultures. To biosynthesize radiolabeled 15-*cis*-phytoene in vitro, the described carotenoid biosynthesis protocol was combined with radiolabeling techniques, which provide uniformly labeled ^{14}C-sucrose as a carbon source to cell suspension cultures (*26*, *27*). Cell cultures were placed in an enclosed Plexiglass labeling chamber, which was constructed to provide safe containment of any respired ^{14}C-radiolabeled CO_2 produced by the cell cultures through utilization of NaOH traps (*26*). Uniformly labeled ^{14}C-sucrose with a specific activity of 370 MBq/mmol (10 mCi/mmol) in a crystalline solid form (ICN Biomedicals Inc., Irvine, CA) was used as a source of radiolabel for the tomato cell cultures. ^{14}C-sucrose stock solutions were prepared in sterile double-distilled water (pH 5.7), and the stock solution was filter sterilized before medium incorporation. Concentrated media containing all components except ^{14}C-sucrose were prepared by bringing the media to 90% of the final volume. The media were then dispensed and autoclaved at 72 mL per 250 mL flask. After creating new cell suspension cultures by inoculating the concentrated media with 6 mL of cells and media from established cultures, 8 mL of the ^{14}C-sucrose stock solution was added bringing the final ^{14}C-radiolabel concentration to approximately 181.3 kBq/mL. Tomato cell cultures were then incubated in the enclosed chamber for 7 days, removed, and treated with norflurazon (0.06 mg/80 mL), and incubated again for another 7 days. All suspension cultures were grown on a 160 rpm rotary shaker at 24 °C in the dark. Two ^{14}C-labeled culture runs were conducted in the labeling chamber, each run containing 11 labeled and one unlabeled control culture flasks.

Tomato Cell Harvest. After a 2 week growth cycle, nonlabeled and ^{14}C-labeled cells were harvested and separated from the growth media using Whatman no. 4 filter paper and gentle vacuum until no liquid was expressed for 30 s. Collected cells were weighed and then frozen at −80 °C until extraction, to prevent degradation of the carotenoids.

Carotenoid Extraction of Tomato Cells. Carotenoids were extracted from nonlabeled and ^{14}C-labeled tomato cells by placement of 0.25−1.0 g of cells and 5 mL of ethanol, containing 0.1% butylated hydroxytoluene, into a 35 mL centrifuge tube. To promote disruption of cell membranes, samples were thoroughly mixed on a vortex, briefly homogenized (<5 s) on low speed (Kinematica PCU1; Brinkmann, Westbury, NY), and then placed in a water bath at 60 °C for 30 min, with intermittent mixing. Samples were then immediately placed on ice, and 2 mL of HPLC grade water was added to provide for separation of layers. Subsequently, 6 mL of hexane was added, and samples were mixed on a vortex for ∼30 s and then centrifuged for 10 min at 4 °C. The organic, hexane phase of the extract, containing the carotenoids, was removed and reserved. The process of hexane addition, mixing, and centrifugation was completed three times. Extracts were pooled and dried in a Speedvac concentrator (model AS160; Savant, Farmingdale, NY), flushed with argon, and stored at −80 °C.

High-Pressure Liquid Chromatography−Photodiode Array (HPLC-PDA) Analysis of Phytoene and Phytofluene. An HPLC-PDA analytical method was adapted from a method employed in our laboratory for the isolation of all-trans and cis isomers of lycopene (*22*, *28*). Samples from each carotenoid-enriched extract were dissolved in 32−40 μL of methyl-*tert*-butyl ether (MTBE) and injected onto a C30, 4.6 mm × 150 mm analytical column (YMC, Wilmington, NC) maintained at 25 °C. The HPLC mobile phases and gradient procedure utilized have been previously described (*28*). Qualitative and quantitative analysis was conducted with a HPLC-PDA system consisting of a Waters 991 detector (Millipore, Milford, MA) monitored at 200−600 nm, a Rainin Dynamics gradient pump system model SD-200 (Walnut

Creek, CA), and a Varian Prostar pump model 210 (Woburn, NC). Phytoene and phytofluene isomers were qualitatively identified through comparison to UV spectra (*11*, *29*) and retention times of analytical phytoene and phytofluene standards (BASF, Ludwigshafen, Germany). The major phytoene isomer and two distinct phytofluene isomers present in the norflurazon-treated tomato cells eluted at approximately 9.0, 10.5, and 12.0 min retention times, respectively. Quantification of phytoene and phytofluene was carried out with analytical standards (phytoene $\lambda_{max} = 286$ nm, $A_{1cm}^{1\%} = 1250$ in petroleum ether; phytofluene $\lambda_{max} = 348$ nm, $A_{1cm}^{1\%} = 1350$ in petroleum ether) (*30*).

Isolation of ^{14}C-15-*cis*-Phytoene from Harvested ^{14}C-Labeled Tomato Cells. ^{14}C-15-*cis*-phytoene was extracted and isolated through preparatory reverse phase, HPLC-PDA. Each ^{14}C-15-*cis*-phytoene-enriched extract was dissolved in 50 μL of MTBE and injected onto a C30 preparative column, S-5 micron (YMC), maintained at 25 °C. The HPLC-PDA system and HPLC mobile phases utilized are described above. The gradient procedure utilized for preparatory HPLC-PDA has been previously described (*31*). HPLC-PDA eluents containing ^{14}C-15-*cis*-phytoene were collected and dried in a Speedvac concentrator (model AS160; Savant), flushed with argon, and stored at −80 °C.

Analysis of ^{14}C in Samples. To measure the amount of radioactivity in the cells or extracts, a known amount of sample (μL or mg) was added to BioSafe II liquid scintillation cocktail (10 mL) (Research Products International Corp., Mount Prospect, IL), and the two components were thoroughly mixed on a vortex. Radioactivity was determined using a Beckman liquid scintillation counter, model LS-6500 (Bakersfield, CA).

Prostate Cancer Cell Culture. DU 145 human prostate adenocarcinoma cells were obtained from American Type Culture Collection (Rockville, MD). Low passage cells (<8 passages) were utilized, and cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium, with 0.25% glucose, 0.238% HEPES, 0.011% sodium pyruvate, 0.15% $NaHCO_3$, and 10% fetal bovine serum and kept in a humidified incubator at 37 °C with 5% CO_2. Cells were subcultured when 75 mm flasks were ∼90% confluent.

Evaluation of Uptake and Metabolism of ^{14}C-15-*cis*-Phytoene by DU 145 Cells. Prior to media incorporation, ^{14}C-labeled phytoene and nonlabeled phytoene were aliquoted into glass tubes to be used in culture media. Three flasks (25 cm^2) of DU 145 cells were plated at $\sim 1 \times 10^9$ cells/L and incubated for 2 days. On day 3, the conditioned media were removed and fresh media containing both ^{14}C-radiolabeled and unlabeled 15-*cis*-phytoene were added to the cells. The ^{14}C-labeled phytoene and nonlabeled phytoene were incorporated into the medium using THF, with a final solvent concentration of 0.1%. The conditioned media taken from each of the cell flasks were also incubated with ^{14}C-radiolabeled and unlabeled 15-*cis*-phytoene in a new, sterile flask (25 cm^2). Each flask of cells and conditioned medium contained ∼0.2 μmol/L 15-*cis*-phytoene, with a total radioactivity of ∼222 Bq. All flasks were incubated for an additional 2 days after carotenoid treatment. At harvest, medium was collected from all flasks, and cells were trypsinized and pelleted. All samples were flushed with argon, parafilmed, and stored in −80 °C in glass, screw cap tubes.

Extraction and HPLC-PDA Analysis of DU 145 Cells and Media. ^{14}C-Labeled phytoene treated DU 145 cells were extracted with the addition of 1 mL of ethanol, containing 0.1% butylated hydroxytoluene, to the cell pellet. Saturated KOH (200 μL) was added, and the mixture was vortexed and saponified for 20 min at 60 °C. After samples were cooled on ice, 200 μL of HPLC grade water was added. After equal amounts of hexane were added, the samples were vortexed thoroughly (30 s) and centrifuged at 4 °C at 2400 rpm for 3 min. The organic, hexane phase of the extract, containing the carotenoids, was removed and reserved. The process of hexane addition, mixing, and centrifugation was completed three times. Extracts were dried in a Speedvac concentrator (model AS160; Savant), flushed with argon, and stored at −80 °C. Culture media (1 mL) were extracted by adding 1 mL of ethanol, containing 0.1% butylated hydroxytoluene. Without saponification or KOH addition, the extraction procedure proceeded for culture media with the addition of equal volumes of hexane as stated above. Quantitative and qualitative analysis were conducted through reverse phase, HPLC-PDA analysis, as described above. HPLC-PDA eluent fractions were collected every 30 s in glass scintillation vials and evaluated for radioactivity using liquid scintillation counting.

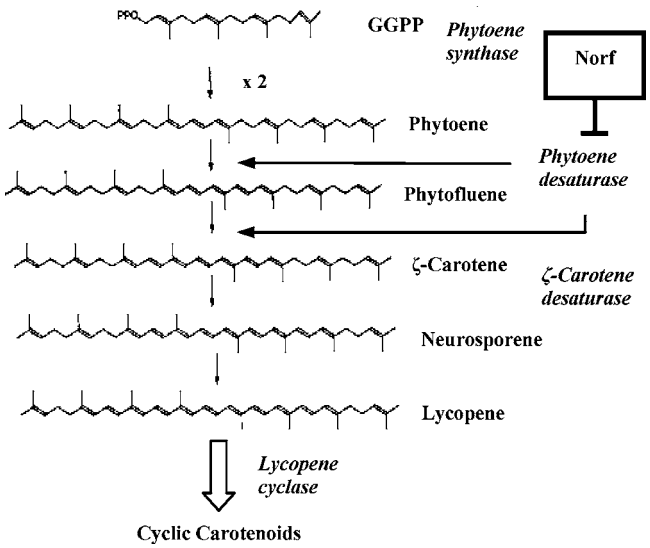

Figure 1. Inhibition of phytoene desaturase by norflurazon for elicitation of phytoene and phytofluene in tomato carotenoid biosynthesis. Phytoene desaturase is used in two consecutive desaturase reactions to insert two double bonds, thus ultimately converting phytoene to ζ-carotene, with phytofluene as an intermediate. Norflurazon (Norf) inhibits both desaturase reactions, thus eliciting phytoene and phytofluene production.

Statistical Analysis. The tomato cell suspension culture experiment examining norflurazon treatments was conducted in duplicate runs, each run contained 3−5 replicates of each treatment, and the data were analyzed together. For this study, mean differences were analyzed by one-way analysis of variance, and group mean comparisons were further analyzed by the posthoc least significant difference test with $\alpha = 0.01$. All statistical analyses were conducted with SAS (version 8.1; SAS Institute, Cary, NC). Results were expressed as means ± standard errors of the mean (SEM), unless otherwise indicated.

RESULTS

Production of Phytoene and Phytofluene in Tomato Cell Suspension Cultures. Figure 1 depicts the biosynthetic scheme of carotenoids in plants. The carotenoid biosynthetic enzyme, phytoene desaturase, causes two consecutive desaturation reactions to convert phytoene to ζ-carotene, with phytofluene as an intermediate product (*32*, *33*). Norflurazon is a known inhibitor of phytoene desaturase (*34*−*36*). Therefore, we hypothesized that through the provision of norflurazon to this tomato cell suspension culture system, this enzyme would be primarily blocked at the first desaturation step, thus ultimately eliciting phytoene production within the tomato cells. Subsequently, phytofluene was expected to accumulate to a lesser extent than phytoene in the tomato cells following norflurazon addition.

In preliminary trials, experiments were conducted to determine whether various concentrations of norflurazon and duration of culture exposure to norflurazon affected tomato cell yield of phytoene. Tomato cells were treated with norflurazon (0.3, 0.03, and 0.003 mg norflurazon/40 mL fresh media, with final media concentration of 0.29% ethanol) for 3, 5, 7, or 9 days. Results indicated that a norflurazon treatment of 0.03 mg norflurazon/40 mL fresh media for 7 days provided the highest phytoene yield (170.3 μg phytoene/L tomato cell suspension), as compared to the other treatments (data not shown). The addition of norflurazon to the tomato cell cultures was effective in the inhibition of phytoene desaturase, as no quantities of ζ-carotene or lycopene were detected in the treated cells. Data from these

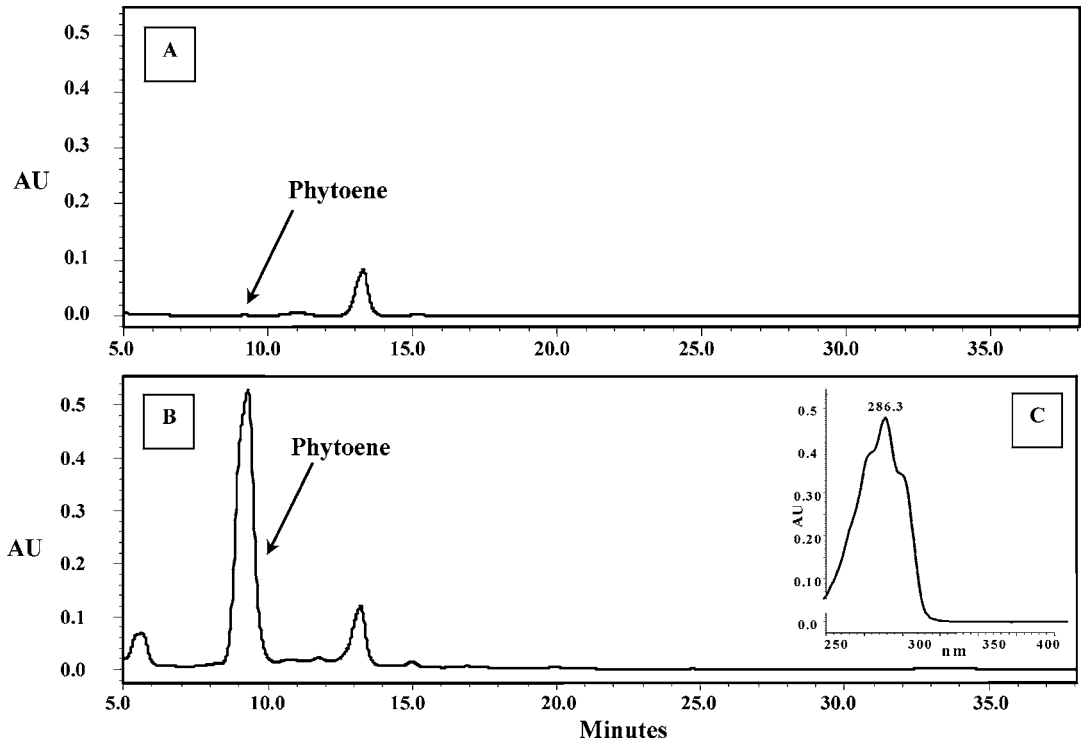

Figure 2. Representative HPLC chromatograms of tomato cell extracts measured at 286 nm of tomato cells treated (**A**) in the absence of norflurazon (control cells) or (**B**) with norflurazon (0.03 mg/40 mL fresh media). Phytoene is elicited with addition of norflurazon to tomato cells, as shown by the large peak with a retention time of approximately 9.0 min. (**C**) Representative UV spectrum of the phytoene isomer present in tomato cells treated with norflurazon (0.03 mg/40 mL fresh media). The spectrum has been identified as 15-*cis*-phytoene, the natural isomer of phytoene present in tomatoes.

preliminary trials were utilized to conduct further experiments in this tomato cell suspension culture system.

As shown in **Figure 2A**,**B**, HPLC separation and analysis of carotenoid extracts from control tomato cells at 286 nm wavelength showed very little production of phytoene; yet, when tomato cells were treated with norflurazon for 7 days (0.03 mg norflurazon/40 mL fresh media), there was a dramatic increase in phytoene accumulation, with a retention time of 9.0 min. The absorbance spectrum of the phytoene isomer present in norflurazon treated cells has inflections and maxima at 276.5, 286.3, and 298.2 nm (**Figure 2C**). Through comparison to analytical standards and a lesser pronounced inflection at 298.2 nm, the compound has been identified as 15-*cis*-phytoene (*11*, *29*, *30*).

Figure 3A,**B** illustrates similar results for phytofluene analysis, monitored at 348 nm wavelength. Extracts from control cells contained only trace quantities of phytofluene isomers, but when tomato cells were treated with norflurazon for 7 days (0.03 mg norflurazon/40 mL fresh media), there was a marked increase in two separate phytofluene isomers, with retention times of 10.5 and 12.0 min. **Figure 3C** shows two distinct phytofluene spectra obtained from cells treated with norflurazon, both with inflections and maxima at 331.5, 348.2, and 365.8 nm. Both isomers appear to be in the *cis*-conformation, due to the appearance of absorption peaks at the lower end of the spectra (∼250−290 nm).

Table 1 represents experiments conducted to determine whether different solvent concentrations or solvent carriers for delivery of the norflurazon have an influence on tomato cell yield of phytoene and phytofluene. Tomato cells were treated with norflurazon (0.03 mg norflurazon/40 mL fresh media) provided in either ethanol or DMSO (final solvent concentration of 0.07 or 0.29% in culture) and incubated for 7 days. Norflurazon treatment significantly increased both phytoene and phytofluene yields, as compared to the nontreated controls. Delivery of norflurazon in the DMSO carrier at the reduced solvent concentration (0.07%) produced the highest phytoene yield, as compared to the other treatments tested. As was the case for phytoene, the yield of phytofluene was enhanced when norflurazon was administered in a lower solvent concentration (0.07 vs 0.29% final solvent concentration). However, while the use of DMSO enhanced the production of phytoene, the type of solvent carrier did not significantly influence phytofluene yield. The norflurazon-treated tomato cells produced greater quantities of phytoene than phytofluene, which was expected.

Production of ^{14}C-Labeled 15-*cis*-Phytoene in Tomato Cell Suspension Cultures. Tomato cells grown for 14 days in the labeling chamber accumulated ∼17.5% of the administered ^{14}C-sucrose dose, as the majority of the ^{14}C-labeled dose remained in the filtered media (**Table 2**). The average total cell weight of the two runs (11 flasks each) was 60.6 (±4.8) g. The average phytoene concentration of the ^{14}C-labeled tomato cell cultures was 1.0 (±0.4) μg/g, and the specific activity of phytoene produced from the ^{14}C-labeled cultures was 407 Bq/μg (±111 Bq). The total recovery of ^{14}C-labeled 15-*cis*-phytoene from both runs was 40.5 μg of 15-*cis*-phytoene with a total radioactivity of 19.1 kBq.

^{14}C-Labeled 15-*cis*-Phytoene Uptake by DU 145 Prostate Tumor Cells. Approximately 23% of the administered 15-*cis*-phytoene was taken up by the DU 145 prostate tumor cells, whereas ∼59% remained in the cell media, as demonstrated by cell and media extract separation and analysis (**Table 3**). These results also suggest that ∼19% of the administered 15-*cis*-phytoene was degraded due to incubation conditions during the 2 day period. In contrast, the conditioned media (flasks without DU 145 cells) contained only ∼28% of the administered 15-*cis*-phytoene after 2 days of incubation, indicating a substantial degradation of 15-*cis*-phytoene in the absence of cells. The

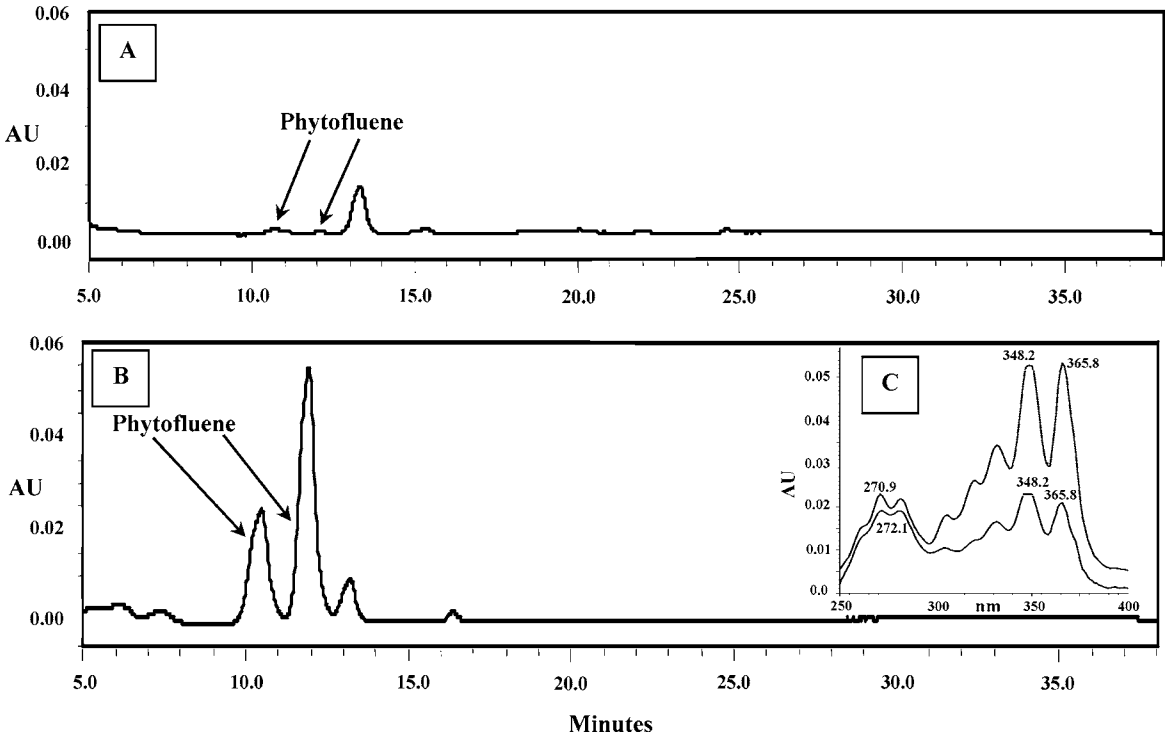

Figure 3. Representative HPLC chromatograms of tomato cell extracts measured at 348 nm of tomato cells treated (**A**) in the absence of norflurazon (control cells) or (**B**) with norflurazon (0.03 mg/40 mL fresh media). (**C**) Two distinct phytofluene isomers are produced with addition of norflurazon to tomato cells, as shown by two separate peaks with retention times of approximately 10.5 and 12.0 min. The spectra displays two separate *cis*-phytofluene isomers, also naturally present in tomatoes.

Table 1. Effects of Different Concentrations of Norflurazon Carrier (Ethanol or DMSO) at a Final Culture Concentration of 0.07 or 0.29% on Yield of Phytoene and Phytofluene

treatment		
0.03 mg norflurazon/ 40 mL culture, 7 days	μg/L suspension culture	
	phytoene[a]	phytofluene[a]
control	5.1 ± 0.8 d	6.1 ± 0.8 d
control (ethanol, 0.07%)	4.8 ± 0.6 d	5.4 ± 0.7 d
control (ethanol, 0.29%)	5.3 ± 1.2 d	7.3 ± 2.3 d
control (DMSO, 0.07%)	3.9 ± 0.5 d	5.1 ± 0.7 d
control (DMSO, 0.29%)	3.4 ± 0.5 d	4.4 ± 0.8 d
norflurazon (ethanol, 0.07%)	522.3 ± 83.5 b	100.9 ± 15.5 b
norflurazon (ethanol, 0.29%)	230.6 ± 15.3 c	51.8 ± 6.2 c
norflurazon (DMSO, 0.07%)	633.7 ± 99.2 b	88.2 ± 18.0 b,c
norflurazon (DMSO, 0.29%)	523.4 ± 59.9 b	83.4 ± 9.9 b,c

[a] Values are mean yields ± SEM; $n = 6$–9. Means within a column without a common letter are statistically different; $P < 0.01$.

Table 2. Percent Distribution of ^{14}C in Cells, Media, or Respired CO_2 Following Growth of Tomato Cell Suspension Cultures in a Labeling Chamber on Media Containing ^{14}C-Sucrose for 14 Days

sample	% of ^{14}C-sucrose dose
final ^{14}C distribution[a]	
cell mass	17.5 ± 3.1
media remainder	69.9 ± 4.9
respiration	12.6 ± 1.8

[a] Values are mean percentages ± SD of total administered radioactivity in two runs (166.5 MBq ^{14}C dose per run).

accumulation of 15-*cis*-phytoene in cells and media was confirmed through detection of the characteristic absorption spectrum of 15-*cis*-phytoene within the cell and media extracts (data not shown) (*11*, *29*, *30*).

Table 3. Percent Distribution of 15-*cis*-Phytoene in DU 145 Prostate Tumor Cells, Cell Media, or Degraded in Flasks Incubated with ^{14}C-Radiolabeled 15-*cis*-Phytoene for 2 Days

sample	% of 15-*cis*-phytoene dose
incubated flasks with DU 145 cells[a]	
DU 145 cells	22.8 ± 4.7
cell media	58.6 ± 12.9
degradation	17.4 ± 6.9
incubated flasks with conditioned media[a]	
media	28.1 ± 4.7
degradation	71.9 ± 4.7

[a] Values are mean percentages ± SEM of total administered 15-*cis*-phytoene (0.2 μmol/L); $n = 3$ flasks.

Prior to culture administration of ^{14}C-labeled 15-*cis*-phytoene, there was a small quantity of ^{14}C-labeled 15-*cis*-phytoene unintentionally degraded to an oxidized ^{14}C-labeled phytoene product (**Figure 4A**). Because this product was in such small quantities, it was not detected by HPLC-PDA analysis (data not shown). Liquid scintillation counting analysis shows the presence of ^{14}C-labeled 15-*cis*-phytoene in the DU 145 cell extract, as well as oxidized or metabolic ^{14}C-labeled polar products of phytoene (**Figure 4B**). These polar products were identified based on relative elution times, in addition to what has been reported for lycopene metabolism, a similar acyclic carotenoid (*22*, *31*). The oxidized ^{14}C-labeled phytoene product present in the administered dose appeared to increase through the 2 day incubation period, as seen in the conditioned media (flasks without DU 145 cells) (**Figure 4C**). Surprisingly, this oxidized product was completely absent in the media from flasks containing DU 145 cells (**Figure 4D**), suggesting that DU 145 cells took up this oxidized product, in addition to 15-*cis*-phytoene.

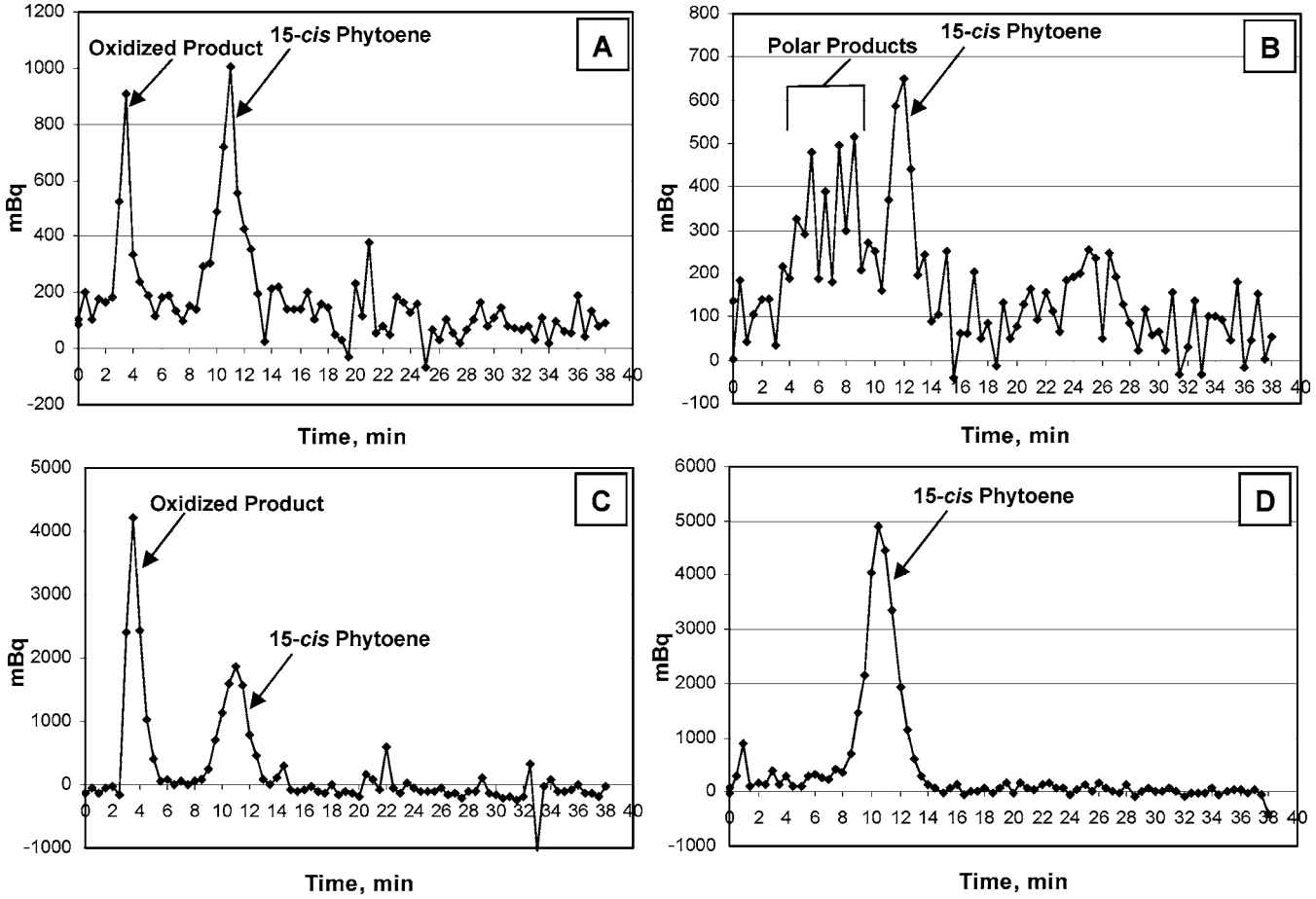

Figure 4. Distribution of radioactivity in (**A**) the media with added ^{14}C-labeled phytoene prior to incubation, (**B**) the DU 145 cellar extract following 2 days of incubation with ^{14}C-labeled phytoene, (**C**) the media incubated with ^{14}C-labeled phytoene for 2 days in the absence of DU 145 cells, and (**D**) the media incubated with ^{14}C-labeled phytoene for 2 days in the presence of DU 145 cells. Pooled extracts of media and cells were separated by HPLC, collected in 30 s aliquots, and analyzed for radioactivity. An oxidized product of 15-*cis*-phytoene eluted at ~3 min, unidentified polar metabolic products eluted at ~4–8 min, and 15-*cis*-phytoene eluted at ~11 min.

DISCUSSION

Results from this work demonstrate that the addition of norflurazon to tomato cell cultures significantly enhances production of both phytoene and phytofluene, as compared to the control tomato cells. Although other methods have been developed previously for lycopene production under artificial laboratory or in vitro conditions (*23*, *37*−*39*), to our knowledge, this is the first reported method specifically designed for phytoene and phytofluene biosynthesis using tomato cell cultures. The carotenoids produced by tomato cells treated with norflurazon have been identified as 15-*cis*-phytoene and two *cis*-phytofluene isomers. The exact locations of cis-double bonds within natural *cis*-phytofluene isomers have not been adequately reported in the literature. It is important to note that 15-*cis*-phytoene is the major natural phytoene isomer present in tomato fruit (*11*−*14*), while several *cis*-phytofluene isomers also naturally accumulate in the tomato (*11*−*14*, *40*). Thus, the application of this methodology makes available, for the first time, a source of these carotenoids in the same natural, isomeric conformations present in tomatoes for additional studies. As predicted, the quantity of phytoene produced from the treated cells was far greater than that of phytofluene; thus, further experiments were focused on production and isolation of radiolabeled 15-*cis*-phytoene.

Previous cell suspension culture studies have established that ~30 and ~34% of the initially supplied ^{14}C-label is effectively incorporated into cell systems of grape (*Vitis vinifera*) and ohelo (*Vaccinium pahalae*), respectively (*26*), whereas ~14−15% of the administered radioactivity was recovered in the flavonoid-rich fractions of these cultures (*27*). Although the efficiency of radiolabeling 15-*cis*-phytoene in this work was less than that of ^{14}C-flavonoid production in other systems, these studies provided sufficient quantities of ^{14}C-labeled 15-*cis*-phytoene for prostate cancer cell uptake studies. Further studies will be conducted by our laboratory to more effectively incorporate the initially supplied ^{14}C-label into this tomato cell culture system, with the goal of increasing the efficiency of ^{14}C-label incorporation into 15-*cis*-phytoene.

To begin elucidating the role of 15-*cis*-phytoene in prostate cancer prevention, it is essential to initially determine if this carotenoid isomer or its metabolites accumulate in prostate cells. Thus, ^{14}C-labeled 15-*cis*-phytoene biosynthesized from tomato cell cultures was further utilized to determine the potential cellular uptake and metabolism of 15-*cis*-phytoene by DU 145 prostate tumor cells. Results from these studies indicate that prostate tumor cells took up ~23% of the administered 15-*cis*-phytoene over a 2 day period. The accumulation of 15-*cis*-phytoene in the tumor cells was confirmed through detection of the characteristic absorption spectrum of the isomer within the cell extract.

In addition, data from this work suggest that prostate tumor cells took up an oxidized product of 15-*cis*-phytoene originally present in the administered dose. Unfortunately, because 15-*cis*-phytoene is highly labile, there was some unintended

degradation of ^{14}C-labeled 15-*cis*-phytoene to an oxidized ^{14}C-labeled phytoene product prior to culture administration of the dose. Because of small quantities, this oxidized product had not been detected through HPLC-PDA analysis. This oxidized ^{14}C-labeled product increased through the 2 day incubation period, as seen in the conditioned media (flasks without DU 145 cells), suggesting increased oxidation of 15-*cis*-phytoene due to incubation conditions. To our surprise, this ^{14}C-labeled oxidized product of phytoene was completely absent in the cell media of the flasks containing prostate tumor cells, suggesting that DU 145 cells took up this oxidized product, in addition to 15-*cis*-phytoene. Furthermore, analyses also illustrate the presence of several unidentified polar ^{14}C-labeled products of phytoene in the cancer cells, likely as a result of the metabolism of ^{14}C-labeled 15-*cis*-phytoene and/or the ^{14}C-labeled oxidized phytoene product present in the administered dose. Because of small quantities of these polar products, decipherable spectra of these metabolic products could not be obtained. Future work will be conducted to more effectively examine the potential metabolism of this compound in this prostate cell culture line and others.

Although limited prior work has been conducted, antioxidant and cancer protective effects of phytoene have been shown with the establishment of phytoene-producing mammalian cells through the transfection of crtB gene, which encodes phytoene synthase, into mouse embryo-derived NIH3T3 fibroblast cells (*18*). In this culture system, phytoene demonstrated a 46% cellular reduction in phospholipid hydroperoxidation when compared to the control cells, thereby protecting the cells against oxidative cellular stress (*18*). In addition, the phytoene-producing cells had a decreased formation of transformed foci induced by oncogene H-*ras* when compared to the control cells, thus demonstrating the potential anticancer effects of phytoene (*18*). However, treatment of prostate cancer cell lines, including PC-3, DU 145, and LNCaP, with phytoene (5, 10, or 20 μmol/L) did not significantly reduce cell growth, suggesting that phytoene may not directly affect cell proliferation (*19*).

Various carotenoid metabolites have been identified in human serum, milk, and prostate (*10*, *16*). In addition, previous results from our laboratory indicate the presence of polar lycopene metabolites in hepatic and extrahepatic tissues, including the prostate and seminal vesicles (*22*), and recent data suggest that polar metabolites of lycopene increase over time in the prostate and seminal vesicles (*31*). Although the overall biological significance remains to be determined, in vitro studies suggest that oxidation products of carotenoids may activate the promoter region of the retinoic acid receptor-β gene (*41*), enhance gap junctional communication (*42*), and induce cellular differentiation (*43*). More specifically, oxidized mixtures of phytofluene, ζ-carotene, and lycopene more strongly inhibited cancer cell growth in HL-60 and LNCaP cells than the intact carotenoids, and this growth inhibition was associated with the induction of apoptosis (*20*, *44*). Collectively, these various studies suggest that oxidative products of carotenoids may have a more profound impact on cancer cells; yet, similar effects for phytoene oxidative products have not been adequately studied (*20*).

Because of the potential accumulation of phytoene polar products, as demonstrated in our work, biological effects of phytoene oxidative products warrant further investigation. It is important to note that although precisely controlled cell culture conditions allow for the potential characterization of cellular and molecular processes modulated by carotenoids, one must take caution when directly correlating in vitro results to in vivo activity. Furthermore, it is essential to quantify phytoene, phytofluene, and their metabolites in the prostate in vivo before a mechanism of action for these carotenoids can be elucidated under in vitro conditions.

It is presently unclear the extent to which phytoene, phytofluene, and their metabolites accumulate in the prostate in vivo. Fisher-344 rats supplemented with a tomato carotenoid extract for 10 weeks had an accumulation of phytoene and phytofluene in the liver, and the uptake of these carotenoids by the liver, relative to lycopene, was much higher than expected, based on the relatively low percent of these compounds in the tomato extract (*17*). Given that the research focus of that work was centered on lycopene, phytoene and phytofluene analysis of the prostate or other tissues was not conducted. Another study demonstrated that female Sprague−Dawley rats fed a phytoene and phytofluene-containing algae diet for 2 weeks accumulate phytoene and phytofluene in the plasma, liver, adrenal, kidney, and spleen (*45*); yet, because this study utilized female rats, analysis of the prostate was not possible. In addition, the algal carotenoid isomers fed to these rats were not the same as the natural phytoene or phytofluene isomers found in tomatoes, as the study utilized all-trans and 9-cis isomers of both phytoene and phytofluene. Because it is known that the type of carotenoid isomers fed can have a profound effect on tissue accumulation of carotenoids (*46*−*48*), it is essential that natural isomeric forms of tomato carotenoids be provided to the animals when assessing metabolic or health effects.

In conclusion, we have successfully developed a novel approach to produce naturally accumulating tomato isomers of phytoene and phytofluene in tomato cell suspension cultures through the incorporation of a phytoene desaturase inhibitor to the culture medium. Moreover, we have biosynthesized and isolated ^{14}C-labeled 15-*cis*-phytoene through this culture system. Preliminary data from a human prostate tumor cell type suggest that indeed 15-*cis*-phytoene is taken up by cells, as well as a potential oxidized product of 15-*cis*-phytoene, and are apparently metabolized to unidentified polar phytoene metabolites. Through biosynthesizing naturally accumulating isomers of radiolabeled phytoene and phytofluene through in vitro plant techniques, further in vitro and subsequent in vivo bioavailability and metabolism experiments can be conducted (*21*, *22*). Only then can studies begin to adequately evaluate the potential roles of phytoene, phytofluene, and/or their metabolites in health, including prostate cancer prevention.

ABBREVIATIONS USED

HPLC, high-pressure liquid chromatography; HPLC-PDA, high-pressure liquid chromatography−photodiode array.

ACKNOWLEDGMENT

We appreciate the donation of tomato seed by Dr. Betty Ishida, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture (Albany, CA). Technical grade norflurazon was kindly donated by Syngenta (Greensboro, NC). Analytical standards of phytoene and phytofluene were generously provided by BASF (Ludwigshafen, Germany) for quantitative and qualitative analysis.

LITERATURE CITED

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

(2) Steinmetz, K. A.; Potter, J. D. Vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes Control* **1991**, *2*, 325−57.

(3) *World Cancer Research Fundamentals*; American Institute for Cancer Research: Washington, DC, 1997.
(4) Dorai, T.; Aggarwal, B. B. Role of chemopreventive agents in cancer therapy. *Cancer Lett.* **2004**, *215*, 129−140.
(5) Miller, E. C.; Giovannucci, E.; Erdman, J. W., Jr.; Bahnson, R.; Schwartz, S. J.; Clinton, S. K. Tomato products, lycopene, and prostate cancer risk. *Urol. Clin. North Am.* **2002**, *29*, 83−93.
(6) Boileau, T. W. M.; Moore, A. C.; Erdman, J. W., Jr. Carotenoids and vitamin A. In *Antioxidant Status, Diet, Nutrition, and Health*; Papas, A. M., Ed.; CRC Press LLC: Boca Raton, 1999; pp 133−158.
(7) Beecher, G. R. Nutrient content of tomatoes and tomato products. *Proc. Soc. Exp. Biol. Med.* **1998**, *218*, 98−100.
(8) Boileau, T. W.; Liao, Z.; Kim, S.; Lemeshow, S.; Erdman, J. W., Jr.; Clinton, S. K. Prostate carcinogenesis in *N*-methyl-*N*-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. *J. Natl. Cancer Inst.* **2003**, *95*, 1578−1586.
(9) Chen, L.; Stacewicz-Sapuntzakis, M.; Duncan, C.; Sharifi, R.; Ghosh, L.; van Breemen, R.; Ashton, D.; Bowen, P. E. Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. *J. Natl. Cancer Inst.* **2001**, *93*, 1872−1879.
(10) Kucuk, O.; Sarkar, F. H.; Sakr, W.; Djuric, Z.; Pollak, M. N.; Khachik, F.; Li, Y. W.; Banerjee, M.; Grignon, D.; Bertram, J. S.; Crissman, J. D.; Pontes, E. J.; Wood, D. P., Jr. Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. *Cancer Epidemiol. Biomarkers Prev.* **2001**, *10*, 861−868.
(11) Jungalwala, F. B.; Porter, J. W. The configuration of phytoene. *Arch. Biochem. Biophys.* **1965**, *110*, 291−299.
(12) Andersson, P. O.; Takaichi, S.; Cogdell, R. J.; Gillbro, T. Photophysical characterization of natural *cis*-carotenoids. *Photochem. Photobiol.* **2001**, *74*, 549−557.
(13) Britton, G.; Liaaen-Jensen, S.; Pfander, H. Natural carotenoids. In *Carotenoids: Handbook*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhauser Verlag: Basel, 2004; pp 42−45.
(14) 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.
(15) Paetau, I.; Khachik, F.; Brown, E. D.; Beecher, G. R.; Kramer, T. R.; Chittams, J.; Clevidence, B. A. Chronic ingestion of lycopene-rich tomato juice or lycopene supplements significantly increases plasma concentrations of lycopene and related tomato carotenoids in humans. *Am. J. Clin. Nutr.* **1998**, *68*, 1187−1195.
(16) Khachik, F.; Carvalho, L.; Bernstein, P. S.; Muir, G. J.; Zhao, D. Y.; Katz, N. B. Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. *Exp. Biol. Med.* **2002**, *227*, 845−851.
(17) Zhao, Z.; Khachik, F.; Richie, J. P., Jr.; Cohen, L. A. Lycopene uptake and tissue disposition in male and female rats. *Proc. Soc. Exp. Biol. Med.* **1998**, *218*, 109−114.
(18) Nishino, H. Cancer prevention by natural carotenoids. *J. Cell. Biochem. Suppl.* **1997**, *27*, 86−91.
(19) Kotake-Nara, E.; Kushiro, M.; Zhang, H.; Sugawara, T.; Miyashita, K.; Nagao, A. Carotenoids affect proliferation of human prostate cancer cells. *J. Nutr.* **2001**, *131*, 3303−3306.
(20) Nara, E.; Hayashi, H.; Kotake, M.; Miyashita, K.; Nagao, A. Acyclic carotenoids and their oxidation mixtures inhibit the growth of HL-60 human promyelocytic leukemia cells. *Nutr. Cancer* **2001**, *39*, 273−283.
(21) Williams, A. W.; Boileau, T. W.; Zhou, J. R.; Clinton, S. K.; Erdman, J. W., Jr. Beta-carotene modulates human prostate cancer cell growth and may undergo intracellular metabolism to retinol. *J. Nutr.* **2000**, *130*, 728−732.
(22) Zaripheh, S.; Boileau, T. W.; Lila, M. A.; Erdman, J. W., Jr. [14C]-lycopene and [14C]-labeled polar products are differentially distributed in tissues of F344 rats prefed lycopene. *J. Nutr.* **2003**, *133*, 4189−4195.
(23) Robertson, G. H.; Mahoney, N. E.; Goodman, N.; Pavlath, A. E. Regulation of lycopene formation in cell suspension culture of VFNT tomato (*Lycopersicon esculentum*) by CPTA, growth regulators, sucrose, and temperature. *J. Exp. Bot.* **1995**, *46*, 667−673.
(24) Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant* **1962**, *15*, 473−497.
(25) Nitsch, J. P.; Nitsch, C. Haploid plants from pollen grains. *Science* **1969**, *163*, 85−87.
(26) Grusak, M. A.; Rogers, R. B.; Yousef, G. G.; Erdman, J. W., Jr.; Lila, M. A. An enclosed-chamber labeling system for the safe 14C-enrichment of phytochemicals in plant cell suspension cultures. *In Vitro Cell. Dev. Biol.: Plant* **2004**, *40*, 80−85.
(27) Yousef, G. G.; Seigler, D. S.; Grusak, M. A.; Rogers, R. B.; Knight, C. T.; Kraft, T. F.; Erdman, J. W., Jr.; Lila, M. A. Biosynthesis and characterization of 14C-enriched flavonoid fractions from plant cell suspension cultures. *J. Agric. Food Chem.* **2004**, *52*, 1138−1145.
(28) Yeum, K. J.; Booth, S. L.; Sadowski, J. A.; Liu, C.; Tang, G.; Krinsky, N. I.; Russell, R. M. Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. *Am. J. Clin. Nutr.* **1996**, *64*, 594−602.
(29) Neudert, U.; Martinez-Ferez, I. M.; Fraser, P. D.; Sandmann, G. Expression of an active phytoene synthase from *Erwinia uredovora* and biochemical properties of the enzyme. *Biochim. Biophys. Acta* **1998**, *1392*, 51−58.
(30) Britton, G. UV/visible spectroscopy. In *Carotenoids: Spectroscopy*; Britton, G., Liaaen-Jensen, S., Pfander, H., Ed.; Birkhauser: Basel, 1995; pp 13−63.
(31) Zaripheh, S.; Erdman, J. W., Jr. The biodistribution of a single oral dose of [14C]-lycopene in rats pre-fed either a control or lycopene-enriched diet. *J. Nutr.* **2005**, *135*, 2212−2218.
(32) Hirschberg, J. Carotenoid biosynthesis in flowering plants. *Curr. Opin. Plant Biol.* **2001**, *4*, 210−218.
(33) Bramley, P. M. Regulation of carotenoid formation during tomato fruit ripening and development. *J. Exp. Bot.* **2002**, *53*, 2107−2113.
(34) Wakabayashi, K.; Boger, P. Target sites for herbicides: entering the 21st century. *Pest Manage. Sci.* **2002**, *58*, 1149−1154.
(35) Giuliano, G.; Bartley, G. E.; Scolnik, P. A. Regulation of carotenoid biosynthesis during tomato development. *Plant Cell* **1993**, *5*, 379−387.
(36) Simkin, A. J.; Breitenbach, J.; Kuntz, M.; Sandmann, G. In vitro and in situ inhibition of carotenoid biosynthesis in *Capsicum annuum* by bleaching herbicides. *J. Agric. Food Chem.* **2000**, *48*, 4676−4680.
(37) Ishida, B. K.; Mahoney, N. E.; Ling, L. C. Increased lycopene and flavor volatile production in tomato calyces and fruit cultured in vitro and the effect of 2-(4-chlorophenylthio)triethylamine. *J. Agric. Food Chem.* **1998**, *46*, 4577−4582.
(38) Fosket, D. E.; Radin, D. N. Induction of carotenogenesis in cultured cells of *Lycopersicon esculentum* [Tomatoes]. *Plant Sci. Lett.* **1983**, *30*, 165−175.
(39) Chang, Y. H.; Raymundo, L. C.; Glass, R. W.; Simpson, K. L. Effect of high temperature on CPTA [2-(4-Chlorophenylthio)-Triethylamine Hydrochloride]-induced carotenoid biosynthesis in ripening tomato fruits. *J. Agric. Food Chem.* **1977**, *25*, 1249−1251.
(40) Britton, G.; Liaaen-Jensen, S.; Pfander, H. *Carotenoids: Handbook*; Birkhauser Verlag: Basel, Boston, 2004; pp 42−45.
(41) Nikawa, T.; Schulz, W. A.; van den Brink, C. E.; Hanusch, M.; van der Saag, P.; Stahl, W.; Sies, H. Efficacy of all-trans-beta-carotene, canthaxanthin, and all-trans-, 9-cis-, and 4-oxoretinoic acids in inducing differentiation of an F9 embryonal carcinoma RAR beta-lacZ reporter cell line. *Arch. Biochem. Biophys.* **1995**, *316*, 665−672.
(42) Stahl, W.; von Laar, J.; Martin, H. D.; Emmerich, T.; Sies, H. Stimulation of gap junctional communication: Comparison of acyclo-retinoic acid and lycopene. *Arch. Biochem. Biophys.* **2000**, *373*, 271−274.

(43) Duitsman, P. K.; Barua, A. B.; Becker, B.; Olson, J. A. Effects of epoxycarotenoids, beta-carotene, and retinoic acid on the differentiation and viability of the leukemia cell line NB4 *in vitro*. *Int. J. Vitam. Nutr. Res.* **1999**, *69*, 303−308.

(44) Nagao, A. Oxidative conversion of carotenoids to retinoids and other products. *J. Nutr.* **2004**, *134*, 237S−240S.

(45) Werman, M. J.; Mokady, S.; Ben-Amotz, A. Bioavailability of the isomer mixture of phytoene and phytofluene-rich alga *Dunaliella bardawil* in rat plasma and tissues. *J. Nutr. Biochem.* **2002**, *13*, 585−591.

(46) Boileau, A. C.; Merchen, N. R.; Wasson, K.; Atkinson, C. A.; Erdman, J. W., Jr. *Cis*-lycopene is more bioavailable than *trans*-lycopene *in vitro* and *in vivo* in lymph-cannulated ferrets. *J. Nutr.* **1999**, *129*, 1176−1181.

(47) Boileau, T. W.; Boileau, A. C.; Erdman, J. W., Jr. Bioavailability of all-trans and *cis*-isomers of lycopene. *Exp. Biol. Med.* **2002**, *227*, 914−919.

(48) Deming, D. M.; Teixeira, S. R.; Erdman, J. W., Jr. All-trans beta-carotene appears to be more bioavailable than 9-cis or 13-cis beta-carotene in gerbils given single oral doses of each isomer. *J. Nutr.* **2002**, *132*, 2700−2708.

Received for review July 20, 2005. Revised manuscript received November 18, 2005. Accepted November 21, 2005. This work was supported in part by USDA/IFAFS Grant 00-52101-9695.

JF0581269