JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Relation between Particle Size and Carotenoid Bioaccessibility in Carrot- and Tomato-Derived Suspensions

Katlijn R. N. Moelants, Lien Lemmens, Marijke Vandebroeck, Sandy Van Buggenhout, Ann M. Van Loey, and Marc E. Hendrickx*

Laboratory of Food Technology and Leuven Food Science and Nutrition Research Centre (LFoRCe), Department of Microbial and Molecular Systems (M2S), KU Leuven, Kasteelpark Arenberg 22, PB 2457, 3001 Leuven, Belgium

ABSTRACT: To study the effect of particle size on the relative *all-E-β*-carotene and *all-E*-lycopene bioaccessibility in carrot- and tomato-derived suspensions, respectively, an *in vitro* digestion approach including oil was used. Adding olive oil (2%) during digestion, especially as an oil-in-water emulsion, resulted in a substantial increase in carotenoid uptake in the micellar phase. Carotenoid bioaccessibility decreased with average particle size. Only particles smaller than an individual cell resulted in high bioaccessibility values, pointing out the importance of the cell wall as the main barrier for carotenoid uptake. The relation obtained between particle size and bioaccessibility was used to predict the carotenoid bioaccessibility in carrot- and tomato-derived purées. These predictions indicated that carotenoid bioaccessibility in plant-based food suspensions is not only determined by the cell wall integrity (related with particle size) but is also affected by interactions between the structural compounds of the complex food matrix.

KEYWORDS: *bioaccessibility, carotenoids, particle size, carrot, tomato, oil emulsion*

INTRODUCTION

Fruits and vegetables are often processed into plant-based food suspensions such as soups, juices and purées. During and certainly after the production process, these suspensions should meet certain specifications, among others microbiological safety and high nutritional quality. To obtain plant-based food suspensions with a high nutritional quality, not only the nutrient concentration (reflecting the amount of nutrients present) but even more the nutrient bioaccessibility is important.[1] In order to be accessible for absorption in the gut, the nutrients have to be released from the food matrix.[2] Nutrient bioavailability additionally includes nutrient absorption, metabolism, tissue distribution and bioactivity.[1] This means that a prerequisite for nutrient bioavailability is its bioaccessibility. Nutrient bioaccessibility is commonly determined by *in vitro* methods simulating the human digestion[3,4] and is assumed to be a good starting point for estimating nutrient bioavailability.[5]

In this study, carotenoids were considered as micronutrients for the evaluation of the nutritional quality of carotenoid-containing plant-based suspensions. Besides their differences in chemical structure, *all-E-β*-carotene and *all-E*-lycopene were selected because they are found in many fruits and vegetables. These pigments are known to have antioxidant activity, and their consumption may protect against cancer or cardiovascular diseases.[6,7] Carotenoid bioaccessibility and bioavailability are affected by both exogenous and endogenous factors, combined in the term "SLAMENGHI": *S*pecies of carotenoids, *L*inkage at molecular level, *A*mount of carotenoids, *M*atrix, *E*ffectors, *N*utrient status, *G*enetics, *H*ost-related factors and *I*nteractions among these variables.[8−10] In this study, the effect of the food matrix and structure in which the carotenoids are incorporated on the carotenoid bioaccessibility was investigated. An *in vitro* digestion approach including oil was used. Since carotenoids are very lipophilic molecules, transfer to mixed micelles during the digestion is a precondition for absorption in the intestinal tract.[11] The presence of fat can increase carotenoid solubilization in mixed micelles and, therefore, oil addition to a plant-based food suspension can have an important effect on the carotenoid bioaccessibility. Several studies demonstrated the beneficial effect of oil on carotenoid bioaccessibility.[12−14] To mimic the emulsifying process as occurring in the gastrointestinal tract, oil was also added as an oil-in-water emulsion during *in vitro* digestion.[15] The latter is a rather new approach, which so far has not commonly been used in the context of *in vitro* carotenoid bioaccessibility studies. Previously, Tydeman et al.[16] sought to mimic *in vitro* the dietary emulsion produced during digestion in terms of droplet size and composition. They reported an increase in carotene uptake in the oil phase when an oil emulsion was used instead of bulk oil, and transfer of carotenoids to micelles was not specifically investigated.

In the first part, the relative *all-E-β*-carotene and relative *all-E*-lycopene bioaccessibility in carrot- and tomato-derived products, respectively, with varying particle size were determined in order to identify the relation between the particle size, resulting from tissue disintegration, and the carotenoid bioaccessibility in two different food matrices. In contrast to most of the previous studies investigating the importance of the cell wall structure and particle size for carotenoid bioaccessibility in real, complex plant-based food suspensions,[13,16−19] the current study used specific model suspensions, composed of isolated particle size fractions, to

Received: August 13, 2012
Revised: November 15, 2012
Accepted: November 16, 2012
Published: November 16, 2012

Table 1. Composition of the Reconstituted Purées Assembled from Several Particle Size Fractions with Different Particle Sizes and Particle Size Distributions of Carrot and Tomato Purées, Obtained by Blending (Followed by High-Pressure Homogenization at 20 MPa), Calculated as the Mass Percentage of Each Particle Size Fraction Collected after Wet Sieving[a]

	mass percentage of each particle size fraction (wt %)									
	RCP 1	RCP 2	RCP 3	RTP 1	RTP 2	RTP 3	CP_B	CP_H	TP_B	TP_H
500−1000 μm	25	25	25	25	0	0	19	0	4	0
250−500 μm	25	0	0	25	25	25	33	24	13	10
125−250 μm	0	0	0	0	0	0	6	22	22	16
40−125 μm	0	25	25	0	25	25	1	4	5	5
<40 μm_B	0	0	50	0	0	50	41	0	57	0
<40 μm_H	0	0	0	0	0	0	0	50	0	69
deionized water	50	50	0	50	50	0	0	0	0	0

[a]RCP, reconstituted carrot purée; RTP, reconstituted tomato purée; CP_B, blended carrot purée; CP_H, carrot purée homogenized at 20 MPa; TP_B, blended tomato purée; TP_H, tomato purée homogenized at 20 MPa.

specifically focus on the effect of particle size. In addition, the investigation of the transfer of different carotenoids to the micellar phase was included as this step in the digestion process of carotenoids is crucial for the absorption of lipophilic micronutrients and has not been studied in previous relevant studies.[20] In a second part, it was evaluated whether the relative carotenoid bioaccessibility in plant-based food suspensions, derived from carrot or tomato, could be predicted and calculated based on the particle size distribution (PSD) of these products and the identified relation between carotenoid bioaccessibility and particle size. Carrot and tomato were chosen as model systems since they are major sources of β-carotene and lycopene, respectively, in the diet. Moreover, previous studies indicated that structure formation during mechanical high-pressure processing was quite different for carrot- or tomato-derived suspensions.[21,22]

MATERIALS AND METHODS

Plant Materials. Fresh carrots (*Daucus carota* cv. Nerac) and tomatoes of a processing variety (*Solanum lycopersicum* cv. Prunella) were purchased from a local supplier in Belgium.

Carrots were stored at 4 °C until further use. Tomatoes, on the other hand, immediately after purchase were washed, cut into slices of 0.5−1 cm and vacuum-packed in a plastic bag. These bags were heated in a temperature-controlled water bath at 95 °C for 8 min (to inactivate enzymes), cooled and stored at −80 °C prior to processing.

Preparation of Carrot- and Tomato-Derived Particles and Purées. Carrots were peeled, cut into pieces, vacuum-packed in plastic bags and heated in a temperature-controlled water bath at 95 °C for 5 min (to inactivate intrinsic enzymes). After heating, the carrot pieces were immediately cooled in an ice water bath. Subsequently, they were mixed with deionized water in a 1:1 ratio to facilitate the blending process, which was performed during 1 min using a kitchen blender (Waring blender 7010G, Torrington, CT, USA). Depending on the desired particle size, the carrot blend was used as such or further disintegrated by high-pressure homogenization (HPH) at 20 MPa (Panda 2K, Gea Niro Soavi, Mechelen, Belgium). The blended and homogenized carrot purée are denoted as CP_B and CP_H, respectively. The relative *all-E-β*-carotene bioaccessibility in these purées was analyzed. Purées were separated in fractions with different particle sizes by using the technique of wet sieving (Retsch, Aartselaar, Belgium) with a set of sieves with pore sizes of 40, 125, 250, 500, and 1000 μm. The sieve shaker was loaded with 300 g of the fresh sample and wet sieving was performed in 2 min (shaking amplitude 0.5 mm). Afterward, pulp retained on each sieves was assembled and drained over a filter to remove excess water until constant weight was reached. By weighing these pulp fractions, the mass distribution of the different particle size fractions present in the purées obtained after blending (followed by HPH) was determined. For the carrot blend (CP_B), particle size fractions with a particle size < 40 μm (denoted as <40 μm_B), 250−500 μm and 500−1000 μm were isolated, whereas for the homogenized carrot purée (CP_H) particle size fractions with a particle size < 40 μm (denoted as <40 μm_H), 40−125 μm, and 125−250 μm were collected. Reconstituted carrot purées (denoted as RCP1-3) were prepared by assembling certain of these particle size fractions as indicated in Table 1. Carrot-derived particles with different sizes as well as the reconstituted carrot purée were further analyzed for, among others, the relative *all-E-β*-carotene bioaccessibility. A single batch of carrots was used to prepare all carrot-derived samples.

For the preparation of the tomato-derived samples, defrosted heat-treated tomato slices were mixed during 1 min using a kitchen blender (Waring blender 7010G, Torrington, CT, USA). Tomato blend was sieved (pore size 1.0 mm) to remove skin and seeds after which the blended tomato purée was used as such or further disintegrated by HPH at 20 MPa. The blended and homogenized tomato purées are denoted as TP_B and TP_H, respectively. The relative *all-E*-lycopene bioaccessibility in these purées was analyzed. By using the technique of wet sieving, fractions with particle size < 40 μm (denoted as <40 μm_H) and 40−125 μm were isolated from the homogenized tomato purée (TP_H), whereas fractions with particle size < 40 μm (denoted as <40 μm_B), 125−250 μm, 250−500 μm and 500−1000 μm were collected from the blended tomato purée (TP_B). Reconstituted tomato purées (denoted as RTP1-3) were prepared by assembling certain of these particle size fractions as indicated in Table 1. Tomato-derived particles as well as the reconstituted tomato purées were further analyzed for, among others, the relative *all-E*-lycopene bioaccessibility. A single batch of tomatoes was used to prepare all tomato-derived particles.

Preparation of the Oil Emulsion. An oil-in-water emulsion (5%) was prepared fresh daily. Thereto, L-α-phosphatidylcholine (1%) (Sigma-Aldrich, Bornem, Belgium) was solubilized in water after which olive oil was added (5%). The solution was mixed using an Ultraturrax mixer for 10 min and homogenized at 100 MPa.

Determination of the Particle Size. The PSD of the blended and homogenized purées was determined by the use of wet sieving (measured as the mass partition of the different particle size fractions) (cf. section Preparation of Carrot- and Tomato-Derived Particles and Purées).

The cumulative volumetric PSDs of all particle size fractions were measured using laser diffraction (Malvern Instrument Ltd., Worcestershire, UK), which can detect particles between 0.05 and 880 μm. Since the particle size fraction 500−100 μm can comprise particles with a size up to 1000 μm, this measurement technique gives an underestimation of the average particle size of this fraction. Approximately 6 g of sample (50 wt. %) was poured in a stirred tank filled with deionized water. The diluted sample was pumped into the measuring cell. The volumetric PSDs were calculated from the intensity profile of the scattered light with the Mie theory by use of the instrument's software.

Determination of the Relative *in Vitro All*-E-β-carotene Bioaccessibility. The relative *all-E-β*-carotene bioaccessibility in a carrot sample was calculated as the ratio of the *all-E-β*-carotene

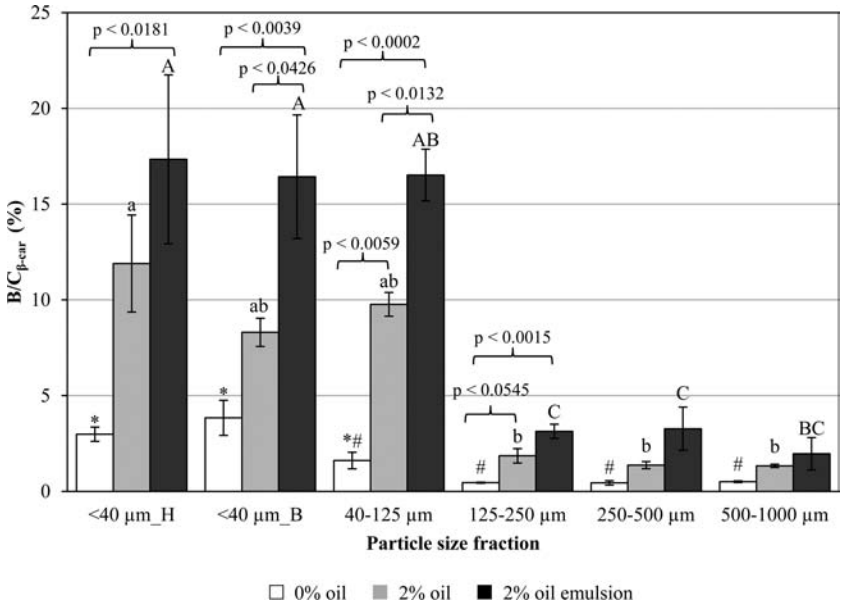

Figure 1. Relative *all-E-β*-carotene bioaccessibility (±standard error, $n = 4$) calculated as the ratio of *all-E-β*-carotene bioaccessibility to the *all-E-β*-carotene concentration ($B/C_{\beta\text{-car}}$) (%) in carrot-derived particles with varying particle size. Significant differences between the bioaccessibility means of samples of a particular particle size without oil addition, with the addition of 2% olive oil as such and with the addition of oil emulsion (2%) are indicated by their *P*-values ($P < 0.05$). Carrot-derived particles with significantly different mean *all-E-β*-carotene bioaccessibility values within the same category of oil addition (without oil, oil as such or oil emulsion) are indicated with different letters or symbols (*, #; a, b and A, B, C respectively).

bioaccessibility to the *all-E-β*-carotene concentration in that particular sample. The relative bioaccessibility refers to the percentage of the tested compound remaining in the bioaccessible fraction related to the original nondigested sample. This data conformation was applied since the use of wet sieving caused an unequal partition of carotenoids in the different sieving fractions.

The *all-E-β*-carotene bioaccessibility in the different carrot-derived particles and carrot purées was determined using a two-step (gastric and small intestinal) static *in vitro* digestion procedure based on the methods of Hedrén et al.[4] and Lemmens et al.[18] with minor modifications. To start, 0.02 g of pure olive oil or 4 mL of a 5% oil emulsion was gently added to 10 g of carrot sample. As a control, samples without oil addition were also considered. The mixture was diluted with a NaCl (0.9% in water)/ascorbic acid (1% in water) solution (5 mL) and stomach electrolyte (0.3% NaCl, 0.11% KCl, 0.15% $CaCl_2 \cdot 2H_2O$, 0.05% KH_2PO_4 and 0.07% $MgCl_2 \cdot 6H_2O$ in water) (5 mL). The pH of the mixture was adjusted to pH 4 ± 0.05 and gastric juice (0.52% porcine pepsin (Sigma-Aldrich) in electrolyte solution) (3 mL) was added. The headspaces of the tubes were flushed with nitrogen and the samples were incubated for 30 min at 37 °C, while shaking end-over-end. Next, pH was lowered to pH 2 ± 0.05, after which the headspaces were flushed again with nitrogen and samples were incubated for 30 min at 37 °C, while shaking end-over-end. To mimic the small intestine digestion, the pH was increased to pH 6.9 ± 0.05, duodenal juice (0.4% porcine pancreatin, 0.2% porcine pancreas lipase, 2.5% porcine bile, 0.5% pyrogallol and 1% tocopherol (Sigma-Adrich) in water) was added and the samples were incubated for 2 h at 37 °C. The aqueous micellar phase was separated from the undigested oil droplets and from the undigested plant material by the use of ultracentrifugation (L7 Ultracentrifuge, Beckman, Palo Alto, California, USA) at 165000*g* during 1 h and 5 min at 4 °C. The aqueous fraction was filtrated (Chromofil PET filters, 0.2 µm pore size, 25 mm diameter) to remove remaining oil and crystalline carotenoids. *β*-Carotene was extracted from the aqueous phase and quantified as described below. For each sample, the *β*-carotene bioaccessibility was determined in quadruple.

The determination of the *all-E-β*-carotene concentration was based on the procedure described by Sadler et al.[23] and Lemmens et al.[18] The micellar phase or 1 g of carrot purée/particle size fraction was stirred with 1 g of $CaCl_2$ and 50 mL of extraction solvent, containing acetone, ethanol, hexane (25:25:50 v:v:v), and butylated hydroxytoluene (0.1%) during 20 min at 4 °C. Then, 15 mL of reagent grade water was added and after stirring during 10 min at 4 °C, an organic phase and an aqueous phase could be separated. The organic phase, comprising *β*-carotene, was filtered (Chromofil PET filters, 0.2 µm pore size, 25 mm diameter) and concentrated under a vacuum using a rotary evaporator at 30 °C. The extraction procedure was performed under subdued light to prevent *β*-carotene degradation. The concentrated *β*-carotene was redissolved in hexane/dichloromethane (4:1), transferred to an amber high-performance liquid chromatography (HPLC) vial and analyzed by a RP-HPLC system (Agilent Technologies 1200 Series, Diegem, Belgium), equipped with a C_{30}-column (25 °C) (5 µm × 250 mm ×4.6 mm, YMC Europe, Dinslaken, Germany) and a diode array detector (450 nm). The separation was carried out by gradient elution with methanol (A), methyl-*t*-butyl-ether (B), and reagent grade water (C). Starting from the initial conditions (81% A, 15% B and 4% C), a linear gradient was built up in 20 min to the end conditions (41% A, 55% B and 4% C) at a flow rate of 1 mL/min. Next, the column was washed at these solvent conditions for 8 min and equilibrated again at the starting conditions. The concentration factor was calculated by adding a known amount of *β*-apo-8′-carotenal prior to the evaporation of the apolar solvent. For the quantification, a calibration curve of *all-E-β*-carotene (CaroteNature, Lupsingen, Switzerland) was prepared. For each particular sample, the extraction of *β*-carotene and the subsequent determination of the concentration were performed in triplicate.

Determination of the Relative *in Vitro All-E*-lycopene Bioaccessibility. The relative *all-E*-lycopene bioaccessibility in a tomato sample was calculated as the ratio of the *all-E*-lycopene bioaccessibility to the *all-E*-lycopene concentration in a particular sample.

Determination of the *all-E*-lycopene bioaccessibility in the different tomato-derived particles and tomato purées was performed as described above for carrot-derived samples. On the basis of the results for carrot samples, it was decided to add oil (2%) as an oil emulsion to all tomato samples at the start of the *in vitro* digestion procedure. To determine the *all-E*-lycopene concentration, 0.5 g of NaCl was added to 1 g of tomato purée/particle size fraction or micellar phase. Next

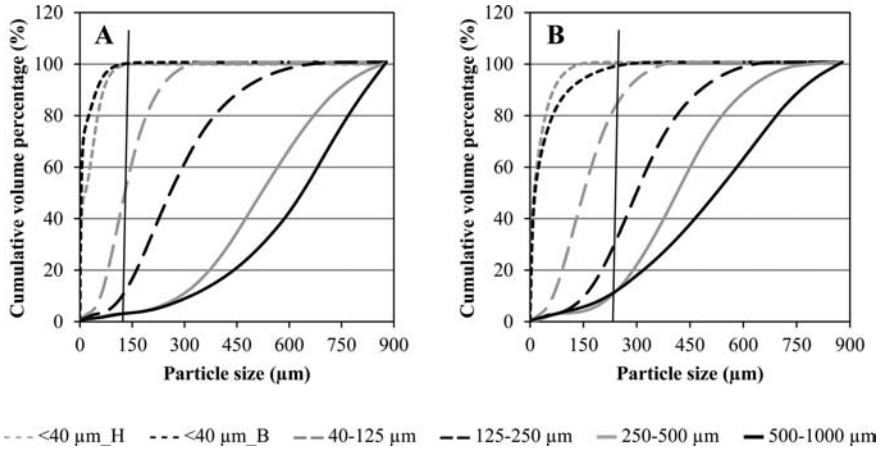

Figure 2. Cumulative volumetric particle size distribution of the different particle size fractions obtained from carrot (A) and tomato purée (B). The vertical line on the graphs indicates the average particle size of a single cell.

steps of the extraction procedure of *all-E*-lycopene were performed as described above for the extraction of *all-E-β*-carotene from carrot-derived samples. The concentrated extract was analyzed by a RP-HPLC system, equipped with a C_{30}-column (25 °C) (3 μm × 150 mm × 4.6 mm, YMC Europe, Dinslaken, Germany). The separation was carried out by gradient elution at a flow rate of 1 mL/min with methanol (A), methyl-*t*-butyl-ether (B), and reagent grade water (C): linear change from 81% A, 15% B and 4% C to 36% A, 60% B and 4% C in 5 min followed by the linear change to 28% A, 68% B and 4% C in another 25 min. Next, the column was washed with 16% A, 80% B and 4% C and equilibrated again at the starting conditions. Detection of lycopene took place at 472 nm instead of 450 nm as used for *β*-carotene. For the quantification, a calibration curve of *all-E*-lycopene (CaroteNature, Lupsingen, Switzerland) was prepared.

Statistical Analysis. Significant differences among the relative *all-E-β*-carotene bioaccessibility means and the relative *all-E*-lycopene bioaccessibility means in carrot-derived particles and tomato-derived particles with different sizes, respectively, were analyzed using Tukey's Studentized Range test (statistical software package SAS, version 9.3, Cary, NC). The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Relative *in Vitro All-E-β*-carotene and *All-E*-lycopene Bioaccessibility in Particles with Varying Particle Size. The current study specifically investigated the effect of particle size, resulting from tissue disintegration, on the *all-E-β*-carotene bioaccessibility and the *all-E*-lycopene bioaccessibility in carrot-derived particles and tomato-derived particles, respectively. In addition, a methodological *in vitro* digestion approach including oil was used. As no substantial changes in isomerization were detected in the HPLC profiles (data not shown), this study was focused on the *all-E-β*-carotene and *all-E*-lycopene bioaccessibility.

In Figure 1, the relative *all-E-β*-carotene bioaccessibility ($B/C_{\beta\text{-car}}$) for each particle size fraction is represented for samples with or without oil addition during *in vitro* digestion. Significant differences between the bioaccessibility means of samples of a particular particle size without oil addition, with the addition of 2% olive oil as such and with the addition of oil emulsion (2%), are indicated by their *P*-values ($P < 0.05$). For each particle size fraction, a clear increase in $B/C_{\beta\text{-car}}$ (>2−10 times) can be observed when oil (2%) was added during the *in vitro* digestion procedure, although this increase turned out to be mainly significant for rather small particle size fractions. Previous studies already demonstrated that carotenoid bioaccessibility can be increased by the presence of oil.[12,14,24,25] However, the way oil is added to the best of our knowledge has not yet explicitly been investigated. Adding oil as an emulsion (90% of the oil droplets were smaller than 9 μm) at the start of the *in vitro* bioaccessibility measurement is, according to us, however highly relevant in the context of mimicking the oil emulsification process in the stomach.[15,26] By emulsifying the oil, the oil surface area increases whereby the transfer of hydrophobic carotenoids to the lipophilic phase can be enhanced.[27,28] Also Tydeman et al.[16] concluded that increasing the surface area of the emulsion resulted in a positive effect on carotene uptake in the oil phase.

Moreover, a clear effect of particle size, in particular for particles becoming smaller than 125 μm, on the relative *all-E-β*-carotene bioaccessibility is noticeable in Figure 1. Since the mean carrot cell diameter is about 125 μm,[29] this implies that $B/C_{\beta\text{-car}}$ becomes small when particles are larger than the size of a single carrot cell. The cell wall of particles smaller than 125 μm is damaged whereby carotenoids can be released easily from the particle and are in that way accessible for uptake in the oil fraction. Results show that cell wall integrity had a large impact on the release of carotenes from the food matrix, as described in previous studies.[16,30−33] Among others, Hedrén et al.[4] noticed the positive effect of reducing particle size on the release of *β*-carotene from the carrot matrix. Knockaert et al.[13] reported an increase in bioaccessible *β*-carotene in carrot purée homogenized at pressures higher than 50 MPa, causing mechanical disruption of carrot tissue. However, studies investigating the relation between particle size, resulting from tissue disintegration, and carotenoid bioaccessibility in plant-based suspensions are rare. Lemmens et al.[20] demonstrated that this relation in carrots was dependent on the particle structural characteristics which in turn were a result of the way of processing prior to tissue disintegration. However, no oil was added during *in vitro* digestion in that study and *all-E-β*-carotene bioaccessibility was determined as the relative amount released from the food matrix; transfer of carotenoids into the micellar phase was not considered.

In Figure 2A, the cumulative volume particle size distributions of the different carrot-derived particle size fractions are represented. The figure clearly shows that the particle size fraction 40−125 μm comprises both particles with a diameter below and above 125 μm, whereas the particle size fractions 125−250 μm, 250−500 μm and 500−1000 μm consisted mostly of particles with a diameter larger than 125

μm. This observation is clearly linked with the fact that $B/C_{\beta\text{-car}}$ was significant lower for these latter particle size fractions as compared to $B/C_{\beta\text{-car}}$ in the particle size fraction 40−125 μm. Tydeman et al.[16,31] and Lemmens et al.[20] also concluded that a single cell wall is sufficient to reduce carotene bioaccessibility from a cell by acting as physical barrier, which cannot be broken down during upper gut digestion.

Although many studies confirm the beneficial effect of oil addition and mechanical disruption on carotenoid bioaccessibility, comparing the results of $B/C_{\beta\text{-car}}$ in the present study (cf. Figure 1) with others in the literature turned out to be quite difficult. Values of $B/C_{\beta\text{-car}}$ in carrot samples observed by, e.g., Hedrén et al.,[4] Hornero-Méndez and Mínguez-Mosquera[12] and Granado-Lorencio et al.[34] were remarkably higher than the ones shown in Figure 1. Deviating methodologies for measuring the *in vitro* carotenoid bioaccessibility (e.g., not including ultracentrifugation to separate the micellar phase or no removal of crystalline carotenes by filtration) or the differences in the relative amount of oil added can be possible explanations for the observed differences.

As the *all-E*-lycopene bioaccessibility in tomato-based products was shown to be rather low without oil addition,[14] and the addition of an oil emulsion turned out to be most suitable to obtain the highest relative *all-E-β*-carotene bioaccessibility in carrot (cf. Figure 1), the *all-E*-lycopene bioaccessibility in all tomato samples in this study was analyzed in the presence of an oil emulsion added at the moment of *in vitro* digestion. Results of the relative *all-E*-lycopene bioaccessibility (B/C_{lyc}) for each particle size fraction are represented in Figure 3. As for carrot-derived particles, a decrease in B/C_{lyc}

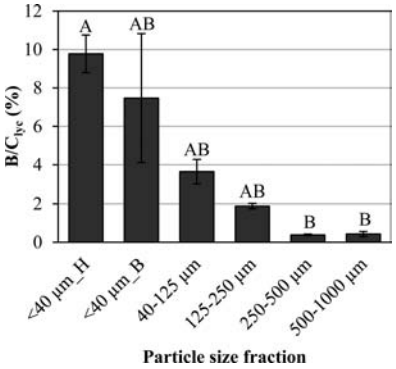

Figure 3. Relative *all-E*-lycopene bioaccessibility (±standard error, n = 4) calculated as the ratio of *all-E*-lycopene bioaccessibility to the *all-E*-lycopene concentration (B/C_{lyc}) (%) for tomato-derived particles with varying particle size. To all samples an oil emulsion (2%) was added during *in vitro* digestion. Significant differences between the relative *all-E*-lycopene bioaccessibility means of samples are indicated with different letters (A and B respectively).

could be observed with increasing particle size. B/C_{lyc} turned out to be especially low for particles obtained on sieves with a pore size larger than 250 μm. Since tomato cells have an average diameter of 300−1000 μm,[21,35] the present results clearly stress the importance of the cell breakage for the release of carotenoids from the cell and the subsequent uptake of carotenoids in micelles. In contrast to carrot-derived particles, no sudden drop in B/C_{lyc} value at a particular particle size fraction was visible and B/C_{lyc} decreased continuously with increasing particle size which can be possibly explained by the broader range of cell size in tomato. Moreover, in Figure 2B, it can be observed that not only the particle size fraction 40−125 μm, but also fractions 125−250 μm, 250−500 μm and 500−1000 μm comprise, to a greater or lesser extent, particles with a diameter beyond 250 μm. As the average particle size is increasing, the relative presence of broken cells is decreasing whereby also the relative *all-E*-lycopene bioaccessibility decreases. Consequently, the particle size can be used as a measure for cell wall destruction and is therefore related to carotenoid bioaccessibility. However, the relation between particle size and carotenoid bioaccessibility is not unique and can be influenced by a number of factors among others by using vegetables of different degrees of ripening or by the use of other heat treatments before destruction of the heated tissue, influencing cell wall firmness (due to e.g. pectin changes) and thereby cell breakage or cell separation during tissue disintegration as was concluded by Tydeman et al.[16] and Lemmens et al.[20]

The relative *all-E*-lycopene bioaccessibility in tomato-derived particles (1−10%) was lower than the relative *all-E-β*-carotene bioaccessibility in carrot-derived particles (2−17%) with a similar average particle size. Also Svelander et al.[22] reported limited lycopene bioaccessibility in tomato-based emulsions as compared to α- and β-carotene bioaccessibility in carrot-based emulsions. However, values of B/C_{lyc} reported by Svelander et al.[22] were higher than those obtained in the present study (cf. Figure 3). This difference can be explained by the lower centrifugation speed used during the procedure to measure the *in vitro* lycopene bioaccessibility as in the present study. On the other hand, similar values of B/C_{lyc} were obtained by Colle et al.[14,25] for tomato soups or pulp. Hampered incorporation of lycopene into micelles can be attributed to the differences in chemical structure between these carotenoids.[36,37] Differences in the food matrix (carrot vs tomato) might also contribute to the observed difference.

Estimation of the Relative *in Vitro All-E-β*-carotene and *All-E*-lycopene Bioaccessibility in Reconstituted Purées Based on the Particle Size Distribution of these Assemblies. In the second part of this study, it was investigated whether the carotenoid bioaccessibility in liquid-like processed particulate products (e.g., soups, juices, sauces and purées), derived from carrot or tomato, could be predicted and calculated based on the PSD (which corresponds to the mass fraction of the different particle size fractions) and the identified relation between the carotenoid bioaccessibility and the particle size (obtained in part 1) of these products. Such a prediction based on structural characteristics that are easily measured would be highly valuable in the context of controlling the nutritional quality of mechanically processed foods. In a first step, particles with well-known particle size were assembled to obtain three different reconstituted purées. Both for carrot and tomato, purées were reconstituted from particle size fractions with rather high and/or low relative bioaccessibility (B/C) (cf. Table 1). B/C of the reconstituted purées was measured as well as calculated. In a second step (discussed in the next section), purées were prepared by destructing intact plant tissue (instead by assembling particle size fractions). In this way, the effect of structural elements (e.g., serum phase with fiber network) created during processing of the purées could be taken into account.

In Table 2A,B, results of the measured and calculated values of B/C are represented for reconstituted purées assembled from carrot- and tomato-derived particles, respectively. Table 2A confirmed that $B/C_{\beta\text{-car}}$ was increased by the addition of oil

Table 2. Relative *All-E-β*-carotene Bioaccessibility ($B/C_{\beta\text{-car}}$) (A) and Relative *All-E*-lycopene Bioaccessibility (B/C_{lyc}) (B) of Reconstituted Purées, Assembled from Carrot- or Tomato-Derived Particles (RCP, Reconstituted Carrot Purée; RTP, Reconstituted Tomato Purée) Respectively, Measured (± Standard Error, $n = 4$) or Calculated as the Sum of the Bioaccessibilities from Each Particle Size Fraction Present in the Reconstituted Purées[a]

				A				
	oil addition		measured $B/C_{\beta\text{-car}}$ (%)				calculated $B/C_{\beta\text{-car}}$ (%)	
RCP1	0% oil	0.15	±	0.02[b]	**[c]	0.24[d]	±	0.38
	2% oil	0.38	±	0.00	c	0.68	±	0.05
	2% oil emulsion	0.95	±	0.19	B	1.31	±	0.35
RCP2	0% oil	0.73	±	0.20	*	0.53	±	0.11
	2% oil	1.16	±	0.23	b	2.78	±	0.16
	2% oil emulsion	2.55	±	0.18	B	4.62	±	0.40
RCP3	0% oil	0.70	±	0.09	*	2.45	±	0.47
	2% oil	2.62	±	0.73	a	6.93	±	0.40
	2% oil emulsion	6.01	±	1.34	A	12.83	±	1.66
				B				
	oil addition		measured B/C_{lyc} (%)				calculated B/C_{lyc} (%)	
RTP1	2% oil emulsion	2.14	±	0.38[e]	C[f]	0.20[g]	±	0.03
RTP2	2% oil emulsion	3.79	±	0.18	B	1.01	±	0.16
RTP3	2% oil emulsion	5.16	±	0.27	A	4.74	±	3.67

[a]For carrot samples, *all-E-β*-carotene bioaccessibility was determined without oil addition during digestion or with the addition of 2% oil as such or added as an oil emulsion. [b]std error. [c]RCP with significantly different mean measured *all-E-β*-carotene bioaccessibility values within the same category of oil addition (without oil, oil as such or oil emulsion) are indicated with different letters or symbols (*, **; a, b, c and A, B, respectively). For a particular RCP, significant differences between the bioaccessibility means for samples without oil addition, with the addition of 2% oil as such, and with the addition of oil emulsion (2%) are mentioned in the text. [d]Calculated based on the mass fraction of each particle size fraction in the reconstituted purée (cf. Table 1) and $B/C_{\beta\text{-car}}$ of that particular particle size fraction (cf. Figure 1); for example, RCP1 contains 25 wt % of the particle size fraction 500−1000 μm, 25 wt % of the particle size fraction 250−500 μm and 50 wt % deionized water, having a $B/C_{\beta\text{-car}}$ of respectively 0.51%, 0.44% and 0%. The calculated $B/C_{\beta\text{-car}}$ then becomes 0.24 = (25·0.51) + (25·0.44) + (50·0). [e]std error. [f]RTP with significantly different mean measured *all-E*-lycopene bioaccessibility values are indicated with different letters (A, B and C). [g]Calculated based on the mass fraction of each particle size fraction in the reconstituted purée (cf. Table 1) and B/C_{lyc} of that particular particle size fraction (cf. Figure 3); for example, RTP1 contains 25 wt % of the particle size fraction 500−1000 μm, 25 wt % of the particle size fraction 250−500 μm and 50 wt % deionized water, having a B/C_{lyc} of respectively 0.43%, 0.38% and 0%. The calculated B/C_{lyc} then becomes 0.20 = (25·0.43) + (25·0.38) + (50·0).

(2%) during digestion, and this increase was larger in the case an oil emulsion was used. $B/C_{\beta\text{-car}}$ in the presence of an oil emulsion was significantly different from $B/C_{\beta\text{-car}}$ in the absence of oil for all reconstituted carrot purées (RCP) (*P*-value of 0.0074, 0.0026, and 0.0128 for RCP1, RCP2 and RCP3, respectively). Only for RCP3, replacing pure oil by an oil emulsion had no significant effect (*P*-value of 0.0743). From this table, it can also be observed that by replacing a particle size fraction with a low $B/C_{\beta\text{-car}}$ by a particle size fraction with a higher $B/C_{\beta\text{-car}}$ (e.g., in RCP2 contains the fraction 40−125 μm instead of the fraction 250−500 μm in RCP1), the measured as well as the calculated values were increased (no significant increase in case an oil emulsion was used). The replacement of the continuous water phase in reconstituted carrot purée with a phase consisting of particles smaller than 40 μm (RCP3 as compared to RCP2) resulted in an increase in $B/C_{\beta\text{-car}}$ (significant when oil was added). Although the overall effects of oil addition and changing particle size were reflected in both the calculated and the measured $B/C_{\beta\text{-car}}$ values, calculated values were consistently higher. Therefore, it can be concluded that the uptake of *all-E-β*-carotene in micelles is lower for mixtures of particles with different sizes as compared to the uptake of *all-E-β*-carotene in micelles from an undiluted particle fraction with a specific particle size. At equilibrium conditions during digestion, the constant ratio of the particle concentration to the oil concentration was similar in both carrot-derived products and should in principle lead to calculated values that are equal to the measured ones. The lower measured values can indicate a slower establishment of equilibrium conditions in the purée. This observation however requires further investigation.

In Table 2B, the measured and calculated values of the relative *all-E*-lycopene bioaccessibility in reconstituted tomato purées (RTP) are shown. As for RCP, the replacement of a particle size fraction with a low B/C_{lyc} by a particle size fraction with a higher B/C_{lyc} resulted in a significantly higher B/C_{lyc} (e.g., RTP1 vs RTP2). Remarkably, in contrast to RCP, the values calculated for B/C_{lyc} were lower as compared to the measured ones. This observation can be explained by the low solubility characteristics of lycopene in oil[14,22] (since dilution of specific particle size fractions into a purée with a continuous aqueous phase causes the relative amount of oil available for uptake of carotenoids during digestion to increase, also B/C_{lyc} can be positively affected). Besides, dilution of specific particle size fractions into a purée with a continuous aqueous phase can lead to the partial removal of factors in the food matrix hindering the transfer of carotenoids to the micellar phase. Further research is required to identify all relevant factors.

Calculating the carotenoid bioaccessibility in reconstituted purées based on the PSD of this purée and the identified relation between the carotenoid bioaccessibility and the particle size turned out to be rather difficult. For the self-assembled purées, it was however shown that it is feasible to predict which of the self-assembled carrot purées will have the highest nutritional quality.

Estimation of the Relative *in Vitro All*-E-*β*-carotene and *All*-E-lycopene Bioaccessibility in Purées Obtained by Blending (Followed by High-Pressure Homogeniza-

Table 3. Relative *All-E-β*-carotene Bioaccessibility ($B/C_{\beta\text{-car}}$) (A) and Relative *All-E*-lycopene Bioaccessibility (B/C_{lyc}) (B) of Purées Obtained by Blending (Followed by High-Pressure Homogenization) (CP_B, Blended Carrot Purée; CP_H, Carrot Purée Homogenized at 20 MPa; TP_B, Blended Tomato Purée; TP_H, Tomato Purée Homogenized at 20 MPa), Measured (± standard error, $n = 4$) or Calculated as the Sum of the Bioaccessibilities from Each Particle Size Fraction Present in the Purée, Considering the Particle Size Distribution of the Purée[a]

		A						
	oil addition	measured $B/C_{\beta\text{-car}}$ (%)				calculated $B/C_{\beta\text{-car}}$ (%)		
CP_H	0% oil	0.78	±	0.08[b]	**[c]	1.76[d]	±	0.19
	2% oil	5.99	±	1.09	a	7.07	±	1.26
	2% oil emulsion	6.00	±	1.45	A	10.81	±	2.21
CP_B	0% oil	1.41	±	0.22	*	1.88	±	0.38
	2% oil	6.06	±	0.91	a	4.35	±	0.31
	2% oil emulsion	8.63	±	1.48	A	8.60	±	1.40
		B						
	oil addition	measured B/C_{lyc} (%)				calculated B/C_{lyc} (%)		
TP_H	2% oil emulsion	2.50	±	0.48[e]	A[f]	7.23[g]	±	0.67
TP_B	2% oil emulsion	2.40	±	0.52	A	4.91	±	1.91

[a]For carrot samples, *all-E-β*-carotene bioaccessibility was determined without oil addition during digestion or with the addition of 2% oil as such or added as an oil emulsion. For tomato samples, *all-E*-lycopene bioaccessibility was determined with the addition of 2% oil as an oil emulsion. [b]std error. [c]Carrot purées with significantly different mean measured *all-E-β*-carotene bioaccessibility values within the same category of oil addition (without oil, oil as such or oil emulsion) are indicated with different letters or symbols (*, **; a and A, respectively). For a particular carrot purée, significant differences between the bioaccessibility means for samples without oil addition, with the addition of 2% oil as such and with the addition of oil emulsion (2%) are mentioned in the text. [d]Calculated based on the mass fraction of each particle size fraction in the purée (cf. Table 1) and $B/C_{\beta\text{-car}}$ of that particular particle size fraction (cf. Figure 1); For example, CP_H contains 0 wt % of the particle size fraction 500−1000 μm, 24 wt % of the particle size fraction 250−500 μm, 22 wt % of the particle size fraction 125−250 μm, 4 wt % of the particle size fraction 40−125 μm and 50 wt % of the particle size fraction <40 μm_H, having a B/C_{lyc} of respectively 0.51%, 0.44%, 0.46%, 1.61% and 2.98%. The calculated B/C_{lyc} then becomes 0.24 = (0·0.51) + (24·0.44) + (22·0.46) + (4·1.61) + (50·2.98). [e]std error. [f]The measured *all-E*-lycopene bioaccessibility means in both tomato purees were not significantly different (indicated with the same letter). [g]Calculated based on the mass fraction of each particle size fraction in the purée (cf. Table 1) and B/C_{lyc} of that particular particle size fraction (cf. Figure 3); for example, TP_H contains 0 wt % of the particle size fraction 500−1000 μm, 10 wt % of the particle size fraction 250−500 μm, 16 wt % of the particle size fraction 125−250 μm, 5 wt % of the particle size fraction 40−125 μm and 69 wt % of the particle size fraction <40 μm_H, having a B/C_{lyc} of respectively 0.43%, 0.38%, 1.88%, 3.64% and 9.78%. The calculated B/C_{lyc} then becomes 7.23 = (0·0.43) + (10·0.38) + (16·1.88) + (5·3.64) + (69·9.78).

tion) Based on the Particle Size Distribution of These Purées. The relative presence of different particle size fractions in the carrot or tomato purées (= PSD of the purées as obtained by wet sieving) is shown in Table 1. Average particle size was clearly reduced by using HPH at 20 MPa, both for carrot and tomato purée. However, the shift in PSD was rather small.

Table 3 contains the measured and calculated values of B/C for these carrot and tomato purées. Based on the PSD of the purées (shown in Table 1), B/C in each purée was calculated by using B/C of the different particle size fractions present in the purée taken into account their relative mass percentage. These values clearly show the significantly (P-value < 0.05) positive effect of oil addition for carrot purées, as comparable to the self-assembled purées. However, replacing pure oil by an oil emulsion did not result in a significant extra increase in $B/C_{\beta\text{-car}}$ in both purées (P-value >0.05). The results also confirm the lower relative *all-E*-lycopene bioaccessibility in tomato purée compared to that of *all-E-β*-carotene in carrot purée.

For both blended purées, the calculated B/C values fairly well approached the measured values. Predicting the relative carotenoid bioaccessibility in the homogenized purées based on particle size only, on the other hand, was less successful. The calculated values of B/C for the homogenized purées were higher than those of the bended purées, both for carrot and tomato. However, since HPH changed the relative presence of particle size fractions with a low B/C (fractions with a size > 125 μm for carrot and fractions > 250 μm for tomato) to the relative presence of particle size fractions with a high B/C (fractions < 125 μm for carrot and fractions < 250 μm for tomato) only to a small extent, the increase in calculated B/C after HPH was rather limited (the effect of HPH is only significant for carrot purée without oil). The latter indicates that besides particle size reduction, HPH of plant-based food purées simultaneously could provoke changes that can have a negative effect on the carotenoid bioaccessibility of these purées. Moelants et al.[38] already demonstrated that HPH of carrot and tomato purées caused pectin solubilization whereby serum viscosity changes. In this way, carotenoid bioaccessibility may be hampered after HPH. However, further work is required to confirm this hypothesis.

Previous investigations on the effect of HPH on the *β*-carotene bioaccessibility in carrot-derived products are rare. Moreover, the experimental setup of other studies often deviate from the one in this study, which makes comparison of results difficult. Svelander et al.[22] noticed an increase in the relative amount of *β*-carotene released from the food matrix due to HPH. However, oil (5%) was added in this study prior to the homogenization process, whereby HPH could decrease the oil droplet size. Knockaert et al.[13] reported an increase in *β*-carotene bioaccessibility in carrot purée homogenized at pressures beyond 50 MPa. Similar as observed in this study, lower pressure levels during HPH did not result in enhanced *β*-carotene bioaccessibility in carrot purée. Also several other studies noticed no increased lycopene bioaccessibility after HPH of tomato purée.[19,22] Colle et al.[19] already reported that HPH of tomato purée not only reduced the average particle size of the purée, but also resulted in lycopene bioaccessibility values lower than expected based on the particle size. This observation was explained by an improved fiber network

strength whereby lycopene may be entrapped. The encapsulation of lipophilic compounds by a polymer network was previously also hypothesized by McClements et al.[28]

In conclusion, particle size reduction caused by HPH (at 20 MPa) did not increase the *all-E-β*-carotene or *all-E*-lycopene bioaccessibility in carrot or tomato purée, respectively. Moreover, predicting the carotenoid bioaccessibility in these homogenized food suspensions based on their PSD was shown to be difficult. Besides particle size, interactions between structural elements (e.g., pectin influencing serum viscosity, fiber network) may also affect B/C and can therefore be important to consider when predicting B/C in plant-based food suspensions.

On the basis of the results of this study, it can be concluded that the *in vitro all-E-β*-carotene and *all-E*-lycopene bioaccessibility in carrot- and tomato-derived particles, respectively, were increased by oil addition (especially an oil emulsion) during *in vitro* digestion as well as by particle size reduction. As it was shown that only particles smaller than an individual cell result in high carotenoid bioaccessibility values, cell wall destruction turned out to be an important prerequisite for carotenoids to be released from the plant particles to enhance the transfer into the micellar phase.

Although the cell wall was shown to be an important barrier in controlling carotenoid bioaccessibility, predicting the latter in plant-based purées based on particle size was rather difficult. In these purées, even when particle size was small and cell walls were destructed, the transfer of carotenoids from the particles to the micellar phase was possibly hindered by the presence of other structural factors (like the presence of a serum phase with a structural network).

The insight obtained in this work with regard to the effect of particle size on the carotenoid bioaccessibility in different food matrices can be useful for future design of plant-based food products with improved nutritional quality.

AUTHOR INFORMATION

Corresponding Author

*Tel: +32 16 321572. Fax: +32 16 321960. E-mail: Marc.Hendrickx@biw.kuleuven.be.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

PSD, particle size distribution; HPH, high-pressure homogenization; CP_B, blended carrot purée; CP_H, carrot purée homogenized at 20 MPa; RCP, reconstituted carrot purée; TP_B, blended tomato purée; TP_H, tomato purée homogenized at 20 MPa; RTP, reconstituted tomato purée; HPLC, high-performance liquid chromatography; RP, reversed phase; B/C, relative bioaccessibility; *β*-car, *β*-carotene; lyc, lycopene

REFERENCES

(1) Parada, J.; Aguilera, J. M. Food microstructure affects the bioavailability of several nutrients. *J. Food Sci.* **2007**, *72*, R21−R32.

(2) Faulks, R. M.; Southon, S. Challenges to understanding and measuring carotenoid bioavailability. *BBA-Mol. Basis Dis.* **2005**, *1740*, 95−100.

(3) Hur, S. J.; Lim, B. O.; Decker, E. A.; McClements, D. J. In vitro human digestion models for food applications. *Food Chem.* **2011**, *125*, 1−12.

(4) Hedrén, E.; Diaz, V.; Svanberg, U. Estimation of carotenoid accessibility from carrots determined by an *in vitro* digestion method. *Eur. J. Clin. Nutr.* **2002**, *56*, 425−430.

(5) Fernandez-Garcia, E.; Carvajal-Lerida, I.; Perez-Galvez, A. *In vitro* bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutr. Res. (N.Y.)* **2009**, *29*, 751−760.

(6) Rock, C. L. Carotenoids: Biology and treatment. *Pharmacol. Therapeut.* **1997**, *75*, 185−197.

(7) Johnson, E. J. The role of carotenoids in human health. *NCC* **2002**, *5*, 56−65.

(8) Yeum, K.-J.; Russell, R. M. Carotenoid bioavailability and bioconversion. *Annu. Rev. Nutr.* **2002**, *22*, 483−504.

(9) Castenmiller, J. J. M.; West, C. E. Bioavailability and bioconversion of carotenoids. *Annu. Rev. Nutr.* **1998**, *18*, 19−38.

(10) van het Hof, K. H.; West, C. E.; Weststrate, J. A.; Hautvast, J. G. A. J. Dietary factors that affect the bioavailability of carotenoids. *J. Nutr.* **2000**, *130*, 503−506.

(11) Deming, D. M.; Erdman, J. W. Mammalian carotenoid absorption and metabolism. *Pure Appl. Chem.* **1999**, *71*, 2213−2223.

(12) Hornero-Mendez, D.; Minguez-Mosquera, M. I. Bioaccessibility of carotenes from carrots: Effect of cooking and addition of oil. *Innovative Food Sci. Emerging Technol.* **2007**, *8*, 407−412.

(13) Knockaert, G.; Lemmens, L.; Van Buggenhout, S.; Hendrickx, M.; Van Loey, A. Changes in *β*-carotene bioaccessibility and concentration during processing of carrot puree. *Food Chem.* **2012**, *133*, 60−67.

(14) Colle, I. J. P.; Van Buggenhout, S.; Lemmens, L.; Van Loey, A. M.; Hendrickx, M. E. The type and quantity of lipids present during digestion influence the *in vitro* bioaccessibility of lycopene from raw tomato pulp. *Food Res. Int.* **2012**, *45*, 250−255.

(15) Armand, M.; Borel, P.; Pasquier, B.; Dubois, C.; Senft, M.; Andre, M.; Peyrot, J.; Salducci, J.; Lairon, D. Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum. *Am. J. Physiol.* **1996**, *271*, G172−G183.

(16) Tydeman, E. A.; Parker, M. L.; Wickham, M. S. J.; Rich, G. T.; Faulks, R. M.; Gidley, M. J.; Fillery-Travis, A.; Waldron, K. W. Effect of carrot (*Daucus carota*) microstructure on carotene bioaccessibilty in the upper gastrointestinal tract. 1. *In vitro* simulations of carrot digestion. *J. Agric. Food Chem.* **2010**, *58*, 9847−9854.

(17) Van Buggenhout, S.; Alminger, M.; Lemmens, L.; Colle, I.; Knockaert, G.; Moelants, K. R. N.; Van Loey, A.; Hendrickx, M. *In vitro* approaches to estimate the effect of food processing on carotenoid bioavailability need thorough understanding of process induced microstructural changes. *Trends Food Sci. Technol.* **2010**, *21*, 607−618.

(18) Lemmens, L.; Van Buggenhout, S.; Oey, I.; Van Loey, A.; Hendrickx, M. Towards a better understanding of the relationship between the *β*-carotene *in vitro* bio-accessibility and pectin structural changes: A case study on carrots. *Food Res. Int.* **2009**, *42*, 1323−1330.

(19) Colle, I.; Van Buggenhout, S.; Van Loey, A.; Hendrickx, M. High pressure homogenization followed by thermal processing of tomato pulp: Influence on microstructure and lycopene in vitro bioaccessibility. *Food Res. Int.* **2010**, *43*, 2193−2200.

(20) Lemmens, L.; Van Buggenhout, S.; Van Loey, A. M.; Hendrickx, M. E. Particle size reduction leading to cell wall rupture is more important for the ß-carotene bioaccessibility of raw compared to thermally processed carrots. *J. Agric. Food Chem.* **2010**, *58*, 12769−12776.

(21) Lopez-Sanchez, P.; Svelander, C.; Bialek, L.; Schumm, S.; Langton, M. Rheology and microstructure of carrot and tomato emulsions as a result of high-pressure homogenization conditions. *J. Food Sci.* **2011**, *76*, E130−E140.

(22) Svelander, C. A.; Lopez-Sanchez, P.; Pudney, P. D. A.; Schumm, S.; Alminger, M. A. G. High pressure homogenization increases the in vitro bioaccessibility of *α*- and *β*-carotene in carrot emulsions but not of lycopene in tomato emulsions. *J. Food Sci.* **2011**, *76*, H215−H225.

(23) Sadler, G.; Davis, J.; Dezman, D. Rapid extraction of lycopene and *β*-carotene from reconstituted tomato paste and pink grapefruit homogenates. *J. Food Sci.* **1990**, *55*, 1460−1461.

(24) Hedrén, E.; Mulokozi, G.; Svanberg, U. *In vitro* accessibility of carotenes from green leafy vegetables cooked with sunflower oil or red palm oil. *Int. J. Food Sci. Nutr.* **2002**, *53*, 445−453.
(25) Colle, I. J. P.; Andrys, A.; Grundelius, A.; Lemmens, L.; Löfgren, A.; Van Buggenhout, S.; Van Loey, A.; Hendrickx, M. Effect of pilot-scale aseptic processing on tomato soup quality parameters. *J. Food Sci.* **2011**, *76*, C714−C723.
(26) Hur, S. J.; Decker, E. A.; McClements, D. J. Influence of initial emulsifier type on microstructural changes occurring in emulsified lipids during in vitro digestion. *Food Chem.* **2009**, *114*, 253−262.
(27) Golding, M.; Wooster, T. J. The influence of emulsion structure and stability on lipid digestion. *Curr. Opin. Colloid Interface Sci.* **2010**, *15*, 90−101.
(28) McClements, D. J.; Decker, E. A.; Park, Y. Controlling lipid bioavailability through physicochemical and structural approaches. *Crit. Rev. Food Sci.* **2009**, *49*, 48−67.
(29) Van Buggenhout, S.; Lille, M.; Messagie, I.; Van Loey, A.; Autio, K.; Hendrickx, M. Impact of pretreatment and freezing conditions on the microstructure of frozen carrots: Quantification and relation to texture loss. *Eur. Food Res. Technol.* **2006**, *222*, 543−553.
(30) Tydeman, E. A.; Wickham, M. S. J.; Faulks, R. M.; Parker, M. L.; Waldron, K. W.; Fillery-Travis, A.; Gidley, M. J. Plant cell walls - A barrier to bioaccessibility of organic micronutrients. In *Proceedings of the International Symposium on Future Technologies for Food Production and Future Food Scientists*; Gothenburg, Sweden, June 2−4, 2003; Swedish Institute for Food and Biotechnology: Gothenburg, Sweden; pp 34−37.
(31) Tydeman, E. A.; Parker, M. L.; Faulks, R. M.; Cross, K. L.; Fillery-Travis, A.; Gidley, M. J.; Rich, G. T.; Waldron, K. W. Effect of carrot (*Daucus carota*) microstructure on carotene bioaccessibility in the upper gastrointestinal tract. 2. *In vivo* digestions. *J. Agric. Food Chem.* **2010**, *58*, 9855−9860.
(32) Castenmiller, J. J. M.; West, C. E.; Linssen, J. P. H.; van het Hof, K. H.; Voragen, A. G. J. The food matrix of spinach is a limiting factor in determining the bioavailability of *β*-carotene and to a lesser extent of lutein in humans. *J. Nutr.* **1999**, *129*, 349−355.
(33) Ellis, P. R.; Kendall, C. W.; Ren, Y.; Parker, C.; Pacy, J. F.; Waldron, K. W.; Jenkins, D. J. Role of cell walls in the bioaccessibility of lipids in almond seeds. *Am. J. Clin. Nutr.* **2004**, *80*, 604−613.
(34) Granado-Lorencio, F.; Olmedilla-Alonso, B.; Herrero-Barbudo, C.; Perez-Sacristán, B.; Blanco-Navarro, I.; Blázquez-García, S. Comparative in vitro bioaccessibility of carotenoids from relevant contributors to carotenoid intake. *J. Agric. Food Chem.* **2007**, *55*, 6387−6394.
(35) Redgwell, R. J.; Curti, D.; Gehin-Delval, C. Physicochemical properties of cell wall materials from apple, kiwifruit and tomato. *Eur. Food Res. Technol.* **2008**, *227*, 607−618.
(36) Boileau, A. C.; Merchen, N. R.; Wasson, K.; Atkinson, C. A.; Erdman, J. W. Cis-lycopene is more bioavailable than trans-lycopene *in vitro* and *in vivo* in lymph-cannulated ferrets. *J. Nutr.* **1999**, *129*, 1176−1181.
(37) Sugawara, T.; Kushiro, M.; Zhang, H.; Nara, E.; Ono, H.; Nagao, A. Lysophosphatidylcholine enhances carotenoid uptake from mixed micelles by Caco-2 human intestinal cells. *J. Nutr.* **2001**, *131*, 2921−2927.
(38) Moelants, K. R. N.; Jolie, R. P.; Palmers, S. K. J.; Cardinaels, R.; Christiaens, S.; Van Buggenhout, S.; Van Loey, A. M.; Moldenaers, P.; Hendrickx, M. The effects of process-induced pectin changes on the viscosity of carrot and tomato sera. *Food Bioprocess Technol.* **2012**, DOI: 10.1007/s11947-012-1004-5.