Food Chemistry 371 (2022) 130821

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Greater bioavailability of xanthophylls compared to carotenes from orange juice (high-pressure processed, pulsed electric field treated, low-temperature pasteurised, and freshly squeezed) in a crossover study in healthy individuals

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ARTICLE INFO

Keyword:
β-cryptoxanthin
β-carotene
Lutein
Pulsed electric field
High-pressure processing
Low-temperature pasteurisation

ABSTRACT

This study examined the effect of the intake of orange juice provided freshly squeezed (FS) or processed using low-temperature pasteurisation (LP), high-pressure processing (HPP), or pulsed electric field (PEF) treatment on the serum carotenoid concentrations of 12 healthy individuals, aged 20–32 years, enrolled in a crossover study. Participants were instructed to consume 500 ml of orange juice/day for 14 days. Carotenoid concentrations in the orange juice as well as serum samples retrieved on days 7 and 14 were analysed via HPLC. A significant increase in serum xanthophyll concentrations, but not serum carotenes, was observed, with the highest increase in α- and β-cryptoxanthin. The processing technologies applied appeared to affect serum carotenoid concentrations, with concentrations being similar in the HPP and FS orange juice types. As high variability in serum carotenoid concentrations was observed, the effect of different technologies on serum carotenoid concentration warrants further studies with larger sample sizes.

1. Introduction

Oranges are popular fruits that are widely consumed worldwide. In the European Union, oranges are primarily eaten fresh (84%), with approximately 16% of production supplied for processing (USDA, 2020). Spain is the largest producer of oranges in the European Union. Oranges are most consumed fruit in Spain (MAPA, 2020). Orange juice is the most consumed fruit juice in Europe and worldwide (Chanson-Rolle, Braesco, Chupin, & Bouillot, 2016), with Europe being the second largest consumer of orange juice in the world, accounting for 36.04% of global consumption (Neves, Trombin, Marques, & Martinez, 2020).

Orange juice contains substantial quantities of several micronutrients and bioactive compounds, including vitamin C, folate, fibre, polyphenols, and carotenoids, and may contribute significantly to consumers' daily intake of these nutrients. The carotenoid profile of orange juice is among the most complex reported for fruit-derived food (Meléndez-Martínez, Vicario, & Heredia, 2007; Giuffrida et al., 2019). Provitamin A carotenoids, lutein, and zeaxanthin contained in orange juice (Dias et al., 2018) are relevant to human health (Granado, Olmedilla, & Blanco, 2003; IOM. Institute of Medicine (US), 2001; Ranard et al., 2017; IOM, 2000). According to data on food consumption from the most recent Spanish National Dietary Intake Survey, oranges and orange juice accounted for 11.8% of the total provitamin A dietary carotenoid intake in the Spanish adult population (Beltrán-de-Miguel,

Abbreviations: FS, freshly squeezed; HPP, high-pressure processing; LP, low-temperature pasteurisation /refrigerate storage; PEF, pulsed electric field.

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https://doi.org/10.1016/j.foodchem.2021.130821
Received 15 May 2021; Received in revised form 23 July 2021; Accepted 6 August 2021
Available online 10 August 2021

Estévez-Santiago, & Olmedilla-Alonso, 2015), as well as 2.7% and 1.6% of the lutein and zeaxanthin dietary intake, respectively (Estévez-Santiago, Beltrán-de-Miguel, & Olmedilla-Alonso, 2016). In oranges, β-cryptoxanthin is present in much higher quantities than β-carotene, and comparatively, evidence suggests that β-cryptoxanthin may be more bioavailable and more efficient in increasing serum levels of retinol in undernourished children than β-carotene, depending on the type of food (de Pee et al., 1998; Burri, Chang, & Neidlinger, 2011; Estévez-Santiago, Olmedilla-Alonso, & Fernández-Jalao, 2016; Olmedilla-Alonso, Rodríguez-Rodríguez, Beltrán-de-Miguel, & Estévez-Santiago, 2020). However, although oranges are a staple food in our diet and are major contributors to the dietary intake of β-cryptoxanthin, one of the main carotenoids in human blood, there are few studies analysing the metabolism of β-cryptoxanthin and the effect of its consumption on human health. Even fewer studies examine the effect of processing on the nutrient content of oranges and orange juice, and moreover, its effect on serum nutrient concentrations in human subjects (Burri, La Frano, & Zhu, 2016).

Commercially available fruit juices are typically processed using industrial technology to preserve and extend their shelf life by deactivating microorganisms and naturally present enzymes. Traditional thermal treatments (e.g., pasteurisation and sterilisation), reduce nutritional and bioactive compounds, change physicochemical properties (colour, flavour, and texture) (Morales-de la Peña, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2010; Zulueta, Esteve, & Frígola, 2010), and can modify their bioavailability (Sánchez-Moreno et al., 2004; Trancoso-Reyes et al., 2016). Thus, traditional thermal processing is being replaced by less intense thermal technologies, such as low-temperature pasteurisation and refrigerated storage, and non-thermal treatments such as high-pressure processing (HPP) and pulsed electric field (PEF) treatment, as alternatives that enhance food safety and shelf life without compromising the organoleptic qualities of the fruit (Sánchez-Moreno et al., 2004; Sánchez-Moreno et al., 2005; McInerney, Seccafien, Stewart, & Bird, 2007; Plaza et al., 2011; De Ancos, Rodrigo, Sánchez-Moreno, Cano, & Zacarías, 2020; Cilla et al., 2020). These developments enable processors to retain the flavour, colour, and health benefits of fresh foods, such as oranges. The beneficial health effects derived from the intake of orange juice are partly related to the bioavailability of their bioactive compounds. However, the carotenoid bioavailability of orange juice consumed by humans has hardly been studied, with most studies focusing on β-cryptoxanthin (Franke, Cooney, Henning, & Custer, 2005; Aschoff et al., 2015; Hornero-Méndez et al., 2018). We hypothesised that carotenoid bioavailability from orange juice would be the same, regardless of the processing method. Therefore, this study aimed to assess the effect of consuming freshly squeezed (FS) orange juice and orange juice processed using a variety of treatments (low pasteurisation and refrigerated storage, HPP, and PEF treatment) on serum carotenoid concentrations in a crossover study in apparently healthy individuals using multiple doses (supplementation approach).

2. Materials and methods

2.1. Orange juice

Oranges (*Citrus sinensis* L.) of the Navel Late variety (Spain) were purchased from a local supermarket and stored at 4 °C before being processed. Orange juice was obtained using a domestic squeezer (Lomi Model 4, Madrid, Spain) and then filtered using a 2 mm steel sieve. Portions of 250 ml of orange juice were packaged in low-permeability plastic bags (Doypack) under light vacuum and divided into two batches. One of the batches was designated as FS (freshly squeezed without treatment) and the other as HPP (high pressure processing).

2.1.1. High pressure processing (HPP)

The freshly squeezed orange juice were treated for 1 min at maximum pressure of 400 MPa at 25 °C (HPP). Two bags of packaged orange juice were placed into the vessel unit filled with water as a pressure-transmitting medium. The compression and decompression rates were both 2.5 MPa/s and this means that the total process time was 6.33 min. These treatment conditions were selected due to the good results in terms of health promoting characteristics demonstrated for this HPP-treated orange juice obtained in previous *in vivo* assays (Sánchez-Moreno et al., 2003). The hydrostatic pressure unit was formed by a vessel with a capacity of 2350 ml (Gec Alsthom ACB 900 HPP, type ACIP 665, Nantes, France). As a result of adiabatic compression, the maximum temperature in the vessel was 40 °C at 400 MPa. The pressure, time, and temperature were controlled by a computer program, and were constantly monitored and recorded during the process.

2.1.2. Pulsed electric field treatment (PEF)

Freshly squeezed orange juice was treated in a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH) using square-wave pulses. The PEF processing conditions were 35 kV/cm electrical field applied in a bipolar mode, 800 Hz pulse frequency, 4 μs pulse width, and 750-μs total treatment time (Sánchez-Moreno et al., 2004). PEF-treated orange juice was also packaged in low-permeability plastic bags (Doypack) under a light vacuum.

The HPP, PEF, and FS orange juice types were stored at 4 °C for a maximum of 2 days until they were delivered to the study participants.

The low pasteurised (LP) orange juice was a refrigerated pasteurised commercial orange juice (Don Simón Naranja Exprimida, Murcia, Spain).

2.2. Participants and study design

Twelve apparently healthy individuals (6 male and 6 female) were enrolled in a multiple-dose crossover study with a two-week duration. The inclusion criteria were as follows: age (20–32 years), body mass index (BMI) within healthy range, serum cholesterol and triglyceride concentrations within normal range, and serum retinol levels within the range of 31–70 μg/dL. The exclusion criteria were as follows: vitamin/mineral supplement intake, regular medication, pregnancy, lactation, chronic disease, and smoking status (current use of tobacco products). The participants received oral and written information about the study and provided written consent. This study was carried out in the Vitamins Unit of the Hospital Universitario Puerta de Hierro (HUPH) in Madrid and was approved by the Clinical Research Ethics Committee of the HUPH (Madrid, Spain).

The participants were instructed to consume 500 ml of orange juice/day in two doses, 250 ml in the morning and 250 ml in the afternoon over three consecutive 14-day periods, separated by 1–1.5-month washouts. The orange juice types assayed were FS, commercially available LP juice, and juices treated by HPP and PEF. All participants consumed the LP and HPP orange juice. Six participants consumed PEF orange juice and six consumed FS orange juice.

Blood samples were collected on the first day of the study, prior to the first intake. Thereafter, participants drank the juice at home in two doses of 250 ml each, one in the morning and one in the afternoon (500 ml/day) for 2 consecutive weeks. Blood was collected at baseline after fasting for 10 h, and on days 7 and 14 to analyse individual serum carotenoid levels. Serum samples were stored at –80 °C and analysed within 6 months of collection. The participants were instructed not to change their diet or lifestyle during the study period. Diet was not controlled during the intervention study.

2.3. Carotenoid analysis in orange juice and serum

Analyses of the carotenoid content in orange juice and in blood samples were performed using an HPLC chromatographic system consisting of an ALC/GPC chromatograph (Model 201, Waters Associates, Milford, MA, USA) equipped with an M45 pump, a manual injector

(Rheodyne), and a data acquisition system (Millenium Station, Waters Assoc., Milford, MA, USA) with a Spheri-5-ODS column (Brownlee Applied Biosystems, San José, CA, USA) and an RP-18 guard column. This column was used with a mobile phase in gradient elution at 1.8 ml/min from acetonitrile: methanol (85:15 v/v) for 5 min, and acetonitrile: dichloromethane: methanol (70:20:10, v/v/v) for 20 min. Ammonium acetate (0.2 g) was added to the methanol. Analytes were detected with a photodiode array detector (PDA 996, Waters Assoc., Milford, MA, USA) set at 425 nm for carotenoids and 325 nm for retinol. Carotenoids and retinol were identified by comparing their retention times and online ultraviolet (UV) spectra with those of authentic standards and quantified against standard calibration curves (Olmedilla, Granado, Gil-Martínez, Blanco, & Rojas-Hidalgo, 1997). The carotenoid analysis was focused on those with provitamin A activity, lutein, and zeaxanthin because of their importance for human eye health. The accuracy and precision of the carotenoid analytical method in serum were periodically checked through our participation in the Quality Assurance Program conducted by the National Institute of Standards and Technology.

The carotenoids and retinols were extracted from the serum as previously described (Olmedilla, Granado, Gil-Martínez, Blanco, & Rojas-Hidalgo, 1997). Briefly, 800 μL of serum, 800 μL of ethanol containing retinyl acetate (0.4 mg/L), and tocopheryl acetate (0.1 g/L) were added as internal standards. After vortex for 45 s, we extracted the serum twice with hexane (2 ml, stabilised with 0.1 g/L butylated hydroxtoluene), vortexing the extracts twice, for 3 min and 2 min, respectively. The organic phases were removed, pooled, evaporated under nitrogen atmosphere, reconstituted with 300 μL of a solution of tetrahydrofurane: ethanol (1:1), and injected (7.5 μL) into the HPLC system. The serum samples from each participant were analysed in duplicate.

Carotenoid extraction from the orange juice was performed in duplicate or triplicate using an extraction method described elsewhere (Olmedilla, Granado, Blanco, & Gil-Martínez, 1998). Briefly, carotenoids were extracted with tetrahydrofuran (THF): methanol (50:50), mixed at 12,000 rpm for 3 min, and partitioned into water: petroleum ether before evaporation to dryness. To hydrolyse the xanthophyll ester forms, extracts were saponified according to Granado, Olmedilla, Gil-Martínez, and Blanco (2001), with an excess of OHK over a short period (vortex 3–5 min). Extracts were reconstituted with THF: ethanol (1:1) and injected into the HPLC system.

2.4. Statistical analysis

Results are expressed as mean ± standard deviation (SD). The normal distribution of the data was assessed using the Kolmogorov-Smirnov test, and not all carotenoid concentrations in the serum and orange juices met the criteria for normal distribution. The differences in serum carotenoids in the orange juice at the beginning of the study were checked using the Student's *t*-test. There were no statistically significant differences ($p > 0.05$) between the mean serum concentrations at the beginning of each period in the intervention study. The GLM (mixed model of repeated measures) followed by the Bonferroni test/Dunnet test was used to assess the statistical differences between periods and the types of juices. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0.

As there are differences in the individual carotenoid content of the four types of orange juice in the study, the concentration in the FS orange juice was considered as the reference for the carotenoid content of the LP-, HPP-, and PEF-treated orange juices. The degree of change in the serum responses to the carotenoid concentrations varied according to the type of juice consumed. The effects of the intake of the different orange juice types were compared to the FS orange juice concentrations and calculated as follows (e.g., lutein):

Serum lutein in each group × lutein intake supplied by FS orange juice / lutein intake in each group.

Carotenoid intake at baseline was not assessed in these participants and the mean carotenoid intake of the adult Spanish population was assigned as 1235.2 μg/d lutein and zeaxanthin (1140 μg/d lutein, 95 μg/d zeaxanthin, applying a factor of 13 based on proportions of these carotenoids in our previous dietary studies), 322 μg/d zeaxanthin, 322 μg/d β-cryptoxanthin, 269 μg/d α-carotene, and 1458 μg/d β-carotene (Beltrán-de-Miguel, Estévez-Santiago, & Olmedilla-Alonso, 2015; Estévez-Santiago, Beltrán-de-Miguel, & Olmedilla-Alonso, 2016). Dietary intake at 7 and 14 days was the sum of the basal intake plus concentrations supplied by each orange juice type.

3. Results and discussion

This study assessed the long-term effects of orange juice intake on serum carotenoid concentrations in a well-defined group of healthy adults (controlled for age, BMI, serum retinol, and cholesterolemia). Baseline characteristics (mean ± SD) of the participants (6 male and 6 female) at the begining of the study were as follows: age, 22.2 ± 3.0 years; weight, 63.3 ± 10.7 kg; height, 1.69 ± 0.11 m; BMI, 22.2 ± 1.6 kg/m^2; total cholesterol, 171 ± 41.4 mg/dl; tryglicerides, 80.4 ± 24.6 mg/dl; uric acid, 4.9 ± 1.3 mg/dl; glucose, 86.7 ± 8.2 mg/ dl; haematocrit, 43.8 ± 3.0 %; and retinol 52.2 ± 9.1 μg/dL. There were no differences between the sexes. The haematological and biochemical profiles of the participants were within the normal range at the beginning and at the end of the study.

The most relevant carotenoids in the context of diet and health (lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, α-carotene, and β-carotene) were analysed in each of the four types of orange juice, as well as in blood samples drawn from the study participants. Table 1 shows the carotenoid concentrations in the orange juice types used in this study and the amount ingested with 500 ml of orange juice consumed daily. β-Cryptoxanthin, the predominant carotenoid, and β-carotene concentrations were similar across the four types of orange juice processing; however, the HPP orange juice contained lower concentrations of lutein and zeaxanthin ($p = 0.000$) than the other three orange juice types, lower α-cryptoxanthin concentration than the PEF orange juice ($p = 0.001$) and lower α-carotene concentration than the FS orange juice ($p = 0.005$). The carotenoid profile of these orange juice types showed a high β-cryptoxanthin content, β-carotene and α-carotene at lower concentrations, as well as the presence of lutein and zeaxanthin in the proportions described (Dias et al., 2018). The three provitamin-A carotenoids (α- and β-carotene and β-cryptoxanthin), and two xanthophylls, lutein and zeaxanthin, play important roles in visual and cognitive function (Johnson, 2014). In addition, we assessed the α-cryptoxanthin concentration, a less abundant provitamin A carotenoid (Solomons, 2016) in the human diet that is present in oranges at higher concentrations than β- and α-carotene, both in the orange juice and in the serum of participants.

The level of two provitamin A carotenoids in oranges, β-cryptoxanthin and β-carotene, were similar in all four of the orange juice types. However, differences in other carotenoids (lutein, zeaxanthin, α-carotene, α-cryptoxanthin) were observed, particularly in that lower concentrations were found in the HPP orange juice. Factors that influenced carotenoid content included the variety and seasonality of the fruit used to produce the orange juice, as well as the processing method. The same variety of oranges was used to prepare the HPP, PEF, and FS juices; however, the collection period (January–February [HPP] and April–May [PEF, FS]), along with the potential effect of climate and weather, could have affected carotenoid concentrations (Dias et al., 2018). Regarding the processing method, HPP treatment leads to increased carotenoid extractability when compared with other methods or untreated juice. Thus, along with PEF, HPP is considered a good option to preserve carotenoids in orange juice during refrigerated storage (Plaza et al., 2011).

Oranges are major contributors to the levels of provitamin A carotenoids found in the Spanish population (Beltrán-de-Miguel, Estévez-Santiago, & Olmedilla-Alonso, 2015), and are the leading contributor to dietary lutein and zeaxanthin intake among fruits (33.4 μg/p/d) (Estévez-Santiago, Beltrán-de-Miguel, & Olmedilla-Alonso, 2016). The

Table 1
Carotenoid concentrations in orange juice (MP, HP, EPF, FS) consumed in the intervention study.

	LP-orange juice [1,2]		HPP-orange juice [3]			PEF-orange juice [2]		FS-orange juice [3]	
	μg/100 ml	Daily supply (μg/500 ml)	μg/100 ml	Daily supply (μg/500 ml)	P-value	μg/100 ml	Daily supply (μg/500 ml)	μg/100 ml	Daily supply (μg/500 ml)
Lutein	71.9 ± 14.4 [a]	359.5[a]	31.8 ± 5.7[b]	158.8[b]	0.000	68.1 ± 15.4 [a]	340.6[a]	78.1 ± 21.0 [a]	390.3[a]
Zeaxanthin	62.2 ± 16.3 [a]	311.1[a]	13.4 ± 4.5[b]	68.5[b]	0.000	73.1 ± 17.4 [a]	365.3[a]	60.2 ± 27.8 [a]	300.8[a]
α-cryptoxanthin	23.1 ± 6.2	115.4	18.5 ± 3.9[b]	90.2[b]	0.001	34.5 ± 10.8 [a]	172.5[a]	27.9 ± 11.5	139.3
β-cryptoxanthin	106.4 ± 7.1	531.9	121.7 ± 9.9	611.1		137.1 ± 18.2	685.7	113.3 ± 37.9	566.3
α-carotene	8.3 ± 3.2	41.3	5.2 ± 1.9[b]	28.4[b]	0.005	9.0 ± 2.4	45.0	9.8 ± 4.1 [a]	49.1[a]
β–carotene (total)	16.6 ± 5.5	83.0	10.8 ± 2.7	54.0		16.2 ± 3.0	81.0	16.3 ± 10.1	81.2
β-carotene (trans)	14.1 ± 4.6	70.5	10.4 ± 2.5	52.0		15.9 ± 2.8	79.5	15.6 ± 9.8	78.0
β-carotene (cis)	2.5 ± 1.1	10.0	0.6 ± 0.2	3.0		0.3 ± 0.2	1.5	0.8 ± 0.8	4.0
∑ Carotenoids	288.4 ± 51.5 [a]	1442.0[a]	201.6 ± 19.6[b]	1008.0[b]	<0.001	338.0 ± 55.7 [a]	1690[a]	305.4 ± 106.0 [a]	1527.0[a]

LP, low-temperature pasteurised orange juice; HPP, high-pressure processed orange juice; PEF, pulsed electric field-treated orange juice; FS, freshly squeezed orange juice.
In each row, superscript letters indicate statistically significant differences.
[1] Don Simón orange juice (squeezed orange. Trademark: J García Carrión, S.A., Jumilla, Murcia, Spain).
[2] mean of three batches, injected in duplicate.
[3] mean of four batches, injected in triplicate (HPP samples) and duplicate (FS).

Table 2
Serum carotenoid concentrations (μg/dL) mean ± ES at baseline, 7 and 14 days (n = 36; 12 participants in LP and HPP groups, 6 in FS and PEF groups).

		Orange juice (MP, HP, EPF-treated, FS)	P-value	LP	HPP	PEF	FS	P-value	Adjusted for the carotenoid concentration supplied by FS orange juice (mean ± SD)				P-value
									LP	HPP	PEF	FS	
Lutein	basal	12.4 ± 0.9[A]		12.1 ± 1.7	14.5 ± 1.6	8.2 ± 1.4	12.8 ± 2.4		12.1 ± 5.9	14.5 ± 5.6	8.2 ± 3.4	12.8 ± 5.9	
	7 d	14.7 ± 0.8[B]		14.2 ± 1.4	15.1 ± 1.4	14.0 ± 2.0	15.6 ± 1.6		14.5 ± 4.0	17.8 ± 5.6	14.5 ± 5.0	15.6 ± 3.8	
	14 d	16.3 ± 1.2[B]	0.012	14.9 ± 2.9	17.4 ± 1.7	14.8 ± 2.0	18.2 ± 2.8		15.2 ± 10.4	20.5 ± 6.8	15.3 ± 5.0	18.2 ± 6.8	
Zeaxanthin	basal	4.0 ± 0.5[A]		3.1 ± 0.5[b]	3.5 ± 0.5[b]	2.6 ± 0.5[b]	8.0 ± 1.7[a]	0.001	3.1 ± 1.6	3.5 ± 1.7[A]	2.6 ± 1.2[A]	8.0 ± 4.2	
	7 d	6.0 ± 0.6[B]		4.6 ± 0.7	4.2 ± 0.6	6.2 ± 0.6	12.0 ± 1.6		4.5 ± 2.2[b]	10.0 ± 5.3[aB]	5.3 ± 1.3[bcB]	12.0 ± 3.8[b]	0.001
	14 d	5.3 ± 0.7[B]	0.028	4.1 ± 0.7	4.3 ± 0.6	6.3 ± 0.6	13.1 ± 1.2		4.0 ± 2.2[d]	10.4 ± 4.8[bB]	5.4 ± 1.3[cB]	13.1 ± 2.9[a]	0.000
α-cryptoxanthin	basal	2.5 ± 0.3[A]		2.0 ± 0.6	2.4 ± 0.5	1.8 ± 0.4	4.2 ± 1.1		2.0 ± 2.1[A]	2.4 ± 1.8[A]	1.8 ± 0.9[A]	4.2 ± 2.6[A]	
	7 d	4.6 ± 0.4[B]		3.1 ± 0.4	4.4 ± 0.7	4.6 ± 0.3	7.8 ± 1.4		3.8 ± 1.8[A]	6.9 ± 3.6[B]	3.7 ± 0.6[B]	7.8 ± 3.3[A]	0.022
	14 d	6.8 ± 0.6[C]	0.000	4.5 ± 0.5	5.6 ± 0.7	7.4 ± 0.8	13.2 ± 1.3		5.4 ± 2.1[bB]	8.7 ± 3.5[B]	6.0 ± 1.6[bC]	13.2 ± 3.2[aB]	0.000
β-cryptoxanthin	basal	16.7 ± 1.7[A]		15.0 ± 2.2	20.6 ± 3.2	9.6 ± 2.4	19.5 ± 5.0		15.0 ± 7.5[A]	20.6 ± 11.0	9.6 ± 5.9[B]	4.2 ± 2.6[A]	
	7 d	25.1 ± 1.9[B]		20.3 ± 2.3	27.6 ± 3.4	19.6 ± 2.0	35.2 ± 6.2		21.1 ± 8.4	26.2 ± 11.2	17.2 ± 4.4	35.2 ± 15.3	
	14 d	30.5 ± 2.1[B]	0.000	23.0 ± 2.7	33.6 ± 3.8	27.5 ± 3.7	42.3 ± 5.4		23.9 ± 9.7[B]	32.0 ± 12.4	24.2 ± 8.0[A]	42.3 ± 13.3[B]	
α-carotene	basal	5.1 ± 0.6		6.0 ± 1.2	5.2 ± 1.0	2.5 ± 0.5	5.8 ± 1.3		6.0 ± 4.0	5.2 ± 3.6	2.5 ± 1.3	5.8 ± 3.2	
	7 d	5.4 ± 0.5		5.6 ± 1.1	5.3 ± 1.1	3.3 ± 0.6	6.9 ± 0.8		5.8 ± 3.8	5.7 ± 3.9	3.3 ± 1.6	6.9 ± 2.0	
	14 d	5.5 ± 0.5		5.4 ± 0.9	5.4 ± 0.8	4.1 ± 0.7	7.6 ± 0.6		5.6 ± 3.1	5.7 ± 3.0	4.1 ± 1.8[b]	7.6 ± 1.5[a]	0.02
β-carotene	basal	23.8 ± 3.2		26.2 ± 5.2	22.9 ± 4.0	24.9 ± 14.8	19.4 ± 3.8		26.2 ± 18.0	23.0 ± 13.7	25.0 ± 36.4	19.4 ± 9.3	
	7 d	22.5 ± 2.4		21.6 ± 3.7	21.7 ± 4.0	23.9 ± 9.8	21.1 ± 2.9		21.6 ± 12.7	22.0 ± 14.0	23.9 ± 24.0	21.1 ± 7.1	
	14 d	22.7 ± 2.4		20.8 ± 4.3	22.2 ± 3.8	25.5 ± 8.8	23.5 ± 2.9		20.8 ± 14.8	22.6 ± 13.6	25.5 ± 21.6	23.5 ± 7.0	

In the results adjusted for carotenoid amount supplied by FS: in each row, superscript letters indicate statistically significant variations in the concentration ($p < 0.05$); in each column, superscript capital letters indicate statistically significant variations in the concentration ($p < 0.05$).

contribution of provitamin A carotenoids (α- and β-carotene, α- and β-cryptoxanthin) to the daily dietary intake is higher than that of non-provitamin A carotenoids (lutein and zeaxanthin).

The intake of the four orange juice types in this study provided β-cryptoxanthin at a concentration that exceeded the average mean daily intake of the Spanish population (165–213%) and contributed to the dietary intake of lutein plus zeaxanthin (18%–57%), α-carotene (34%–64%), and a lower percentage of β-carotene (3.7–5.5%) (Beltrán-de-Miguel, Estévez-Santiago, & Olmedilla-Alonso, 2015; Estévez-Santiago, Beltrán-de-Miguel, & Olmedilla-Alonso, 2016). In this interventional study, the intake of provitamin A carotenoids was between two and three times the average daily intake of the Spanish population,

mainly due to β-cryptoxanthin. The contribution of α-cryptoxanthin could not be compared, as there was no dietary intake data for the Spanish population. The supply of additional dietary provitamin A did not affect the serum retinol concentrations in the participants as expected, given that the vitamin A status was within the normal reference range.

To assess the effect of processing orange juice on carotenoid bioavailability, concentrations in serum were monitored in a 14-day intervention study. The serum carotenoid concentrations at the beginning of each study and at 7 and 14 days of orange juice intake are shown in Table 2. At baseline, there were no differences in the serum carotenoid and retinol concentrations in the participants assigned to the different types of orange juice, except for zeaxanthin (higher in FS orange juice group than in any of the other groups, $p = 0.001$). There was an increase in the carotenoid serum concentration (lutein, zeaxanthin, α- and β-cryptoxanthin) at 7 and 14 days (p values at 14 days: 0.012, 0.028, 0.000, and 0.000, respectively) in response to the orange juice intake, but not in the concentrations of α-carotene ($p = 0.789$) and β-carotene ($p = 0.923$) (Table 2, Fig. 1). The intake of orange juice, regardless of the processing method, resulted in an increase in serum concentrations of xanthophyll concentration (two-fold of α- and β-cryptoxanthin, and to a lesser extent, lutein and zeaxanthin) but not the concentrations of carotenes (Fig. 2A, B). However, the contribution of α-carotene to dietary intake was similar to that of lutein and zeaxanthin. Lutein and zeaxanthin serum concentrations increased by 31.5% and 32.5% from baseline, α-carotene increased by 7.8%, and β-carotene decreased by 5.4%. Serum α- and β-cryptoxanthin were the carotenoids that showed the greatest increase (172% and 82.6%, respectively). These xanthophylls are mostly esterified in oranges (Giuffrida et al., 2019), and these forms seem to have equal or higher bioavailability than the corresponding free forms (Olmedilla-Alonso & Estévez-Santiago, 2019). Moreover, β-cryptoxanthin, mainly supplied in the diet in ester forms, seems to have a greater bioavailability than β-carotene (Burri, Chang, & Neidlinger, 2011; Olmedilla-Alonso, Rodríguez-Rodríguez, Beltrán-de-Miguel, & Estévez-Santiago, 2020).

However, considering that the four orange juice types contained different carotenoid concentrations and that the degree of the changes in serum concentrations of some carotenoids (net increments) varied according to the type of orange juice consumed, the serum carotenoid concentration responses were compared to those supplied by FS orange juice (Table 2). There were no significant variations in serum concentrations of lutein (except in response to PEF orange juice, with a slight increase at 14 days, $p = 0.055$) and β-carotene. We observed an increase in the concentrations of zeaxanthin (in response to the HPP and PEF orange juice types), α-cryptoxanthin (in response to all four juice types), and β-cryptoxanthin (with the exception of HPP orange juice) at 7 and 14 days.

The increases in the concentration of serum zeaxanthin after 7 days were (FS = HPP) > LP. The response to FS was higher than that of PEF orange juice at 7 days ($p = 0.001$). After 14 days, serum zeaxanthin increased with (FS = HPP) > (PEF = LP) ($p = 0.000$). Serum α-cryptoxanthin increased ($p = 0.022$) at 7 and 14 days ($p = 0.000$) (FS) > (PEF = LP). Serum α-cryptoxanthin concentrations increased on day 14 with the intake of LP, FS, and PEF orange juices ($p = 0.05$, $p = 0.03$, and $p = 0.02$, respectively), but not with HPP orange juice.

The serum β-cryptoxanthin concentration increased at 7 and 14 days with the intake of all the orange juice types, and no statistically significant differences were found in relation to the processing methods used, probably due in part to the high variability of the participants' responses within each of the groups.

The FS orange juice intake resulted in higher or equal responses in serum zeaxanthin, α-cryptoxanthin, and α-carotene concentrations than the LP and PEF orange juices, with zeaxanthin concentration at 7 and 14 days ($p = 0.001$ and 0.000), α-cryptoxanthin concentration at 14 days ($p = 0.000$), and α-carotene at 7 and 14 days ($p = 0.02$). No significant variations in β-carotene serum concentrations were found with the intake of any of the orange juice types over the 14-day study period.

Despite the high global consumption of oranges and the contribution of this fruit to provitamin A intake in many population groups, a limited number of studies on the bioavailability of carotenoids from oranges or orange juice have been published. Regarding the bioavailability of β-cryptoxanthin, the main carotenoid in oranges, a postprandial study supplying 744 μg/d found the bioavailability of β-cryptoxanthin was higher in a pasteurised orange juice than in a fresh orange juice (Aschoff et al., 2015). In another postprandial study comparing the carotenoid bioavailability in fresh orange juice to that of fermented orange juice (500 ml), β-cryptoxanthin and lutein absorption was significantly higher in the fermented orange juice (Hornero-Méndez et al., 2018). In the

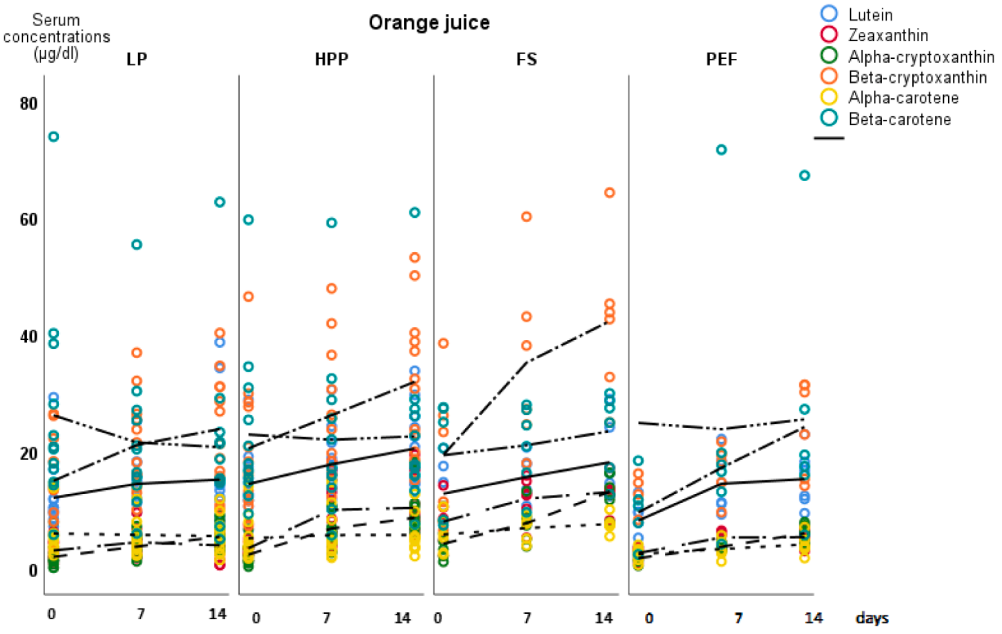

Fig. 1. Mean serum carotenoid concentrations (μg/dL) responses to the orange juice intake. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

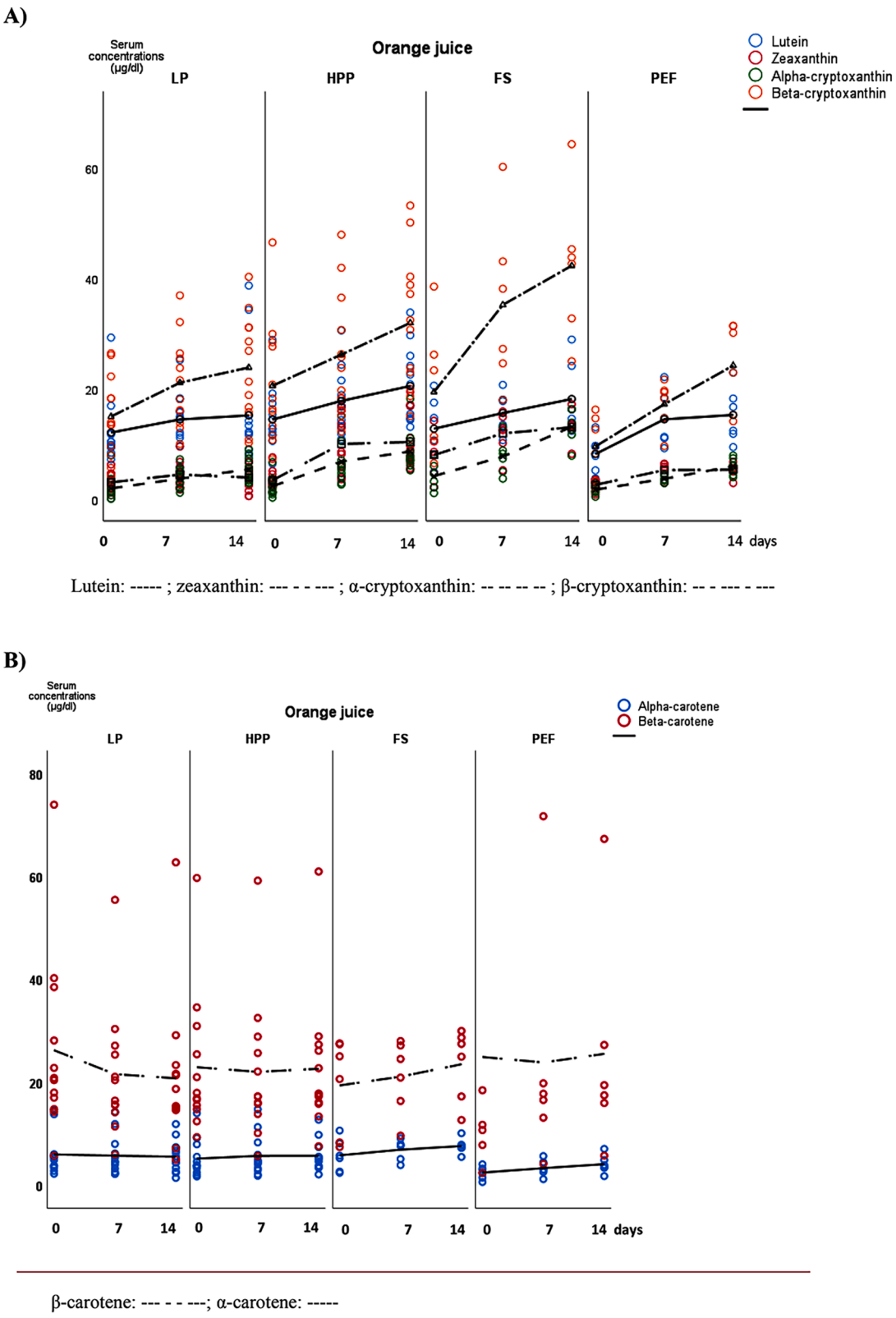

Fig. 2. Mean serum carotenoid (µg/dL) responses to the orange juice intake: A) xanthophylls, B) carotenes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

present study, no differences in baseline carotenoid concentrations were observed among the four groups (LP, HPP, PEF, and FS), and a higher increase was noted in the α-cryptoxanthin and β-cryptoxanthin serum concentrations (172% and 82.6%, respectively), which corresponds with the study by Franke, Cooney, Henning, and Cuser (2005) in which participants aged 28–51 years consumed 711 ml/day of commercially available orange juice, resulting in an increase in α-cryptoxanthin and β-cryptoxanthin of 112% and 94%, respectively. In accordance with the present study, the percentage increase in lutein and zeaxanthin concentrations in this study was approximately 32%. There were no

variations in the α- and β-carotene serum concentrations, as observed in the present study, which might be due to the adequate nutritional status of the participants in both studies. All these studies were performed in healthy adults with characteristics similar to those of the participants in the present study (e.g., age, sex, and normal BMI), with a similar sample size (between 7 and 13 volunteers), supplying similar amounts of orange juice or oranges, and a similar study duration (postprandial and up to 22 days).

In this study, no differences in the baseline concentrations among the four groups were observed. Although there was a higher increase in xanthophyll concentrations, as in β-cryptoxanthin, no significant differences were observed in some of the pairwise comparisons among the four types of orange juice provided. This might be due to the high variability in serum responses to intake of the different orange juice types included in the study. In general, the responses to FS orange juice (zeaxanthin, α- and β-cryptoxanthin, and α-carotene) were similar to the responses to HPP, and higher than the responses to LP. Moreover, responses to FS were higher than those to PEF. As previously described, HPP increased the extractability of carotenes in orange juice, while PEF treatment retained similar levels to those of untreated juice, and both technologies are considered a good option to preserve carotenoids in orange juice during refrigerated storage (Sánchez-Moreno et al., 2005; Plaza et al., 2011). The serum carotenoid responses to the LP orange juice intake in this study were similar to those described by Franke, Cooney, Henning, and Cuser (2005) in a study that included 13 volunteers consuming 711 ml/day of minimally processed, chilled orange juice for three weeks (a slightly higher volume of juice than in the present study). In both studies, the highest increase was observed for α-cryptoxanthin (94% vs. 125% in the present study), followed by that of β-cryptoxanthin (94% versus 53%) and lutein plus zeaxanthin (32%, reported together in Franke, Cooney, Henning, and Cuser (2005) and 32% zeaxanthin and 23% lutein in the present study). In all the orange juice types, the amount of β-crypoxanthin supplied was similar to that of lutein and zeaxanthin; however, the responses were quite different.

4. Conclusions

The daily intake of orange juice (500 ml) for 14 days in healthy adults resulted in a significant increase in xanthophylls but not in the carotene serum concentrations, with the highest increase being in α- and β-cryptoxanthin concentrations. The processing methods applied to obtain orange juice appeared to have different effects on serum carotenoid concentrations, with the effect induced by HPP orange juice, a technology with a wide industrial application, being similar to that of FS orange juice. However, due to the high variability in the serum carotenoid concentrations observed during this study, the effects of the different technologies should be verified in studies involving a larger sample size.

CRediT authorship contribution statement

Begona Olmedilla-Alonso: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Supervision, Validation, Writing – original draft, Writing - review & editing. **Fernando Granado-Lorencio:** Formal analysis, Investigation. **Begoña de Ancos:** Methodology, Investigation, Writing - review & editing. **Concepción Sánchez-Moreno:** Methodology, Investigation, Writing - review & editing. **Olga Martín-Belloso:** Conceptualization, Funding acquisition, Methodology, Investigation, Formal analysis, Writing - review & editing. **Inmaculada Blanco:** Investigation. **Carmen Herrero-Barbudo:** Investigation. **Pedro Elez-Martínez:** Investigation. **Lucía Plaza:** Investigation. **M. Pilar Cano:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Formal analysis, Resources, Supervision, Validation, Writing - review & editing. **:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to thank Teresa Motilla and Pilar Martínez for blood collection and Laura Barrios and Belén Garzón, from the Subdirección General de Apoyo a la Investigación of the CSIC, for statistical analysis support.

Begoña Olmedilla-Alonso participates in the Spanish Carotenoid Network (CaRed) funded by the Spanish MINECO (Ministry of Economy, Industry and Competitiveness) grant BIO2015-71703- REDT. Begoña Olmedilla-Alonso and M Pilar Cano participate in the European Carotenoid Network (EuroCaroten) funded by the European Commission COST Action CA15136.

Funding

This work was supported by the Consejería de Educación, Comunidad Autónoma de Madrid, Spain (grants: CAM 07G/0040/2000 and 07G/0041/2000) and by the Instituto de Salud Carlos III, Spain (grant RCMN C03/08).

Compliance with ethical standards

Ethics approval and consent to participate. The study was approved by the Clinical Research Ethics Committee of Hospital Universitario Puerta de Hierro (Madrid, Spain). The study was carried out in accordance to the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments and the Good Clinical Practices in the Spanish laws have been observed. Signed informed consent was obtained from all volunteers.

Ethics approval

Conduction of the study was approved by the Clinic Research Ethics Committee of the HUPH (Madrid, Spain).

Informed consent

All persons gave their informed consent prior to their inclusion in the study.

Availability of data and material

Raw data can be provided upon e-mail request.

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