

β -Cryptoxanthin is more bioavailable in humans from fermented orange juice than from orange juice

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ABSTRACT

Carotenoids, especially β -cryptoxanthin, exert multiple biological activities in the organism. Various processing techniques can improve carotenoid bioavailability in relation to the food matrix. The study objective was to compare the bioavailability of carotenoids from orange juice (OJ) with that from a beverage obtained by alcoholic fermentation of orange juice (FOB). Seven volunteers were recruited for a randomized, controlled, and crossover study. Post-intake plasma carotenoid concentrations were measured by HPLC in the subjects at 0-8 h after their consumption of OJ or FOB. β -Cryptoxanthin and lutein absorption was significantly higher from FOB than from OJ, but no significant difference in zeaxanthin absorption was found. The mean baseline-corrected area under the concentration curve ($AUC_{0-8\text{ h}}$) for β -cryptoxanthin, lutein and zeaxanthin was 24.6-, 1.3- and 4.65-fold larger, respectively, after FOB *versus* OJ consumption. This fermented orange beverage could be an abundant source of bioavailable carotenoids, and its regular consumption may exert healthy effects.

Keywords: Carotenoids, β -Cryptoxanthin, Bioavailability, Orange juice, Alcoholic fermentation, Humans.

Chemical compounds studied in this article:

β -Cryptoxanthin (PubChem CID: 5281235)

Zeaxanthin (PubChem CID: 5280899)

Lutein (PubChem CID: 5281243)

1. Introduction

Carotenoids are an important group of natural pigments in fruits and vegetables and have been attributed with multiple biological properties. These include provitamin A activity, antioxidant capacity, macular protection, bone health promotion, and anti-carcinogenic action (Chatterjee, Roy, Janarthan, Das, & Chatterjee, 2012; Wu, Cho, Willett, Sastry, & Schaumberg, 2015; Cicero & Colletti, 2017), all potentially related to the prevention/treatment of cardiovascular disease, cancer, and aging-related disorders (Fernández-García, Carvajal-Lérida, Jarén-Galán, Garrido-Fernández, Pérez-Gálvez, & Hornero-Méndez, 2012).

Carotenoids must be bioavailable in order to exert their positive health effects, i.e. accessible for digestion and absorption (bioaccessibility), metabolism, transport, tissue distribution, and bioactivity. The bioaccessibility of dietary carotenoids depends on multiple factors, including the characteristics of the food matrix (van het Hof, West, Weststrate, & Hautvast, 2000a). Thus, the ratio of plasma carotenoid concentrations to carotenoid intake is generally lower when raw rather than processed food is consumed (Fernández-García et al., 2012). A study by Rock, Lovalvo, Emenhiser, Ruffin, Flatt, and Schwartz (1988) in eight females reported a mean plasma β -carotene concentration of 0.83 $\mu\text{mol/L}$ after daily consumption of processed carrots and spinach for 4 weeks *versus* 0.60 $\mu\text{mol/L}$ after daily consumption of the fresh vegetables under the same conditions.

Various authors have evaluated the influence of different matrices in processed foods on carotenoid bioaccessibility. For example, carotenoid release was found to be enhanced by mechanical disruption of the spinach matrix (puree) in comparison to whole-leaf spinach (Eriksen, Luu, Dragsted, & Arrigoni, 2017), and the bioaccessibility of carotenoids from milk and soymilk was improved by high-intensity pulsed electric fields and high-pressure processing in comparison to the untreated products (Rodríguez-Roque et al., 2016).

The carotenoid profile of orange juice (OJ) is among the most complex reported for any fruit-derived food (Meléndez-Martínez, Vicario, & Heredia, 2007; Cerrillo, Escudero-López, Hornero-Méndez, Martín, & Fernández-Pachón, 2014). In common with persimmons, tangerines,

and papayas, among others, oranges and OJ are a rich source of β -cryptoxanthin (USDA, 2015). For numerous populations worldwide, the consumption of oranges and OJ is estimated to provide a majority (64.2–67.6%) of their intake of this carotenoid pigment (Murphy, Barraj, Herman, Bi, Cheatham, & Randolph, 2012; Beltrán-de-Miguel, Estévez-Santiago, & Olmedilla-Alonso, 2015). β -cryptoxanthin is a vitamin A precursor and has been found to exert antioxidant and anticancer effects, besides playing a role in bone health, immune function, cholesterol and glycemic homeostasis, liver function, and vision physiology, among other biological processes (Burri, La Frano, & Zhu, 2016).

In previous studies, our group developed and characterized a novel fermented orange beverage (FOB) obtained by the alcoholic fermentation of OJ and its subsequent heat treatment, finding no differences between FOB and OJ in qualitative or quantitative carotenoid profile (Cerrillo et al., 2014; Escudero-López, Cerrillo, Gil-Izquierdo, Hornero-Méndez, Herrero-Martín, Berná, Medina, Ferreres, Martín, & Fernández-Pachón, 2016). However, it is plausible that the bioavailability of carotenoids may be higher from FOB than from OJ due to food matrix modifications. Improved carotenoid bioaccessibility could result from the lesser content of soluble fiber in FOB, its heat treatment and consequent facilitation of matrix disruption, and the presence of ethanol and resulting increase in hydrophilicity, among other factors. The main objective of this study was to investigate whether the bioavailability of carotenoids, especially β -cryptoxanthin, is higher from FOB than from OJ, based on plasma carotenoid levels in healthy humans after a single intake of OJ or FOB.

2. Materials and methods

2.1. Reagents and chemicals

Deionised water (HPLC-grade) was produced with a Milli-Q Advantage A10 system (Merck Millipore, Madrid, Spain). HPLC-grade methanol and acetone were supplied by BDH Prolabo (VWR International Eurolab, Barcelona, Spain). Diethyl ether containing 7 ppm of butylated

hydroxytoluene (BHT) was purchased from Scharlau (Scharlab, Barcelona, Spain). Remaining reagents were all of analytical grade and purchased from Sigma-Aldrich Química (Madrid, Spain).

2.2. Subjects

The study included seven healthy subjects (five females and two males) aged 21-25 years with a mean (\pm SD) body mass index (BMI) of $20.7 \pm 2.53 \text{ kg m}^{-2}$. Eligibility was based on routine hematological and biochemical laboratory tests, medical history, anthropometric measurements, and a health and lifestyle questionnaire. Exclusion criteria were: 1) the presence of chronic disease (cardiovascular disease, diabetes, cancer, chronic obstructive pulmonary disease, metabolic syndrome), overweight/obesity, and kidney or liver failure; 2) abnormal baseline blood test results; 3) intake of any medication or nutritional supplement containing carotenoids in the previous 4 weeks; 4) any smoking habit; and/or 5) alcohol consumption of >2 drinks day^{-1} . All participants gave written informed consent to participate in the study, which followed the principles of the Declaration of Helsinki and was approved by the Clinical Research Ethical Committees of Virgen del Rocío Hospital (IEC 2013PI/022; Seville, Spain) and Pablo de Olavide University (Seville, Spain).

2.3. Fermented orange beverage: Production and composition

Grupo Hespérides Biotech S.L. (Seville, Spain) and Mitra Sol Technologies S.L. (Alicante, Spain) produced FOB using a commercial pasteurized OJ from *Citrus sinensis* L. var. *Navel late*. Controlled alcoholic fermentation of OJ was carried out for 10 days at 22 °C in 100 L stainless steel tanks (semi-industrial scale) under aseptic conditions using *Pichia kluyveri* var. *kluyveri* (previously isolated from the natural microbiota in orange fruit). This yeast strain ferments only reducing-sugars, resulting in a final product with low alcohol content (1% v/v) and sweet taste. The fermented juice was pasteurized (25 L h^{-1}) at 80°C for 30 s in a semi-industrial tubular pasteurizer (Mipaser Prototype, Murcia, Spain) and then cooled to 10°C in an ice-water-bath. Next, the

beverage underwent carbonation up to a pressure of 0.44×10^5 Pa and was aseptically poured into aluminum containers (250 mL), which were stored at 4°C until their consumption. Quality parameters of OJ and FOB were obtained using the methodology described by OIV (2017).

2.4. Experimental design

The study was a randomized, controlled, and crossover intervention performed at the Sevilab S.L. clinical laboratory (Seville, Spain). Volunteers were given a list of carotenoid-rich fruits and vegetables to avoid for 48 h before the study and throughout its duration. Subjects arrived at the clinical laboratory after overnight fasting for 12 h. Following a baseline blood draw (0 h), 500 mL of OJ was served at 8:00 h and consumed under supervision within 15 min. Consecutive blood samples were then drawn at 1, 2, 3, 4, 5, 6, and 8 h. At 13:00 h (after blood draw at 4 h post-intake), subjects consumed a standardized lunch low in carotenoids and fat (sandwich with two slices of bread (60 g), cooked turkey (40 g), and slice of low-fat cheese (20 g) plus nonfat yogurt (125 g)), which provided 245 kcal from 3.2 g total fat, 20 g total protein, and 34.2 g total carbohydrates. No other food or beverage except water (*ad libitum*) was allowed during the eight-hour blood sampling period. After a two-week wash-out period, the intervention was repeated but with the consumption of 500 ml FOB instead of OJ. All participants completed the study, and no adverse effects were reported during the clinical trial.

2.5. Blood sampling

Blood samples (18 mL) were drawn from a forearm vein into EDTA tubes, which were immediately centrifuged for plasma separation at 3,500 rpm for 3 min at 4 °C. Plasma samples were stored at – 80 °C until carotenoid analysis.

2.6. Carotenoid quantification

The methodology utilized to analyze carotenoid pigments in OJ and FOB samples was previously reported (Escudero-López et al., 2013). Briefly, an aliquot (10 mL) of sample was centrifuged at $10,000\times g$ and $4\text{ }^{\circ}\text{C}$ for 10 min, and the pellet containing carotenoids was extracted with acetone (3 mL). The extract was dried under nitrogen stream and subsequently dissolved in 3 mL of diethyl ether. Then, 0.5 mL of 20% (w/v) KOH-MeOH was added for saponification during a 20-min period under periodic agitation. After neutralization with water, the upper (organic) phase was collected by centrifugation at $5,000\times g$ and $4\text{ }^{\circ}\text{C}$ for 5 min, dried under nitrogen stream, dissolved in 0.5 mL acetone (containing 0.1% BHT), and stored at $-30\text{ }^{\circ}\text{C}$ until HPLC analysis. For the plasma analysis, samples were processed according to Pérez-Rodríguez, García-de Blas, Martínez-Padilla, Mougeot, and Mateo (2016). Briefly, 100 μL of each sample was deproteinized by mixing with 200 μL deionized water and 150 μL absolute ethanol, vortexing for 5 min, and centrifuging at $5,000\times g$ and $4\text{ }^{\circ}\text{C}$ for 5 min. The supernatant was extracted twice with 1 mL n-hexane for 2 min. Hexane phases were collected by centrifugation at $2,500\times g$ and $4\text{ }^{\circ}\text{C}$ for 2 min and were then combined and evaporated to dryness under nitrogen stream. The dry residue was dissolved in 100 μL of N,N-dimethylformamide (NNDMF) and kept at $-30\text{ }^{\circ}\text{C}$ until HPLC analysis. All procedures were performed under dimmed light to prevent isomerization and photodegradation of carotenoids. Chromatographic analysis of extracts was carried within 24 h of sample extraction. Carotenoids were analysed by HPLC using a C18 reversed-phase column (Mediterranea SEA18, $20\times 0.46\text{ cm}$ I.D., 3 μm particle size; Teknokroma, Barcelona, Spain) and binary gradient elution system of acetone–deionized water at a flow of 1.0 mL/min. The mobile phase started at 75% acetone, rose linearly to 95% within 10 min, continued isocratically for 7 min, became 100% within 3 min, and remained at 100% for 3 min. Injection volume was 10 μL for OJ or FOB samples and 50 μL for plasma extracts. Detection was carried out at 450 nm and also, in the case of OJ/FOB samples, at 402 and 424 nm. The column was maintained at $25\text{ }^{\circ}\text{C}$. HPLC analyses were performed with a Waters e2695 Alliance quaternary pump equipped with a Waters 2998 diode array detector and were controlled with Empower2 data acquisition software (Waters Corporation, Milford,

Massachusetts, USA). Pigments were quantified in the saponified extracts using calibration curves (6-8 concentration levels) prepared with standard stock solutions for each carotenoid in the concentration range 5-100 µg/mL; *cis* isomers were quantified using the calibration curve of the corresponding all-*trans* counterpart.

2.7. Statistical analysis

SPSS 22 (IBM SPSS Statistic 22) was used for statistical analyses. Data non-normally distributed according to the Shapiro-Wilk test were log-transformed. A linear mixed model, with carotenoid food source (OJ and FOB) and time as fixed-effects terms and subjects as random-effects term, was developed to investigate the effect of alcoholic fermentation on the bioavailability of carotenoids from orange juice. $P < 0.05$ was considered significant. The baseline-corrected area under the concentration curve (AUC) *versus* time over the 8-h period after OJ or FOB consumption was considered to represent post-intake carotenoid bioavailability. The AUC was calculated from the corresponding concentration/time data points by trapezoidal approximation using Excel 2016.

3. Results and discussion

Since the influence of alcoholic fermentation on the bioavailability of carotenoids has not previously been studied in any fruit or juice derived, we have carried out the current pilot study. The quality parameters of OJ and FOB are exhibited in Table 1. We highlight: the lower percentage of pulp in FOB (5.7%) than in OJ (12%), with its consequently reduced fiber content; its lesser reducing-sugar content (20.3 g/L vs. 48.5 g/L, respectively), and the presence of ethanol (0.9% v/v) due its fermentation. The carotenoid profiles of OJ and FOB were highly similar and were consistent with previous reports on OJ (Lee & Coates, 2003; Gama & Sylos, 2005; Stinco, Fernández-Vázquez, Escudero-Gilete, Heredia, Meléndez-Martínez, & Vicario, 2012; Cerrillo et al., 2014). As shown in Table 2, the total carotenoid content of FOB was 10.32 µg/mL and the provitamin A value was 70.97 RAEs. β -cryptoxanthin, zeaxanthin, lutein, ζ -carotene and β -

carotene were among the major pigments, together with a group of carotenoids characterized by 5-8-epoxide groups in their structures (karpoxanthin, neochrome, latochrome, mutatoxanthin, luteoxanthin and auroxanthin). These latter pigments are derived from pre-existing 5,6-epoxide carotenoids by contact with the organic acids in the juice. However, because epoxy-xanthophylls such as neoxanthin and violaxanthin are known to be poorly absorbed by humans (Barua & Olson, 2001), this group of pigments was not considered in the present study. Moreover, a preliminary qualitative analysis of some of the plasma samples failed to detect any of these carotenoids.

Figure 1 depicts the post-intake absorption curves for β -cryptoxanthin, lutein, and zeaxanthin after consumption of OJ or FOB. Concentrations (nmol \times h/L plasma) were baseline-corrected, and baseline-corrected mean AUC values for all carotenoids are summarized in Table 3. All carotenoids followed a similar time course after the consumption of either OJ or FOB: an increase in plasma concentration at 1 h post-intake (more moderate after OJ vs. FOB), a transient decrease at 2 h (except for β -cryptoxanthin levels after OJ). Post-intake absorption curves showed maximum plasma levels at 4 h after OJ consumption (β -cryptoxanthin: 25.3 nmol/L; lutein: 14.3 nmol/L; zeaxanthin: 3.17 nmol/L) and at 3 h after FOB consumption (β -cryptoxanthin: 52.3 nmol/L; lutein: 11.3 nmol/L; zeaxanthin: 2.89 nmol/L). Previous studies have reported maximum carotenoid peaks after OJ intake at 5 h, 5-6 h, 6 h, 7 h, and 6-12 h (Unlu, Bohn, Clinton, & Schwartz, 2005; Unlu, Bohn, Francis, Nagaraja, Clinton, & Schwartz, 2007a; Unlu, Bohn, Francis, Clinton, & Schwartz, 2007b; Schweiggert et al., 2014; Aschoff et al., 2015; Bub, Möseneder, Wenzel, Rechkemmer, & Briviba, 2008; Breithaupt, Weller, Wolters, & Hahn, 2003). This could be due to differences in the carotenoids evaluated, dietary sources, intake doses or analytical techniques (plasmatic concentration of pure compound, levels in triacylglycerol-rich lipoprotein fraction, etc.).

Baseline-corrected concentrations of β -cryptoxanthin were higher after FOB *versus* OJ intake throughout the 8-h post-intake period (Fig. 1A). β -cryptoxanthin absorption was significantly higher from FOB than from OJ ($P=0.004$). The mean AUC for plasma β -cryptoxanthin after FOB

intake (208.7 nmol×h/L) was 24.6-fold larger than after OJ (8.5 nmol×h/L) (Table 3). Hence, the bioavailability of β -cryptoxanthin was significantly higher from FOB than from OJ.

Lutein absorption was also greater (Fig. 1B) from FOB than from OJ ($P=0.019$). The mean AUC for lutein after FOB (12.3 nmol x h/L plasma) was 1.3-fold larger than after OJ (9.6 nmol x h/L plasma) (Table 3). The bioavailability of lutein was therefore also significantly higher from FOB than from OJ.

In the case of zeaxanthin, no statistically significant difference in absorption was observed between FOB and OJ (Fig. 1C); however, the mean AUC was larger after FOB (4.65 nmolxh/L plasma) *versus* OJ (1.00 nmolxh/L plasma) intake (Table 3). Aschoff et al. (2015) also reported no significant difference in zeaxanthin absorption after a single intake of oranges *versus* pasteurized OJ.

The greater absorption of β -cryptoxanthin and lutein from FOB than from OJ may be attributable to factors that enhance the bioaccessibility of these compounds. These may be related to properties of FOB derived from its processing, including the lower soluble fiber content, the presence of ethanol, and modification/disruption of the carotenoid matrix, as noted above.

With regard to soluble fiber content, the increase in intestinal viscosity produced by pectin consumption and the consequent reduction in biliary salt diffusion have been reported to reduce the efficacy of micellarization (van den Berg et al., 2000), while soluble fibers have also been found to diminish micelle formation by increasing the fecal excretion of bile acids (Priyadarshani, 2017). It has been proposed by various authors that the rate of carotenoid absorption may be limited by a delay or reduction in micelle formation (Rock & Swendseid, 1992; Riedl, Linseisen, Hoffmann, & Wolfram, 1999; Fernández-García et al., 2012; Aschoff et al., 2015). In the present study, the fiber content of FOB was reduced by the production process in comparison to the original OJ. In one study, carotenoid bioavailability was found to be enhanced by a reduction of 6% in the fiber content of OJ (Aschoff et al., 2015).

The improved digestibility of plant foods after heat treatment is well documented (van het Hof et al., 2000b; Rock et al. 1998; Priyadarshani & Chandrika, 2007). Thus, heating softens and disrupts plant cell membranes and cell walls, further disassembling protein-carotenoid complexes (Erdman, Poor, & Dietz, 1988), and the action of digestive enzymes therefore becomes more effective (Priyadarshani, 2017). Unlu et al. (2007a) found that plasma lycopene levels were higher in adults consuming lycopene from heat-induced *cis*-isomer-rich *versus* all-*trans*-rich tomato sauce. Likewise, higher plasma β -cryptoxanthin and lutein levels were observed after consuming pasteurized fresh OJ *versus* fresh oranges, although the difference was only significant for β -cryptoxanthin (Aschoff et al., 2015). FOB underwent pasteurization (85 °C for 30 sec) to inactivate microorganisms and guarantee its preservation.

A hydrophilic environment can facilitate the digestion and absorption of carotenoids (Fernández-García et al., 2012), which are lipophilic and therefore poorly dispersed in the aqueous medium of the digestive tract. The moderate alcohol content of FOB (0.9% v/v) may increase the hydrophilicity of the medium and thereby improve carotenoid bioaccessibility.

The bioaccessibility of carotenoids is also favored by disruption of the juice matrix during FOB processing (Britton, 1995; Aschoff et al., 2015; Priyadarshani, 2017). The internal structure of suspended carotenoid-containing solids is changed by alcoholic fermentation, enhancing their extractability and bioaccessibility. All of the above factors may play important roles in the enhanced bioavailability of β -cryptoxanthin and lutein from FOB.

Investigation with larger sample size is necessary to obtain more definitive results.

4. Conclusions

A significantly higher increase in plasma β -cryptoxanthin and lutein levels was observed after the single intake of FOB *versus* OJ. This improved bioavailability may be related to modifications in the matrix of OJ due to alcoholic fermentation and pasteurization. The regular consumption of FOB could be a good dietary source of bioavailable β -cryptoxanthin and exert

healthy effects derived from the multiple biological activities of this bioactive compound. This novel food can be considered a functional beverage. Further research is warranted on the impact of the different matrix characteristics of FOB (e.g., reduced soluble fibers, moderate alcohol content, thermal pasteurization, and mechanical disruption of cell wall) at each stage of the digestive process. It should also be investigated whether the β -cryptoxanthin metabolized after repeated FOB consumption exerts its activity *in vivo*, exploring the mechanisms involved.

Conflict of interest

All authors declare that there are no conflicts of interest.

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FIGURE CAPTIONS

Fig. 1. Baseline-corrected concentrations (nmol/L plasma) of β -cryptoxanthin (A), lutein (B) and zeaxanthin (C) over 8 h after the intake of fermented orange beverage (FOB) (–) or orange juice (OJ) (--). Data points represent means (n=7), and vertical bars indicate standard errors of the mean.

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Table 1

Quality parameters of orange juice (OJ) and fermented orange beverage (FOB).

Parameters	OJ	FOB
pH	3.48 ± 0.20	3.48 ± 0.03
TA (g citric acid/L)	8.48 ± 0.02	8.60 ± 0.01
TSS (°Brix)	11.0 ± 0.50	9.01 ± 0.14
% Pulp	12.0 ± 2.00	5.65 ± 0.22
Alcohol (% v/v)	0	0.90 ± 0.15
Ascorbic acid (mg/L)	423 ± 1.80	197 ± 6.80
Total sugars (g/L)	78.2 ± 5.64	47.9 ± 4.10
Reducing sugars (g/L)	48.5 ± 3.63	20.3 ± 2.40
Non-reducing sugars (g/L)	29.7 ± 2.01	27.1 ± 2.61

Values are expressed as means ± SD of 3 replicates.

Abbreviations: TA: titratable acidity; TSS: total soluble solids.

Table 2

Carotenoid profile of test beverages (orange juice (OJ) and fermented orange beverage (FOB)).

Compound	OJ ($\mu\text{g/mL}$)	FOB ($\mu\text{g/mL}$)
(all- <i>E</i>)-Zeaxanthin	1.41 ± 0.02	1.03 ± 0.05
(all- <i>E</i>)-Lutein	0.66 ± 0.01	0.48 ± 0.02
(9 <i>Z</i>)-Lutein	0.06 ± 0.00	0.04 ± 0.00
(13 <i>Z</i>)-Lutein	0.19 ± 0.01	0.21 ± 0.01
β -Cryptoxanthin	1.07 ± 0.03	1.34 ± 0.06
ζ -Carotene	0.77 ± 0.03	0.76 ± 0.04
(all- <i>E</i>)- α -Carotene	0.08 ± 0.00	0.06 ± 0.00
(all- <i>E</i>)- β -Carotene	0.18 ± 0.01	0.15 ± 0.01
Σ Carotenoid epoxides and derivatives ^a	6.62 ± 0.09	6.26 ± 0.21
RAEs ($\mu\text{g/L}$)	62.93 ± 1.85	70.97 ± 3.26

Values are expressed as means \pm SD of 3 replicates.

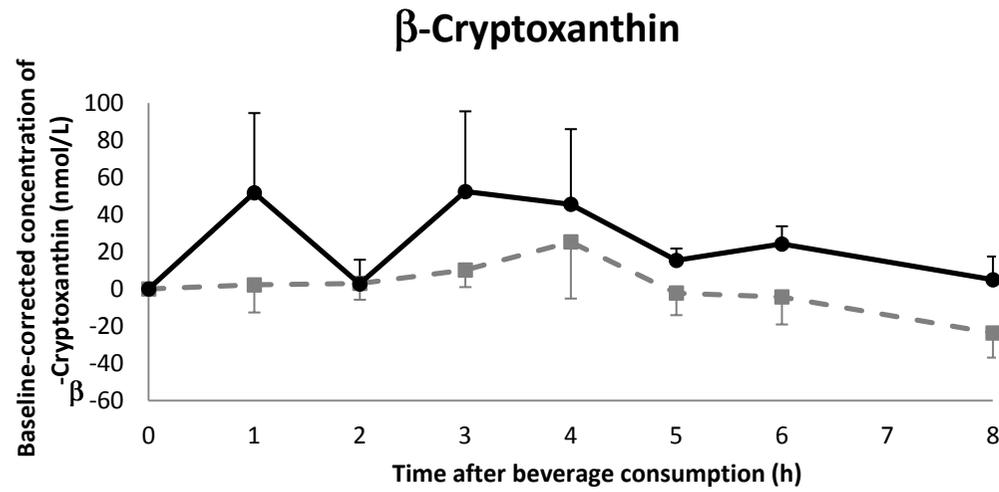
^a Σ Carotenoid epoxides and derivatives includes: luteoxanthin, kryptoxanthin, neochrome, luteoxanthin, auroxanthin and mutatoxanthin.

Table 3

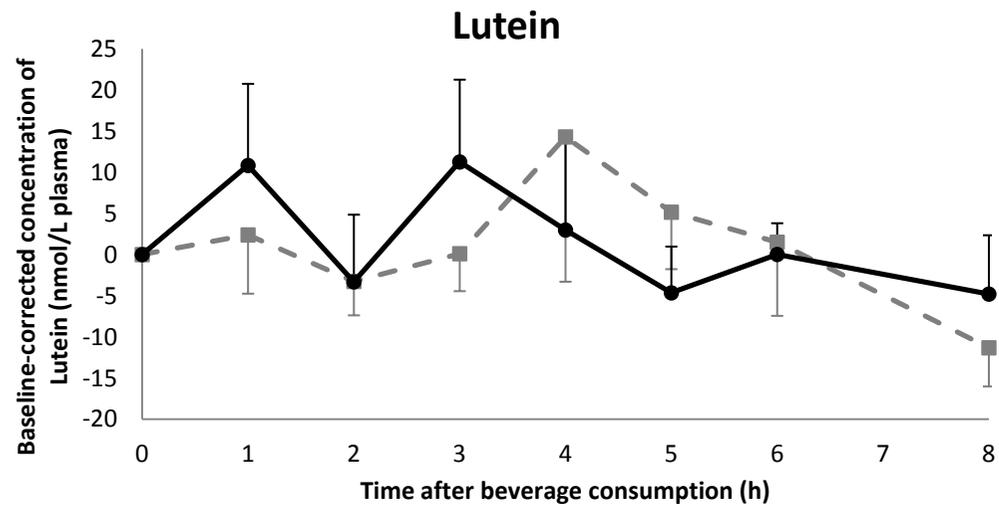
Baseline-corrected carotenoid AUC levels (nmol×h/L plasma) after consumption of orange juice (OJ) and fermented orange beverage (FOB) by 7 individuals (means, standard error of mean, medians, and range).

	OJ				FOB			
	Mean	SEM	Median	Range	Mean	SEM	Median	Range
Zeaxanthin	1.00	7.88	5.61	-23.90–29.04	4.65	14.50	-0.51	-46.72–65.81
β-Cryptoxanthin	8.50	40.77	7.12	-161.34–137.99	208.69	144.43	92.36	-100.62–1047.52
Lutein	9.64	25.06	25.13	-124.99–70.67	12.35	45.40	-9.01	-177.24–184.29

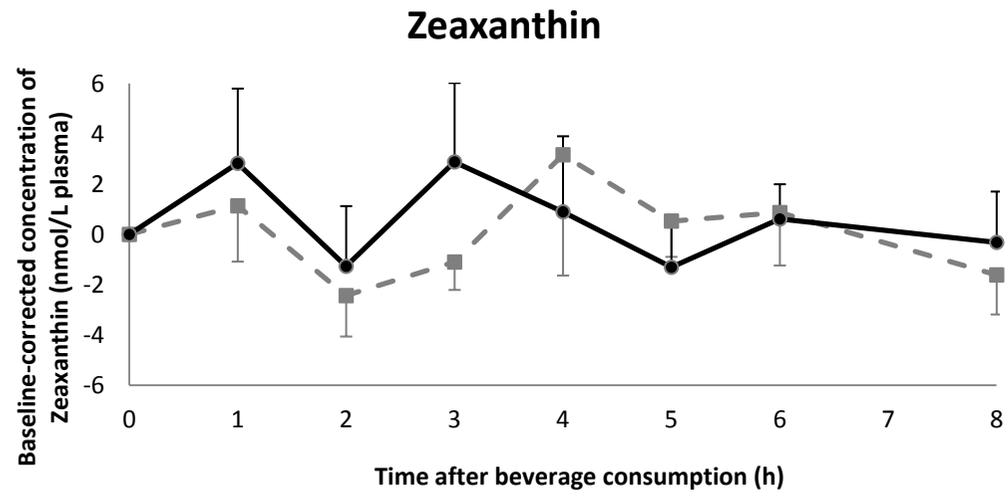
A



B



C



Highlights

- Alcoholic fermentation improves carotenoids bioavailability from orange juice.
- Plasma β -cryptoxanthin level is higher from fermented orange juice than from juice.
- Plasma lutein level is higher from fermented orange juice than from juice.
- The AUC_{0-8h} of carotenoids were greater from fermented orange juice than from juice.
- Fermented orange beverage could be a good source of bioavailable carotenoids.