

# Carotenoids in Nonthermally Treated Fruit Juices

I. Odriozola-Serrano, G. Oms-Oliu, R. Soliva-Fortuny, O. Martín-Belloso

Department of Food Technology, University of Lleida, Lleida, Spain

## CHAPTER POINTS

- Nonthermal technologies allow obtaining fruit juices with minor changes or increased content in carotenoids.
- Temperature during processing and storage period is a determinant factor affecting the carotenoid content of fruit juices.
- Compared to thermally-pasteurized tomato juices, nonthermally-treated fruit juices kept higher carotenoids content during storage.
- Different results may be obtained for different fruit juices because the presence of antagonistic agents in the matrix may substantially compromise carotenoids stability.
- Future research should focus on identifying the role of biochemical transformations of carotenoid precursors as influenced by processing.

## INTRODUCTION

Consumption of plant-based minimally processed foods such as fruit juices is increasing because of the evidence about the many benefits for human health that have been attributed to the dietary intake of fresh-like fruits and vegetables (Kaur and Kapoor, 2001). It is widely known that these products are outstanding sources of bioactive compounds, such as carotenoids. Carotenoids (carotens and xanthophylls) are yellow, orange, and red pigments present in many commonly eaten fruits and vegetables. Their importance to human health is related to their role as provitamin A, antioxidants, cell differentiation and proliferation regulators, cell-cell communication stimulators, immune function

and carcinogen metabolism modulators, and blue light filters. There are many studies showing a strong correlation between carotenoids intake with both the improvement of the immune system and the reduction of the incidence of some degenerative diseases such as cancer, cardiovascular diseases, cataract and macular degeneration (Cooper *et al.*, 2004). However, carotenoids are highly unsaturated compounds with an extensive conjugated double-bonds system and are therefore susceptible to oxidation, isomerization, and other chemical changes during processing and storage (Shi and Le Maguer, 2000). Despite carotenoids being affected by abusive temperatures, thermal processing remains the most commonly used technology for inactivating microorganisms and enzymes in processed food. In this context, nonthermal technologies such as high intensity pulsed electric fields (HIPEF) and high-pressure (HP) are being introduced by food processors as alternative or complementary to conventional thermal treatments. Both technologies have potential to improve the quality and freshness character of processed food while assuring its microbiological safety and stability (Odriozola-Serrano *et al.*, 2009; Plaza *et al.*, 2011). In recent years, the impact of nonthermal technologies on health-related compounds has been extensively evaluated. Based on these results, the effect of HIPEF and HP on the stability of carotenoids in fruit juices will be reviewed.

## HOW COMPOSITION IS ALTERED

### Pulsed Electric Field Treatment

Processing by pulsed electric fields may allow keeping the stability of carotenoids. Some studies have demonstrated that carotenoid content is significantly increased

after HIPEF processing compared to the untreated juice (Table 77.1). [Odriozola-Serrano et al. \(2007\)](#) reported an enhancement of up to 46.2% in the lycopene concentration of tomato juices after applying different HIPEF treatments (35 kV/cm for 1000  $\mu$ s). Consistently, slight increases in lycopene content of HIPEF-treated watermelon juices had been previously reported by [Oms-Oliu et al. \(2009\)](#). It has been hypothesized that thermal treatments may imply an increase in some individual carotenoids, owing to greater stability, inactivation of oxidative and hydrolytic enzymes, and unaccounted losses of moisture, which concentrate the sample ([Rodríguez-Amaya, 1997](#)). [Nguyen and Schwartz \(1999\)](#) suggested that homogenization and heat treatment disrupt cell membranes and protein-carotenoids complex, making carotenoids more accessible for extraction. Results obtained by [Odriozola-Serrano et al. \(2009\)](#) offer some controversy, provided that changes in the relative amounts of carotenoids are not consistent for similar compounds. Although the reason for these results is not well understood, it was speculated that carotenoid conversions could be triggered by PEF treatments. This

would explain the increase in lycopene at the expense of its precursors. HIPEF processing may accelerate lycopene synthesis in tomatoes, involving the conversion of geranyl-geranyl diphosphate (GGPP) to phytoene by phytoene synthase and the conversion of phytoene to phytofluene,  $\beta$ -carotene, and lycopene by phytoene desaturase ([Fraser, et al., 2004](#)). [Cortés et al. \(2006a\)](#) stated that the effects of treatments on carotenoid composition mainly depended on processing conditions. Process parameters such as electric field strength and treatment time are the most relevant variables to be controlled in HIPEF treatments for attaining microbial reduction and have also been shown to play a key role in carotenoids retention. In this regard, the greater the electric field strength and treatment time, the higher the carotenoid content ([Odriozola-Serrano et al., 2008](#)). Consistently, [Cortés et al. \(2006b\)](#) observed that the carotenoids content in HIPEF-treated orange juice increased significantly when the most intensive treatments (35 or 40 kV/cm) were conducted. Carotenoids concentration in orange-carrot juice rose as after prolonged HIPEF treatments at 25 or 30 kV/cm ([Torregrosa et al., 2005](#)). Furthermore, other treatment variables such as pulse frequency, pulse width, and polarity may also matter. Higher frequency and pulse width resulted in a greater carotenoid content in tomato juices compared to untreated samples, whereas the use of bipolar pulses led to a greater rise in the carotenoids content of tomato juices with respect to juices subjected to monopolar treatments ([Odriozola-Serrano et al., 2007](#)).

Other studies have been focused on determining the influence of HIPEF on vitamin A precursors such as  $\beta$ -carotene,  $\alpha$ -carotene,  $\gamma$ -carotene, and  $\alpha$  and  $\beta$ -cryptoxanthin. HIPEF-treated carrot juice exhibited higher  $\beta$ -carotene concentrations than untreated juice, which is related to greater vitamin A contents in the samples ([Quitão-Teixeira et al., 2009](#)). [Odriozola-Serrano et al. \(2009\)](#) observed that  $\beta$ -carotene in treated tomato juice underwent a significant increase (31%–38%), whereas  $\gamma$ -carotene content depleted (3%–6%) when HIPEF treatments (35 kV/cm for 1000  $\mu$ s) were applied. It was suggested that  $\gamma$ -carotene may undergo cyclization to form six membered rings at one end of the molecule, giving  $\beta$ -carotene as a product after HIPEF processing. In contrast, other studies have not reported major effects of HIPEF treatments on the carotenoid content of orange juices ([Sánchez-Moreno et al., 2005](#)).

Several authors have studied the evolution of some carotenoids in HIPEF-treated juices during storage, reporting higher stability of these health-related compounds in comparison to thermally-pasteurized tomato juices ([Min et al., 2003](#); [Odriozola-Serrano et al., 2007](#)), orange juices ([Cortés et al., 2006a](#)), carrot juices ([Quitão-Teixeira et al., 2009](#)), and fruit juice-milk beverages ([Morales-de la Peña et al., 2011](#)). Most differences

**TABLE 77.1** Effect of High Intensity Pulsed Electric Field Treatment on Carotenoid Compounds of Fruit Juices

| Product          | Treatment Conditions  | Major Finding  | Reference                                       |
|------------------|---|--|---|
| Orange juice     | 35–40 kV/cm/30–340 $\mu$ s                                  | Slight increase in the concentration of some carotenoids (13- <i>cis</i> -violaxanthin and 7,8,7',8'-tetrahydrolycopene) | <a href="#">Cortés et al. (2006b)</a>           |
|                  | 40 kV/cm/30–60 $\mu$ s                                      | Increase in Vitamin A content (4.5–20%)  | <a href="#">Torregrosa et al. (2005)</a>        |
| Tomato juice     | 35 kV/cm/1000 $\mu$ s (bipolar 7- $\mu$ s pulses at 250 Hz) | Enhancement of lycopene concentration (46.2%)  | <a href="#">Odriozola-Serrano et al. (2007)</a> |
|                  | 35 kV/cm/1000 $\mu$ s (bipolar 4- $\mu$ s pulses at 100 Hz) | Increase in several individual carotenoids such as lycopene (10%), $\beta$ -carotene (38%) and phytofluene (5%)          | <a href="#">Odriozola-Serrano et al. (2009)</a> |
| Carrot juice     | 35 kV/cm/1500 $\mu$ s (bipolar 6- $\mu$ s pulses at 200 Hz) | Substantial increase in $\beta$ -carotene concentration (23%)  | <a href="#">Quitão-Teixeira et al. (2009)</a>   |
| Watermelon juice | 35 kV/cm/50 $\mu$ s (bipolar 7- $\mu$ s pulses at 200 Hz)   | Slight increase in lycopene content (113%)   | <a href="#">Oms-Oliu et al. (2009)</a>          |

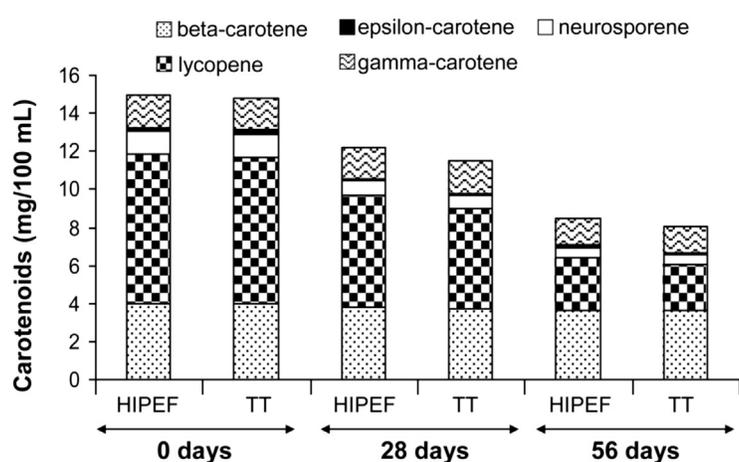
between HIPEF and heat treatments can be explained in terms of the temperature/time binomial. Hence, mild processing temperatures during HIPEF-processing might explain the higher retention of carotenoids in fruit juice samples. According to [Kidmose \*et al.\* \(2002\)](#) the major cause of carotenoid losses in vegetable products is the oxidation of the highly unsaturated carotenoid structure. Oxidation may occur by autooxidation, which is a spontaneous free-radical chain reaction in the presence of oxygen, or by photooxidation produced by oxygen in the presence of light. These oxidative reactions may result in carotene bleaching, which is the cause of formation of colourless end-products ([Gross, 1991](#)). During autooxidation of carotenoids, alkylperoxyl radicals are formed and these radicals attack the double bonds resulting in formation of epoxides. The severity of oxidation depends on the structure of carotenoids and the environmental conditions, and the compounds being formed depend on the oxidation process and the carotenoids structure ([Ramakrishnan and Francis, 1980](#)). Lutein has been shown to be the most susceptible carotenoid to isomerisation or oxidation processes, thus exhibiting the greatest losses in HIPEF and thermally treated orange juice after 40 days of storage at 4°C ([Plaza \*et al.\*, 2011](#)). [Zulueta \*et al.\* \(2007\)](#) mentioned that lutein and zeaxanthin are highly susceptible to degradation during thermal treatments due to the presence of oxygen in their chemical structures. Regarding carotenes, [Odrizola-Serrano \*et al.\* \(2009\)](#) pointed out that lycopene content in HIPEF and heat processed tomato juices decreased much more considerably than other carotenes (neurosporene,  $\gamma$ -carotene,  $\xi$ -carotene, and  $\beta$ -carotene) through storage at 4°C ([Figure 77.1](#)).

## High Pressure Processing

Several research efforts have assessed the suitability of HP technology for fruit juice processing ([Sánchez-Moreno \*et al.\*, 2009](#)). It seems that high pressure influences

the extraction yield of carotenoids in fruit juices ([Table 77.2](#)). HP-processed orange juice exhibited a significant increase in total carotenoids (49.19%) and vitamin A content (30.89%) compared to an untreated juice ([Plaza \*et al.\*, 2011](#)). According to [De Ancos \*et al.\* \(2002\)](#) orange juices treated at 350 MPa/30°C/5 min exhibited a higher carotenoid content ( $\alpha$ -carotene, 60%;  $\beta$ -carotene, 50%;  $\alpha$ -cryptoxanthin, 63%;  $\beta$ -cryptoxanthin, 42%) than freshly squeezed juices, which was attributed to the pressure-induced denaturation of carotenoid-binding proteins. HP processing (500–700 MPa, 100°C) increased the extractability of lycopene from tomato juice, whereas thermal treatment had a negligible effect on its concentration ([Gupta \*et al.\*, 2011](#)). These results are in agreement with those found by [Hsu \(2008\)](#), who reported a significant increase in lycopene and total carotenoids (up to 60 and 62%, respectively) as a result of HP treatment of tomato juice (300–500 MPa/25°C/10 min). Carotenoids are localized in subcellular organelles (plastids), in chloroplasts and chromoplasts, mainly associated with macromolecules such as proteins and membrane lipids ([Gärtner \*et al.\*, 1997](#)). It has been reported that high pressure treatments above 300 MPa cause irreversible protein denaturation at room temperature leading to irreversible damage in vegetable cell membranes ([Hendrickx \*et al.\*, 1998](#)). Therefore, HP treatment could improve the extractability of carotenoids owing to a better release of these bioactive compounds from the fruit matrix, leading to different release rates for specific carotenoid types.

Storage may cause the instability of the polyene chain of carotenoids. As a consequence, these compounds may undergo geometric isomerisation, which is promoted by heat, light, and acids, as well as oxidation, which is stimulated by light, heat, metals, enzymes, and peroxides, and inhibited by antioxidants ([Rodríguez-Amaya, 1997](#)). Non significant changes in carotenoids concentration have been reported for at least 10 days of refrigerated storage in an orange juice treated at 100 MPa/60°C/5 min, whereas substantial losses have been found at the

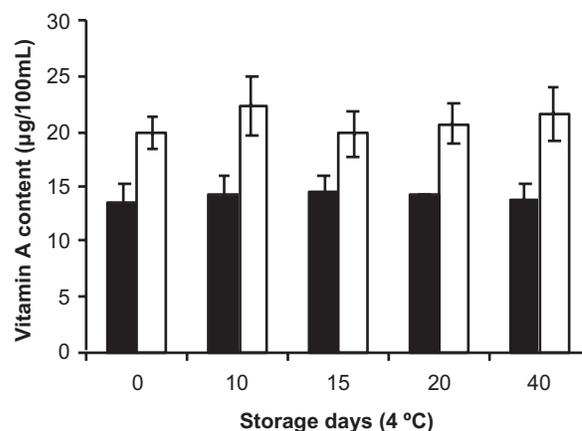


**FIGURE 77.1** Contents of the main carotenoids in HIPEF-treated and thermally-treated tomato juice through storage at 4°C. HIPEF: 35 kV/cm for 1500  $\mu$ s. TT: 90°C for 1 min. Data shown are mean 4 set of analysis of tomato juices. (Unpublished).

**TABLE 77.2** Effect of High Pressure Processing on Carotenoids Compounds of Fruit Juices

| Product      | Treatment Conditions          | Major Finding  | Reference                           |
|--------------|-------------------------------|--|-------------------------------------|
| Tomato juice | 300 MPa/4°C and 25°C          | 62 and 56% increase in total carotenoids and lycopene contents, respectively                                     | Hsu (2008)                          |
| Orange juice | 50–350 MPa/30–60°C/2.5–15 min | Up to 23% increase in total carotenoids after 100- to 350-MPa treatments   | De Ancos <i>et al.</i> (2002)       |
|              | 100–400 MPa/30–60°C/1–5 min   | 10–32% enhancement of total carotenoids content<br>Zeaxanthin content increased regardless the treatment applied | Sánchez-Moreno <i>et al.</i> (2003) |
|              | 600 MPa/20°C/60 s             | No modifications in $\beta$ -carotene levels   | Bull <i>et al.</i> (2004)           |
|              | 400 MPa/40°C/1 min            | Increase in the extractability of individual carotenoids   | Plaza <i>et al.</i> (2011)          |

end of the storage period of samples processed at 350 MPa/30°C/2.5 min or 400 MPa/40°C/1 min (20.56% and 9.16%, respectively) (Sánchez-Moreno *et al.*, 2003). According to De Ancos *et al.* (2002), the vitamin A content of HP-treated orange juice significantly decreased throughout storage (42% and 24% reduction after 30 days storage of samples treated at 50 MPa/30°C/5 min and 100 MPa/30°C/5 min, respectively). This decrease significantly surpassed the 9% reduction observed in untreated juice samples. In contrast, Bull *et al.* (2004) reported that the  $\beta$ -carotene content of HP-treated (600 MPa/20°C/60 s) and heat-treated (85°C, 25 s) orange juices did not significantly decrease during storage at 4°C. Thus, the extent of the oxidation depends on the carotenoid involved. HP treatments have been reported to better maintain carotenoids than traditional thermal treatments in many juices. Gupta *et al.* (2010) reported a higher retention of carotenoids in HP treated tomato juice compared to thermally processed samples. HP-treated orange juice exhibited higher carotenoids concentration than heat-pasteurized juice during refrigerated storage at 4°C. In consequence, vitamin A values increased above 40% with respect to those in untreated samples (Plaza *et al.*, 2011) (Figure 77.2). The inactivation of enzymes involved in carotenoid losses during storage



**FIGURE 77.2** Vitamin A value of thermally processed and HP-treated orange juices. Vitamin A content: retinol activity equivalents per 100 mL. Data shown are mean  $\pm$  standard deviation of 4 set of analysis of orange juices. Thermally processed: 70°C/30s (black square). HP-treated: 400 MPa/4°C/1 min (white square). (Unpublished)

and the facilitation of extraction due to HP treatments are the reasons that some authors have put forward to explain those results (De Ancos *et al.*, 2002).

The *all-trans* isomers of carotenoids are the predominant form in nature (94–96%) in fruits and vegetables. However, the high concentrations of *in vivo cis* isomers suggest that they may be more bioavailable and/or more bioactive than the *all-trans* form, possibly due to the higher solubility and/or preferential incorporation of the *cis* isomers into chylomicrons. Varma *et al.*, (2010) reported that HP processing causes conformational changes from the *all-trans* to *cis* isomer form, indicating that high pressure application can induce isomerization, increasing the availability of the carotenoids in the sample.

## ANALYTICAL TECHNIQUES

Carotenoids have a long chain of unsaturated double bonds which confers instability and facilitates their reaction, namely with acids, oxygen, and active radicals in the presence of light. To avoid artifacts formation and quantitative losses, the analysis should be completed within the shortest time possible, at relatively low temperature, in exclusion of oxygen, using protecting atmosphere, under protection from light, excluding the light wavelengths absorbed by carotenoids, avoiding contact with acids, using high-purity solvents free from harmful impurities such as chlorinated compounds and peroxides, and using material that does not adsorb carotenoids (Britton, 1991). Applicability of an analytical method depends on the matrix, the analytes present, and their levels. Various methods have been reported for the determination of carotenoids in nonthermally processed fruit juices including chromatographic and spectroscopic procedures.

## Extraction Techniques

For extracting carotenoids from fruit juices, there is not an universally accepted method, mainly due to the great number of possible carotene/matrix combinations. For food samples that contain large amounts of water such as fruit juices, it is advisable to use organic solvents that are miscible with water, to allow a better release of carotenoids from the matrix. Diluted solutions of ethanol, hexane, ethyl acetate, acetone, and tetrahydrofuran, alone or in combination, are generally used for the extraction of carotenoids from fruit juices. The conjugated double bonds system found in carotenoids is the main source of problems associated with carotenoids manipulation. To avoid this issue, butylhydroxytoluene (BHT) is used to avoid oxidation at concentrations ranging between 0.05 and 0.1% (Cortés *et al.*, 2006a,b; Odriozola-Serrano *et al.*, 2007; Oms-Oliu *et al.*, 2009).

## Saponification

Saponification (alkaline hydrolysis) has been widely used to facilitate carotenoid analysis because it is an effective method to remove chlorophylls and unwanted lipids that may interfere in the chromatographic separation, or to hydrolyze the carotenoid esters, therefore simplifying the chromatographic profiles. In several studies related to nonthermally treated juices, saponification is conducted by mixing carotenoids with methanolic potassium hydroxide (5–10%) under nitrogen at room temperature for a few hours in darkness. After this, the carotenoid solution is transferred to a separatory funnel,

diethyl ether is added, the solution is then washed several times with water to remove the alkali and eventually evaporated (De Ancos *et al.*, 2002; Sánchez-Moreno *et al.*, 2005; Cortés *et al.*, 2006a,b; Morales-De la Peña *et al.*, 2011)

## Separation, Identification and Quantification by HPLC

High-performance liquid chromatography (HPLC) methods are advantageous compared to direct measurement techniques, especially in what regards to specificity, sensitivity, or easiness of operation. Reversed-phase C<sub>18</sub> columns have been widely used for carotenoids separation because of the weak hydrophobic interaction with the analytes, their compatibility with most carotenoid solvents, and the polarity range of carotenoids. Mobile-phases used in HPLC methods for the determination of carotenoids in different fruit juices are summarized in Table 77.3. Carotenoids are easily identified at wavelengths between 290nm and 500nm. Quantification can be carried out by comparison with internal or external standards.

## Spectrophotometric Methods

Carotenoids may be easily identified since the majority exhibit a maximum in the long UV range. Absorbance spectroscopy is the simplest method to identify carotenoids because the absorbance spectrum is the fingerprint of each carotenoid molecule. Total lycopene content has been measured spectrophotometrically in nonthermally treated tomato juices

TABLE 77.3 Mobile Phase Used for Carotenoids Analysis of Nonthermally Treated Fruit Juices

| Product             | Carotenoids  | Mobile Phase                             | Reference   |
|---------------------|--|--|---|
| Tomato juice        | Lycopene   | MeOH:methyl tert-butyl ether             | Min <i>et al.</i> (2003)<br>Gupta <i>et al.</i> (2010, 2011)                                    |
|                     | Total carotenoids and lycopene   | 1-butanol:ACN: dichloromethane           | Hsu (2008)  |
|                     | Lycopene, neurosporene, $\gamma$ -, $\zeta$ -, $\beta$ -, phytofluene and phytoene   | ACN:MeOH:hexane<br>Dichloromethane       | Odriozola-Serrano <i>et al.</i> (2009)  |
| Carrot juice        | $\beta$ -Carotene  | ACN:MeOH:hexane<br>dichloromethane       | Quitão-Teixeira <i>et al.</i> , (2009)  |
| Orange juice        | Lutein, zeaxanthin, $\alpha$ - and $\beta$ -criptoxanthin, $\alpha$ - and $\beta$ -carotene  | ACN:MeOH:water:<br>dichloromethane       | De Ancos <i>et al.</i> (2002) Sánchez-Moreno <i>et al.</i> (2003)<br>Plaza <i>et al.</i> (2011) |
|                     | $\beta$ -Carotene  | Acetonitrile:MeOH:1,<br>2-dichloroethane | Bull <i>et al.</i> (2004)   |
|                     | Lutein, zeaxanthin, isolutein, $\beta$ -criptoxanthin, phytoene + phytofluene, $\alpha$ - $\beta$ and $\zeta$ -carotene  | MeOH: methyl tert-butyl ether:water      | Cortés <i>et al.</i> (2006ab)   |
| Orange–carrot juice | Antheraxanthin, mutatoxanthin, violaxanthin, lutein, zeaxanthin, isolutein, $\beta$ -criptoxanthin, phytoene + phytofluene, $\alpha$ - $\beta$ and $\zeta$ -carotene | MeOH: methyl tert-butyl ether:water      | Torregrosa <i>et al.</i> (2005)   |

(Odrizola-Serrano *et al.*, 2007) and watermelon juices (Oms-Oliu *et al.*, 2009). Extraction of lycopene from juices was carried out with a mixture of acetone, ethanol, and hexane. The lycopene content of each sample was estimated using the absorbance at 503 nm and the sample weight.

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