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In Vitro Bioaccessibility of β-Carotene from Heat-Processed Orange-Fleshed Sweet Potato

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Orange-fleshed sweet potato (OFSP) is currently promoted in parts of sub-Saharan Africa as a biofortified staple food with large potential to provide considerable amounts of provitamin A carotenoids. However, the bioaccessibility of provitamin A carotenoids from OFSP has not been widely investigated, especially not as an effect of different preparation methods. In this study, we used an in vitro digestion model to assess the bioaccessibility of β-carotene from differently heat-processed OFSP. The fraction of carotenoids transferred from the food matrix to a micellar phase obtained after microfiltration and to a supernatant obtained after low-speed centrifugation was investigated. The percentage of accessible *all-trans*-β-carotene in the micellar phase varied between 0.5 and 1.1% in the heat-processed OFSP without fat and between 11 and 22% with the addition of 2.5% (w/w) cooking oil. In comparison with the micellar phase, the percentage of accessible *all-trans*-β-carotene in the supernatant phase was significantly higher ($P < 0.001$), between 24 and 41% without fat and between 28 and 46% with fat. These results support the importance of fat for an improved micellarization of β-carotene. Overall, the high in vitro bioaccessibility of β-carotene from heat-processed OFSP indicates that sweet potato might be a promising dietary approach to combat vitamin A deficiency.

KEYWORDS: Bioaccessibility; in vitro digestion; β-carotene; orange-fleshed sweet potato; *Ipomoea batatas*

INTRODUCTION

Vitamin A deficiency (VAD) has traditionally been addressed by several intervention strategies, including food fortification, supplementation, and dietary diversification (*1*−*3*). Despite these targeted approaches, VAD is still recognized as a central public health problem in large parts of sub-Saharan Africa, affecting predominantly preschool children and women of reproductive age (*4*). The failure of these intervention methods to prevent and treat this micronutrient deficiency is, in rural areas, mainly due to limited financial resources and distributional problems (*5*, *6*). A more sustainable approach may be to promote the production and consumption of biofortified plant foods. Biofortification refers to the process of breeding food crops with desirable properties such as disease tolerance, increased dry matter content, and high content of micronutrients (*7*).

With an average annual production of 84 kg per capita in Uganda (*8*), mainly used for human consumption, sweet potato (*Ipomoea batatas*) is considered to be an important staple food targeted for increased provitamin A content in biofortification programs. Orange-fleshed sweet potato (OFSP) has been reported to contain considerable amounts of provitamin A carotenoids (*9*−*13*), in particular β-carotene, but there is limited information about the release of these components from the cell matrix and their subsequent absorption in the small intestine. However, according to two recently performed dietary intervention studies in school-aged children, vitamin A status was significantly improved after consumption of OFSP (*14*, *15*).

Carotenoid bioaccessibility is generally defined as the fraction of carotenoids transferred from the food matrix to mixed micelles and, thereby, made available for subsequent uptake by the intestinal mucosa (*16*, *17*). To be absorbed in the intestine, carotenoids need to be incorporated into mixed micelles formed by the action of bile salts, phospholipids, and the hydrolysis products of dietary lipids. The fraction of bioaccessible carotenoids that are absorbed and available for utilization and storage in the body is referred to as the bioavailability of carotenoids.

Carotenoid bioaccessibility is known to be affected by a number of dietary factors, including the matrix in which the carotenoids are incorporated and the presence and type of lipid and fiber co-ingested with the carotenoids. The bioaccessibility can also be influenced by the heat treatment and the degree of homogenization during food processing. Cooking may enhance the release of carotenoids from the vegetable matrix by softening or degradation of the cell walls and by dissociating the structural components with which carotenoids are complexed (*18*, *19*). However, there is still limited information regarding the extent of carotenoid release as a result of different cooking methods.

The structural properties of sweet potato may influence the release of carotenoids from the matrix during digestion. Low-temperature blanching (LTB) prior to cooking has been shown to

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improve firmness of cooked vegetables, thereby providing a suitable method for preserving texture, which can be applied in the industrial processing of sweet potato (*20*, *21*). This blanching step is normally performed at 55−80 °C for time periods ranging from a few minutes to several hours (*20*). The firming effect of LTB in sweet potato is probably due to the combined effects of enzymatic pectin demethylation (*22*) and breakdown of starch into sugar oligomers that can escape from the cell without causing cell separation (*23*). However, to our knowledge there is no information whether this firming effect also may influence the accessibility of carotenoids from sweet potato.

Previous in vivo studies have shown the importance of dietary fat for an improved bioavailability of carotenoids (*16*, *24*, *25*), and the amount of fat normally contained in the diet is probably sufficient to achieve an enhanced absorption. Dietary fat facilitates carotenoid absorption by supplying a hydrophobic domain within which the carotenoids can be solubilized during digestion and by stimulating secretion of bile salts and pancreatic lipase required for micelle formation (*26*).

In vitro digestion models are considered to be appropriate analytical tools to estimate the bioaccessibility of bioactive ingredients from different food matrices (*27*). In vitro carotenoid bioaccessibility is measured as the fraction of carotenoid transferred from the food matrix either to a micellar phase obtained after ultracentrifugation/microfiltration (*28*−*31*) or to a supernatant obtained after low-speed centrifugation/decantation (*32*−*34*). Both in vitro digestion protocols have recently been suggested to estimate in vivo bioavailability of carotenoids in different plant foods (*17*, *35*). One of the main advantages of in vitro models is the possibility to screen for factors and components that may have an effect on the carotenoid bioaccessibility in a fairly cost-efficient manner.

The main objective of the present study was to estimate the bioaccessibility of β-carotene from OFSP using an in vitro digestion method and to evaluate the effect of commonly used cooking methods, both with and without addition of fat. Furthermore, we wanted to investigate whether the firming effect of an initial low-temperature blanching of sweet potatoes had an impact on the β-carotene in vitro bioaccessibility. In the present study we assessed the amount of β-carotene released into the supernatant and the amount incorporated into the micellar phase after in vitro digestion. Both measurements of in vitro bioaccessibility might reflect the bioavailability of β-carotene and also provide a possible means to compare the findings of in vitro studies with differently obtained bioaccessibility values.

MATERIALS AND METHODS

Chemicals and Standards. Pepsin (porcine), α-amylase, pancreatin (porcine), and bile extract (porcine) were purchased from Sigma-Aldrich (Schnelldorf, Germany). All other reagents used for in vitro digestion were obtained from Fischer Scientific GTF (Göteborg, Sweden) as were extraction and HPLC solvents. *all-trans*-β-Carotene standard was from Sigma-Aldrich.

Preparation of Samples. Sweet potatoes from Israel were purchased in a local store in Göteborg, Sweden. Under these circumstances the cultivar could not be identified, but this was not considered to be of importance because the objective of the present study was to evaluate the effect of different preparation methods on the in vitro bioaccessibility of β-carotene, not to obtain data on the actual cultivar used.

Sweet potatoes were peeled and cut into cylinders (35 mm ∅ × 50 mm long). Sweet potato cylinders were prepared either by boiling, steaming, or microwave cooking. In Uganda, sweet potato is commonly prepared by boiling or steaming and subsequently mashed into a purée. Samples were boiled in distilled water in a pot covered with a lid until the center temperature of the sweet potato reached 100 °C and was then maintained for an additional 5 min at this temperature. The total boiling time was approximately 20 min. Steamed sweet potato samples were prepared according to the same procedure with a total steaming time of 20 min. The samples were placed above the surface of boiling water in a pot covered with a lid. Sweet potato cylinders were microwaved (800 W) for 90 s in a closed container to minimize loss of moisture. A center temperature of 100 °C was reached within 45 s of microwave heating.

In a separate experiment sweet potato samples (10 mm thick sweet potato slices) were prepared either with or without an initial LTB step. This procedure was done by immersing sweet potato slices in a water bath at 70 °C. This temperature was reached in the center of the slices within 7 min. Samples were removed after 30 min and allowed to cool at room temperature before they were boiled in water for an additional 10 min. Another portion was boiled for 10 min (100 °C in the sample center was reached within 5 min) without the LTB step.

For each heat treatment three separate samples were prepared, each sample obtained from two OFSP roots. Sample temperatures were monitored with a T-type thermoelement coupled with a PC-logger 3100 and Easy view software (INTAB Interface-Teknik AB, Stenkullen, Sweden). The software permitted real-time data gathering and storage, allowing heating curves to be obtained.

The heat-processed sweet potato roots were homogenized with a hand mixer until puréed to similar particle size (> 60% of the particles were in the size of 125−500 μm for all preparation methods). Sunflower oil (2.5% (w/w)) was added and mixed into half of the purée while still warm. Sunflower oil is locally produced in certain areas of Uganda and can therefore be considered an advantageous means for addition of small amounts of fat to the normal diet. Sunflower oil has been reported not to contain any β-carotene (*36*). The resulting purée, both with and without added fat, was intended to represent a realistic ready-to-eat meal for small children in developing countries. Aliquots of each purée were transferred to screw-capped polypropylene tubes, overlaid with nitrogen, and stored at −80 °C. The carotenoid content of the samples was determined within 3 months.

In Vitro Digestion. The in vitro digestion procedure was based on previous studies (*28*, *32*), but several minor modifications were made to adjust the protocol to the specific plant material in this study. Most importantly, an oral phase of digestion was included due to the high starch content of sweet potato. All solutions for the in vitro digestion were prepared on the same day as the experiment was performed.

Duplicate samples (~3 g) were weighed into screw-capped polypropylene tubes, and a saliva solution (10 mL) consisting of 50 mM NaCl, 10 mM KH_2PO_4, 2 mM $CaCl_2 \cdot 2H_2O$, 40 mM $NaHCO_3$, and 1 mg/mL α-amylase was added. This enzyme concentration corresponds to approximately 1000 units of α-amylase/g of sweet potato sample. The pH was adjusted to 6.7 before the samples were blanketed with nitrogen and incubated at 37 °C on an orbital shaker (500 rpm; DOS-10 L, Techtum Lab AB, Umeå, Sweden) for 15 min. Next, the pH was decreased to 4.2 with 1 M HCl before the addition of 5 mL of gastric solution containing 50 mM NaCl, 14 mM KCl, 3.5 mM KH_2PO_4, 10 mM $CaCl_2 \cdot 2H_2O$, 3.6 mM $MgCl_2 \cdot 6H_2O$, and pepsin (21 g/L, 914 units/mg of protein). The pH of the samples reached 4.0 after the addition of gastric solution. Samples were overlaid with nitrogen and incubated at 37 °C for 30 min. The gastric digestion was continued by adjusting the pH to 2.0 with 1 M HCl and incubating for another 30 min. The intestinal phase was simulated by raising the pH to 6.3 with 1 M $NaHCO_3$ followed by the addition of 3 mL of pancreatin/bile extract solution (4.5 g/L pancreatin, 28 g/L bile extract in 100 mM $NaHCO_3$). The pH was adjusted to 6.9 before the samples were blanketed with nitrogen and incubated at 37 °C for 2 h. After digestion had been completed, the samples were centrifuged at 5000*g* for 20 min to obtain the supernatant fraction. An aliquot of the supernatant was microfiltered (Millex-MF, 0.22 μm pore size, Millipore, Billerica, MA) to obtain the micellar fraction. The whole procedure was performed under dimmed light. No antioxidant was included in the in vitro digestion procedure.

The β-carotene content was measured both in the supernatant and in the micellar fraction. In vitro digestion experiments were performed within 3 months after sample preparation. The coefficient of variation (CV) for the in vitro digestion protocol was below 5%, measured on six replicates of boiled and homogenized samples.

Extraction of β-Carotene from OFSP Samples. Carotenoids were extracted from the heat-processed OFSP samples as described previously (*13*). Briefly, sweet potato purée was thawed, and ~0.5 g samples in duplicate were added to test tubes and mixed with acetone containing

0.1% (w/v) butylated hydroxytoluene. The test tubes were vortexed and then centrifuged at 4750*g* for 3 min. Each resulting supernatant was transferred into a new test tube. The residue was repeatedly extracted with acetone up to five times until the residue and supernatant were colorless. Petroleum ether (3 mL) was added to the resulting acetone extract together with distilled water (5 mL) to facilitate phase separation. The organic and water phases were separated by centrifugation at 4750*g* for 4 min, and the organic phase was transferred to a new test tube. This step was repeated once before the pooled organic phase was evaporated to dryness in a water bath at 35 °C under a stream of nitrogen. The residue was dissolved in 5 mL of mobile phase consisting of methanol/methyl *tert*-butyl ether (60:40, v/v). Samples were protected from light throughout the extraction and analysis.

Extraction of β-Carotene from in Vitro Digested Samples. Carotenoids in the supernatant and micellar fraction from the in vitro digested samples were extracted twice with hexane/acetone/ethanol (50:25:25) containing 0.1% (w/v) butylated hydroxytoluene. Samples were vortexed and centrifuged at 4750*g* for 3 min to facilitate phase separation. Organic fractions were combined and dried under a stream of nitrogen in a water bath held at 35 °C, after which the residue was dissolved in 1 mL of mobile phase (methanol/methyl *tert*-butyl ether (60:40, v/v)). Recovery of β-carotene in the digesta was assessed from a subset of the samples prior to centrifugation according to the same extraction procedure.

β-Carotene Analysis by HPLC. Carotenoids were analyzed by reversed phase HPLC (Waters 600 multisolvent delivery system and controller equipped with a Waters 996 UV−visible photodiode array detector; Waters, Stockholm, Sweden). Absorption spectra were recorded between 250 and 500 nm. Separations were carried out on a C30 carotenoid column (5 μm, 250 × 4.6 mm i.d., YMC Europe GMBH, Schermbeck, Germany). The injection volume was 20 μL. Analytes were eluted from the column with differing proportions of methanol (solvent A) and methyl *tert*-butyl ether (solvent B) with a flow rate of 1 mL/min. The solvent gradient was the following: 0−17 min, 70 to 30% A; 18−35 min, 30% A; 36−37 min, 30 to 70% A; 38−40 min, 70% A. The gradient allowed separation of *all-trans*-β-carotene and its geometrical isomers (9-, 13-, and 15-*cis*-β-carotene). Quantification of *cis*- and *all-trans*-β-carotene was carried out at 452 nm based on linear calibration curves with eight points constructed with *all-trans*-β-carotene and using the response factor 0.806 for 13-*cis*-β-carotene (*37*).

Statistical Analysis. Three independent in vitro digestions were made from separate samples for each preparation method, with and without the addition of fat. Each in vitro digestion was performed in duplicate. All statistical analyses were performed using SPSS (version 14.0, SPSS Inc., Chicago, IL). Data are presented as mean values ± standard deviation (SD). Mean values were compared by analysis of variance, and determination of significant differences between groups was made with Tukey's post hoc test. Differences were considered to be significant at $P < 0.05$.

In vitro carotenoid bioaccessibility was calculated as the percentage of carotenoid transferred from the food matrix both to the supernatant obtained after low-speed centrifugation and to the micellar phase obtained after microfiltration of the supernatant following simulated digestion.

RESULTS AND DISCUSSION

β-Carotene Content in OFSP. The *all-trans*-β-carotene content in the heat-processed OFSP samples ranged from 50 to 104 μg/g of fresh weight (FW) (**Table 1**). This is in line with previous studies on OFSP (*12*, *13*). The amount of 13-*cis*-β-carotene varied considerably with the type of heat treatment. The lowest amounts were obtained after microwave cooking, whereas the highest were obtained after boiling and steaming, ranging from 1.8 to 6.1 μg/g of FW. This corresponds to between 2.4 and 7.8% of the total β-carotene content. The lower amount of 13-*cis*-β-carotene after microwave cooking was expected, given the considerably shorter heating time compared with the other preparation methods. No other cis-isomers were detected in any of the heat-processed samples. The recovery of *all-trans*-β-carotene after simulated oral, gastric, and small intestinal digestion, measured on a subset of the samples, was at least 85%. The recovery of 13-*cis*-β-carotene was on average somewhat lower, but still exceeded 70%.

Table 1. Amount of *all-trans*-β-Carotene and 13-*cis*-β-Carotene in Heat-Processed OFSP[a]

sample/preparation method	*all-trans*-β-carotene (μg/g of FW)	13-*cis*-β-carotene	
		μg/g of FW	% of total β-carotene[b]
cylinders[c]			
boiling	57.06 (50.03−64.84)	4.13 (3.70−4.41)	7.3 a
steaming	68.24 (66.37−71.49)	5.04 (4.46−5.68)	7.4 a
microwave heating	92.94 (75.12−103.94)	2.51 (1.84−3.20)	2.7 b
slices[c]			
LTB + boiling	91.52 (78.05−99.05)	5.41 (5.20−5.52)	6.0 a
boiling	86.69 (65.22−98.70)	5.48 (4.30−6.14)	6.4 a

[a] Data are the mean of three independent samples (lowest and highest values shown in parentheses) analyzed in duplicate. [b] Values in the same column not sharing the same letters (a, b) are significantly different ($P < 0.001$) using one-way ANOVA and Tukey's post hoc test. [c] OFSP cylinders were 35 mm ∅ × 50 mm long, whereas OFSP slices were 10 mm in thickness.

Comparison of in Vitro Methodology. Reported values on carotenoid bioaccessibility from fruits and vegetables differ to a large extent. The type of plant matrix probably accounts for most of these differences. A study by de Pee et al. (*38*) showed that fruits were more effective than green leafy vegetables in increasing serum concentrations of β-carotene in school children. Apart from this matrix effect, there are still considerable variations between reported values of carotenoid in vitro bioaccessibility from different plant foods. A contributing factor to these discrepancies is most likely the various methods applied to obtain the accessible carotenoid fraction. In the literature, in vitro carotenoid bioaccessibility is measured either as the fraction of carotenoid transferred from the food matrix to a supernatant obtained after low-speed centrifugation/decantation or to a micellar phase obtained after ultracentrifugation/microfiltration. Hence, to facilitate the comparison of bioaccessibility values obtained with these two methods, we here present the accessibility of β-carotene from OFSP both before and after microfiltration. The supernatant was obtained after in vitro digestion followed by low-speed centrifugation, whereas the micellar phase was obtained by microfiltering the supernatant. There was a significant difference ($P < 0.001$) between the two fractions both in the presence and in the absence of dietary fat (**Table 2**). This apparent difference illustrates the necessity for a standardized procedure for measuring the in vitro bioaccessibility of carotenoids during simulated digestion. The supernatant can be interpreted to contain both the carotenoids incorporated into micelles and soluble aggregates of nonmicellarized carotenoids. These nonmicellarized carotenoids might have the potential to be transferred into a micellar phase under in vivo conditions (*39*). However, until additional data from studies in humans are available, neither of the two methods can be proposed as a reference method to predict the bioavailability of carotenoids.

The effect of microfiltration on the carotenoid content in the micellar fraction after in vitro digestion has previously been evaluated (*28*, *40*). In contrast to the present study, microfiltration did not affect the amount of β-carotene in the filtrate. However, the diverging results between these studies are most likely due to the ultracentrifugation step, which was used prior to microfiltration both by Garrett et al. (*28*) and by Wright et al. (*40*), but not in the present study.

Bioaccessibility of *all-trans*-β-Carotene in Heat-Processed OFSP. We have previously explored the effects of different preparation methods on the β-carotene content in OFSP (*13*). The retention of *all-trans*-β-carotene after boiling, steaming, and deep-frying was similar and as high as nearly 80%. Even though the type of cooking seems to have a minor effect on the amount of β-carotene

Table 2. In Vitro Bioaccessible *all-trans*-β-Carotene in Differently Heat-Processed OFSP (Measured in the Supernatant and in the Micellar Phase)[a]

	all-trans-β-carotene in supernatant (%)		*all-trans*-β-carotene in micellar phase (%)	
sample/preparation method	no fat	2.5% fat (w/w)	no fat	2.5% fat (w/w)
cylinders[b]				
boiling	40.77 ± 4.79 a	45.54 ± 3.36 a	0.86 ± 0.07 ab	19.87 ± 1.72 a
steaming	38.15 ± 4.75 a	44.77 ± 5.10 a	0.88 ± 0.17 ab	20.20 ± 0.76 a
microwave heating	23.71 ± 2.06 b	27.55 ± 2.32 b	0.53 ± 0.08 b	11.25 ± 1.00 b
slices[b]				
LTB + boiling	32.05 ± 5.68 ab	43.80 ± 1.51 a	1.10 ± 0.31 a	21.75 ± 1.56 a
boiling	37.62 ± 3.69 a	40.41 ± 2.24 a	0.95 ± 0.14 ab	18.73 ± 2.19 a

[a] Data are the mean ± SD of duplicate measurements of three independent in vitro digestions ($n = 3$). The presence of different letters (a, b) within a column indicates that the preparation methods are significantly different ($P < 0.05$) using one-way ANOVA and Tukey's post hoc test. All percentages are calculated in relation to the total amount of *all-trans*-β-carotene in the corresponding heat-processed samples. [b] OFSP cylinders were 35 mm ∅ × 50 mm long, whereas OFSP slices were 10 mm in thickness.

retained in the sweet potato, the potential impact of the preparation method on the in vitro bioaccessibility of β-carotene in sweet potato needs to be investigated. In the present study, OFSP was subjected to various domestic cooking procedures, including boiling, steaming, and microwave cooking. The effects of these cooking methods on the transfer of β-carotene from the food matrix to the supernatant and micellar fraction were investigated. The proportion of accessible β-carotene in the supernatant and micellar fraction, respectively, did not vary to a large extent between the different preparation methods except for microwave cooking (**Table 2**). Even though the retention can be expected to be higher in the microwaved samples as a result of the shorter preparation time, the fraction of accessible β-carotene was significantly lower ($P < 0.05$) compared with the other preparation methods in the presence of fat. These data suggest that the short heating period for the microwaved samples was not sufficient to obtain an adequate breakdown of the sweet potato cell matrix and, subsequently, the release of β-carotene from the matrix to the supernatant/micellar fraction was impaired. Ryan et al. (*41*) studied the influence of various cooking methods on the micellarization of β-carotene, lycopene, β-cryptoxanthin, and lutein in courgette, red pepper, and tomato. They observed a significantly enhanced β-carotene micellarization after cooking in comparison with the corresponding raw vegetable.

With no added fat, the fraction of *all-trans*-β-carotene obtained in the supernatant after in vitro digestion of the differently prepared samples varied between 23.7 and 40.8% in the present study (**Table 2**). There was a significant difference ($P < 0.05$) between the microwaved samples and the other preparation methods except for LTB. The transfer of *all-trans*-β-carotene into the micellar fraction in samples without fat ranged from 0.5 and 1.1%. Mills et al. (*42*) recently reported correspondingly low values on the efficiency of micellarization in boiled OFSP in the absence of fat.

To investigate the impact of fat on the transfer of β-carotene from the food matrix to the supernatant and micellar phase in the present study, sunflower oil was added in the proportion of 2.5% (w/w) to the differently heat-treated OFSP samples. The fraction of accessible *all-trans*-β-carotene in the microwave-heated samples was significantly lower ($P < 0.05$) compared with all other preparation methods in presence of fat, measured either in the supernatant or in the micellar phase. There was a significant linear relationship ($R^2 = 0.75$, $P < 0.001$) between the percentage of accessible *all-trans*-β-carotene in the micellar phase and the supernatant, respectively, with the addition of oil in the samples. Both methods may therefore be useful in the estimation of the bioaccessible amount of β-carotene in heat-treated OFSP with the addition of fat.

In the supernatant, the accessibility of *all-trans*-β-carotene increased between 7 and 37% by the addition of fat. However, the amount of *all-trans*-β-carotene transferred into the micellar fraction increased by a factor of 20 as a result of fat addition. The results in the present study clearly indicate that dietary fat is necessary to obtain an efficient micellarization of *all-trans*-β-carotene, which is in accordance with previous in vitro studies (*43*−*45*). However, due to methodological differences between different in vitro models such as content and activity of digestive enzymes and bile salts, it is difficult to make direct comparisons with previously described studies. Huo et al. (*43*) reported an improved micellarization of carotenes from a salad meal in the presence of 0.5% (v/w) triolein and trioctanoin, but did not notice any further increase with lipid amounts up to 2.5% (v/w). However, the requirement of fat seems to be higher for relatively mildly processed vegetables in comparison with cooked foods, as was shown in a human study where carotenoid responses were higher in subjects consuming salads containing 28 g of oil compared with the same salad containing 6 g of oil (*16*).

LTB has been reported to be an appropriate method to increase firmness retention in cooked and industrially processed sweet potato (*20*, *21*). Van Dijk et al. (*46*) reported a higher preservation of the pectin structure as a result of blanching. The presence of pectin has been shown to reduce carotene bioavailability in humans (*47*). Similar findings were reported in an in vitro study by Ornelas-Paz et al. (*45*), in which pectin isolated from ripening mangoes had a negative impact on the micellarization of β-carotene from an oil-based supplement. Although we noticed a firmer texture of the blanched and subsequently boiled samples compared with the samples that were boiled directly, there was no significant difference in β-carotene accessibility. Boiling of OFSP cylinders for 20 min or of OFSP slices for 10 min had no significant effect on the percentage of accessible *all-trans*-β-carotene or 13-*cis*-β-carotene, respectively.

Bioaccessibility of 13-*cis*-β-Carotene in Heat-Processed OFSP. The addition of cooking oil significantly improved ($P < 0.001$) the in vitro bioaccessibility of 13-*cis*-β-carotene, both in the supernatant and in the micellar phase (**Table 3**). The percentage of accessible 13-*cis*-β-carotene showed a linear relationship ($R^2 = 0.90$, $P < 0.001$) between the two phases. The quantity of 13-*cis*-β-carotene that partitioned into micelles during in vitro digestion of the differently heat-processed OFSP samples without oil ranged from 0.14 to 0.45 μg/g, which corresponds to an efficiency of micellarization between 5.6 and 8.8% (**Table 3**). The incorporation into micelles was significantly higher ($P < 0.001$) for 13-*cis*-β-carotene compared with *all-trans*-β-carotene in all samples without fat. In the presence of fat, the same effect was observed, but to a lower degree. In the supernatant fraction, the same relationship was observed but only in samples with added fat.

A more efficient micelle incorporation of 13-*cis*-β-carotene than the corresponding all-trans-form during digestion was also observed in a study by Tyssandier et al. (*39*), in which 10 healthy subjects were fed a vegetable test meal containing carotenoids and

Table 3. In Vitro Bioaccessible 13-*cis*-β-Carotene in Differently Heat-Processed OFSP (Measured in the Supernatant and the Micellar Phase)[a]

	13-*cis*-β-carotene in supernatant (%)		13-*cis*-β-carotene in micellar phase (%)	
sample/preparation method	no fat	2.5% fat (w/w)	no fat	2.5% fat (w/w)
cylinders[b]				
boiling	29.28 ± 3.69 a	69.56 ± 3.76 a	8.48 ± 1.36 a	56.01 ± 1.91 a
steaming	25.11 ± 0.65 ab	62.72 ± 1.18 ab	8.84 ± 0.39 a	56.21 ± 2.50 a
microwave heating	18.42 ± 1.81 b	38.38 ± 3.50 c	5.62 ± 1.15 b	28.40 ± 1.32 b
slices[b]				
LTB + boiling	29.43 ± 1.59 a	56.71 ± 5.27 b	7.64 ± 1.29 ab	44.90 ± 7.42 a
boiling	30.22 ± 4.38 a	63.57 ± 5.30 ab	7.89 ± 0.36 ab	49.15 ± 5.39 a

[a] Data are the mean ± SD of duplicate measurements of three independent in vitro digestions ($n = 3$). The presence of different letters (a−c) within a column indicates that the preparation methods are significantly different ($P < 0.05$) using one-way ANOVA and Tukey's post hoc test. All percentages are calculated in relation to the total amount of 13-*cis*-β-carotene in the corresponding heat-processed samples. [b] OFSP cylinders were 35 mm Ø × 50 mm long, whereas OFSP slices were 10 mm in thickness.

sunflower oil. Likewise, more efficient incorporation of *cis*-lycopene than of *trans*-lycopene has been reported both into synthetically prepared micelles (*48*) and into mixed micelles following in vitro digestion (*49*). Moreover, the transfer from emulsion droplets to mixed micelles could be affected by different localization of the cis- and trans-forms of β-carotene within the droplets. In contrast to the linear shape of *all-trans*-β-carotene, the cis-configuration has a bent structure that decreases the space occupied by the molecule. This likely prevents aggregation and crystallization of cis-isomers, thereby facilitating incorporation into mixed micelles (*50*).

Potential Provitamin A Activity of OFSP. The amount of in vitro bioaccessible β-carotene obtained in the micellar fraction ranged from 0.5 to 1.0 μg/g of FW without added fat, whereas the amount varied between 10.6 and 19.8 μg/g of FW in the presence of 2.5% (w/w) sunflower oil. The recommended safe intake level of vitamin A for 1−10-year-old children is estimated to be 400 μg of RE/day (*51*). Although there is a lack of human data to verify whether the in vitro model can predict β-carotene bioavailability, the present results suggest that it can be a valuable tool to estimate relative bioaccessibility of carotenoids from different dietary sources. Hence, assuming a complete absorption of the in vitro bioaccessible β-carotene incorporated in mixed micelles and a 50% conversion to retinol in the mucosa would indicate that a 100 g ready-to-eat portion of the OFSP purée without added fat could provide from 6 to 12% of the daily vitamin A requirements. The same purée with added fat, on the other hand, could more than well cover the daily vitamin A requirements (132−248%), indicating the importance of fat in a provitamin A-rich vegetable diet. The accessibility of *all-trans*-β-carotene in the micellar phase varied between 11.3 and 21.8%. Assuming 50% conversion to retinol in the mucosa, these data correspond to conversion factors of 18 μg of β-carotene:1 μg of retinol and 9 μg of β-carotene:1 μg of retinol, respectively, which is in line with the vitamin A equivalence factor of 13:1 that was estimated for a meal of sweet potato sautéed in corn oil measured in a human isotopic absorption study on Bangladeshi men (*52*).

In summary, the in vitro bioaccessibility of β-carotene from heat-processed OFSP was significantly higher as measured in the supernatant than in the micellar phase. The results in the present study clearly indicate the need for a reference method to determine the in vitro accessibility of carotenoids that has been correlated with data from human studies. In addition, the present study shows the importance of fat for an enhanced in vitro bioaccessibility of β-carotene, which is in agreement with several human studies. A similar in vitro bioaccessibility of β-carotene from OFSP was achieved after the different heat treatment methods except for microwave cooking, which resulted in significantly lower percentage of accessible β-carotene in the presence of fat.

ABBREVIATIONS USED

VAD, vitamin A deficiency; OFSP, orange-fleshed sweet potato; LTB, low-temperature blanching.

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