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RESEARCH ARTICLE

Bioavailability of β-cryptoxanthin is greater from pasteurized orange juice than from fresh oranges – a randomized cross-over study

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Scope: Orange fruits and products thereof represent important dietary sources of carotenoids, particularly β-cryptoxanthin. Since previous studies reported a positive effect of vegetable processing on carotenoid absorption, our objective was to compare the bioavailability of β-cryptoxanthin from either fresh navel oranges (*Citrus sinensis* L. Osbeck) or pasteurized orange juice.

Methods and results: The study was designed as a randomized 2-way cross-over study. Twelve volunteers consumed two meals delivering 744 μg of β-cryptoxanthin from either fresh navel oranges or pasteurized orange juice. Eight blood samples were collected over 9.5 h after test meal consumption and analyzed using HPLC-DAD. Additionally, carotenoid bioaccessibility was assessed after in vitro digestion of the same test foods. β-cryptoxanthin bioavailability from pasteurized orange juice was 1.8-fold higher than from fresh oranges ($P = 0.011$). Similarly, mean absorption of the non-dose adjusted carotenoids lutein ($P = 0.301$), zeaxanthin ($P = 0.216$), and zeinoxanthin ($P = 0.090$) were slightly higher from orange juice, although not reaching statistical significance. The in vitro digestion revealed a 5.3-fold higher bioaccessibility of β-cryptoxanthin from orange juice. Dietary fiber contents in the test foods were inversely associated with carotenoid bioavailability.

Conclusion: Orange juice represents a more bioavailable source of β-cryptoxanthin than fresh oranges.

Keywords:
bioaccessibility / Carotenoids / Citrus / Dietary fiber / Fruit matrix / Processing

1 Introduction

While the worldwide production of orange fruits has increased by 19% over the past decade [1], a decreasing annual per capita consumption of orange juice has simultaneously been observed in both the United States and the European Union (EU) [2, 3]. However, orange juice still represents the most popular fruit juice, reaching annual per capita consumptions of 4.9–13.1 L [2, 3]. Both orange fruits and juice are commonly perceived as natural and healthy sources of nutrients and vitamins, although the overall nutritional value of orange juice has recently been questioned due to its high intrinsic sugar level of 8.3–8.8% [4]. Additionally, epidemiological trials associated the excessive consumption of fruit juices with a higher risk of type 2 diabetes [5].

Despite these findings, several health benefits of orange fruit and juice constituents have been clearly shown in the past. Their high content in readily accessible vitamin C (ca. 50 mg/100 mL [6]) may be labeled according to current EU regulations [7] and, in the EU, a total of 15 health claims regarding vitamin C have been authorized based on substantial evidence of its health benefits [8]. Furthermore, prospective studies associated orange juice consumption with the prevention of endotoxin level increases after meals high in fat and carbohydrate [9], a reduction of total cholesterol levels [10], and an improvement of vascular function [11].

A controversial discussion about the active compounds responsible for these effects is ongoing. For instance, both

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Abbreviations: **AUC**, area under the curve; $\mathbf{C_{max}}$, concentration maximum; **TRL**, triacylglycerol-rich lipoprotein

orange fruits and juice provide a rich source of potentially health-promoting carotenoids [6, 12–14]. While lutein and zeaxanthin were reported to support the prevention of age-related macular degeneration and cognitive impairment in elderly people [15], the provitamin A carotenoids α-carotene, β-carotene, and β-cryptoxanthin can be metabolized to vitamin A. β-cryptoxanthin constitutes a major carotenoid in sweet oranges [6, 13] and was estimated to represent 14% of the total carotenoids consumed annually in European countries, mainly derived from citrus fruits and products thereof [16]. In comparison to β-carotene from carrots and green leafy vegetables or lycopene from tomato products, β-cryptoxanthin has been scarcely investigated to date. In vitro studies postulated a positive effect on osteoblastic bone formation [17]. Likewise, epidemiological studies suggested a potential role of β-cryptoxanthin among other carotenoids for nutritionally improving bone health [18, 19]. Its bioavailability has been shown to be notably higher as compared to other carotenoids from the same food [20, 21], thus possibly compensating its by 50% lower provitamin A activity as compared to β-carotene [22].

Despite the dietary abundance of β-cryptoxanthin, postprandial human studies assessing carotenoid bioavailability from fresh oranges and processed orange juice are lacking. After comparing particularly β-cryptoxanthin bioavailability, our second aim was to evaluate the potential correlation of our in vivo results with those obtained from a frequently used in vitro digestion model for determining bioaccessibility of carotenoids. Furthermore, the absorption of further citrus carotenoids such as lutein, zeaxanthin, and zeinoxanthin was monitored.

2 Materials and methods

2.1 Chemicals and materials

Chemicals and solvents used were of analytical- or HPLC-grade and were obtained from Merck (Darmstadt, Germany) or VWR International (Leuven, Belgium) unless stated otherwise. Porcine pancreatic α-amylase (46.4 U/mg), porcine bile extract, cholesterol esterase from porcine pancreas (42.9 U/mg), pancreatin from porcine pancreas, and pepsin from porcine gastric mucosa were purchased from Sigma Aldrich Chemie (Steinheim, Germany). Antheraxanthin (95.0%), α-carotene (98.9%), lutein (99.9%), mutatoxanthin (95.5%), violaxanthin (99.3%), zeaxanthin (99.8%), and zeinoxanthin (97.7%) were from CaroteNature (Ostermundingen, Switzerland), whereas β-carotene (≥97%), β-cryptoxanthin (≥97%), and 3-*tert*-butyl-hydroxyanisol (BHA) were purchased from Fluka Chemie (Buchs, Switzerland).

2.2 Subjects

Twelve healthy, non-pregnant, and non-smoking volunteers (6 men and 6 women) aged 23–30 years (median 27.5 years) were recruited. The number of subjects was calculated from previously published data [20], providing a power of >80% with $\alpha = 0.05$. Subjects had a BMI of 23.0 ± 1.9 kg/m^2, and fulfilled the following criteria, which were assessed by a health and lifestyle questionnaire: no history of chronic gastrointestinal disease, liver disease, and cancer, no use of medications affecting lipid metabolism, no regular use of nutritional supplements containing carotenoids, and no frequent alcohol consumption. Written informed consent was obtained. The study was approved by the ethics committee of the State Chamber of Physicians of Baden-Württemberg (Stuttgart, Germany, project F-2013-097) and registered at clinicaltrials.gov (NCT02380144).

2.3 Study design

The study was conducted at the University of Hohenheim between February and April 2014, following a randomized, two-way cross-over design. Age and BMI of the subjects were recorded during their initial visit, at which all clinical procedures were explained to them. Subjects consumed the test meals described below on two different days, each preceded by a washout period of at least 2 weeks, in which they were required to strictly follow a diet low on carotenoids. The selection of carotenoid-rich foods to be avoided was based on the USDA-Nutrition Coordinating Center and National Cancer Institute Nutrient Database [23]. A list of these foods was handed to the volunteers during the initial visit.

Subjects arrived at the Institute of Nutritional Medicine of the University of Hohenheim on each clinical day at 0800 after overnight fasting for at least 12 h. A catheter was installed into a forearm vein and a baseline blood sample was taken at time point 0 h. Subjects then consumed a breakfast consisting of one slice of toast (25 g), two slices of fried bacon (25 g), scrambled egg white (80 g) prepared in 20 g soy oil, and one optional cup of coffee or black tea (total caloric value of the breakfast: 433.8 kcal, total fat: 30.1 g, total protein: 14.1 g, total dietary fiber: 0.7 g). Subsequently, subjects ingested the test foods consisting of either fresh oranges (400 g) or orange juice (719 g). The amount of test food was adjusted to deliver an equal amount of β-cryptoxanthin (744 μg). Subjects were given 20 min for consumption of the entire breakfast. Further blood samples were collected consecutively after 2, 3, 4, 5, 6, 8, and 9.5 h. A standardized lunch low in carotenoids was served after 4.5 h, comprising a pizza-like dish (without tomato sauce) and a chocolate pudding (total caloric value of the lunch: 860.5 kcal, total fat: 45.7 g, total protein: 23.5 g, total dietary fiber: 4.2 g). Subjects consumed water ad libitum, while no other foods or beverages were allowed during the test. After 2 weeks of further washout, subjects returned to the institute to proceed with the respective test meal they had not consumed before. All subjects completed the study, and no adverse effects were reported during the clinical trial.

2.4 Blood sampling and carotenoid analyses

Blood samples (~10 mL) were drawn into K_2EDTA tubes (S-Monovette® EDTA/K2-Gel, Sarstedt, Nümbrecht, Germany) and centrifuged at 2300 × *g* and 4°C for 10 min using a Heraeus Labofuge 400R (Thermo Scientific, Langenselbold, Germany). Triacylglycerol-rich lipoprotein (TRL) fractions were isolated as previously described [20] with the following modification. After ultracentrifugation and separation of the TRL fraction, polyallomer tubes were rinsed twice with 0.25 mL NaCl solution (density 1.006 kg/L) to obtain a final TRL fraction volume of 1 mL. The TRL samples were stored at –80°C until carotenoid analysis as reported before [24] with slight modifications. Briefly, 1 mL of TRL fraction was mixed with 1 mL ethanol and vortexed for 30 s. 2 mL of hexane were added and the sample was probe-sonicated (Sonoplus HD 3080 with MS 72 sonotrode, Bandelin, Berlin, Germany) three times for 8 s at 75% amplitude. After centrifugation at 3000 rpm (966 × *g*) for 3 min (Labofuge 200 Heraeus, Hanau, Germany), the upper organic phase was collected, and the samples were re-extracted once with 2 mL of hexane. The combined organic phases where dried under a stream of nitrogen gas at $T \leq 25$°C. Subsequently, the dried extracts were re-dissolved in 200 μL of 2-propanol and membrane-filtered (0.45 μm) into amber glass vials prior to HPLC analysis. Carotenoid identification and quantitation were performed according to Kopec et al. [24]. Zeinoxanthin, which was not investigated by Kopec et al. [24], was identified and quantitated using an authentic standard.

2.5 Preparation of test foods

Fresh, untreated oranges (*Citrus sinensis* (L.) Osbeck cv. "Navel Late") were bought from a wholesale market (Stuttgart, Germany) 2 weeks prior to the first intervention day, whereas all other foods served during the clinical trial were purchased from a local supermarket. A single batch of oranges (200 kg) originating from one single supplier was used for the study, which was designed and organized to be finished within 4 weeks. On each clinical day, 12 oranges were randomly sampled from this batch, peeled, quartered, and carefully pooled. The quarters were randomly distributed and administered to the respective subjects. An aliquot of the pooled quarters was frozen in liquid nitrogen and stored at –80°C until further analysis.

Using the same batch of navel oranges as used for fresh fruit consumption, the orange juice was produced 1 week prior to the first intervention day using industrial scale equipment. A total of 120 kg of oranges was washed with cold water and the peel was rasped off using a CITRORAP essential oil extractor (Bertuzzi Food Processing, Busto Arsizio, Italy). Extraction of the juice was performed with a JBT-391B juice extractor (JBT Food Tech, Madrid, Spain). The fresh juice was continuously heated to 90°C at a flow rate of 120 L/min (total heat exposure at 90°C: 54 s) using a tubular heater (Schmidt-Bretten, Bretten, Germany), hot-filled into 500 mL glass bottles, cooled to room temperature in a cooling tunnel (Anlagenbau, Kirchberg, Switzerland), and stored at 6°C until further use. A pasteurization value of 0.383 was calculated using *Bacillus coagulans* as the reference germ ($T_{Ref} = 93.3$°C, $z = 8.9$ K) according to Hirsch et al. [25]. Finishing of the juice was conducted on the morning of each respective treatment day using a commercial paddle pulper (PAP 0533, Bertuzzi, Busto Arsizio, Italy) with a mesh size of 0.5 mm. An aliquot of the finished juice was frozen in liquid nitrogen and stored at –80°C until further analysis.

2.6 Characterization of the test foods

Frozen orange quarters and frozen orange juice were milled under liquid nitrogen using a model 38BL41 blender (Waring, Torrington, CT, USA) prior to all carotenoid analyses. Carotenoids were extracted and saponified as previously described [6], and HPLC-PDA analyses were performed as described for TRL samples. Using the same method, carotenoid concentrations in both test foods were monitored at 4 time points during the entire study period to ensure constant β-cryptoxanthin dosage on each treatment day.

Dietary fiber contents were determined gravimetrically by means of a "BIOQUANT® total dietary fiber kit" according to the instructions of the supplier (Merck, Darmstadt, Germany). All samples were analyzed in quadruplicate. Pectin and sugar contents as well as titratable acidity, total soluble solids, and centrifugable pulp were determined using the methods of the International Fruit Juice Union [26].

2.7 In vitro bioaccessibility of carotenoids

The in vitro digestion was conducted as previously described [6], using a static model comprising oral, gastric, and small intestinal digestion. The orange fruit segments were cut into cubic pieces of 5 mm edge length to mimic coarse comminution obtained during chewing, whereas the finished orange juice was subjected to the digestion model without any pretreatment. After digestion, carotenoid levels in the digests were analyzed by HPLC-PDA as described for TRL samples. In vitro bioaccessibility was calculated as the amount of the respective carotenoid in the micellar fraction (obtained by microfiltration (0.2 μm) of the supernatant) divided by the amount of the respective carotenoid in the test food. The in vitro digestion was performed in quadruplicate.

2.8 Statistics

Tukey's test was used to identify statistically significant differences of means (significance level: $P < 0.05$) regarding test food parameters and bioaccessibility, using SAS 9.1.3 (SAS Institute, Cary, NC, USA). Standard deviation of the *in vitro* bioaccessibility of carotenoids was calculated by Gaussian law of error propagation from the standard deviations

of the concentrations in the test foods and those of the total bioaccessible amounts of the respective analyte.

Trapezoidal approximation (Excel 2010, Microsoft Corporation) was used to calculate the baseline-corrected area under the concentration versus time curve (area under the curve; AUC) over 9.5 h from the respective data points of the TRL samples. The AUC was used as a representative parameter for postprandial carotenoid bioavailability as previously described [20, 27]. An ANOVA was carried out to model carotenoid absorption using a linear mixed model with the covariates food sequence (two combinations), period (two clinical visits), participants ($n = 12$), and carotenoid food source (orange fruit, orange juice). Additionally, the nonparametrical Cochran–Mantel–Haenszel test was used with the different food sequences as strata for the overall and pairwise identification of significant differences between means, controlling for carry-over, period and time effects. *P*-values quoted in the text are from ANOVA unless stated otherwise.

3 Results

3.1 Carotenoid and dietary fiber contents in the test foods

In agreement with previous reports [6, 13], (9*Z*)-violaxanthin, 2 mutatoxanthin epimers (8*R*/8*S*), lutein, zeaxanthin, zeinoxanthin, β-cryptoxanthin, α-carotene, and β-carotene were identified in both fresh oranges and orange juices. While lutein, zeinoxanthin, and β-cryptoxanthin were present in major concentrations (Table 1, Fig. 1), quantitation of zeaxanthin was intricate due to the previously reported coelution with an antheraxanthin isomer [6]. Therefore, the test meals were adjusted to deliver the same amount of β-cryptoxanthin, being the predominant carotenoid in both orange fruit (186 μg/100 g FW) and juice (104 μg/100 g FW). Since β-cryptoxanthin concentration in the fruits was higher than in the juice, a total of 400 g fresh fruit and 719 g of orange juice was administered during the clinical trial to achieve equal doses. Variations in the carotenoid contents of test foods caused by oxidative degradation or isomerization during the intervention period may hamper the accuracy of bioavailability studies, particularly when using fresh fruits with respiratory activity. Therefore, we monitored carotenoid levels in the test foods over the entire course of the intervention, revealing only slight and insignificant variations ($P > 0.05$) in their β-cryptoxanthin concentrations.

Noteworthy, violaxanthin and mutatoxanthin isomers have not been observed to be absorbed to human plasma yet [28]. Since we were also unable to detect them in our human TRL fractions, these carotenoids were no further considered. Expectedly, the absorption of α- and β-carotene was low (concentration maximum; $C_{max} \leq 2$ nmol/L) due to their negligible concentrations in the test foods and, consequently, no data is shown for their postprandial concentrations in blood plasma.

Table 1. Carotenoid concentrations in test foods and in the supernatant and micelles after in vitro digestion (based on fresh weight)

	Carotenoid concentration [μg/100 g test food][a)]	
	Orange fruit	Orange juice
In test meal		
Sum (C_P)	328.7 ± 17.7a	230.5 ± 5.8b
Lutein	61.2 ± 1.6a	58.9 ± 0.8a
Zeinoxanthin	49.4 ± 3.6a	47.0 ± 1.4a
β-Cryptoxanthin	186.0 ± 9.5a	103.5 ± 1.5b
α-Carotene	11.0 ± 1.3a	10.0 ± 1.1a
β-Carotene	21.1 ± 1.6a	11.1 ± 0.9b
ZEA + (9*Z*)-ANT[d)]	251.7 ± 10.1a	79.4 ± 0.6b
In supernatant[b)]		
Sum (C_L)	34.2 ± 2.0a	143.9 ± 4.7b
Lutein	9.2 ± 0.7a	39.5 ± 1.0b
Zeinoxanthin	6.7 ± 0.4a	28.0 ± 0.8b
β-Cryptoxanthin	14.8 ± 1.2a	64.5 ± 2.6b
α-Carotene	1.5 ± 0.1a	5.3 ± 0.2b
β-Carotene	2.0 ± 0.1a	6.6 ± 0.2b
In micelles[c)]		
Sum (C_M)	20.8 ± 0.8a (6.3%)	63.3 ± 3.8b (27.5%)
Lutein	5.5 ± 0.4a (9.0%)	17.7 ± 1.0b (30.1%)
Zeinoxanthin	4.0 ± 0.1a (8.1%)	12.7 ± 0.8b (27.0%)
β-Cryptoxanthin	9.1 ± 0.6a (4.9%)	26.8 ± 1.7b (25.9%)
α-Carotene	0.9 ± 0.0a (8.2%)	2.7 ± 0.2b (27.0%)
β-Carotene	1.3 ± 0.1a (6.2%)	3.4 ± 0.2b (30.6%)

a) Mean ± standard deviation, $n = 2$ for carotenoid analyses, $n = 4$ for in vitro digestion. Different letters within rows indicate significant differences (Tukey adjusted $P < 0.05$). Bioaccessibility is displayed in % behind the respective carotenoid concentration in micelles.
b) Obtained after centrifugation of the digesta.
c) Obtained after centrifugation and microfiltration (0.2 μm) of the digesta.
d) ZEA, zeaxanthin; (9*Z*)-ANT, (9*Z*)-antheraxanthin.

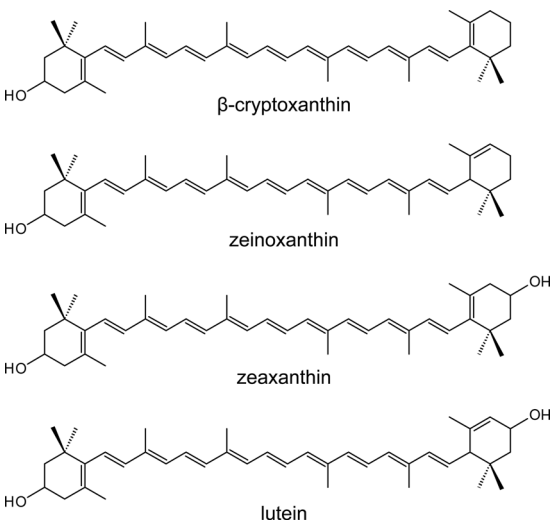

Figure 1. Chemical structures of β-cryptoxanthin, zeinoxanthin, lutein, and zeaxanthin. Note the structural similarities of β-cryptoxanthin and zeinoxanthin as well as lutein and zeaxanthin.

Table 2. Dietary fiber, pectin, and sugar contents as well as titratable acidity, total soluble solids and centrifugable pulp in the test foods (based on fresh weight)

	Orange fruits	Orange juice
Dietary fiber contents [g/100 g][a)]		
Soluble	0.90 ± 0.43a	0.07 ± 0.06b
Insoluble	1.26 ± 0.34a	0.06 ± 0.05b
Total	2.16 ± 0.53a	0.13 ± 0.10b
Pectin content [mg/100 g][a)]	320.4 ± 8.4a	59.2 ± 0.7b
Sugar contents [g/100 g][b)]		
Sucrose	4.1 ± 0.1a	4.6 ± 0.0b
Glucose	2.0 ± 0.1a	2.0 ± 0.0a
Fructose	2.2 ± 0.1a	2.1 ± 0.0a
Total soluble solids [°Brix][b)]	-	11.1 ± 0.0
Total titratable acidity [g/L][b)]	-	8.8 ± 0.0
Centrifugable pulp [% v/v][a)]	-	12.3 ± 0.6

a) Mean ± standard deviation, $n = 4$.
b) Mean ± standard deviation, $n = 2$. Different letters within rows indicate significant differences (Tukey adjusted $P < 0.05$).

Table 2 displays the contents of soluble, insoluble, and total dietary fibers, showing a significant reduction upon dejuicing to 5–8% ($P < 0.05$) of the initial content of the fresh fruit. Similarly, the pectin content in the orange juice decreased to 18% compared to that of the fresh fruit (Table 2). Sugar contents, total soluble solids, and total titratable acidity were within the ranges given by the reference guidelines for orange juice published by the Association of the Industry of Juices and Nectars from Fruits and Vegetables of the EU [29]. The amount of centrifugable pulp (12.3%) indicates a rather pulp-rich juice, since pulp contents in orange juices usually vary between ca. 8 and 12% [30].

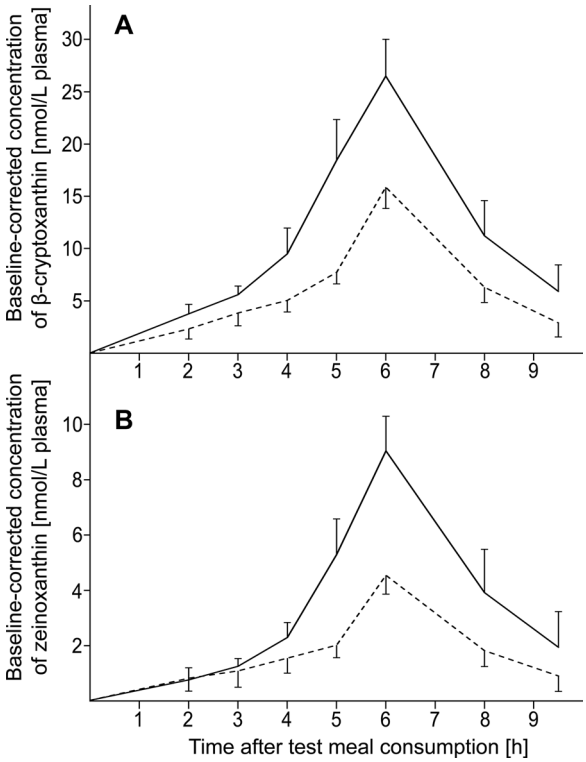

Figure 2. Baseline-corrected concentrations of β-cryptoxanthin (A) and zeinoxanthin (B) in the triacylglycerol-rich lipoprotein fraction [nmol/L plasma] after consumption of either orange fruit (- - -) or orange juice (—). Data points represent means ($n = 12$), vertical bars indicate standard errors of mean. β-cryptoxanthin AUC after consumption of orange juice was significantly higher than that after consumption of orange fruit ($P = 0.011$). The higher AUC of zeinoxanthin after orange juice consumption ($P = 0.090$) almost reached significance level when the non-parametrical Cochran–Mantel–Haenszel test was applied ($P = 0.054$).

3.2 Carotenoid response in the TRL fraction after ingestion of the test foods

Figure 2A shows the baseline-corrected concentration versus time curves of β-cryptoxanthin in the TRL fraction [nmol/L plasma]. The baseline-corrected mean AUC was 103(SEM15) nmol × h/L plasma (Table 3) when subjects had consumed orange juice, being 1.8-fold higher than the AUC determined when subjects consumed an equal dose of β-cryptoxanthin from fresh oranges (57(SEM9) nmol × h/L plasma). Thus, the postprandial bioavailability of β-cryptoxanthin was significantly higher from orange juice than from orange fruit ($P = 0.011$). The highest baseline-corrected concentrations of β-cryptoxanthin in the TRL fraction (C_{max}) were found 6 h after test food consumption, corresponding to 26.5 (SEM 3.5) and 15.9 (SEM 2.1) nmol/L plasma for orange juice and orange fruit, respectively.

By analogy to β-cryptoxanthin, the mean AUC of the structurally similar zeinoxanthin (Fig. 1) was 1.9-fold ($P = 0.090$) greater after orange juice consumption (Fig. 2B, Table 3), although not reaching statistical significance. However, when applying the non-parametrical Cochran–Mantel–Haenszel test, the significance level was almost reached ($P = 0.054$).

The absorption curves of the non-dose adjusted carotenoids, zeaxanthin, and lutein are shown in Fig. 3A and B, respectively, demonstrating the better absorption of both carotenoids from orange juice than from fresh fruit. The mean AUC of lutein was 1.6-fold higher after orange juice consumption, however not reaching statistical significance ($P = 0.301$), possibly due to an insufficient number of participants. As noted above, the exact levels of zeaxanthin in the test foods remained unknown and, thus, the higher bioavailability of zeaxanthin from orange juice as compared to that from orange fruits (Fig. 3A, Table 3) should be interpreted with caution.

The applied statistical analyses indicated the absence of carry-over, time, and period effects on carotenoid absorption.

Table 3. Baseline corrected carotenoid AUC levels [nmol × h/L] in the triacylglycerol-rich lipoprotein fraction of human plasma after consumption of orange fruit and orange juice test meals by 12 subjects (medians, 25th–75th percentiles, means, and standard error of mean)

	Lutein		Zeaxanthin		Zeinoxanthin		β-cryptoxanthin	
	Fruit	Juice	Fruit	Juice	Fruit	Juice	Fruit	Juice
Median	32.6	36.1	9.6	10.3	16.0	22.6	60.5	90.3
25th–75th perc.	14.9–47.8	28.9–70.8	4.3–14.4	8.5–18.7	12.5–23.2	18.2–45.0	48.4–75.6	62.9–144.8
Mean	31.6	49.5	7.2	13.7	16.3	31.7	56.8	102.7
SEM	9.6	9.3	3.3	2.9	4.1	6.1	9.2	14.8

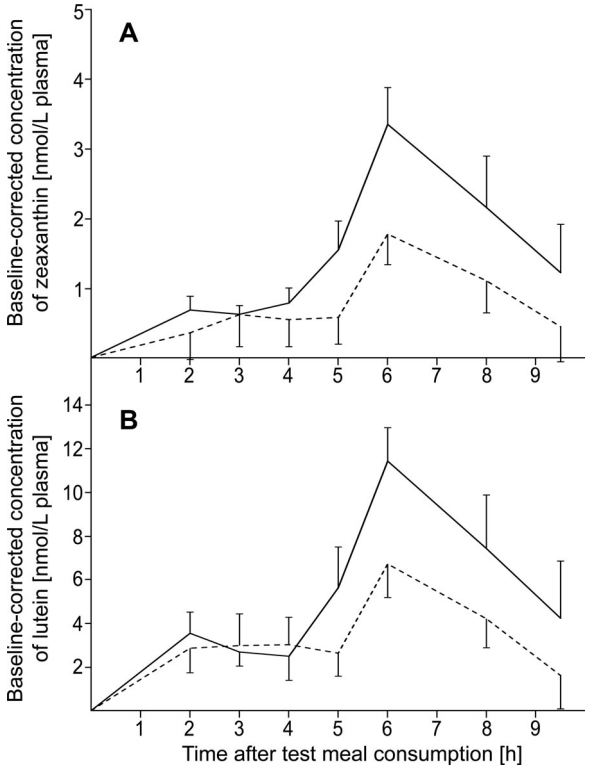

Figure 3. Baseline-corrected concentrations of the non-dose adjusted carotenoids zeaxanthin (A) and lutein (B) in the triacylglycerol-rich lipoprotein fraction [nmol/L plasma] after consumption of either orange fruit (- - -) or orange juice (—). Values are mean ($n = 12$), vertical bars indicate standard errors of means. We observed higher AUCs of zeaxanthin and lutein ($P = 0.216$ and 0.301, respectively) after orange juice consumption as compared to that after fresh fruit consumption. However, statistical significance was not reached.

Furthermore, no correlation between the volunteers' BMI or sex and carotenoid absorption was found.

3.3 In vitro carotenoid bioaccessibility

The concentrations of all monitored carotenoids in both the supernatant and the micelles were higher after in vitro digestion of orange juice than after digestion of orange fruits ($P < 0.05$, Table 1). Zeaxanthin was excluded from the shown data due to the hampered quantitation in the test foods, thus not allowing valid determination of its bioaccessibility. Total carotenoid bioaccessibility from orange fruits was 6.3 ± 0.2% compared to 27.5 ± 1.7% from orange juice. The β-cryptoxanthin bioaccessibility was 4.9 ± 0.3% and 25.9 ± 1.6% from orange fruits and juice, respectively, corresponding to a 5.3-fold increase due to juice processing.

4 Discussion

4.1 Bioavailability of β-cryptoxanthin from orange fruits and juice

In the present study, an equal dose of β-cryptoxanthin (0.744 mg) was 1.8-fold more bioavailable from orange juice than from fresh oranges. Establishing a clear-cut hypothesis which processing step caused the observed effect may be intricate, since the production of orange juice comprises multiple steps known to modulate carotenoid bioavailability. Previous studies have pointed out the hindering effect of dietary fibers, particularly citrus pectin, on carotenoid absorption [31, 32]. Total dietary fiber contents of our orange juice test food were reduced to only 6% of that of the orange fruit test food, being of potential importance for the enhanced carotenoid bioavailability from the juice.

In agreement, a previous study with seven participants reported that the addition of 12 g citrus pectin to a test meal led to a substantially decreased postprandial β-carotene plasma response from a 25 mg β-carotene capsule, reaching only half (0.39 μmol/L) of the maximum concentration as compared to a test meal consumed without dietary fiber (0.94 μmol/L) [31]. Riedl et al. [32] also reported a significantly decreased postprandial β-carotene response when subjects co-consumed 0.15 g/kg body weight citrus pectin, guar, or alginate (AUC of 57–67% as compared to that without fibers). The co-consumption of cellulose and wheat bran reduced the mean AUC to 80–82%, although not reaching significant difference to the meal without dietary fiber. The authors proposed that water soluble fibers might have a more pronounced negative effect on carotenoid absorption as compared to insoluble fibers, mostly due to disturbed micelle formation and slower diffusion processes during intestinal digestion. In the above mentioned studies [31, 32], isolated carotenoids were ingested as a dietary supplement, without being incorporated into the complex food matrix. In contrast, Castenmiller et al. [33]

evaluated the effect of intrinsically present and artificially added dietary fibers on carotenoid absorption from spinach over a 3 week period. While notable increases of the serum β-carotene concentration were observed after the consumption of both whole leaf (0.12 μmol/L) and minced (0.13 μmol/L) spinach, enzymatic liquefaction and concomitant removal of intrinsic dietary fibers further boosted β-carotene absorption (0.20 μmol/L). However, similar serum β-carotene concentrations (0.20 μmol/L) were reached when dietary fibers were added to the liquefied spinach meal. Noteworthy, the liquefied and added amount of dietary fibers in the above mentioned study were relatively low (3.1–4.3 g/day) as compared to our and the above mentioned study. In addition, only one third of the dietary fibers in Castenmiller et al.'s study consisted of water soluble fibers. Therefore, the increased β-carotene response was at least partly attributed to the complete disintegration of the plant cells and the carotenoid-containing chloroplasts rather than the sole removal of dietary fibers [33].

In our study, subjects ingested 8.6 and 0.9 g of dietary fibers from the orange fruit and orange juice, respectively, of which approximately 42–54% accounted for water soluble fibers (Table 2). As the amount of dietary fiber in the accompanying breakfast was 0.7 g, subjects ingested about 5.8-times more dietary fibers from the orange fruit test meal. Thus, in accordance with the above mentioned studies, the consumption of 9.3 g of dietary fibers in the orange fruit test meal was associated with a reduced β-cryptoxanthin mean AUC of 55% as compared to that after consumption of 1.6 g of dietary fibers in the orange juice test meal. Lastly, the study of Kay and Truswell [34] adds further evidence to the negative effect of dietary fibers on the intestinal absorption of lipophilic compounds. They observed a reduction of plasma cholesterol concentrations by 13% when subjects consumed 15 g of citrus pectin per day with a controlled diet over a period of 3 week. Concomitantly, the fecal fat excretion increased by 44%, indicating a lower fat absorption. These results may be transferred to the absorption of lipid soluble carotenoids, mainly following the absorption pathway of dietary fat (i.e. primary liberation from the food matrix, incorporation into mixed micelles, and subsequent absorption by the enterocyte [35, 36]). A mechanistic hypothesis for the hampering effect of dietary fibers on carotenoid bioavailability may be the viscosity increase due to water soluble polysaccharides. As a consequence, carotenoid liberation and micellarization might be limited due to a reduced diffusion rate of the required emulsifying bile salts into the gel phase. Hampered or delayed formation of micelles might be rate-limiting for carotenoid absorption, as only micellarized carotenoids are taken up during digestion [31]. In addition, gastric emptying may be delayed by the presence of additional dietary fibers, although Bennink et al. [37] reported no differences for solid and liquid test meals by means of a single radionuclide imaging study.

Besides differences in dietary fiber content, the mechanical disruption of the cell matrix during juice production and the thermal treatment during pasteurization are two more factors previously discussed to enhance carotenoid bioavailability. Gärtner et al. [38] reported a 3.8-fold higher lycopene mean AUC after consumption of a tomato paste compared to the ingestion of an equal lycopene dose from fresh tomatoes. However, tomato paste is produced by straining boiled tomatoes, partially removing dietary fibers. Thus, evaluating the respective contributions of thermal and mechanical treatment versus the removal of dietary fibers on carotenoid bioavailability may be difficult. In a feeding study by Rock et al. [39], eight female subjects showed higher β-carotene plasma concentrations (0.83 μmol/L) after daily consumption of processed carrots and spinach over a 4 weeks period when compared with the consumption of fresh vegetables (0.60 μmol/L). Surprisingly, the thermal effect of vegetable processing was much smaller than the authors anticipated, without even reaching the significance level ($P = 0.09$). Furthermore, Tassi et al. [40] found no difference in postprandial plasma β-carotene concentrations when five subjects ingested raw and cooked arugula, indicating a minor role of mere heat treatment on carotenoid bioavailability. Conversely, it is widely accepted that dietary lipids have an enhancing effect on carotenoid absorption [36]. However, the amount of ingested fat (30 g) was similar for both test foods in our study, annulling its effect on the increased bioavailability of β-cryptoxanthin from orange juice.

As mentioned before, quantifying the positive effects of dietary fiber removal, thermal pasteurization, and mechanical cell wall disintegration on carotenoid bioavailability is highly intricate in a 2-way cross-over study. Therefore, we previously evaluated carotenoid bioaccessibilities of differently processed orange products compared to that of the fresh fruit [6]. Homogenization of the fresh orange segments had no effect on carotenoid bioaccessibility, thus indicating only a minor role of mechanical cell disruption. The greatest increase in carotenoid bioaccessibility (+162%) was observed upon the removal of dietary fibers during juice extraction. Finishing and thermal pasteurization of the fresh juice further increased carotenoid bioaccessibility as compared to the fresh juice (+40%), although to a minor extent [6]. These findings indicate the reduction of dietary fibers to be the most important step for improving carotenoid bioavailability during orange juice processing. When transferring the present findings to a common western diet, it should be noted that navel oranges as used in our study commonly contain comparably low amounts of carotenoids (116 μg/100 g FW, [23]) as compared to the carotenoid contents in regular industrial juices (148 μg/100 g FW, [23]), produced mainly from carotenoid-rich Valencia oranges [41]. However, navel oranges are widely distributed both in the US and the EU, being mostly consumed as fresh fruit. Thus, transferring our results to an exemplary diet, the consumption of one glass (200 mL) of canned orange juice would even provide 3-times more β-cryptoxanthin than one serving of fresh navel orange (154 g) [23].

4.2 Comparison of the in vitro versus the in vivo model

Randomized cross-over trials are still considered to be the "gold standard" for assessing the bioavailability of both micro- and macronutrients. However, their high cost, long duration, and limited sample throughput often make preliminary in vitro experiments indispensable. Although the increase in β-cryptoxanthin bioavailability from orange juice was not as high as expected from its respective bioaccessibility, consistency of the results was still given. A possible explanation for the different magnitude of β-cryptoxanthin bioaccessibility and bioavailability might be the insufficient simulation of the peristalsis when applying the in vitro model, resulting in comparatively lower carotenoid bioaccessibilities from solid foods.

Using a very similar in vitro digestion model, Reboul et al. [42] reported the in vitro bioaccessibility of β-carotene from carrot puree to be similar to that determined in vivo after aspiration of duodenal content (~5%). The in vitro bioaccessibility of α-carotene was 2-fold higher (8.9%) compared to that obtained in vivo (4.7%). Similarly, a significant qualitative correlation was found between the results of in vitro bioaccessibility studies and those of postprandial carotenoid bioavailability in humans, indicating the in vitro digestion model to be a valuable preliminary tool for finding carotenoid sources worthwhile for investigation [42].

However, the suitability of in vitro experiments for assessing carotenoid bioaccessibility may not be generalized, since both the digestion model used as well as the investigated foods may have pronounced influence on the findings. For instance, Schweiggert et al. [43] previously reported insignificant difference in lycopene bioaccessibility from fresh papaya (0.3%) and tomato (0.3%). The addition of 2.5% oil to the test food resulted in an increased lycopene bioaccessibility from tomato (0.8%), but not from papaya (0.2%). Conversely, their recently published clinical trial revealed a 2.6-fold greater lycopene mean AUC after consuming fresh papaya compared to the consumption of an equal lycopene dose from tomatoes [20]. Thus, there is a need for further improvement and standardization of in vitro digestion models, as proposed by the consensus methodology agreed within the INFOGEST COST Action [44].

JKA, RC, and RMS designed the research; JKA, CLR, NB, ABW, and RMS conducted the research; JKA and RMS enrolled the participants; JKA and CLR analyzed the data; JKA and JH carried out the statistical analysis; JKA wrote the paper and designed figures/tables; ABW, RC and RMS revised the paper and contributed substantially to the discussion. All authors read and approved the final paper. RC had previous projects with juice producers, however not relating to the present study. Funding for the study was provided solely by the University of Hohenheim. We gratefully acknowledge the technical assistance of Svenja Baur, Julia Kahlhöfer, and Judith Karschin during the clinical trial.

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