JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

Combined Pressure−Temperature Effects on Carotenoid Retention and Bioaccessibility in Tomato Juice

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ABSTRACT: This study highlights the changes in lycopene and β-carotene retention in tomato juice subjected to combined pressure−temperature (P-T) treatments ((high-pressure processing (HPP; 500−700 MPa, 30 °C), pressure-assisted thermal processing (PATP; 500−700 MPa, 100 °C), and thermal processing (TP; 0.1 MPa, 100 °C)) for up to 10 min. Processing treatments utilized raw (untreated) and hot break (∼93 °C, 60 s) tomato juice as controls. Changes in bioaccessibility of these carotenoids as a result of processing were also studied. Microscopy was applied to better understand processing-induced microscopic changes. TP did not alter the lycopene content of the tomato juice. HPP and PATP treatments resulted in up to 12% increases in lycopene extractability. *all-trans*-β-Carotene showed significant degradation ($p < 0.05$) as a function of pressure, temperature, and time. Its retention in processed samples varied between 60 and 95% of levels originally present in the control. Regardless of the processing conditions used, <0.5% lycopene appeared in the form of micelles (<0.5% bioaccessibility). Electron microscopy images showed more prominent lycopene crystals in HPP and PATP processed juice than in thermally processed juice. However, lycopene crystals did appear to be enveloped regardless of the processing conditions used. The processed juice (HPP, PATP, TP) showed significantly higher ($p < 0.05$) *all-trans*-β-carotene micellarization as compared to the raw unprocessed juice (control). Interestingly, hot break juice subjected to combined P-T treatments showed 15−30% more *all-trans*-β-carotene micellarization than the raw juice subjected to combined P-T treatments. This study demonstrates that combined pressure−heat treatments increase lycopene extractability. However, the in vitro bioaccessibility of carotenoids was not significantly different among the treatments (TP, PATP, HPP) investigated.

KEYWORDS: high-pressure processing, pressure-assisted thermal processing, thermal processing, tomato, lycopene, β-carotene, retention, micellarization, bioaccessibility, electron microscopy, light microscopy

INTRODUCTION

Dietary intake of lycopene (80% of which is derived from tomato and tomato products) has been shown to be inversely correlated with the risk of certain types of cancer and chronic diseases.[1−3] However, lycopene ($C_{40}H_{56}$) with 11 conjugated double bonds is particularly susceptible to oxidative degradation and isomerization upon exposure to light, oxygen, elevated temperatures, extremes in pH, and active surfaces.[4]

Combined pressure−temperature (P-T) treatments can be utilized for food pasteurization (high-pressure processing (HPP); 400−600 MPa treatment at chilled or mild process temperatures) and sterilization (pressure-assisted thermal processing (PATP); 500−700 MPa, 90−120 °C).[5,6] These technologies help food processors produce quality foods with minimal effects on taste, texture, appearance, or nutritional value.

Although lycopene is fairly stable to degradation and isomerization during conventional thermal processing,[4,7] high-pressure processing has shown an increase in the lycopene extractability (i.e., the amount of carotenoid extracted from the product matrix after processing) from tomato products.[8−10] Krebbers et al.[9] studied the fate of lycopene in tomato puree subjected to high pressure (300, 500, and 700 MPa at 20 °C for 2 min) and combined pressure−thermal treatments (700 MPa, 90 °C for 30 s) and found that such treatments increased the amount of extractable lycopene from the tomato puree. Similar results were reported for high-pressure processing of tomato puree (100−600 MPa, 20 °C, 12 min) by Qiu et al.[8] and for tomato juice (300−500 MPa, 25 °C, 10 min) by Hsu et al.[10] However, none of the prior studies investigated the effects of different juice-processing methods (raw juice vs hot break juice) on carotenoids. In addition, the impact of combined pressure−heat treatments (at elevated process temperatures, >60 °C) on carotenoids is not well understood. Hence, a comprehensive study is needed to understand the changes to carotenoids during combined P-T processing. In addition, a microscopy study of changes in the microstructure has been performed to assist in identifying factors that may be responsible for increased lycopene extractability after HPP and PATP treatments.

Understanding how novel processing technologies affect the ability of the body to obtain vitamins and other compounds from a food matrix is an important consideration in evaluating the processing method. Once consumed, lipophilic compounds such as lycopene and β-carotene must first be incorporated into a micelle in the digestive tract if they are to be absorbed. The amount of carotenoid incorporated into the micelle is defined as the "bioaccessible" fraction. Several studies in the literature have reported limited micellarization of lycopene from unprocessed

Received: February 9, 2011
Revised: June 8, 2011
Accepted: June 17, 2011
Published: June 17, 2011

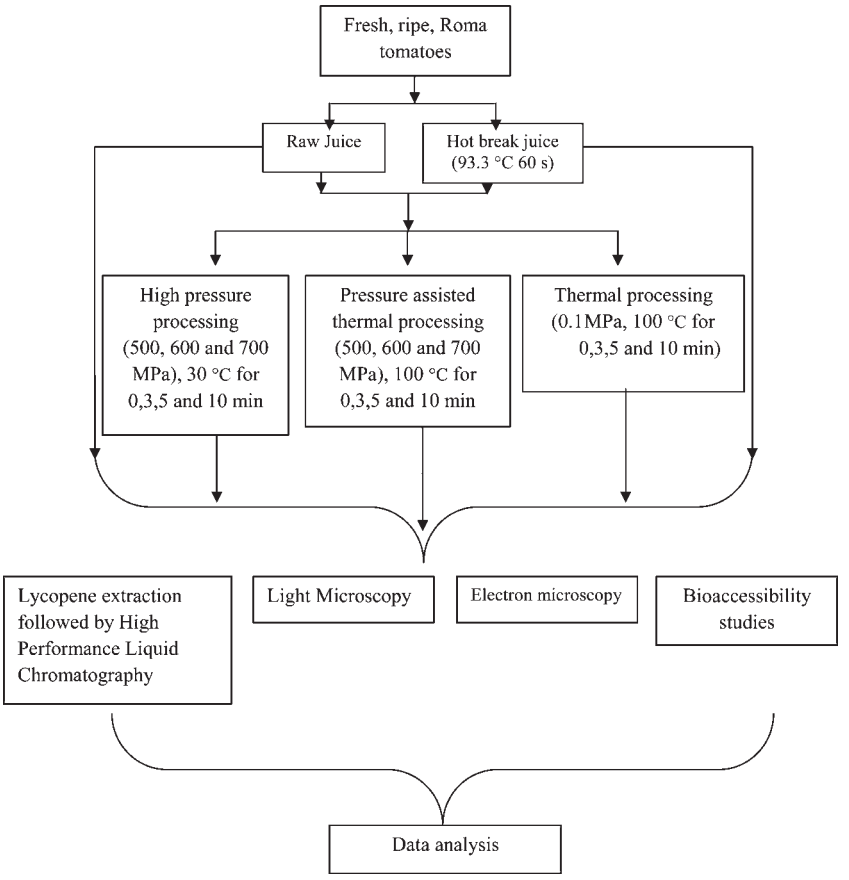

Figure 1. Flowchart outlining the steps involved in the experiment.

and thermally processed tomato-based test meals.[11−17] However, the impact of combined pressure−temperature treatments on micellarization of lycopene has not yet been studied. Hence, one of the goals of this research was to study the bioaccessibility (micellarization) of lycopene from tomato juice processed using combined pressure−temperature treatments.

The primary objective of this study was to investigate the effect of a range of pressure−thermal treatments on postprocessing extractability, isomerization, and bioaccessibility of lycopene and β-carotene in tomato juice and to study the effects of these treatments on the microstructural changes within the cell tissue.

MATERIALS AND METHODS

Red ripe Roma tomatoes were purchased from a local store and processed within 48 h of procurement. Figure 1 presents the overall experimental approach. The study utilized raw (untreated) tomato juice and hot break juice as controls.

Juice Preparation. Tomato juice was extracted at ambient conditions (22 °C) on the day of purchase using a laboratory-scale juicer (Juiceman Jr.), immediately filled into polypropylene pouches (5 cm × 3 cm) (76.2 μm, Thomson Equipment and Supply, Cincinnati, OH), and sealed using a hand impulse heat sealer (American International Electric, Whittier, CA) after manual removal of any trapped air bubbles. To minimize undesirable effects of active enzymes, the juice-containing pouches were immediately immersed in an ice bath, stored in a refrigerator (4 °C), and processed within 6 h.

To prepare hot break juice, the raw juice was rapidly heated to 93.3 °C in a hot pan with constant stirring, held for 60 s to inactivate the enzymes, and rapidly cooled to 21 ± 0.6 °C. Subsequently, 4 g of hot break juice was packaged in polypropylene pouches. The packaged juice samples were stored in a refrigerator at 4 °C and subjected to various pressure−heat treatments within 1 day of juice preparation. The initial pH and percent total soluble solids (%TSS) of the fresh tomato juice were 4.45 and 5.1, respectively. Hot break juice had pH and %TSS values of 4.44 and 5.3, respectively (Table 1).

High-Pressure Kinetic Tester. Packaged tomato juice was treated in a high-pressure kinetic tester (pressure test unit PT-1, Avure Technology Inc., Kent, WA).[18] A 54 mL stainless steel (SS-316) pressure chamber was immersed in a temperature-controlled bath to maintain the desired process conditions (30 °C for HPP and 100.5 °C for PATP). Propylene glycol (57-55-6, Avatar Corp., University Park, IL) was used as the pressure and heat transmitting medium in the temperature-controlled bath. The desired pressure was generated at the rate of 18.42 MPa/s using an intensifier (M-340 A, Flow International, Kent, WA) connected to a hydraulic pump (model PO45/45-OGPM-120, Interface Devices, Milford, CT). The depressurization rate was approximately 2 s. The unit was used for both high-pressure processing and pressure-assisted thermal processing experiments as outlined below.

High-Pressure Processing. The tomato juice samples were pressure treated at 500, 600, and 700 MPa for 0, 3, 5, and 10 min at 30 °C. Before pressure treatment, the juice samples were chilled in an ice−water mixture for 5 min. The chilled samples in the pouch were placed inside a 10 mL polypropylene syringe (model 309604, Difo, Becton Dickinson), which served as the sample holder. The sample holder was also filled with ∼6 mL of chilled water to ensure that the immediate vicinity of the sample pouch had temperature and heat of compression characteristics similar to those of the tomato juice. To minimize heat exchange with the surrounding glycol, the sample holder was wrapped with two layers of insulating material (Sports Tape, CVS

Table 1. Selected Attributes of Fresh Raw Juice and Hot Break Juice Obtained from Roma Tomatoes[a]

			lycopene content (mg/100 g juice)			
	°Brix (%TSS[b])	pH	*all-trans*	*cis*	total lycopene (mg/100 g juice)	*all-trans*-β-carotene (mg/100 g fresh juice)
fresh juice	5.1 ± 0.06 f	4.45 h	6.86 ± 0.26 a	0.44 ± 0.01 b	7.30 ± 0.27 a	0.29 ± 0.002 d
hot break juice	5.3 ± 0.10 g	4.44 h	6.54 ± 0.42 a	0.55 ± 0.02 c	7.09 ± 0.44 a	0.24 ± 0.007 e

[a] Values are the mean ± SD of three replicates. Means with different letters within the same column are significantly different ($P < 0.05$). [b] Percent total soluble solids present in the juice.

Table 2. Temperature Histories at Different Stages of High-Pressure Processing (HPP; 500, 600, and 700 MPa at 30 °C), Pressure-Assisted Thermal Processing (PATP; 500, 600, and 700 MPa at 100 °C), and Thermal Processing (TP; 0.1 MPa at 100 °C) of Tomato Juice Samples

			temperature at different stages during processing (°C)					time required at different stages of preprocessing (s)	
treatment	processing pressure (MPa)	processing time (s)	preprocess (T_1)	immediately before pressurization (T_2)	immediately after pressurization (T_3)	holding ($T_3 - T_4$)	depressurization (T_5)	preprocess (t_1)	come up time (t_2)
HPP 30 °C	500	0	2.0 ± 1	13.9 ± 0.5	28.9 ± 1.2	30.0 ± 0.6	17.1 ± 0.8	329 ± 5	23 ± 1
		600	2.0 ± 1	13.9 ± 0.5	28.9 ± 1.2	30.0 ± 0.6	17.1 ± 0.8	329 ± 5	23 ± 1
	600	0	2.0 ± 1	10.1 ± 0.6	28.2 ± 1.1	30.3 ± 0.4	15.2 ± 0.6	329 ± 5	30 ± 1
		600	2.0 ± 1	10.3 ± 0.6	28.2 ± 1.1	30.3 ± 0.4	15.2 ± 0.6	329 ± 5	30 ± 1
	700	0	2.0 ± 1	7.3 ± 0.5	29.3 ± 0.9	30.6 ± 0.6	13.9 ± 0.5	329 ± 5	35 ± 2
		600	2.0 ± 1	7.3 ± 0.5	29.3 ± 0.9	30.6 ± 0.6	13.9 ± 0.5	329 ± 5	35 ± 2
PATP 100 °C	500	0	72.0 ± 0.4	78.0 ± 0.5	99.6 ± 0.7	100.4 ± 0.7	79.6 ± 0.9	329 ± 5	23 ± 1
		600	72.0 ± 0.4	78.0 ± 0.5	99.6 ± 0.7	100.4 ± 0.7	79.6 ± 0.9	329 ± 5	23 ± 1
	600	0	68.0 ± 0.5	72.0 ± 0.6	100.4 ± 0.5	100.1 ± 0.6	74.9 ± 0.8	329 ± 5	30 ± 1
		600	68.0 ± 0.5	72.0 ± 0.6	100.4 ± 0.5	100.1 ± 0.6	74.9 ± 0.8	329 ± 5	30 ± 1
	700	0	65.0 ± 0.4	68.8 ± 0.8	99.2 ± 0.8	100.3 ± 0.4	73.1 ± 1.5	329 ± 5	35 ± 2
		600	65.0 ± 0.4	68.8 ± 0.8	99.2 ± 0.8	100.3 ± 0.4	73.1 ± 1.5	329 ± 5	35 ± 2
TP 100 °C	0.1	0	21.7 ± 0.3			100.0 ± 0.1			73 ± 3
	0.1	600	21.7 ± 0.3			99.9 ± 0.2			73 ± 3

Pharmacy Inc., Woonsocket, RI).[19] The initial temperature of the samples was determined using the following formula[19] and verified by preliminary experiments:

$$T_3 = T_2 + \frac{(\sum_i (CH_m \times M_f)}{M}\left(\frac{\Delta P}{100}\right) + \Delta T_H \quad (1)$$

T_3 is the target temperature, T_2 is the sample temperature prior to pressurization, CH is the heat-of-compression value of the sample (defined as the temperature increase per 100 MPa during sample pressurization), and ΔP is the process pressure. Owing to its high moisture content, the CH value of tomato juice was assumed to follow that of water.[20] ΔT_H is the temperature gain by the test sample from the surrounding glycol bath during pressure process time and early stages of pressure-holding time.

Pressurization was initiated when the sample temperature reached the predetermined value T_2. Sample temperature history at various stages of combined pressure−temperature treatment is given in Table 2. After processing, the samples were immediately withdrawn and stored at 4 °C until analyzed.

Pressure-Assisted Thermal Processing. PATP experiments were carried out at 500, 600, and 700 MPa at 100 °C for 0, 3, 5, and 10 min. Before PATP, the samples within the sample holder (10 mL syringe) were preheated (Table 2) in a hot water bath (Isotemp 128, Fisher Scientific, Pittsburgh, PA) for a period of 5 min. The water bath was maintained at respective predetermined temperatures for each of the pressures. The preheated samples were then transferred to the pressure chamber, which was maintained at the desired process temperature. The pressurization started when the sample temperature reached the predetermined value T_3 (Table 2). More details of the experimental technique are described in sections above and elsewhere.[19] After processing, the samples were immediately cooled in an ice−water bath and subsequently stored at 4 °C until analysis. Details of the pressure−thermal history during various combined pressure-heat treatment are summarized in Table 2.

Thermal Processing. Boiling water in a steam-jacketed kettle was used for thermal processing (100 °C) of tomato juice. Four grams of juice vacuum packaged in (5 cm × 3 cm) PP pouches was immersed in the boiling water and held for 0, 3, 5, or 10 min, after which it was immediately cooled in an ice−water bath and refrigerated at 4 °C until analyzed.

TP and HPP process conditions selected were also helpful to differentiate between thermal- or pressure-only effects on the tomato samples against that of PATP (600 MPa, 100 °C and 0−10 min) treatment.

Analysis. *Carotenoid Extraction and High-Performance Liquid Chromatography−Photodiode Array (HPLC-PDA Analysis).* Carotenoids were extracted from tomato juice using a method developed by

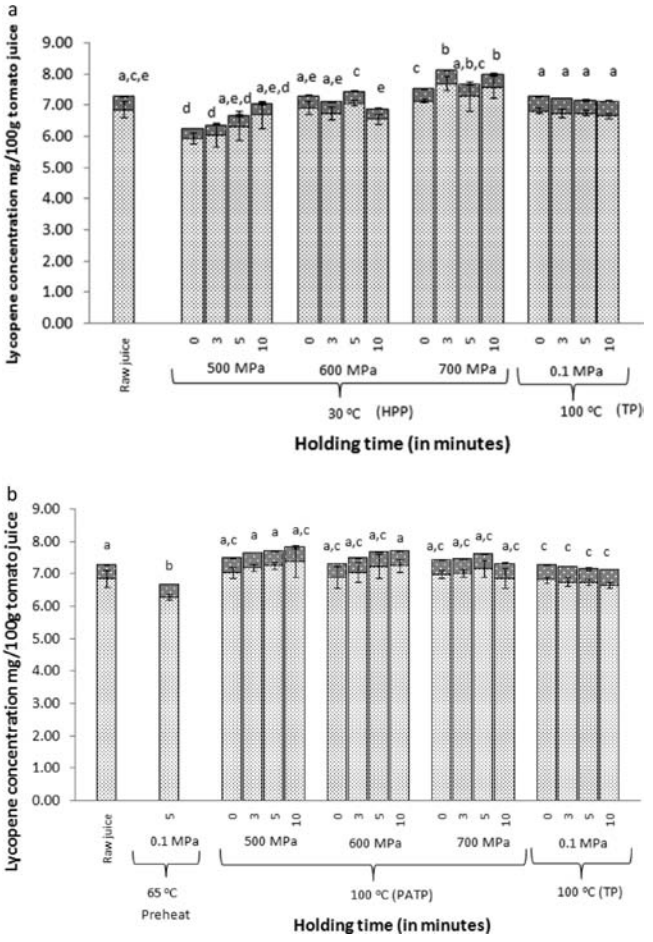

Figure 2. Lycopene retention (lightly shaded bars, *all-trans*-lycopene; heavily shaded bars, *cis*-lycopene) in fresh raw tomato juice subjected to (a) high-pressure processing (500−700 MPa, 30 °C for 0, 3, 5, and 10 min) or (b) pressure-assisted thermal processing (500−700 MPa, 100 °C for 0, 3, 5, and 10 min) and thermal processing (0.1 MPa, 100 °C for 0, 3, 5, and 10 min). Values are the mean ± SD of three replicates. Time = 0 min (zero time) represents the process come-up time at the respective pressure−temperature conditions.

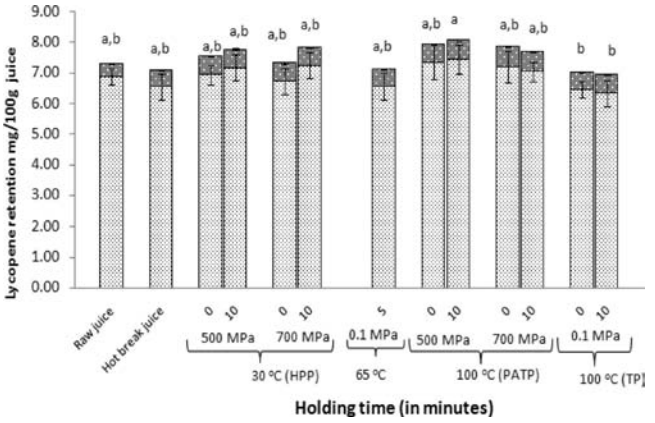

Figure 3. Lycopene retention (lightly shaded bars, *all-trans*-lycopene; heavily shaded bars, *cis*-lycopene) in hot break tomato juice subjected to combined pressure−temperature processing: HPP (500−700 MPa, 30 °C for 0, 3, 5, and 10 min), PATP (500−700 MPa, 100 °C for 0, 3, 5, and 10 min), and TP (0.1 MPa, 100 °C for 0, 3, 5, and 10 min). Values are the mean ± SD of three replicates. Time = 0 min (zero time) represents the process come-up time at the respective pressure−temperature conditions.

Ferruzzi et al.[21] The HPLC-PDA method used was previously reported by Gupta et al.[22]

all-trans-Lycopene was identified by spectra and retention time coincident with those of authentic standard (Chromadex Inc., Irvine, CA). To quantify total lycopene in tomato juice, an external calibration curve was generated using *all-trans*-lycopene standard. *cis*-Lycopene isomers were identified using isomerized lycopene (details provided in Gupta et al.[22]) and were quantified using *all-trans*-lycopene equivalents.

β-Carotene was identified by spectra and retention time coincident with those of authentic standard (Sigma-Aldrich, St. Louis, MO) and quantified using an external calibration curve.

Transmission Electron Microscopy (TEM). Selected samples of fresh and processed tomato juice were examined by transmission electron microscopy using the procedure reported by Nguyen et al.[7] Briefly, tomato juice samples were centrifuged in 2 mL vials at 2000 rpm (24 × 3.75*g*) using a 5424 centrifuge (Eppendorf, Hauppauge, NY). The tomato juice cells were then resuspended for 30 min in a fixative consisting of 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 and then centrifuged. The supernatant was removed and discarded, and the pelleted cells were rinsed three times with 0.1 M phosphate buffer containing 0.1 M sucrose buffer. The cells were resuspended in a small amount of warm 2% agarose, centrifuged, and chilled in an ice−water bath for 10 min to set the agarose. The cloudy portion of the agarose, which contained the cells, was cut into 1 mm size blocks and fixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h. The fixed samples were then dehydrated using 10 min transfers through a graded ethanol series (50, 70, 80, 95, 100, 100%) followed by propylene oxide. After the samples had been embedded in Epon resin and polymerized overnight at 60 °C, they were sliced and the resulting sections transferred to carbon-reinforced grids. The sections were examined using a FEI Tecnai spirit transmission electron microscope (FEI, Hillsboro, OR) at The Ohio State University Microscopy and Imaging Facility.

Light Microscopy. Processed and unprocessed tomato juice samples were examined using Zeiss AxioskopWidefield LM (Carl Zeiss Microimaging GmbH, Goettengen, Germany) at 100× with oil immersion objectives.

Bioaccessibility Studies. The bioaccessibility studies were performed by using the method published by Failla et al.[23] with minor modifications. After simulated digestion, the aqueous digesta (containing micelles) was then filtered through a 0.22 μm syringe filter (25 mm diameter, Fisher Scientific, Pittsburgh, PA) to remove microcrystalline nonmicellarized carotenoids. Aliquots (1 mL) of the filtrate (containing micelles) were combined with 1 mL of ethanol and extracted three times with 2 mL of 1:1 acetone/hexane. One milliliter of distilled water was added to the pooled extracts, and the samples were re-extracted into 2 mLof hexane three times. The hexane extracts were pooled and evaporated under nitrogen gas. The dried samples were reconstituted in 1 mL of 1:1 MTBE/methanol (v/v) and analyzed by HPLC.

Total Soluble Solids (°Brix) and pH. %TSS was measured using an Atago digital hand-held pocket refractometer (Cole-Parmer Instrument Co., Vernon Hills, IL). The pH of raw tomatoes and tomato juice was measured using a portable hand-held pH-meter (model PHH-81A, Omega Engineering, Stamford, CT).

Statistical Data Analysis. Data were analyzed with Minitab software, version 14.1 (Minitab, State College, PA). Data are expressed as the mean ± SD of three replicates for postprocessing lycopene stability and of five replicates in the case of bioaccessibility studies. Pairwise comparisons for the means of factors were evaluated with Tukey's test at a 5% significance level ($P < 0.05$).

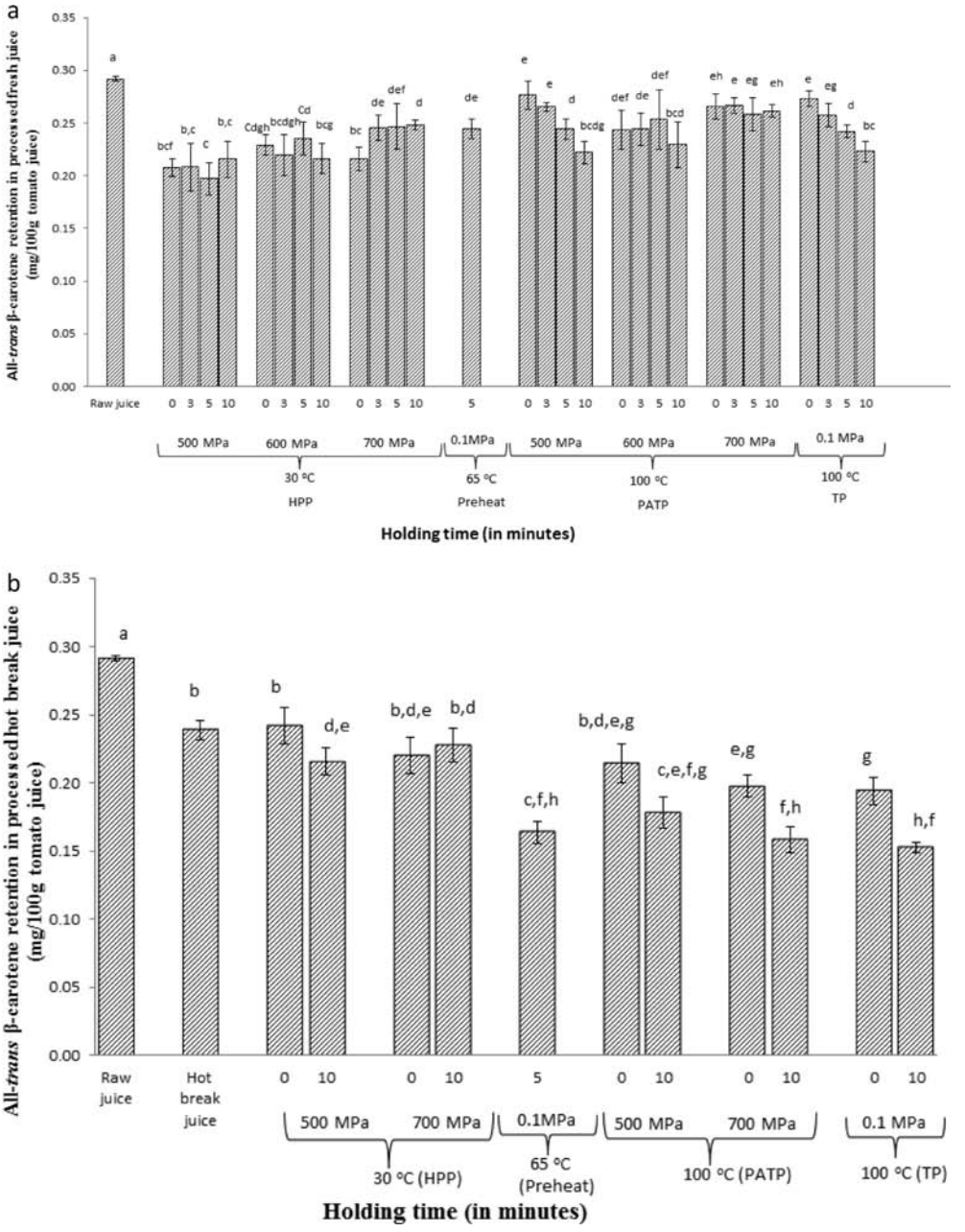

Figure 4. Percent *all-trans-β*-carotene retention in (a) raw and (b) hot break tomato juice after combined pressure−heat processing: HPP (500−700 MPa, 30 °C for 0, 3, 5, and 10 min), PATP (500−700 MPa, 100 °C for 0, 3, 5, and 10 min), and TP (0.1 MPa, 100 °C for 0, 3, 5, and 10 min). Values represent the mean ± SD of three replicates. Time = 0 min (zero time) represents the process come-up time at the respective pressure−temperature conditions.

RESULTS AND DISCUSSION

Juice Extraction and Its Effect on Carotenoids (Lycopene and *β*-Carotene). The total lycopene contents (*all-trans* + *cis* isomers) of raw and hot break tomato juices did not differ significantly. However, the lycopene *cis* isomer content of hot break juice was approximately 25% greater than that of raw juice. Hot break processing reduced the *all-trans-β*-carotene content of the juice by 15% as compared to raw juice. Selected attributes of fresh and hot break juice are given in Table 1.

Impact of Pressure−Thermal Effect on Lycopene Degradation. The stability of tomato lycopene as influenced by processing (HPP, PATP, and TP) and juice preparation (untreated raw and hot break) is presented in Figures 2 and 3. Raw juice had 6.86 mg/100 g *all-trans*-lycopene and 0.44 mg/100 g *cis*-lycopene. Pressure treatment (500 MPa 30 °C) for 0 min yielded approximately 13% less *all-trans*-lycopene and a 14% decrease in total lycopene as compared to the control (Figure 2a). However, prolonged pressure holding time at 500 MPa or elevated pressures did not cause any additional degradation. Pressure treatment at 600 MPa and 30 °C resulted in *all-trans*-lycopene values similar to those of the control. Pressure treatment at 700 MPa and 30 °C resulted in increased *all-trans*-lycopene extractability (up to 12%) as compared to the control. The reasons for the decrease in lycopene content at 500 MPa are unclear.

Studies in the literature have previously reported anomalous behavior in lycopene extractability from tomato products exposed to certain pressure conditions.[9] Pressure is known to cause a decrease in activation volume, and reactions that are favored by increase in pressure and decrease in volume proceed more rapidly under high-pressure conditions. Pectinases and lipoxygenases are enzymes naturally present in tomatoes that are difficult to inactivate under high pressures and ambient temperatures.[9,24,25] Peeters et al.[24] discovered that approximately 25% of lipoxygenase activity is retained in tomato juice treated at 500 MPa for 5 min at 20 °C. Krebbers et al.[9] reported a 5.5−6.5-fold increase in tomato pectinmethylesterase activity at ambient process temperatures regardless of the pressures used (≤700 MPa). Up to 36% residual polygalacturonase activity was reported under same treatment conditions. Past in vitro studies on the enzymatic degradation of lycopene show that addition of soybean lipoxygenase to lycopene significantly decreased the level of lycopene.[26] Likewise, Biacs et al.[27] studied lipoxygenase-catalyzed in vitro co-oxidation of tomato carotenoids in the presence of vitamins C and E. They reported approximately 25% degradation of lycopene during a 15 min holding time. In addition, several studies have discovered the presence of carotenoid cleavage enzymes in plants[28−30] and animals.[29] The increased interaction of one or a combination of these enzymes with their respective substrates due to decrease in activation volume (under pressure), coupled with changes in their conformation and activity, might explain some of the lycopene degradation observed at lower pressure and temperatures.

PATP of raw juice at 500, 600, or 700 MPa at 100 °C for different holding times up to 10 min did not change the *all-trans*-lycopene extractability from the juice (Figure 2b). Both PATP and thermally (0.1 MPa, 100 °C for up to 10 min) processed juice samples showed *all-trans*-lycopene contents similar to that of the raw juice. Preheating fresh juice at 65 °C for 5 min resulted in an up to 8% decrease in lycopene (Figure 2b). However, this decrease was not observed in the PATP-treated samples analyzed for lycopene content, possibly due to the increased extractability of lycopene from tomato juice after PATP.

Combined pressure−temperature treatment of hot break juice resulted in a minor (up to 10%) increase in the *all-trans*-lycopene extractability as compared to the hot break and raw juice controls. PATP and thermally treated samples showed marginally higher *cis*-lycopene isomers compared to hot break juice and HPP-processed samples (Figure 3).

all-trans-Lycopene in tomato products is fairly stable during traditional thermal processing.[4,31,32] Furthermore, *cis*-lycopene levels have been reported to be fairly constant after pressure treatments.[8] It has been proposed that isomerization and degradation of carotenoids are structurally and thermodynamically specific phenomena. Also, the differences in the three-dimensional shape of the molecule influence its ability to exist in a crystalline state, hydrophobicity, solubility, and other such properties, which might affect stability.[7] *all-trans*-Lycopene, as a linear molecule, forms multilayers or aggregates,[33] and this aggregated form might be able to resist further structural changes.[7] Because pressure is known to decrease the activation volume and compress food components, it is possible that pressure favors the formation of compact lycopene aggregates. Formation of *all-trans*-lycopene aggregates might inhibit the ability of lycopene molecules to be isomerized to nonlinear *cis* forms during combined pressure−heat treatments.

Table 3. Percent Lycopene and β-Carotene from Tomato Juice Transferred to the Micelles during in Vitro Bioaccessibility Studies

	concentration[a] (μg/100 g juice)			
	lycopene		β-carotene	
treatment	digesta	micelles	digesta	micelles
Raw Juice				
control[b]	321.5 ± 38.1 b	25.4 ± 1.8 a	35.0 ± 2.6 c	25.2 ± 1.3 c
HPP[c]	307.4 ± 24.5 bc	27.3 ± 2.2 a	42.2 ± 2.1 b	30.0 ± 2.1 b
preheat[d]	257.9 ± 10.7 d	22.8 ± 1.3 b	29.2 ± 2.5 d	25.6 ± 2.2 c
PATP[e]	442.6 ± 51.9 a	27.2 ± 1.6 a	45.4 ± 1.6 a	31.0 ± 2.9 b
TP[f]	274.1 ± 16.3 cd	26.7 ± 1.2 a	34.3 ± 1.1 c	33.9 ± 1.4 a
Hot Break Juice				
control[b]	370.8 ± 6.1 ab	21.4 ± 0.1 d	44.4 ± 2.4 cd	35.9 ± 0.9 b
HPP[c]	300.9 ± 41.9 c	24.3 ± 0.3 c	45.4 ± 0.3 b	37.5 ± 0.4 a
preheat[d]	348.2 ± 17.7 b	24.9 ± 0.8 b	42.9 ± 0.7 d	33.5 ± 0.4 c
PATP[e]	386.4 ± 15.0 a	23.8 ± 0.2 c	48.7 ± 0.4 a	36.1 ± 0.3 b
TP[f]	366.2 ± 3.3 ab	26.2 ± 0.2 a	46.3 ± 0.3 b	37.9 ± 0.6 a

[a] Values represent the mean ± SD of five replicates. Means with different letters within the same column for the same juice type (raw or hot break) are significantly different ($P < 0.05$). [b] Respective unprocessed raw juice and hot break (~93 °C, 60 s) juice. [c] High pressure processed (HPP) juice (700 MPa, 30 °C, 5 min). [d] Preheated for PATP processing (0.1 MPa, 65 °C, 5 min). [e] Pressure-assisted thermally processed (PATP) juice (700 MPa, 100 °C, 5 min). [f] Thermally processed (TP) juice (0.1 MPa, 100 °C, 5 min).

Pressure−Thermal Effects on β-Carotene Degradation. Raw and hot break juice samples subjected to pressure treatment at 30 °C retained 75−93% of *all-trans*-β-carotene as compared to control (Figure 4). PATP or TP treatment of raw juice at 100 °C better retained *all-trans*-β-carotene than pressure treatment at 30 °C (Figure 4a).

PATP and thermal treatments showed similar *all-trans*-β-carotene retention. Under PATP and thermal conditions, *all-trans*-β-carotene retention was inversely correlated with processing time, with higher processing times yielding lower levels (Figure 4b).

There is no consensus within the literature on the effect of processing on β-carotene in tomato juice. Conversion of *all-trans*-β-carotene to *cis*-β-carotene has been reported in tomato products subjected to thermal processing at 100 °C for 30 min.[7,32] However, the magnitude of these changes is quite different between these two studies. In contrast, no change in β-carotene extractability was observed after thermal (0.1 MPa, 95 °C, 60 min) and high-pressure processing (600 MPa, 20 °C, 60 min) of tomato homogenate.[34] It has also been proposed that *all-trans*-β-carotene crystal aggregates in the Langmuir−Blodgett film may not be able to easily assemble into an ordered structure and stabilize and, thus, be more susceptible to isomerization.[33] In another study, a 35% increase in *all-trans*-β-carotene extractability was reported after 400 MPa pressure treatment at 25 °C for 15 min and a significant increase (9%) in *all-trans*-β-carotene extractability after thermal processing at 90 °C for 1 min followed by immediate freezing.[35] However, the effect of freezing on the increase in β-carotene extractability is not clear. The results from the present study show that the

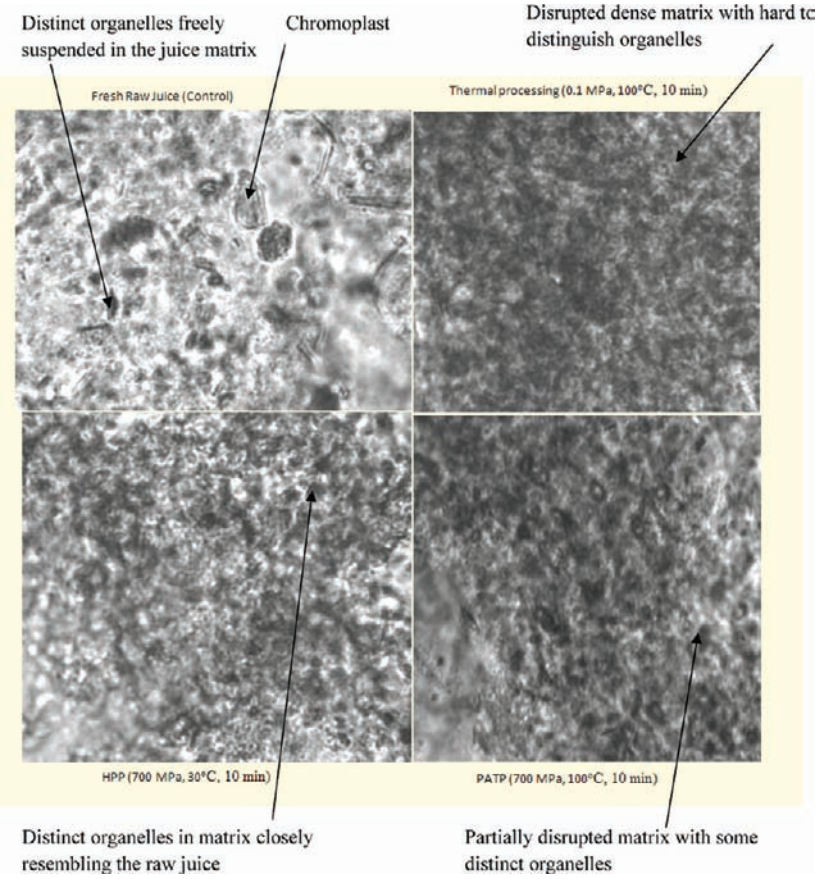

Figure 5. Representative light microscopy images (using 100× oil immersion objectives) of raw tomato juice samples processed using combined pressure−temperature treatments: HPP (500−700 MPa, 30 °C for 0, 3, 5, and 10 min), PATP (500−700 MPa, 100 °C for 0, 3, 5, and 10 min), and TP (0.1 MPa, 100 °C for 0, 3, 5, and 10 min).

stability of β-carotene is influenced by type of juice (raw vs hot break), processing method (HPP, PATP, and TP), and holding time under these processing conditions. Differences among different studies could be attributed to variations in the tomato cultivars utilized, method of juice preparation, extraction, and processing.[36]

In Vitro Bioaccessibility of Carotenoids. On the basis of earlier investigation (Figures 2−4) on the impact of various treatments (HPP, TP, PATP) on lycopene and β-carotene retention, additional sets of experiments (see Table 3) were carried out to evaluate the treatment efficacy on carotenoid bioaccessibility. Bioaccessibility was assessed using an in vitro model, which mimics digestion and determines the micellarization of carotenoids from the food matrix (raw and hot break tomato juice in this study). This technique is a cost-effective method for screening the bioaccessibility of carotenoids from a large number of food samples,[11] and good correlation has been found between in vitro bioaccessibility data and in vivo human data.[12] Table 3 compares the amount of β-carotene and lycopene transferred from the tomato juice into the micelles during a simulated digestion process.

Effect of Processing on Bioaccessibility of *all-trans*-β-Carotene. Up to 16% of *all-trans*-β-carotene from the raw tomato juice was observed in the digesta (i.e., the tomato juice sample after the in vitro digestion, composed of the micellar and nonmicellar fractions), and up to 12% of tomato juice β-carotene appeared in the micelles. Significant differences were observed in the amount of *all-trans*-β-carotene present in the digesta prepared from raw juice processed using different techniques (TP, PATP, and HPP). Digesta from HPP and PATP (700 MPa for 5 min at 30 and 100 °C, respectively) samples had significantly higher ($P < 0.05$) levels of *all-trans*-β-carotene than the digesta from raw juice and TP juice (0.1 MPa, 100 °C for 5 min) (Table 3).

However, no significant difference was observed in the amount of micellarized *all-trans*-β-carotene in HPP, PATP, and thermally processed samples. Micelles from processed samples (HPP, PATP, and thermal) had significantly greater ($P < 0.05$) *all-trans*-β-carotene levels as compared to the raw juice (Table 3).

Similarly, when hot break juice was processed, no significant difference was found in the amount of *all-trans*-β-carotene present in the micelles obtained by in vitro digestion of HPP, PATP, and thermally treated samples. However, the amount of *all-trans*-β-carotene present in the micellar fraction obtained from processed hot break juice samples was significantly greater than that present in the micellar fraction of the processed raw juice (Table 3). Also, the percent micellarization of β-carotene ranged between 70 and 100% of that present in the digesta, with maximum percent micellarization observed in hot break juice thermally treated at 0.1 MPa and 100 °C for 5 min.

Effect of Processing on Bioaccessibility of Lycopene. As compared to β-carotene, significantly less ($P < 0.05$) lycopene was observed in the digesta of raw tomato juice (up to 6% of that present in the raw tomato juice), and only 0.4% of tomato juice

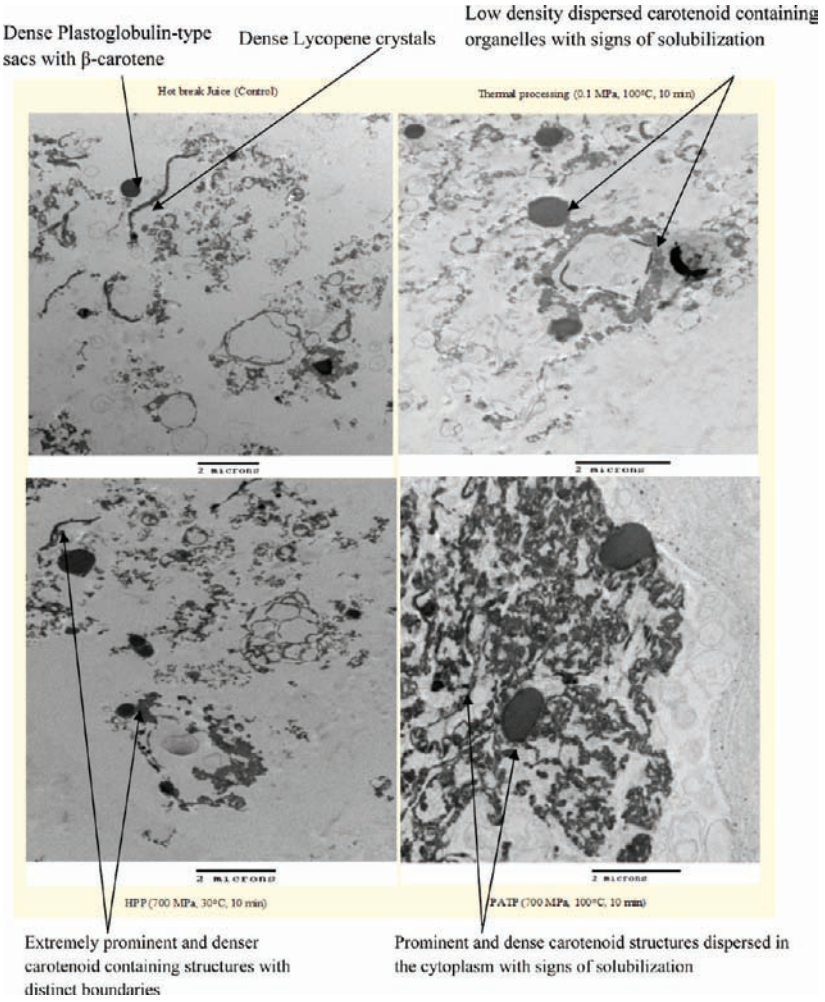

Figure 6. Representative electron microscopy images of hot break tomato juice samples processed using combined P-T treatments: HPP (500−700 MPa, 30 °C for 0, 3, 5, and 10 min), PATP (500−700 MPa, 100 °C for 0, 3, 5, and 10 min), and TP (0.1 MPa, 100 °C for 0, 3, 5, and 10 min).

lycopene was observed in the micelles. In addition, no significant difference was found between the quantities of lycopene in the digesta of hot break and raw juice. The same was true for micellarized lycopene. Lycopene content in the digesta of raw juice (control), HPP, and thermal treatments was not significantly different. However, the lycopene content in the digesta of PATP-processed samples was significantly higher (Table 3). In contrast, lycopene micellarization was below 0.5% for all treatments. Our results are similar to those reported by Garrett et al.,[11] who observed only 5% lycopene appearing in the digesta and <0.5% lycopene appearing in the micellar fraction as compared to the lycopene originally present in the test meal.

Whereas the bioaccessibility of lycopene varies depending upon the type of product, processing conditions, and method of assessment, it is widely accepted that the bioaccessibility of lycopene is generally low.[11−14,17] In vitro digestibility studies on raw and thermally processed tomatoes showed that micellarization of lycopene increased from 0.1% (raw tomatoes) to 1.60% (processed tomatoes) and β-carotene increased from <0.1% (raw tomatoes) to 5.97% (processed tomatoes).[12] It is interesting to note that percent micellarization during TP might be further enhanced by incorporating a greater amount of lipid or different chain lengths of lipid in the product before in vitro digestion is initiated,[14] or by using harsher processing conditions (stir-frying at 177 °C for 4 min).[11] However, milder preservation conditions, such as those observed in combined pressure−temperature processing, have a distinct advantage of improving the product quality and preserving heat-sensitive nutrients.[9,10,22,34,37] Combined P-T processing has been found to produce a shelf-stable tomato juice with a holding time of 10 min at pressures above 600 MPa and temperature ≥45 °C.[22] However, holding times in thermal processing methods commercially used to achieve shelf stability of tomato juice are significantly greater (up to 35 min at 100 °C[38]) than times used in this study (10 min at 100 °C). Longer processing time during thermal processing could lead to adverse effects on nutrients and quality.

Microscopic Changes in Tomato Juice Due to Combined Pressure−Heat Processing. Both raw and hot break tomato juice processed using combined pressure−temperature treatments were observed microscopically for changes in the microstructure (Figure 5). Differences between the juice matrix of all four treatments are evident. Freshly extracted raw juice (Figure 5) showed distinct cellular components including chromoplasts dispersed in the cytoplasm. HPP (700 MPa, 30 °C, 10 min) treated juice showed closer resemblance to the control untreated juice. A denser matrix with less resolution between cell components was observed. In contrast, thermal processing

(0.1 MPa, 100 °C, 10 min) showed a continuous matrix with little or no resemblance to the control juice and indistinguishable cellular components. PATP (700 MPa, 100 °C, 10 min) exhibited the characteristics of both HPP and thermally processed juices.

Unprocessed hot break juice showed a more continuous network with no separation of phases (data not shown). HPP- and PATP-processed hot break juice samples show a matrix different from that observed in unprocessed hot break juice. However, the thermally processed sample shows little or no matrix with indistinguishable cellular components.

Carotenoid biosynthesis and development of carotenoid-bearing structures begin during ripening of tomato tissue and maturation of chromoplasts.[39] A rapid increase in lycopene and its subsequent accumulation result in crystallization,[40] and the lycopene crystals, which are known to be associated with the thylakoid membrane, remain enveloped in the chromoplast. Likewise, β-carotene crystals associated with plastoglobulin-type structures are also enveloped by a membrane and dissolve in the lipid material of the globules. However, the membrane that envelopes β-carotene crystals has been suggested to be different from the one that envelopes lycopene.[41] Although processing conditions are sufficiently rigorous in disrupting cell walls and organelles, from the electron micrographs (Figure 6) it appears that lycopene and β-carotene remain enveloped even after combined pressure−temperature processing. Similar findings with only thermal processing have been reported by Nguyen et al.[7]

It can be seen from Figure 6 that changes in the microstructure due to different processing conditions did not necessarily affect lycopene crystals. The dispersion of lycopene crystals in the matrix is evident regardless of the processing method used. However, it is worth noting the strong contrast between lycopene crystals in the HPP-, PATP-, and TP-processed samples. We hypothesize that increased lycopene extractability observed after HPP and PATP treatments could be attributed to the differences in the tomato juice matrix observed in microscopy images (Figure 6). In addition, the resistance of lycopene to micellarization may be attributed to the fact that the majority of lycopene is still found in the crystalline form even after processing (Figure 6).

In conclusion, this study suggests that the type of juice (raw juice vs hot break juice) has a significant impact on the stability of β-carotene in the processed juice and a minimal impact on lycopene. Combined pressure−temperature processing (HPP, 700 MPa, 30 °C; PATP, 500−700 MPa, 100 °C) increased the extractability of lycopene from the tomato juice (both raw and hot break), whereas thermal processing (TP, 0.1 MPa, 100 °C) had negligible effect on its extractability. β-Carotene degradation was dependent on the processing temperature, processing time, processing pressure, and type of juice (raw vs hot break). In general, increasing the holding time during PATP (500−700 MPa, 100 °C) and TP (0.1 MPa, 100 °C) had an adverse effect on β-carotene content of the juice. The bioaccessibility of lycopene was limited regardless of the processing method used, and microscopic evaluations revealed that the treatments were not severe enough to solubilize the lycopene crystals and facilitate its micellarization. β-Carotene showed better micellarization, and processing (HPP, PATP and TP) further improved its micellarization. On the basis of the results of this study and studies on quality of tomato juice reported in the literature, combined pressure−temperature processing poses a promising alternative for producing good-quality tomato products.

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Funding Sources

Support for this research was provided by USDA-CSREES-NRICGP Grant 2006-35503-17571, by the Ohio Agricultural Research and Development Center (OARDC), and by The Ohio State University. References to commercial products or trade names are made with the understanding that no endorsement or discrimination by The Ohio State University is implied.

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