Dietary and host-related factors influencing carotenoid bioaccessibility from spinach (Spinacia oleracea)

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A B S T R A C T

Several dietary and host related factors potentially influencing carotenoid (beta-carotene, lutein and zeaxanthin) bioaccessibility from spinach, including different concentrations of sodium, calcium and magnesium, were systematically investigated by means of an in vitro digestion model. Bioaccessibility was highest when milk (4% fat) and lowest when skimmed milk or more complex food matrices such as sausage were added to the meal. Micellarisation significantly depended on the presence and concentration of bile salts and pancreatin (p < 0.001, Bonferroni) but was unaffected by pepsin. Micellarisation significantly decreased to 61.4 ± 3.0% of control (p < 0.001, Dunnett’s) at high cholesterol (114 mg/test meal) but not at similar stigmasterol concentrations. Calcium and magnesium ≥ 13.8 mM individually inhibited micelle formation (>40% on average), presumably due to the generation of insoluble soaps with fatty acids and bile salts. Increased sodium concentrations (280 and 460 mM) altered carotenoid micellarisation patterns, favoring beta-carotene isomers (p < 0.001, Bonferroni) but decreasing lutein and zeaxanthin (p < 0.001 and p < 0.05, respectively, Bonferroni). This study suggests that minerals may impact carotenoid bioavailability.

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1. Introduction

Carotenoids are C-40 polyene structures with antioxidant properties, being synthesised by plants, many microorganisms and fungi. Animals including humans rely on dietary intake, especially of the provitamin A precursors, which are essential for the vision cycle (Bendich & Olson, 1989) and the immune response (Hughes et al., 2001). Recent evidence has emerged that a variety of non-provitamin A carotenoids, such as lycopene, may protect from cardiovascular diseases (Voutilainen, Nurmi, Mursu, & Rissanen, 2006) and delay the onset and development of several types of cancer, such as cancer of the prostate (Giovannucci, Rimm, Stampfer, & Willett, 2002). It has also been suggested that other non-provitamin-A carotenoids, such as zeaxanthin and lutein, play an important role in protecting the human retina (Bernstein et al., 2001).

In contrast to the accumulating information on beneficial health effects and presence in foods, knowledge regarding carotenoid bioavailability is still incomplete. Human studies, doubtless the most appropriate approach to investigate uptake and distribution of dietary compounds, are time consuming and ethically disputable, similarly as for animal studies. As a rapid and low-cost alternative, an in vitro model to simulate gastro-intestinal passage was developed in 1981 (Miller, Schricker, Rasmussen, & Van Campen, 1981). This model has later been exploited and modified, amongst other purposes, for carotenoid research (Garrett, Failla, & Sarma, 1999; Hedren, Diaz, & Svanberg, 2002; Reboul et al., 2006). Although only focusing on partial aspects of human digestion, such as release of carotenoids from food matrix, solubility and emulsion into mixed micelles, it has been shown that results from carotenoid bioaccessibility experiments seem to be well correlated with data obtained from human studies (Reboul et al., 2006). Thus, this in vitro model has aided in understanding the impact of various dietary and host-related factors on carotenoid availability, including gastric and intestinal conditions (Garrett et al., 1999; Wright, Pietrangelo, & MacNaughton, 2008), the food matrix (Hedren et al., 2002), the impact of dietary fibre (Mills et al., 2009) and the type of ingested fat (Huo, Ferruzzi, Schwartz, & Failla, 2007). However, many potential factors impacting carotenoid bioaccessibility have not been thoroughly and systematically explored, including mineral concentrations. Minerals might, in theory, affect micelle formation or stability by influencing ion strength of the digesta, or by forming insoluble complexes with food components, such as lipids.

Abbreviations: BHT, butylated hydroxytoluene; HPLC, high performance liquid chromatography; MTBE, methyl tert-butyl ether; SD, standard deviation; LOD, limit of detection; IQD, limit of quantification.

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The current study aimed to systematically investigate factors impacting carotenoid bioaccessibility from spinach based meals, including composition of the meal, the presence of digestive enzymes and bile salts as well as the presence and concentration of potential inhibitors, such as fat-soluble vitamins, cholesterol and phytosterol. In addition, emphasis was given to the impact of mineral concentrations, i.e. sodium, calcium, and magnesium, and their effect on carotenoid micellarisation.

2. Material and methods

2.1. Chemicals and carotenoid standards

Methyl-tert-butyl ether (MTBE), porcine pepsin, porcine bile mixture and porcine pancreatin, stigmasterol, cholesterol and alpha-tocopherol were purchased from Sigma–Aldrich (Bornem, Belgium); methanol and ammonium acetate from BioSolve (Valkenswaard, the Netherlands) and hexane, sodium, calcium and magnesium chloride from VWR (Haasrode, Belgium). Carotenoids were obtained from several suppliers: β-carotene, neoxanthin, violaxanthin from CaroteNature (Lupsingen, Switzerland), lutein and zeaxanthin from Sigma–Aldrich and β-cryptoxanthin from Extrasynthese (Lyon, France).

2.2. Test meals and inhibitors

Test meal ingredients were bought in a local supermarket (CAC-TUS S.A., Esch-sur-Alzette, Luxembourg) and a common meal replacement drink (Fresubin®, Fresenius-Kabi, Bad Homburg, Germany); methanol and ammonium acetate from BioSolve (Valkenswaard, the Netherlands) and hexane, sodium, calcium and magnesium chloride from VWR (Haasrode, Belgium). Carotenoids were obtained from several suppliers: β-carotene, neoxanthin, violaxanthin from CaroteNature (Lupsingen, Switzerland), lutein and zeaxanthin from Sigma–Aldrich and β-cryptoxanthin from Extrasynthese (Lyon, France).

2.3. Carotenoid extraction from spinach

Spinach samples were extracted as described earlier (Biehler, Mayer, Krause, Hoffmann, & Bohn, 2009). All procedures were carried out on ice and dim light as far as possible. In brief, spinach samples were homogenised in a household blender and mixed for 5 min. Pigments were extracted with methanol once and aliquots of 30 g were stored at −80 °C until analysis. A detailed composition of test meals is given in Table 1.

2.4. Dietary and host factors studied (Table 1)

Impact of food matrix (dietary related factors): 0.5 g liver sausage, 2 g Fresubin, 2 g semi-skimmed milk, 2 g regular milk and 2 g condensed milk as well as 2 g soy milk were added in independent experiments to the spinach to test the influence of different food matrices.

Presence or absence of bile/enzymes (host-related factors): standard test meals (4 g of spinach and 2 g of condensed milk) were treated under control conditions without adding bile, enzymes or both.

Impact of inhibitors: alpha-tocopherol (0.25–1 mg), stigmasterol (108 mg) and cholesterol (1–114 mg) were blended into the standard test meals prior to digestion.

Impact of minerals: standard saline solution (0.15 M), which constituted ca. 50% of the total volume during digestion, was exchanged with sodium chloride solutions of higher concentrations (0.4 M and 0.9 M), yielding final sodium chloride concentrations of 280 and 460 mM. Furthermore, the influence of CaCl₂ and MgCl₂ was investigated. In these trials, saline (25 mL) was exchanged with 25 mL of CaCl₂/MgCl₂ solution with the following concentrations: 0.1/0.05/0.025/0.01/0.005 M (see Fig. 4). Considering the total volume of 50 mL, final calcium and magnesium chloride concentrations ranged hence from 50 to 2.5 mM. The 2 g of condensed milk in the standard meal contributed to an additional 1.3 mM of calcium (taken from food labels).

2.5. Simulation of the gastro-intestinal passage

The in vitro digestion protocol was adapted from earlier studies (Bohn et al., 2007; Garrett et al., 1999; Miller et al., 1981).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Ingredients, description and composition of the test meals subjected to in vitro digestion.</th>
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</thead>
<tbody>
<tr>
<td>Product</td>
<td>Description/composition</td>
</tr>
<tr>
<td>Spinach</td>
<td>Chopped, frozen. Protein 2.4%, dietary fibre 1.2%, carbohydrates 1.6%, fat 0.4%</td>
</tr>
<tr>
<td>Cream spinach</td>
<td>Crude, frozen. Protein 3.3%, dietary fibre 2%, carbohydrates 2.3%, fat 3.2%</td>
</tr>
<tr>
<td>(1) Condensed milk</td>
<td>Protein 3.4%, carbohydrates 2.0%, fat 4.0%</td>
</tr>
<tr>
<td>(2) Whole milk</td>
<td>Protein 3.5%, carbohydrates 4.8%, fat 3.5%</td>
</tr>
<tr>
<td>(3) Skimmed milk</td>
<td>Protein 3.8%, carbohydrates 5.8%, fat 1.5%</td>
</tr>
<tr>
<td>(4) Soy milk</td>
<td>Protein 2.9%, carbohydrates 4.8%, fat 2%</td>
</tr>
<tr>
<td>(5) Liver sausage</td>
<td>Protein 13.1%, carbohydrates 0%, fat 31.9%</td>
</tr>
<tr>
<td>(6) Fresubin</td>
<td>Protein 3.8%, carbohydrates 13.8%, fat 3.4%</td>
</tr>
<tr>
<td>(7) Inhibitors</td>
<td>Alpha-tocopherol</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
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<tr>
<td></td>
<td>Stigmasterol</td>
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<tr>
<td></td>
<td>Bile + enzymes</td>
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a Food composition data were taken from food labels.
b Total volume 50 mL.
c Used instead of pure spinach.
d Tested as additive to spinach during method development.
e Added to standard meal to investigate micellarisation inhibitor potential.
2.5.1. Gastric phase

Frozen spinach was thawed and approx. 4 g of spinach was weighed into a 50-mL screw-top polyethylene tube, combined with 2 g of semi-skimmed condensed milk (4% fat) and homogenised by gentle shaking. 25 mL of 0.15 M NaCl containing 150 μM butylated hydroxytoluene (BHT) were then added (either freshly prepared or stored for a max. of 1 week at −25 °C). The samples were additionally sealed with Parafilm® M (Brand GmbH + CO KG, Wertheim, Germany) and put into a closed plastic bag. The tubes were then transferred to a shaking water bath (GFL 1083 from VEL®, Leuven, Belgium; 100 rpm, 37 °C, 1 h). The pH of the post-gastric meal was then increased to 5.5 with 0.7–0.9 mL of 0.9 M NaHCO₃.

2.5.2. Intestinal phase

Enzyme and bile solutions were either prepared or stored at −25 °C until usage (max. 1 week). 9 mL of a mixture of pancreatin and porcine bile extract (2 mg/mL pancreatin and 12 mg/mL bile extract dissolved in 0.1 M NaHCO₃) were added to each digestion tube. The pH of the samples was finally increased to 7–7.5 with the addition of 700–800 μL of 1 M NaOH. The final volume of the samples was adjusted to 50 mL with 0.15 M NaCl. The 50-mL tubes were sealed with Parafilm® and incubated in a shaking water bath (100 rpm, 37 °C) for two hours.

2.5.3. Carotenoid extraction from digesta

Aliquots (12 mL) were conveyed to Beckmann Ultra Clear tubes and centrifuged in a Ti-40 rotor at 16400g at 4 °C for 35 min (Beckmann Optima™ C-90U Ultracentrifuge, Beckmann Coulter, Palo Alto, CA) to separate the solid and oil contents from the aqueous micellar phase. Using a needle and syringe, 8 mL of the aqueous phase were extracted by addition of 4 mL hexane and centrifuged in a Ti-40 rotor at 16400g at 4 °C for 35 min. Aliquots (12 mL) were then added (either freshly prepared or stored for a max. of 1 week at −25 °C). The samples were additionally sealed with Parafilm® M and put into a closed plastic bag. The tubes were then transferred to a shaking water bath (GFL 1083 from VEL®, Leuven, Belgium; 100 rpm, 37 °C, 1 h). The pH of the post-gastric meal was then increased to 5.5 with 0.7–0.9 mL of 0.9 M NaHCO₃.

2.5.4. HPLC analysis

Carotenoid quantification was carried out as described previously (Biehler et al., 2009). In short, a C30 column (Waters Inc, Milford, MA) was equilibrated at 28 °C and used in combination with a binary elution gradient consisting of solvent A) methanol/MTBE/ammonium acetate/water (88%:5%:2%:5% by vol.) and solvent B) MTBE/methanol/water/ammonium acetate (79%:16%:3%:2% by vol.). The gradient started at 100% A (0 to 5 min), changed to 65% B (during min 5 to 26), and up to 100% B until min 34, holding this constant for 5 min, changing back to 100% A until min 40, and keeping this constant for 4 min. The flow rate was kept constant at 1.2 mL/min and the injection volume was 25 μL.

2.6. Statistical analysis

Micellarisation experiments were based on n = 3–6 replicates. Data were analysed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). Normal distribution of data was verified by Q-Q-plots and Kolmogorov–Smirnoff tests and equality of variance by box-plots. Separate linear mixed models were created to analyse the impact of enzyme concentrations, presence of bile salts and enzymes, presence and concentrations of inhibitors and different salt concentrations. Where indicated, Fisher F tests were followed by Post-Hoc tests (Dunnett’s for the inhibitors and Bonferroni for all other comparisons). P values <0.05 were considered as statistically significant (2-sided). Values and errors in text and figures are given as means ± standard deviation (SD).

3. Results

3.1. Repeatability, reproducibility and detection/quantification limits

Five independent samples were treated under control conditions and the micellarisation rate was determined (‘repeatability’). We found an average micellarisation of 30.8 ± 2.1% for total carotenoids, 42.0 ± 6.8% for zeaxanthin, 57.6 ± 4.1% for lutein, 7.1 ± 0.4% for total beta-carotene (7.0 ± 0.5% and 7.7 ± 0.3%, resp. for the (all-E)- and the major cis beta-carotene isomer, tentatively identified as (9Z)-beta-carotene by comparing absorption spectra and retention times with literature (Thakkar, Maziya-Dixon, Dixon, & Failla, 2007)). The epoxycarotenoids neoxanthin and violaxanthin were not present in the digesta, likely due to degradation during gastric phase.

The micellarisation of total carotenoids from different sets of experiments (reproducibility) was determined as 29.8 ± 4.8% for the control samples (standard conditions) on six different days. Micellarisation of the controls was set at 100% and samples were normalised to the control to minimise day-to-day variations.

The limit of detection (LOD: 0.29 ng) and limit of quantification (LOQ: 0.59 ng) were assessed following the method proposed by the US Environment Protective Agency (Zorn, Gibbons, & Sonzogni, 1997), investigating seven independent beta-carotene standard injections of low concentrations (20 ng/mL) with three times SD yielding the LOD and six times SD the LOQ.

3.2. Carotenoid quantification in spinach

Total carotenoid content in pureed spinach and pureed cream spinach was 18.8 ± 1.3 and 10.8 ± 0.4 mg/100 g, respectively, with lutein (6.0 ± 0.4 and 3.4 ± 0.2 mg/100 g) and total beta-carotene (5.9 ± 0.4 and 3.4 ± 0.2 mg/100 g) showing the highest concentrations, followed by violaxanthin (4.8 ± 0.5 and 2.4 ± 0.1 mg/100 g), zeaxanthin (2.4 ± 0.1 and 0.6 ± 0.05 mg/100 g) and neoxanthin (0.4 ± 0.04 and 0.2 ± 0.04 mg/100 g).

3.3. Carotenoid availability in dependency of food matrix

The addition of condensed milk to spinach resulted in highest total carotenoid micellarisation (Fig. 1) and was consequently chosen as the basis for further micellarisation experiments. The addition of milk containing less fat (3.5% and 1.5%) and the addition of liver sausage and soya milk resulted in significantly reduced carotenoid micellarisation (p < 0.001, Bonferroni). Lowest total micellarisation was obtained with the addition of 2 g of the Fresubin drink (7.1 ± 0.7%).

3.4. Impact of the presence of enzymes and bile

A series of experiments was conducted, omitting single digestive enzymes or bile salts, while all other basic conditions, such as pH, incubation time, shaking speed and temperature were kept constant. The omission of pepsin during gastric phase did only...
slightly, but not significantly decrease micellarisation (Fig. 2), while the omission of either bile salts, pancreatin, or both resulted in drastic changes. Results obtained for total beta-carotene, (9Z)-beta-carotene, lutein and zeaxanthin showed the same pattern compared to total carotenoids (data not shown).

3.5. Presence of inhibitors

The addition of low amounts of inhibitors did not result in significantly altered micellarisation rates (data not shown). Only cholesterol at high concentrations reduced total carotenoids, lutein and zeaxanthin fractional micellarisation, to approx. 61, 57 and 58% compared to the control, respectively (p > 0.001, Dunnet’s), while (9Z)-beta-carotene and total beta-carotene were not impacted (Table 2). The addition of stigmasterol led to a slight, but non-significant decrease in micellarisation of approx. 15% compared to the control.

3.6. Impact of calcium and magnesium

Calcium and magnesium chloride at concentrations above 0.1 M (i.e. 0.051 M final concentration) completely inhibited carotenoid micellarisation. Below 0.1 M, the impact of calcium and magnesium was similar, with magnesium showing slightly weaker effects. At 0.05 M of Ca and Mg (26.3 mM final concentration), very low amounts of carotenoids could be extracted from the aqueous phase (Fig. 3), while at 0.025 M (13.8 mM final concentration), between 45% (Ca) to 75% (Mg) of total carotenoids were micellarised compared to the control. At 0.01 and 0.005 M (6.3 and 3.6 mM final concentration, respectively), similar values for total carotenoid fractional micellarisation for both ions were found compared to the control.

3.7. Different concentrations of sodium

Sodium chloride solutions at 0.4 and 0.9 M significantly increased the micellarisation of (9Z)-beta-carotene and total beta-carotene compared to the control. Both xanthophylls showed a reduced micellarization compared to the control, with lutein being stronger affected than zeaxanthin (Fig. 4).

4. Discussion

In the present study, we aimed at systematically investigating selected dietary and to host-related factors potentially influencing carotenoid bioaccessibility from spinach, employing an in vitro gastro-intestinal model, similarly as described earlier (Bohn et al., 2007; Garrett et al., 1999). This is, to our knowledge, the first study highlighting negative effects of dietary abundant minerals, such as magnesium and calcium, on carotenoid bioaccessibility.

Carotenoid release from the food matrix is deemed to be one of the most important steps determining carotenoid bioavailability, consisting of breakdown of plant cell walls for carotenoid release and transfer of carotenoids into lipid droplets. Disruption of the matrix (Castenmiller, West, Linsen, van het Hof, & Voragen, 1999; Gärtner, Stahl, & Sies, 1997) and presence of lipids (Hedren et al., 2002; Huo et al., 2007; Ornelas-Paz, Failla, Yahia, & Gardea-Bejar, 2008) thus have shown to improve the release of different carotenoid species in vitro and in vivo. In the present study, we found that the addition of milk (4% fat) resulted in highest micellarisation and significantly increased carotenoid solubility compared to low fat milk. Other meal components tested, such as liver sausage and soy milk, although delivering comparable or higher amounts of fat (2 g of milk containing 2% or 0.5 g of sausage containing 32% fat), were less effective in increasing carotenoid bioaccessibility. It is possible that the complex nature of the matrix, i.e. fibre (for a soya milk) attenuated carotenoid accessibility (Ornelas–Paz et al., 2008). Fresubin as an example of a ready-to-drink meal used e.g. as a meal replacement or as part of a balanced diet resulted in strongest reduction of carotenoid micellarization (>50%) compared to other meals tested, which may be due to highly increased viscosity of the digesta, which has also been suggested to negatively impact micellarisation (Yonekura & Nagao, 2009). Although the static in vitro digestion model does not fully mimic the gastro-intestinal conditions in vivo, especially the dynamics of bile/enzymes secretion in relation to the food matrix composition, the results suggest that carotenoids may be better accessible from simple, unprocessed matrices compared to more complex sources.

Another crucial step for carotenoid availability is the formation of mixed micelles, requiring emulsification by means of bile salts and digested lipids. Carotenoid micellarisation was strongly
decreased when omitting either bile salts, pancreatin, or both, being comparable, if not stronger, to previous studies (Garrett et al., 1999; Hedren et al., 2002; Wright et al., 2008). This situation may occur in vivo, such as in cystic fibrosis patients lacking the ability to secrete sufficient amounts of pancreatin, possibly resulting in inadequate uptake of lutein, zeaxanthin and beta-carotene (Rust, Eichler, Renner, & Elmadfa, 1998; Schupp et al., 2004). Pancreatin is required to cleave triglycerides into mono- and diglycerides, which are part of the formed mixed micelles. The low carotenoid micellarization observed in the absence of both pancreatin and bile salts is in line with earlier studies indicating a reduction of carotenoid micellarization by 80–100% when omitting bile salts (Garrett et al., 1999; Hedren et al., 2002; Wright et al., 2008). The omission of pepsin during the gastric phase, however, did not significantly influence carotenoid micellarisation, similar as found by Garrett et al. (1999). It appears that, at least in an in vitro model with a relatively simple, low-protein meal as investigated in our study, the enzymatic activity of pepsin was not required for matrix disruption.

When stratifying for individual carotenoids, xanthophylls showed higher micellarisation compared to carotenes, being in line with results from previous studies (Chitchumroonchokchai, Schwartz, & Failla, 2004; Garrett et al., 1999; Reboul et al., 2006). The potential (9Z)-beta-carotene isomer was equally micellarised decreased when omitting either bile salts, pancreatin, or both, being comparable, if not stronger, to previous studies (Garrett et al., 1999; Hedren et al., 2002; Wright et al., 2008). This situation may occur in vivo, such as in cystic fibrosis patients lacking the ability to secrete sufficient amounts of pancreatin, possibly resulting in inadequate uptake of lutein, zeaxanthin and beta-carotene (Rust, Eichler, Renner, & Elmadfa, 1998; Schupp et al., 2004). Pancreatin is required to cleave triglycerides into mono- and diglycerides, which are part of the formed mixed micelles. The low carotenoid micellarization observed in the absence of both pancreatin and bile salts is in line with earlier studies indicating a reduction of carotenoid micellarization by 80–100% when omitting bile salts (Garrett et al., 1999; Hedren et al., 2002; Wright et al., 2008). The omission of pepsin during the gastric phase, however, did not significantly influence carotenoid micellarisation, similar as found by Garrett et al. (1999). It appears that, at least in an in vitro model with a relatively simple, low-protein meal as investigated in our study, the enzymatic activity of pepsin was not required for matrix disruption.

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as the (all-E)-isomer suggesting that the higher bioavailability of the (all-E)-isomer found in vivo (Deming, Teixeira & Erdman, Jr., 2002) might be due to preferential uptake or due to cis–trans isomerisation during or following absorption. Another factor influencing the different micellarisation of xanthophylls and carotenoids might be the distribution in the micelles, as the more hydrophobic beta-carotene seems to preferably reside in the core of the micelles as opposed to lutein and zeaxanthin, mainly situated on the surface of the micelles (Borel et al., 1996).

Lipophilic compounds with sufficiently high concentrations in the diet, including alpha-tocopherol, cholesterol and stigmasterol, may compete for micellarisation, hence potentially attenuating carotenoid bioaccessibility. A significant reduction of total carotenoids, lutein and zeaxanthin was only found for high but still realistic cholesterol concentrations of 1900 mg/100 g test meal, as it is present in e.g. animal entrails (Souci, Fachmann, & Kraut, 2000). Cholesterol has already been described to attenuate beta-carotene incorporation in artificial systems (Socaci, Jessel, & Diehl, 2000), similar to certain phytosterols in Caco-2 cells (Fahy, O’Callaghan & O’Brien, 2004). Interestingly, at similar high concentrations of 1800 mg/100 g test meal, the cholesterol resembling free stigmasterol did only slightly but non-significantly reduce xanthophyll micellarisation. It can be speculated that a reduction of carotenoid serum levels, found in an in vivo trial following the consumption of phytosterols (Mensink, Ebeling, Lindhout, Plat, & van Heugten, 2002), might be governed by additional factors, such as by the regulation of the uptake into the enterocytes, sequestration into chylomicrons, or by increased excretion into the gastro-intestinal tract.

We hypothesised that minerals could impact carotenoid availability, by complexation and/or precipitation of bile salts, fatty acids, or by compromising micelle stability by e.g. ion–micelle interactions. When conducting experiments adding high sodium concentrations of 0.9 M (460 mM sodium chloride final concentration) as opposed to physiological standard conditions (150 mM), a reduced bioaccessibility of xanthophylls was found (15%), while beta-carotene isomers were significantly better micellarised compared to the control (60%). As we did not observe bile or fatty acid precipitation at high sodium concentrations, other mechanisms such as viscosity or ionic interactions between aqueous phase and micelles must play a role. It is possible that the increased ion strength of the aqueous phase especially favours the incorporation of the hydrophobic carotenoids into the micelles, while the interaction with the micelle surface and the xanthophylls is disturbed. The high sodium concentration is well out of a typical dietary range and was tested to investigate whether the differentiation of carotenoid micellarisation between xanthophylls and carotenoids extends to high salt concentrations. However, at concentrations of 0.4 M NaCl (280 mM final concentration), similar findings compared to 0.9 M were obtained. Although this concentration cannot be easily reached from the diet, the consumption of a meal mostly containing sausages or salty ham is likely to reach sodium concentrations that may impact micellarisation patterns in a similar way.

In addition to sodium and potassium, calcium and magnesium are the most commonly consumed minerals, with daily intakes of approx. 1.0 and 0.3 g, respectively (DGE et al., 2000). Both calcium and magnesium showed strong inhibitory effects on carotenoid solubility at concentrations above 0.01 M (6.3 mM final calcium concentration including calcium from the milk), as well as preparitory effects when pure mineral solutions were added to pure bile salt solutions (results not shown). Both calcium and magnesium have been reported to form insoluble salts at neutral pH as in the small intestine, including glycine-conjugated bile (Hoffmann & Mysels, 1992), bilirubin (Apstein, 1998), and insoluble soaps with especially long-chain fatty acids (Graham & Sackman, 1983). We thus hypothesised that these insoluble salts would not be available any more during mixed micelle formation, reducing the amount and/or size of remaining micelles and therefore soluble carotenoids, or alternatively, occluding carotenoids during precipitation, resulting in a complete unavailability of carotenoids at concentrations exceeding 50 mM (26.3 mM final concentration) calcium or magnesium. When comparing this molarity with the amount of bile salts present (4.4 mM) and the amount of fat (0.1 mM), it appears that a surplus of calcium/magnesium ions (~5-fold) is needed to result in significant inhibition of micellarisation. At calcium concentrations of 25 mM (13.8 mM final concentration), still a significant reduction of carotenoids in the micellar phase, of around 40% was observed. In the future, it would be interesting to also study additional, frequently consumed divalent salts, such as zinc or iron, and mixtures of minerals or trace elements. Even though, concentrations of zinc and iron in the diet are much lower (15 mg daily dietary intake (DGE et al., 2000)) compared to calcium and magnesium, supplement intake could impact carotenoid bioavailability, and these elements have also been reported to be able to result in precipitation of fatty acids (Borzellini & Cunder, 1981).

In summary, we were able to highlight the importance of the interaction of carotenoid digestion and present minerals originating from the diet are warranted.

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References


