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ARTICLE

Effects of Thermal Processing on the in Vitro Bioaccessibility and Microstructure of β-Carotene in Orange-Fleshed Sweet Potato

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The effects of different preparation methods on the bioaccessibility of β-carotene in orange-fleshed sweet potato (OFSP), an important food crop in sub-Saharan Africa, have been evaluated using an in vitro digestion procedure. The preparation methods included, on fresh roots, boiling followed by puréeing and oil addition (BOL) and homogenization followed by boiling and oil addition (HOM); on milled flour from freeze-dried fresh roots, cooking of porridge followed by oil addition (POA) and oil addition to flour followed by cooking of porridge (POB). The retention of *all-trans*-β-carotene ranged from 58% (POB) to 72% (BOL). The presence of oil during heating resulted in a significantly higher formation of 13-*cis*-β-carotene for the POB-treated samples than for the other samples. The efficiency of micellarization of *all-trans*-β-carotene after in vitro digestion was 50% (HOM), 48% (POB), 31% (POA), and 16% (BOL). Brightfield microscopy of the cell structure after processing and in vitro digestion showed a high degree of cell-wall rupture for the HOM-treated samples, whereas cells appeared intact for the BOL samples. Also, coherent anti-Stokes Raman scattering (CARS) microscopy showed smaller β-carotene bodies residing in the HOM samples than in the BOL samples after digestion. These results suggest that the in vitro bioaccessibility of β-carotene in an OFSP meal can be improved by processing methods that promote cell-wall rupture.

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

INTRODUCTION

Sweet potato [*Ipomoea batatas* (L.) Lam] is one of the most widely cultivated root crops in sub-Saharan Africa, where most of the traditionally grown cultivars contain negligible amounts of provitamin A carotenoids and therefore have white flesh color (*1*, *2*). However, biofortification programs initiated by several countries in sub-Saharan Africa gathered into the Vitamin A for Africa Initiative (VITAA) have contributed to the release of new, yellow- and orange-colored cultivars with increased provitamin A carotenoid content as well as optimized traits such as enhanced disease tolerance and early maturity (*3*−*5*). Replacing white-fleshed cultivars with orange-fleshed sweet potato (OFSP) has been shown to improve the vitamin A status of school-aged children (*6*, *7*), without negatively affecting consumer acceptance (*8*, *9*). Hence, diet diversification through the introduction of biofortified OFSP cultivars may be considered a promising approach for alleviating vitamin A deficiency in sub-Saharan Africa. However, there is still a great need for further information concerning the bioaccessibility and bioavailability of provitamin A carotenoids in OFSP to be able to fully evaluate the nutritional potential of sweet potato consumption for reducing vitamin A deficiency.

The bioaccessibility of carotenoids in plant foods is widely accepted to be influenced by their physicochemical state within the plant matrix and the type and degree of heat processing as well as the presence of additional components in the meal such as fat and fiber (*10*). However, to what extent the plant matrix is degraded during thermal processing and how this may influence the release of carotenoids are not well characterized. Degradation of plant tissue under physical stress can generally be divided into cell-wall rupture and cell−cell separation (*11*). Thermal treatments of plant foods soften the cells by turgor loss and degradation of pectin in the middle lamella located between the cells (*12*, *13*). Whereas the softened plant matrix is generally disrupted by separation of cells across the middle lamella, the high turgor pressure normally observed in raw plant tissue favors cell-wall rupture (*11*, *14*). Although deformation behavior is known to strongly affect the textural properties of plant tissues, the effects on the bioaccessibility of carotenoids are less investigated. Furthermore, current knowledge of the physicochemical state of carotenoids in OFSP is limited, and we have recently used coherent anti-Stokes Raman scattering (CARS) microscopy to

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obtain information on the subcellular location and morphology of β-carotene in OFSP (*15*). Structural information may contribute to a more profound understanding of the factors involved in the release of carotenoids during food preparation and digestion, which in turn would facilitate the development of improved processing techniques and thus be of importance for an enhanced bioaccessibility of provitamin A carotenoids in OFSP. Our objective in the present study is therefore to study the effects on the β-carotene retention and in vitro bioaccessibility in OFSP of alternative preparation methods to the commonly used boiling and steaming of whole roots. For this purpose, quantitative data on β-carotene content and in vitro bioaccessibility are related to structural observations of OFSP after thermal processing and in vitro digestion using combined brightfield and CARS microscopy.

MATERIALS AND METHODS

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

Sample Preparation. OFSP roots originating from the United States were purchased locally in Göteborg, Sweden. No information on the cultivar, exact growth location, or age of maturity could be retrieved, but all roots were purchased in a single batch and used for experiments within 2 days after the purchase.

OFSP was subjected to four different treatments, of which two were prepared using fresh roots: boiling followed by puréeing and oil addition (BOL), and homogenization followed by boiling and oil addition (HOM). Two were prepared with milled flour from freeze-dried fresh roots: cooking of porridge followed by oil addition (POA), and oil addition to flour followed by cooking of porridge (POB). Fresh roots were homogenized prior to or after boiling, and dried roots were milled into flour before cooking of porridge with the objective to obtain finely decomposed purée-like products (>80% of the particles were <500 μm in size for all preparation methods). Each preparation method was repeated three times to generate three independent samples for in vitro digestion. Each sample consisted of three randomly chosen OFSP roots of uniform size and form that were peeled and cut into 10 mm thick slices. Fresh reference samples for each treatment were obtained by freeze-drying an aliquot of the fresh OFSP slices used for thermal processing. As part of the preparation process, deionized water was added to the differently treated samples in relation to the weight and dry matter content of the OFSP to generate samples of similar dry matter content for in vitro digestion.

For treatment BOL, OFSP slices were submerged in boiling deionized water and subsequently boiled for 10 min. The boiled slices were homogenized with a hand mixer (Philips, HR1364) until a purée was obtained. Deionized water was added in the same amount (w/w) as the purée, after which sunflower oil [2.5% (w/w)] was mixed into the resulting purée while still warm. For treatment HOM, OFSP slices and deionized water (1:1, w/w) were homogenized in a household blender (Philips, HR2090) for 60 s. The resulting mixture was boiled for 10 min, and sunflower oil [2.5% (w/w)] was added to the boiled purée.

Samples (OFSP slices from three roots) for both treatments POA and POB were freeze-dried and then milled separately to a finely ground flour. In treatment POA, the OFSP flour was rehydrated with deionized water to obtain a dry matter content similar to that of the BOL and HOM purées, and the mixture was boiled for 10 min. After boiling, sunflower oil [2.5% (w/w)] was mixed into the resulting porridge. In treatment POB, the OFSP flour was mixed with sunflower oil [2.5% (w/w)] prior to the addition of deionized water to the same water content as in the POA sample and then boiled for 10 min to obtain a porridge. The 10 min boiling period included in all treatments was carried out in a stainless steel pot covered with a lid to prevent evaporation.

The dry matter content in the thermally processed OFSP samples was determined using a moisture balance (310 M mass balance and HA300 dryer; Precisa, Dietikon, Switzerland). Aliquots of each sample were transferred to screw-capped polypropylene tubes, overlaid with nitrogen, and stored at −80 °C for a maximum of 2 months until analysis. Retention of *all-trans*-β-carotene in the thermally processed samples was calculated on a dry weight basis, and moisture losses during boiling were accounted for.

In Vitro Digestion. OFSP samples were subjected to simulated oral, gastric, and small intestinal phases of digestion with some minor modifications to the procedure described previously (*16*). Briefly, 3 g of thermally processed OFSP (in duplicate) was mixed with 10 mL saliva solution (pH ∼6.8) containing 0.1 mg/mL α-amylase and incubated at 37 °C on an orbital shaker (480 rpm; DOS-10 L, Techtum Lab AB, Umeå, Sweden) for 10 min. Samples were blanketed with nitrogen prior to the incubations to minimize oxygen exposure. The gastric phase was initiated by decreasing the pH to 4.0 with the addition of 1 M HCl and 5 mL of gastric solution including 19.5 g/L pepsin. Samples were incubated at this pH for 30 min and for an additional 30 min at pH 2.0. To simulate small intestinal conditions, the pH in the samples was adjusted to 6.9 with 1 M $NaHCO_3$, and 3 mL of pancreatin/bile extract solution (4.5 g/L pancreatin, 28 g/L bile extract) was added prior to incubation at 37 °C for 2 h. After this incubation period, the samples were centrifuged at 5000*g* for 30 min. Similar to the procedure used in the previous study (*16*), two different fractions (supernatant phase/micellar phase), which may both represent the accessible fraction of β-carotene for uptake in the small intestine, were obtained and subsequently analyzed for their β-carotene content on the same day as the experiment. Recovery of β-carotene in the digesta was assessed from a subset of the samples prior to centrifugation and calculated according to the method of Failla et al. (*17*). In vitro carotenoid bioaccessibility was calculated as the percentage of carotenoid transferred from the food matrix both to a supernatant obtained after centrifugation and to a micellar phase obtained after microfiltration of the supernatant following simulated digestion. All enzyme solutions for in vitro digestion were prepared on the same day as the experiment. All digestion experiments and the subsequent extractions were performed under low-light conditions to minimize carotenoid losses.

Extraction and HPLC Analysis of β-Carotene. The extraction of β-carotene from fresh (∼0.2 g of freeze-dried sample), thermally processed (∼0.5 g of purée/porridge), and in vitro digested (∼1 mL aliquot of the supernatant and micellar phase) OFSP was made according to previously described protocols (*16*, *18*).

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). Separation of carotenoids was achieved using a C_{30} carotenoid column (5 μm, 250 × 4.6 mm i.d.; YMC Europe GMBH, Schermbeck, Germany), and the analytes were eluted from the column using gradient elution at a flow rate of 1 mL/min. The solvent gradient consisted of methanol (eluent A) and methyl *tert*-butyl ether (eluent B) with the following proportions: 0−17 min, from 70 to 30% A; 18−35 min, 30% A; 36−37 min, from 30 to 70% A; 38−40 min, 70% A. This gradient facilitated separation of *all-trans*-β-carotene and 13-*cis*-β-carotene (**Figure 1**). Quantification of these isomers was carried out using calibration curves of *all-trans*-β-carotene at five concentration levels and with a response factor of 0.806 for 13-*cis*-β-carotene (*19*).

CARS and Brightfield Microscopy. The setup for CARS microscopy and detection of β-carotene in OFSP has been described in more detail previously (*15*). Briefly, the setup is composed of a pulsed, near-infrared laser system combined with an inverted Nikon Eclipse TE2000-E microscope and a single-photon-counting detection system. Laser beams with wavelengths of 1064 and 916.5 nm were used for excitation of the C=C vibration mode in β-carotene at 1520 cm^{-1}. The focused laser beams were scanned over the sample surface, and the signal generated at 805 nm was detected in the forward propagation direction. β-Carotene CARS microscopy images with a field of view of 50 × 50 μm were measured for OFSP samples at different stages of thermal processing and in vitro digestion. In addition to β-carotene visualization by CARS microscopy, information on the general structure of the cellular matrix was obtained by concurrently measured brightfield differential interference contrast images. A collection of between five and nine images was taken for each treatment.

Statistical Analysis. Statistical analysis was carried out using PASW Statistics 18 (SPSS Inc., Chicago, IL). Results are given as the mean value of three independent samples (n = 3) ± standard deviation (SD) analyzed

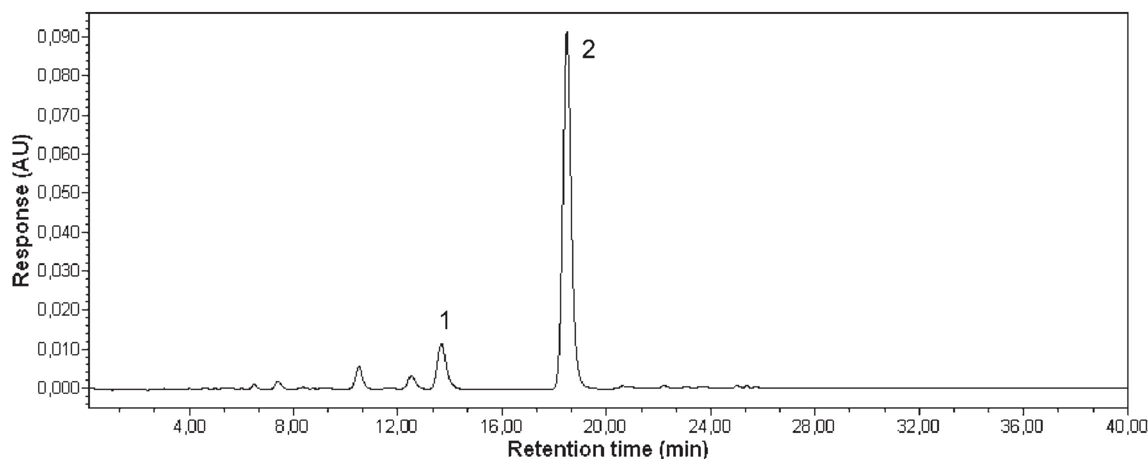

Figure 1. Representative HPLC chromatogram of a BOL-treated sample showing the separation of (peak 1) 13-*cis*-β-carotene and (peak 2) *all-trans-β*-carotene.

Table 1. Amount of *all-trans*-β-Carotene and 13-*cis*-β-Carotene in Fresh and Thermally Processed OFSP and Retention of *all-trans*-β-Carotene after Thermal Processing[a]

	all-trans-β-carotene			13-*cis*-β-carotene	
treatment	fresh (μg/g of DW)	processed (μg/g of DW)	retention (%)	processed (μg/g of DW)	% of total β-carotene
BOL[b]	324.0 ± 28.9	232.0 ± 13.5	71.8 ± 3.8 a	22.3 ± 0.1	8.8 ± 0.4 b
HOM[c]	344.2 ± 19.2	221.6 ± 1.9	64.5 ± 3.9 ab	9.9 ± 1.9	4.3 ± 0.8 c
POA[d]	300.2 ± 11.9	209.8 ± 8.8	69.9 ± 1.6 a	11.7 ± 0.9	5.3 ± 0.4 c
POB[d]	305.8 ± 23.3	178.9 ± 26.6	58.3 ± 4.5 b	26.7 ± 5.7	12.9 ± 0.7 a

[a] Data are the mean ± SD of three independent samples ($n = 3$) analyzed in duplicate. Values in the same column not sharing the same letters (a−c) are significantly different ($P < 0.05$) using one-way ANOVA and Tukey's post hoc test. [b] Boiling of fresh roots followed by puréeing and oil addition. [c] Homogenization of fresh roots followed by boiling and oil addition. [d] Cooked porridges were prepared from freeze-dried OFSP milled into flour; POA, oil added after cooking; POB, oil added to flour prior to cooking.

in duplicate. Treatments were compared using one-way analysis of variance (ANOVA), and significant differences between the means were determined by Tukey's post hoc test. Linear regression analysis was done to compare the two phases obtained after in vitro digestion. Differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Retention of *all-trans-β*-Carotene in Thermally Processed OFSP. The dry matter content of the fresh OFSP roots was on average 21 ± 0.9% (data not shown). A negligible amount of 13-*cis*-β-carotene, on average corresponding to 0.6% of the total β-carotene content, was detected in the fresh OFSP samples. Whereas the *all-trans*-β-carotene contents in the fresh samples were similar and on average 319 ± 20 μg/g of dry weight (DW), the impact of the different preparation methods varied. After thermal processing, the average amount of *all-trans*-β-carotene decreased and varied between 179 μg/g of DW (POB) and 232 μg/g of DW (BOL). Indeed, the retention in the POB (oil added to flour prior to cooking of porridge) samples was significantly lower ($P < 0.05$) than in the BOL and POA samples, but not compared with the HOM samples (**Table 1**). Retention in the BOL, POA, and HOM samples was not significantly different, however. Compared with a number of traditional processing methods in sub-Saharan Africa (*18*), the retention was somewhat lower in the present study, on average 66% for the different treatments.

In addition to the lower retention of *all-trans*-β-carotene, the POB-treated samples showed a higher fraction of 13-*cis*-β-carotene relative to the total β-carotene content. A fraction of 13-*cis*-β-carotene of 12.9% was observed for this treatment, significantly higher ($P < 0.05$) than for the other preparation methods and similar to other studies (*20*, *21*). Thus, the presence of oil during heating seems to induce isomerization of the *trans*-form to the *cis*-form and subsequently also result in lower retention of the *trans*-form. A similar effect of oil has been observed in high-temperature-treated carrot juice (*22*) and after in vitro digestion of OFSP (*17*), which in the latter paper was suggested to be a result of increased isomerization and/or greater stability of 13-*cis*-β-carotene during digestion. Generally, the formation of *cis*-isomers from *all-trans*-β-carotene during thermal processing is related to the severity and length of the heat treatment (*20*).

Bioaccessibility of *all-trans-β*-Carotene in Thermally Processed OFSP. In the present study, the effects of four different preparation methods on the in vitro bioaccessibility of β-carotene in OFSP were investigated. These preparation methods, intended as alternatives to the commonly used boiling and steaming of whole roots, all have the potential to be applied in rural households in sub-Saharan Africa and, in fact, OFSP flour is already used for the preparation of, for example, porridge in Uganda (*23*). Despite exposure to sunlight and oxygen, traditional drying methods applied in sub-Saharan Africa have been shown to cause limited degradation of the *all-trans*-β-carotene (*18*) and total carotenoid content (*24*) in OFSP and can therefore be considered as appropriate alternatives for the preparation of dried OFSP to be used in bread and porridge. However, weather conditions did not allow for open-air sun-drying in the present study, and freeze-drying was therefore used to produce dried samples of OFSP that was milled into flour and used to prepare the porridges. Owing to the importance of fat for an enhanced in vitro bioaccessibility of carotenoids (*25*, *26*), a minor amount of sunflower oil was added to the different samples in the present study. The differently prepared samples with sunflower oil may represent OFSP meals mainly intended for small children with the potential to be prepared in a rural setting in low-income countries, on condition

Table 2. Amount of *all-trans-β*-Carotene in Thermally Processed OFSP and Partitioning of *all-trans-β*-Carotene into Supernatant and Micellar Phase after in Vitro Digestion[a]

treatment	processed (μg/g of WW)	supernatant phase μg/g of WW	supernatant phase %	micellar phase μg/g of WW	micellar phase %
BOL[b]	28.4 ± 0.8 a	8.0 ± 0.1 c	28.8 ± 0.6 c	4.3 ± 0.3 c	15.7 ± 1.1 c
HOM[c]	27.4 ± 1.0 ab	20.3 ± 2.6 a	76.0 ± 9.1 a	13.3 ± 2.0 a	49.8 ± 7.0 a
POA[d]	25.4 ± 1.3 b	14.3 ± 1.4 b	57.9 ± 4.9 b	7.8 ± 1.2 b	31.3 ± 4.3 b
POB[d]	20.8 ± 1.2 c	14.1 ± 1.3 b	69.6 ± 2.7 ab	9.7 ± 0.5 b	47.7 ± 0.6 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 *all-trans-β*-carotene in the corresponding heat-processed samples. [b] Boiling of fresh roots followed by puréeing and oil addition. [c] Homogenization of fresh roots followed by boiling and oil addition. [d] Cooked porridges were prepared from freeze-dried OFSP milled into flour; POA, oil added after cooking; POB, oil added to flour prior to cooking.

that alternative drying methods and homogenization procedures are applied, for example, open-air sun-drying and grating. To avoid a potential influence of the moisture content on the in vitro bioaccessibility of β-carotene for the differently prepared OFSP samples, deionized water was added to generate samples with similar dry matter content, on average 12% (data not shown), for in vitro digestion. This resulted in somewhat different viscosities for the samples of the four treatments, where the HOM-treated samples had a thicker consistency. The recovery of *all-trans-β*-carotene after in vitro digestion, measured on a subset of the samples, was at least 78%.

Due to the lack of consensus concerning estimates of in vitro bioaccessibility of carotenoids using different methods, we have previously compared the transfer of β-carotene from the OFSP matrix to a supernatant obtained after centrifugation with the transfer to a micellar phase obtained after microfiltration (*16*). Whereas the supernatant can be interpreted to contain micellarized carotenoids, soluble aggregates of nonmicellarized carotenoids, and oil droplets, the filtration of the supernatant separates the fraction containing carotenoids incorporated into micelles (*27*). Hence, in the present study, the in vitro bioaccessibility of β-carotene was estimated by measuring the β-carotene content in both of these fractions after in vitro digestion. The *all-trans-β*-carotene content in the supernatant was significantly higher ($P < 0.001$) compared with the micellar phase. However, the amount of *all-trans-β*-carotene in the supernatant was highly correlated with the content in the micellar phase ($R^2 = 0.95$, $P < 0.001$).

The amount of *all-trans-β*-carotene in the supernatant obtained after in vitro digestion ranged from 8.0 to 20.3 μg/g of wet weight (WW) for the different treatments, which corresponds to between 29 and 76% of the content in the undigested OFSP samples (**Table 2**). Even though the percentage of in vitro bioaccessible *all-trans-β*-carotene was higher in the POB samples than in the POA samples, similar amounts of *all-trans-β*-carotene were transferred to the supernatant phase after in vitro digestion. This was due to a correspondingly lower amount of *all-trans-β*-carotene in the POB-treated samples as a result of larger losses during preparation. However, the presence of fat during the cooking of porridge (POB) obviously had a positive effect on the incorporation of *all-trans-β*-carotene into micelles, as the fraction of *all-trans-β*-carotene in the micellar phase was significantly higher ($P < 0.05$) in the POB samples compared with the POA samples. The amount of *all-trans-β*-carotene in the supernatant and micellar phase for the HOM-treated samples was significantly higher ($P < 0.05$) than in the corresponding phases in the samples for the other treatments (**Table 2**). On the other hand, the amount of *all-trans-β*-carotene in the supernatant and micellar phase was significantly lower ($P < 0.05$) for the BOL samples compared with the POA and POB samples. In the micellar phase, the amount of *all-trans-β*-carotene varied from 4.3 to 13.3 μg/g of WW for the various treatments, representing fractions ranging from 16 to 50% in relation to the amount in the processed samples. Consequently, the type of preparation method may have a large impact not only on the retention of *all-trans-β*-carotene but also on the in vitro bioaccessibility of *all-trans-β*-carotene. van het Hof et al. (*28*) observed a significantly higher carotenoid bioavailability in extensively homogenized compared with canned whole tomato, indicating that the disruption of the food matrix is an important determinant of bioavailability. Similar differences have been reported between raw and processed carrot and spinach (*29*).

In a previous study from our group, in which the in vitro bioaccessibility of β-carotene in traditionally prepared OFSP was investigated (*16*), only moderate differences were observed between the various treatments. The most likely explanation for these findings was that the processing conditions may not have had a different impact on the OFSP matrix. On the other hand, the presence of oil was shown to have a significant effect on the transfer of *all-trans-β*-carotene from the processed samples to the micellar phase. Similar results have been observed in another study on OFSP (*30*), in which the transfer to the micellar phase was higher for deep-fried samples containing approximately 5% fat than for boiled and steamed samples without added fat. Likewise, the partitioning of *all-trans-β*-carotene in micelles was recently reported to be limited to a few percent for boiled OFSP digested without added oil (*16*, *17*, *31*).

Microscopy Observations on Cell Structure and β-Carotene Distribution. **Figure 2** presents brightfield and CARS microscopy images of the cellular matrix and β-carotene distribution in OFSP treated according to the BOL and HOM procedures. The left column shows brightfield images covering a field of view of 340 × 220 μm, and the right column shows overlay images of close-up brightfield images (gray color scale) and β-carotene CARS microscopy images (yellow color scale). The microstructure of the BOL samples after thermal processing was characterized by intact cells that were separated across the middle lamella (**Figure 2A**), whereas the thermally processed HOM samples mostly consisted of cell clusters with disrupted cell walls (**Figure 2B**). The corresponding CARS microscopy images showed β-carotene bodies with dimensions of up to 20 μm for the BOL samples, as exemplified in the overlay image of **Figure 2A**, whereas the HOM samples showed β-carotene bodies with dimensions of ≤5 μm (**Figure 2B**). During boiling in the HOM treatment, aggregates of β-carotene bodies were formed. These β-carotene clusters were visible with the unaided eye as small red clusters in the obtained sample and also clearly visualized by CARS microscopy. We speculate that these β-carotene aggregates were highly soluble in the fat emulsion formed during in vitro digestion and, hence, resulted in the high partitioning of β-carotene into the supernatant and micellar phase for the HOM samples.

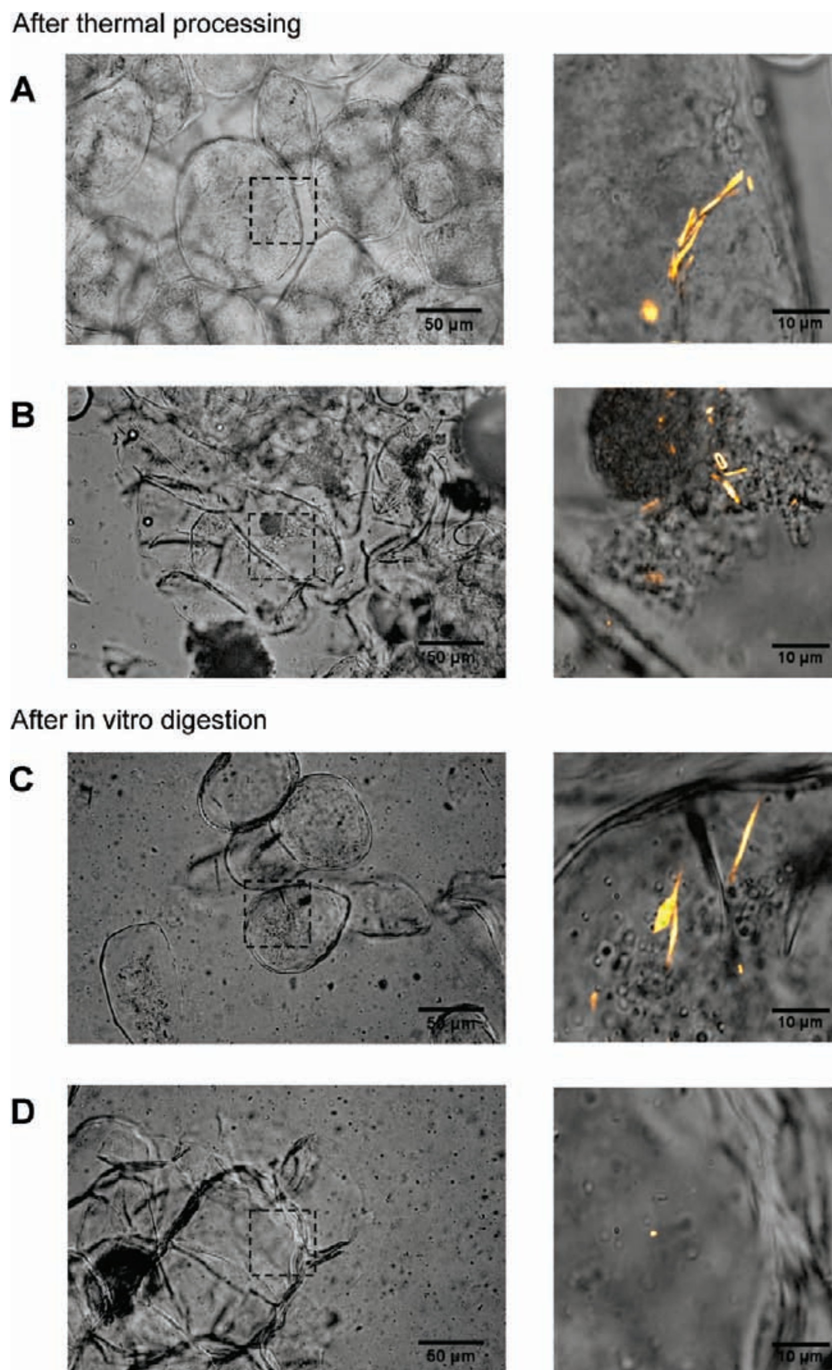

Figure 2. Brightfield differential interference contrast and CARS microscopy images measured in OFSP samples: (**A**) BOL-treated sample after thermal processing, showing a relatively dense matrix with intact cells separated across the middle lamella; (**B**) HOM-treated sample after thermal processing, where cells with disrupted cell walls can be seen in the left part of the image. Corresponding samples obtained after in vitro digestion were less dense: (**C**) BOL sample with intact cells; (**D**) HOM sample with disrupted cells. Disrupted cells favored the release of β-carotene from the cell matrix; for details see Results and Discussion. The left column shows brightfield images ($340 \times 220\,\mu$m) of the cellular matrix. CARS microscopy images ($50 \times 50\,\mu$m) measured in the regions indicated by the dashed black squares are shown in the right column in overlay montages with the region of the brightfield images.

Panels **C** and **D** of **Figure 2** show images measured in the digesta of BOL and HOM samples, respectively, retrieved after in vitro digestion. Similar observations were made as for the undigested samples with intact cells in the BOL samples and cells with disrupted cell walls in the HOM samples. The structure of individual cells in the BOL samples did not seem to be affected during the in vitro digestion process. Similar findings have been obtained in vivo (*32*), and according to Faulks and Southon (*33*), there is no apparent effect of intestinal digestion on the plant cells that remain intact after food preparation and mastication. Thus, the preparation step is highly important to increase the in vitro bioaccessibility of β-carotene in OFSP. Whereas the undamaged cells of the BOL samples contained large β-carotene bodies after in vitro digestion (**Figure 2C**), only relatively small β-carotene bodies in a limited number were observed in the HOM samples (**Figure 2D**). This indicates that the majority of the β-carotene content in the HOM samples was released from the matrix during thermal processing. Hence, the differences observed in size and number of β-carotene bodies residing in the digested samples correlate qualitatively very well with the results on the in vitro

Table 3. Amount of 13-*cis*-β-Carotene in Thermally Processed OFSP and Partitioning of 13-*cis*-β-carotene into Supernatant and Micellar Phase after in Vitro Digestion[a]

		supernatant phase		micellar phase	
treatment	processed (μg/g of WW)	μg/g of WW	%	μg/g of WW	%
BOL[b]	2.7 ± 0.1 a	1.1 ± 0.1 b	39.8 ± 2.3 c	0.8 ± 0.1 b	29.1 ± 1.8 c
HOM[c]	1.2 ± 0.2 b	1.1 ± 0.2 b	90.7 ± 11.0 a	0.8 ± 0.1 b	69.9 ± 10.8 a
POA[d]	1.4 ± 0.1 b	1.0 ± 0.1 b	69.9 ± 5.7 b	0.7 ± 0.1 b	52.4 ± 3.5 b
POB[d]	3.1 ± 0.4 a	2.2 ± 0.3 a	73.4 ± 4.0 ab	1.7 ± 0.2 a	56.2 ± 2.3 ab

[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] Boiling of fresh roots followed by puréeing and oil addition. [c] Homogenization of fresh roots followed by boiling and oil addition. [d] Cooked porridges were prepared from freeze-dried OFSP milled into flour; POA, oil added after cooking; POB, oil added to flour prior to cooking.

bioaccessibility of β-carotene in the thermally processed OFSP. Furthermore, the observation of relatively small β-carotene bodies in the HOM samples suggests an initial decomposition of the larger bodies. Similar observations were made from images measured on samples prepared according to the POA and POB procedures (data not shown) and, similar to the HOM treatment, these samples contained clusters of cells with disrupted cell walls. In summary, compared with the intact cells in the BOL samples, the other treatments showed disrupted cells walls and smaller β-carotene bodies, confirming the observation of higher amounts of *all-trans*-β-carotene in the supernatant and micellar phases retrieved after in vitro digestion.

Opposite to industrial processing of sweet potato, in which the aim generally is to obtain a product with firmer texture (*34*, *35*), household preparation procedures ought to be designed to facilitate the degradation of the sweet potato matrix and thereby the release of carotenoids. We suggest that these procedures should involve a grating step prior to a heating step to induce cell-wall degradation, thereby enhancing the accessibility of β-carotene.

Bioaccessibility of 13-*cis*-β-Carotene in Thermally Processed OFSP. The percentage of accessible 13-*cis*-β-carotene was significantly higher ($P < 0.05$) in the HOM-, POA-, and POB-treated samples in comparison with the BOL-treated samples for both the supernatant and micellar phase (**Table 3**), which is in agreement with the results for *all-trans*-β-carotene. However, as a result of the relatively large quantity of 13-*cis*-β-carotene in the BOL-treated samples after preparation, the amounts of 13-*cis*-β-carotene in the supernatant and micellar phase after in vitro digestion were similar to the amounts found in the HOM and POA samples, being approximately 1.1 and 0.8 μg/g of WW in the supernatant and micellar phase, respectively. However, approximately twice that amount was observed in the POB samples after in vitro digestion, which was due to the large amount of 13-*cis*-β-carotene, 3.1 μg/g of WW, present after this treatment and its relatively high in vitro bioaccessibility.

For all treatments, the percentage of accessible 13-*cis*-β-carotene was significantly higher ($P < 0.001$) than that of *all-trans*-β-carotene in both the supernatant and the micellar phase obtained after in vitro digestion. Similarly, Ferruzzi et al. (*36*) observed higher micellarization of *cis*-isomers of β-carotene than the corresponding *all-trans*-form from meals containing either water-soluble beadlets or the algae *Dunaliella salina* as β-carotene source. *cis*-Isomers of carotenoids have been suggested to be less likely to crystallize in comparison with the *trans*-form, thus having an increased solubility and a higher tendency to be incorporated into mixed micelles (*37*).

Potential Provitamin A Activity of OFSP. Due to the high efficiency of micellarization of *all-trans*-β-carotene and 13-*cis*-β-carotene in the HOM- and POB-treated samples and the moderate efficiency in the BOL- and POA-treated samples, the amounts in the micellar phase ranged from 4.3 to 13.3 μg/g of WW for *all-trans*-β-carotene and from 0.7 to 1.7 μg/g of WW for 13-*cis*-β-carotene, respectively. Assuming a complete absorption of β-carotene in the micellar phase and 50 and 25% conversions to retinol for *all-trans*-β-carotene and 13-*cis*-β-carotene in the epithelial cells of the small intestine, respectively, would imply that a 100 g portion of OFSP prepared according to these treatments can provide between 59 and 172% of the recommended daily vitamin A requirements (*38*). Given the low dry matter content of around 12% in the differently prepared samples, these values indicate that the bioefficacy of OFSP is high enough to contribute substantially to the vitamin A intake for those populations for whom sweet potato is an important staple food.

To conclude, the type of preparation has a large impact not only on the retention of *all-trans*-β-carotene but also on the in vitro bioaccessibility of *all-trans*-β-carotene. Homogenization of fresh OFSP tissue followed by boiling (HOM) gave a significantly higher ($P < 0.05$) accessibility, measured both in the supernatant phase and in the micellar phase, than boiling prior to puréeing (BOL). These results were also in agreement with observations from microscopy of the cellular matrix and β-carotene distribution in OFSP. The results in the present study indicate that the extent of cell-wall rupture is the most important determinant of the in vitro bioaccessibility of carotenoids in OFSP. In addition, the presence of fat during heating was shown to increase isomerization of *all-trans*-β-carotene to 13-*cis*-β-carotene. Hence, we suggest that homogenization prior to boiling is an advantageous approach to increase the release of β-carotene from the OFSP matrix during digestion.

ABBREVIATIONS USED

CARS, coherent anti-Stokes Raman scattering; OFSP, orange-fleshed sweet potato; BOL, boiling followed by pureeing and oil addition; HOM, homogenization followed by boiling and oil addition; POA, cooking of porridge followed by oil addition; POB, oil addition to flour followed by cooking of porridge.

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