EFFECTS OF HIGH INTENSITY PULSED ELECTRIC FIELDS OR THERMAL TREATMENTS AND REFRIGERATED STORAGE ON ANTIOXIDANT COMPOUNDS OF FRUIT JUICE-MILK BEVERAGES. PART II: CAROTENOIDS

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ABSTRACT

Effects of High Intensity Pulsed Electric Fields (HIPEF) or thermal treatments over carotenoids of mixed beverages were evaluated after processing and during refrigerated storage. Nine carotenoids were identified in all samples, being β-carotene the major compound. Just after processing, concentration of most individual carotenoids diminished around 6-30% in fruit juice-skim milk (FJ-SM) and fruit juice-whole milk (FJ-WM) beverages; except, 9-*cis*-violaxanthin-neoxanthin, antheraxanthin and phytoene-phytofluene which increased in the thermally treated FJ-SM (5-19%) and 9-*cis*-violaxanthin-neoxanthin which also augmented in HIPEF processed FJ-SM (24%). Total carotenoid (TC) content decreased (6-13%) in both beverages, irrespectively of the treatment applied. Through the storage, individual carotenoid concentration varied widely in all beverages, depending on the carotenoid structure; however, TC tended to decrease. Nonetheless, changes observed HIPEF treated beverages were less, than those in the conventionally pasteurized ones. Hence, HIPEF is as effective treatment, or even more, than heat processing to preserve bioactive compounds in mixed beverages.

Key words: HIPEF, thermal treatments, carotenoids, mixed beverages

PRACTICAL APPLICATIONS

Nowadays, modern population is claiming for products with high antioxidant properties and attractive flavor. In this sense, mixed beverages formulated with fruit juices and milk are attracting the attention of consumers due to their exotic taste and their elevated concentration of bioactive substances, such as carotenoids. However, these compounds are very susceptible to heat and are easily degraded during processing and storage. Hence, finding preservation technologies for mixed beverages capable to maintain their carotenoid concertation as high as possible after treatment and during shelf-life, is one of the greatest challenges for the food industry. In this sense, the relevance of this study is to demonstrate that High Intensity Pulsed Electric Fields (HIPEF) processing, is a potential alternative to thermal pasteurization in order to obtain not only safe but nutritious fruit juice-mixed beverages with elevated concentration of carotenoids, especially those with pro-vitamin A activity, such as β-carotene.

INTRODUCTION

Nowadays, consumers have become more and more aware about their eating habits. There is a marked trend toward the consumption of fresh-like products rich in health-related compounds. These facts have motivated food scientists and technologists to develop new and convenient products in order to meet the claims of modern population, while contributing to their welfare. Mixed beverages, containing fruit juices and milk, are a great source of antioxidants and bioactive compounds. Hence, they may be considered as an appropriate medium to include functional ingredients in human diet since they are a convenient and widely accepted products. Regular consumption of fruit-based beverages has been associated with reduced incidence of chronic and degenerative diseases (Neuhouser, 2004). These beneficial properties have been associated in part to their content of various bioactive compounds such as carotenoids.

The importance of carotenoid compounds is based in two main properties, their antioxidant capacity and their pro-vitamin A activity, with β-carotene having the highest activity (van der Berg et al., 2000; Marco et al., 1997). In addition, it has been proved that carotenoids modulate the pathogenesis of cancer and coronary hearth diseases (Giovanucci, 1999; Kritchevsky, 1999) and decrease the aged-related macular degeneration (Krinsky and Johnson, 2005). Due to their constrained polyene structure, carotenoids appear in *cis* and *trans* isomeric forms (Britton et al., 1995). Researches indicates that more than 50% of the carotenoids in the human body are found in the *cis* isomeric form, leading to the hypothesis that the *cis* form is the most available in the human body (Boileau et al., 2002). However, since carotenoids are highly unsaturated molecules with an extensive conjugate double-bonds system, they are susceptible to oxidation, isomerisation and other chemical changes during processing and storage (Shi and Le Maguer, 2000).

Although conventional thermal processing ensure safety and extend the shelf-life of beverages by the inactivation of microorganisms and deteriorative enzymes, it often leads to detrimental changes in their nutritional, sensory and physicochemical characteristics (Wolbang et al., 2008). Consequently, novel technologies for food preservation, such as High Intensity Pulsed Electric Fields (HIPEF), are being developed as alternative to heat pasteurization. Up to now, different studies have suggested that HIPEF treatment is efficient enough to destroy microorganisms and enzymes at equivalent levels to those reached by thermal treatments (Mosqueda-Mergar et al., 2008; Aguiló-Aguayo et al., 2010). Moreover, it has been reported that HIPEF process greatly retain health-related compounds on different products such as milk, fruit and vegetable juices as well as mixed beverages (Bendicho et al., 2002; Cortés et al., 2006; Odriozola-Serrano et al., 2009; Sánchez-Moreno et al., 2003; Morales-de la Peña et al., 2010; Salvia-Trujillo et al., 2011a and 2011b; Zulueta et al., 2013). Up to now, most of the available information related to the effect of HIPEF over bioactive compounds in mixed beverages has been focused on different vitamins, polyphenols, flavonoids and antioxidant capacity. However, little is known about how HIPEF treatment can alter or modify the carotenoid composition of mixed beverages. In this sense, the purpose of the present work was to evaluate and compare the effects of HIPEF and thermal treatments on the carotenoid profile of mixed beverages formulated as a blend of fruit juices and whole or skim milk. In addition, the effect of storage (56 days at 4 °C) on the concentration of these bioactive compounds was investigated.

MATERIAL AND METHODS

Beverage preparation

Orange, mango, kiwi and pineapple fruits were purchased in a local supermarket (Lleida, Spain) at commercial ripeness stage. Fruit was sanitized in a 200 ppm sodium hypochlorite solution for 2 min and rinsed with tap water. Then the juice from each fruit was extracted, mixed and added with commercial pasteurized whole (3.5% fat) or skim (0.3% fat) bovine milk and sugar in the following proportions: orange (30%), kiwi (25%), mango (10%), pineapple (10%), milk (17.5%) and sugar (7.5%). These proportions were selected in basis of previous studies in order to maximize the content of vitamin C in the beverages (Salvia-Trujillo et al., 2011a). Whole and skim milk were selected in order to prove if the content of fat in the milk interferes with the effects of the treatments over bioactive compounds. Fruit juice-whole milk (FJ-WM) or –skim milk (FJ-SM) beverages were filtered through a cheese cloth, and their pH was adjusted to 3.35 with citric acid in order to have an acidified product and avoid microbial growth. Beverages where filled in 25 mL sterile plastic flasks at 4 ± 1 °C with minimal headspace volume and stored at 4ºC. Because of microbial growth, the treated and untreated samples were stored for no more than 56 and 14 days, respectively (Salvia-Trujillo et al., 2011a).

HIPEF processing

A continuous flow bench-scale system OSU-4F (The Ohio State University, Colombus, OH), delivering square-wave pulses, was used for HIPEF processing. On the basis of previous work (Salvia-Trujillo et al., 2011a), the HIPEF treatment was conducted at 35 kV/cm electric field strength for 1800 μs, a pulse frequency of 200 Hz, and 4 μs bipolar pulses. Electric field strength, pulse duration and frequency were controlled through a pulse generator (model 9410, Quantum Composers, Inc., Bozeman, MT) and measured with an oscilloscope (TEKScope, Tektronix Inc., Beaverton, OR). The samples were pumped through the system at a flow rate of 760 mL/min with a variable gear pump (model 752210-26, 106 Cole Palmer Instrument Co., Vermon Hills, IL). The system was composed of eight collinear treatment chambers serial connected. Each treatment chamber has two stainless steel electrodes separated by 0.292 cm, a diameter of 0.23 cm and a volume of 0.0121 cm^3. Between each treatment chamber the product was refrigerated in an ice-water bath, so that the temperature of the product was always below 40 °C.

Thermal treatment

Beverages were thermally treated at 90°C for 60 seconds to ensure the inactivation of spoilage microorganisms and to simulate a conventional preservation treatment based on literature (Nagy et al., 1993). Afterwards, they were pumped with a peristaltic pump (model D-21 V, Dinko, Barcelona, Spain) at a flow rate of 40 mL/min and passed through a tubular stainless steel heat exchanger coil system (0.037 cm^2 section and 1100 cm long) submerged in a hot water bath at 90 °C (Universitat de Lleida, Spain). After thermal processing, the beverage was immediately cooled in a heat exchange coil immersed in an ice water-bath.

Carotenoid compounds

Carotenoids were extracted and quantified by HPLC, following a procedure validated by Cortés et al. (2004), with some modifications.

Extraction and saponification

Thirty milligrams of each beverage were mixed with 50 mg magnesium hydroxide carbonate, 25 mg BHT and 35 mL ethanol:hexane solution (4:3, v/v) in an amber round-bottom flask under N_2 atmosphere. After 45 min of continuous agitation, the sample was filtered through Whatman No. 1 filter paper. The residue was washed with 35 mL of ethanol:hexane solution (4:3, v/v) and filtered twice with 12.5 mL of ethanol and once with 12.5 mL of hexane (until it was colorless). All of the liquid filtrates were combined and washed twice with 50 mL of sodium chloride solution (10%) and then, three times with 50 mL of water in an amber decanting funnel. The organic phase was evaporated at 40 °C in a rotary evaporator (200 mbar). The residue obtained was saponified under N_2 atmosphere (30 min) adding 10 mL of diethyl ether, 10 mL of methanolic KOH 0.5 M with 0.1% BHT (w/v). At the end of this time, 20 mL of diethyl ether were added. The obtained solution was washed again with 50 mL of sodium chloride solution (10%) and then, three times with 50 mL of water until neutral pH was obtained. In order to ensure that the water was totally eliminated, 10 mL of absolute ethanol was added to the samples. The solution was then evaporated at 45 °C until dryness. The obtained residue was dissolved with 4 ml of diethyl ether and placed in an amber glass vial. The solvent was evaporated under N_2 and the residue was stored at -18 °C until chromatographic analysis. Before injection in to the HPLC system, the carotenoid extract was reconstituted with 1 mL of methanol:*tert*-butyl methyl ether solution (70:30, v/v).

Chromatography conditions

An aliquot of 20 µL of the extracted samples was injected into the HPLC system, which was equipped with a 600 Controller, a 486 Absorbance Detector programmed to scan from 200 to 350 nm, a thermostatic column compartment, and a 717 Plus Auto Sampler with cooling system (Waters, Milford, MA). Carotenoids were separated using a reverse-phase C18 Spherisorb ODS2 (5 µm) stainless steel column (4.6 mm x 250 mm). Gradient elution was needed for complete separation of the analytes (Table 1). The mobile phase consisted on four eluents: (A) methanol/ammonium acetate 0.1M, (B) milli-Q water, (C) methyl-*tert*-butyl ether and (D) methanol. The flow rate was fixed at 1 mL/min and the total run time was 60 min. The column was set at 30 °C, while sample vials (amber) on the auto sampler were preserved at 4 °C. Carotenoids were identified by UV-vis spectral data and their specific retention times (Cortés et al., 2004; Mouly et al., 1999). It is important to highlight that 9-cis-violaxanthin and neoxanthin, as well as phytoene and phytofluene, have similar chemical structures and therefore, related spectral characteristics (Cortés et al., 2004). Hence, this procedure was not capable to completely resolve their peaks and they were quantified together. Quantification of the identified carotenoids was carried out by integration of the peak areas. Data were compared to calibration curves of each carotenoid and results were expressed as µg of carotenoid per 100 mL of beverage.

Statistical analysis

Treatments were conducted in duplicate and two replicate analyses were carried out for each sample. Analysis of the variance (ANOVA) was performed to compare different formulations and treatments. Least significance difference (LSD) test was employed to determine differences between means immediately after processing and throughout the storage. The confidence interval was set at 0.95 for analysis and procedures. Results were analysed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md).

RESULTS AND DISCUSSION

Carotenoid profile

The initial carotenoid profile in the freshly prepared FJ-WM and FJ-SM beverages, as well as in those HIPEF and thermally treated, is presented in Fig. 1. Regardless of the treatment applied, β-carotene, lutein and zeaxanthin were the carotenoids present at the highest concentration in both whole and skim milk mixed beverages, reaching values of 155 – 190.53 µg/100 mL, 65.14 – 98.87 µg/100 mL and 28.26 – 41.35 µg/100 mL, respectively. In a previous study, Morales-de la Peña et al. (2011) evaluated the carotenoid profile of a mixed beverage containing a blend of fruit juices (orange, kiwi and pineapple) and soymilk. Different to our results, these authors reported that lutein was the major carotenoid present in the beverage, followed by zeaxanthin and β-carotene. However, Zulueta et al. (2007) stated that β-carotene was the predominant carotenoid in different commercial fruit juice-milk beverages containing mango among other fruits. They mentioned that mango contributed significantly to the content of this compound. At the same time, the authors reported that a beverage containing orange and pineapple juices blended with skim milk had a significant concentration of lutein and zeaxanthin. On the other hand, it is well known that β-carotene is the predominant carotenoid in dairy products, comprising approximately 90% of total carotenoid content (Hulshof et al., 2006). Then, the highest β-carotene content in the mixed beverages evaluated in this study could be mainly attributed to the mango and the milk used as ingredients of the FJ-WM and FJ-SM beverages formulations; whereas, the elevated concentration of lutein and zeaxanthin may be related to the orange and pineapple juices.

As can be observed in Fig. 1, the concentration of most individual carotenoids was higher in FJ-WM beverages, than in those prepared with skim milk (FJ-SM). In agreement with Jensen (1995) and Castenmiller and West (1998), carotenoid compounds are stored in fat globules; then, during skimmed milk processing a significant amount of these compounds may be lost. Hulshof et al. (2006) proved that full fat milk (standardized to 3.5% fat) had a higher carotenoid concentration that semi-skimmed milk (standardized with 1.5% fat). Hence, the type of milk used in fruit juice-milk beverage formulations is directly related with the carotenoid content in the end product.

Effects of processing on carotenoid profile

Initial concentration of most individual carotenoids identified in the FJ-WM and FJ-SM beverages diminished just after HIPEF or thermal treatments (Fig. 2, 3, 4, day 0), except for *cis*-antheraxanthin, in HIPEF-treated FJ-WM beverage; and 9-*cis*-violaxanthin-neoxanthin, antheraxanthin and phytoene-phytofluene in FJ-SM beverages, which slightly increased after HIPEF or heat processing. To the best of the authors' knowledge, information related to the effects of HIPEF or thermal treatments on individual carotenoid compounds in mixed beverages is scarce; but there are some works conducted in individual fruit or vegetable juices and a fruit juice-soymilk beverage (Cortés et al., 2006; Odriozola-Serrano et al., 2009; Torregrosa et al., 2005; Vallverdú-Queralt et al., 2013; Morales-de la Peña, 2011; Plaza et al., 2011).

Reported results from the above mentioned studies vary widely regarding to the food matrix. Namely, Torregrosa et al. (2005) stated that the concentration of some carotenoids rose, whereas the content of others decrease when thermal pasteurization or HIPEF treatments were applied to an orange-carrot juice. Otherwise, Plaza et al. (2011) observed that a HIPEF processing applied to an orange juice did not exert any change on the carotenoid content in comparison with the freshly squeezed juice. However, Cortés et al. (2006) reported that the concentration of most of the carotenoids identified in orange juice decreased after thermal or HIPEF processing; but observed losses were higher in pasteurized samples than in those HIPEF processed. On the other hand, Odriozola-Serrano et al. (2009) evaluated the effects of HIPEF and heat pasteurization in tomato juices. These authors observed that lycopene content significantly increased after processing, irrespectively of the treatment applied. However, the concentration of other minor individual carotenoids such as phytoene, phytofluene, and neurosporene diminished after processing. In addition, Morales-de la Peña et al. (2011) observed that, immediately after HIPEF or heat processing, lutein, zeaxanthin and cryptoxanthin concentration in fruit juice-soymilk beverages was significantly reduced; whereas β-carotene content slightly rose in the HIPEF treated beverage.

As can be observed from the different studies carried out during the last years, the concentration of individual carotenoids in fruit based beverages is highly related with their chemical structure and processing conditions. These compounds may undergo geometric isomerization (promoted by heat, light and acids) and oxidation (stimulated by light, heat, metal, enzymes and peroxides) processes which are the main causes of carotenoid changes described in the literature (Melendez-Martinez et al., 2007; Rodríguez-Amaya, 1997). Hence, it might be possible that different biochemical reactions took place during HIPEF or thermal treatments resulting in an augment or a loss of the individual carotenoids compounds identified in the FJ-WM and FJ–SM beverages. Furthermore, it has been reported that thermal treatment may imply an increase in some individual carotenoids, owing to enzymatic degradation, and unaccounted losses of moisture, which concentrate the sample (Rodríguez-Amaya, 1997). According to Ngyuen and Schwartz (1999), the different treatments applied to the beverages might disrupt cell membranes and protein-carotenoids complex, making carotenoids more accessible for extraction. However, on the other hand, treatments could also cause instability on the polyene chain of the carotenoids resulting in degradation. According to this, it is suggested to do more specific studies in order to better understand the possible mechanisms of individual carotenoids changes during processing.

The initial total carotenoid (TC) concentration of the FJ-WM and FJ-SM beverages, obtained by the sum of the nine individual carotenoids, ranged between 344.10 ± 5.35 and 477.95 ± 0.19 µg/100 mL. These range was higher than that reported by Zulueta et al. (2013) in an orange juice-milk beverage, which varied from 309.49 ± 23.31 - 365.65 ± 21.14 µg/100 mL. Mendiola et al. (2008) and Zulueta et al. (2007) stated that fruit-based drinks have widely different carotenoid content, which mainly depend on the variety and ripeness stage of the fruits used as ingredients of the beverage under study. As a result of the changes observed in the concentration of individual carotenoids in the untreated and HIPEF or thermally treated FJ-WM and FJ-SM beverages, their TC content significantly decreased just after processing, irrespectively of the treatment applied (Table 2). However, TC content in both beverages treated by HIPEF (90.2 – 94.4%) was better retained than in those that were thermally treated (87 – 91.1%). In agreement to our results, it has been reported that immediately after HIPEF or heat processing the TC concentration of a fruit juice-soymilk beverage (Morales-de la Peña et al., 2011) and an orange juice (Cortés et al., 2006) was significantly diminished. However, Zulueta et al. (2013) applied a HIPEF (25kV/cm and 280 μs) or thermal (90°C and 15s) treatment, to an orange juice-milk beverage and their results showed no significant changes in TC content regarding to the just prepared beverage. It might be possible that lower processing conditions applied by Zulueta et al. (2013), compared to those used in this study, lead to less effects on carotenoid compounds; therefore, no significant changes on their concentration were observed. Furthermore, the severity and extent of degradation reactions of carotenoids depend on their chemical structure and the environmental conditions (Bitton and Hornero-Mendez, 2001; Ramakrishnan and Francis, 1980). In addition, it has been reported that the application of various treatments can lead to the formation of *cis* isomers, which do not have the same bioactive activity as the *trans* isomers (Zulueta et al., 2007). In this way, HIPEF or thermal processes applied in this research caused more degradation reactions of the different carotenoid identified in the beverages resulting in a lower concentration TC.

Effects of storage on carotenoid profile

As can be seen in Fig. 2, 3 and 4, the concentration of each carotenoid identified in both beverages varied widely throughout the storage, depending on its structure and stability. It was observed that the content of some of them, such as 9-*cis*-violaxanthin-neoxanthin, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin and phytoene-phytofluene, tended to increase or remain with no significant changes along the time; while, the content of *cis*-antheraxanthin, antheraxanthin, lutein and β-carotene decreased throughout the storage, irrespectively of the treatment applied (Fig. 2, 3, 4).

A significant increase of 9-*cis*-violaxanthin-neoxanthin content was observed in HIPEF and thermally treated FJ-WM beverages leading to levels of 26.58 – 27.45 µg/100 mL after 56 days of storage (Fig. 2). Conversely, there were no significant differences in the content of 9-*cis*-violaxanthin-neoxanthin in the treated FW-SM beverage at the end of the storage regarding its initial values. Different to our results, it has been reported that there was a sharp decrease in the 9-*cis*-violaxanthin-neoxanthin content in an untreated and HIPEF-treated orange juice and an orange-carrot juice stored at 10 °C, and it was not detected in the beverages after sixth and three weeks, respectively (Cortés et al., 2006; Giger, 2002). Since carotenoids are dissolved in fat matrix, where they are protected from oxidizing action by vitamin E and other antioxidants (Ball, 1988), it may be possible that the fat content of the whole milk used as ingredient of the FJ-WM beverages acted as a protector of this carotenoid from degradation or, inclusive leads to other reactions that induce to its formation.

As Fig. 2 shows, the concentration of *cis*-antheraxanthin and antheraxanthin in the untreated, HIPEF and thermally processed FJ-WM and FJ-SM beverages decreased abruptly with time. Similarly, Morales-de la Peña et al. (2011) observed that antheraxanthin content in fresh fruit juice-soymilk beverages as well as in those HIPEF or thermally treated was significantly depleted after 56 days of refrigerated storage. In this way, Cortés et al. (2006) and Torregrosa et al. (2005), reported that the concentration of antheraxanthin in an orange juice and a blend of orange and carrot juices, respectively, decreased during the storage at 2 and 10 °C. As stated by Giger (2002), carotenoid 5,6-epoxides may be transformed to carotenoid 5,8-epoxides under acidic conditions, so antheraxanthin can be converted into furanoid structure mutatoxanthin.

Regarding lutein (Fig. 3), it was observed that independently of the treatment applied and the type of milk used in the beverage formulation, the concentration of this carotenoid significantly decreased along the storage period; reaching values, at 56 days, of 33.02 – 37.96 μg/100 mL in FJ-WM beverage and 33.35 – 40.26 μg/100 mL in FJ-SM beverage. These results agree with those reported by Morales-de la Peña et al. (2011), Wolbang et al. (2008), Sánchez-Moreno et al. (2003) and Varvelldú-Queralt et al. (2013) who also observed that lutein showed the major losses among all carotenoids throughout storage in a fruit juice-soymilk beverage, melon, orange and tomato juice, respectively. According to Zulueta et al. (2007), owing to the presence of oxygen in its chemical structure, lutein is more susceptible to degradation with time. Likewise, Plaza et al. (2011) mentioned that among all the carotenoids identified in orange juice, lutein showed the highest losses after 40 days of storage at 4 °C, irrespectively of the treatment applied, being the carotenoid more susceptible to isomerization or oxidation processes.

The concentration of zeaxanthin, α-cryptoxanthin, β-cryptoxanthin (Fig. 3) and phytoene-phytofluene (Fig. 4) in both untreated and treated beverages slightly increased over time. Different results have been reported related to the concentration of these carotenoids during shelf-life of some fruit juices and beverages treated and stored at different conditions. Namely, Cortés et al. (2006) observed that, whereas zeaxanthin concentration decreased in an orange juice stored at 2 or 10 °C during the first week, followed by an increase during the rest of the storage period; the concentration of β-cryptoxanthin diminished along the time. Conversely, there was a slight but significant increase in the phytoene-phytofluene at the end of the storage in the pasteurized and HIPEF treated orange juices. Otherwise, α-cryptoxanthin and β-cryptoxanthin content in a fruit juice-soymilk beverage was kept constant through the storage at 4 °C; nonetheless, zeaxanthin concentration significantly decreased as storage time increased (Morales-de la Peña, et al. 2011). Similar to the effects observed in 9-*cis*-violaxanthin-neoxanthin in both mixed beverages evaluated in this study, it could be possible that the fat content of the milk used in the mixed beverages formulation protected these carotenoids from degradation; as well, other reactions could take place during the storage of this kind of beverages which lead to the formation of some carotenoids.

Finally, β-carotene content of HIPEF treated FJ-WM and FJ-SM beverages remained with no significant changes at the end of the storage; conversely, those beverages conventionally treated showed a significant decrease in the concentration of this carotenoid (Fig. 4). In this sense, Morales-de la Peña et al. (2011) also reported that fruit juice-soymilk beverages treated with HIPEF or heat had lower concentration of β-carotene at 56 days of refrigerated storage (4 °C); nevertheless the degradation was higher in the heat treated beverage. In agreement to these results, Vallverdú-Queralt et al. (2013) observed that the β-carotene content decreased 10 – 11% in tomato juices treated with HIPEF and 14 – 15% in heat processed tomato juices. Other authors have studied the changes of this carotenoid in HIPEF-treated juices through the storage, reporting a higher maintenance in comparison to thermally treated juices (Odriozola-serrano et al., 2008).

Overall, HIPEF treatment had less effects than heat processing on the concentration of individual carotenoids in FJ-WM and FJ-SM beverages under chilled storage. In this sense, it can be stated that different reactions amongst individual carotenoids in mixed beverages might take place along the time, which are mainly influenced by the ingredients used for the formulation, the processing applied and the storage conditions. However, further studies are needed to better explain the mechanisms of individual carotenoid reactions in mixed beverages containing different fruit juices and milk as affected by storage time.

Regarding TC concentration of HIPEF or thermally treated FJ-WM and FJ-SM beverages, it can be observed in Fig. 5 that it decreased as storage time increased, while the content of TC in untreated beverages was only diminished in 5% after 14 days. At the end of the storage (56 days), HIPEF treated beverages maintained TC concentration better (FJ-WM-83.4%; FJ-SM-90.1%) than those heat treated (FJ-WM-76.6%; FJ-SM-89.6%). Morales-de la Peña et al. (2011) reported that the TC content of untreated and HIPEF or thermally treated fruit juice-soymilk beverages tended to decrease with storage time approximately 27 to 33%. Even though there were no significant differences between treated beverages, those HIPEF processed showed slightly higher TC content than the thermally treated samples at the end of the storage. In a similar way, Zulueta et al. (2013) observed that the values of TC in an orange juice-milk beverage diminished with the elapse of storage time, and the concentrations found at the end of the storage were significantly lower in the thermally pasteurized beverages than in those HIPEF-treated. Also, Esteve et al. (2009) found that, in refrigerated orange juice, the concentration of TC was less affected by HIPEF than by conventional thermal process. The instability and degradation of carotenoids is due to susceptibility to oxidation and geometric isomerization of their polyene chain (Zulueta et al., 2013).

Moreover, Shi and Le Maguer (2000) stated that carotenoids are susceptible to oxidation in the presence of light, oxygen and low pH. Then, the changes observed in the TC content of mixed beverages throughout the storage might be due to the low pH of both beverages (FJ-WM, 3.31 ± 0.03; FJ-SM, 3.32± 0.01), and the light and oxygen that might passed through the bottles in which the samples were stored.

CONCLUSIONS

Nine carotenoids were identified in FJ-WM and FJ-SM beverages. Among them, β-carotene was found in highest concentration (155 – 190.53 μg/100 mL), followed by lutein and zeaxanthin. Immediately after HIPEF or thermal processes, the concentration of most individual carotenoids in FJ-WM and FJ-SM beverages diminished (6-30%), while others slightly increased or remained with no significant changes. As a result, the TC content significantly decreased in both beverages irrespectively of the treatment applied. However, HIPEF treated beverages (356.55 – 404.02 μg/100mL) had higher concentration of carotenoids than those thermally treated (344.1 – 389.14 μg/100mL). Along the storage period, individual carotenoids in both beverages underwent different changes; whereas most of them suffered significant losses in their concentration, others remained with no changes or even increased. Nonetheless, the total carotenoid content depleted with time, irrespectively of the treatment applied. At the end of the storage, the concentration of carotenoids was higher in HIPEF treated beverages (83.4 – 90.1%), compared the heat processed samples (76.6 – 89.6%). Hence, the application of HIPEF process could lead to mixed beverages with higher carotenoid concentration than those thermally processed. Then, the food industry could apply this non-thermal technology in order to meet current consumers' claims for quality food with high nutritional value and antioxidant compounds. However, further research should be conducted in order to understand the associated mechanisms of the degradation, interconversion or generation of carotenoid compounds during processing and storage. In this sense, it will be possible to better optimize the preservation treatments to obtain healthy products.

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