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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 ($AUC_{0\text{-}8\ h}$) 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.

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 et al., 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 et al., 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 et al. (1998) in eight females reported a mean plasma β-carotene concentration of 0.83 μmol/L after daily consumption of processed carrots and spinach for 4 weeks *versus* 0.60 μmol/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,

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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 et al., 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 et al., 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 (± SD) body mass index (BMI) of 20.7 ± 2.53 $kg\,m^{-2}$. 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 × g and 4 °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 × g and 4 °C for 5 min, dried under nitrogen stream, dissolved in 0.5 mL acetone (containing 0.1% BHT), and stored at −30 °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 × g and 4 °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 × g and 4 °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 °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