Carotenoid and polyphenol bioaccessibility and cellular uptake from plum and cabbage varieties

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A B S T R A C T

Plum and cabbage are rich in carotenoids and polyphenols. However, their bioactivity depends on their release and intestinal uptake. Four varieties of Brassicaceae (Duchy, Scots Kale, Kale, Kalorama) and Prunus (Cherry Plum, Plum 620, Ersinger, Italian Plum) were studied: bioaccessibility following in vitro digestion, cellular uptake (Caco-2 vs. co-culture cell model: Caco-2:HT-29-MTX (90:10%) and colonic fermentation were determined for carotenoids/polyphenols; the influence of certain kitchen preparations was likewise studied. Carotenoids were non-significantly influenced by the latter, while for polyphenols, boiling and steaming significantly reduced total phenolics (p < 0.05). Carotenoid bioaccessibility did not differ significantly between Prunus vs. Brassicaceae varieties, but xanthophyll was higher than carotene bioaccessibility (p < 0.01). Polyphenol bioaccessibility was low (<10%), possibly compromised by the cream containing test meal. Total carotenoid cellular uptake varied between varieties (0.3–4.1%), being higher for carotenes (4.1%) than for xanthophylls (1.6%, p < 0.01), and were higher for the co-culture cell model compared to Caco-2 cells (p < 0.01). Total carotenoid recovery in the colonic fraction varied from 4% to 25%. Lower bioaccessibility of carotenes thus appeared to be somewhat counterbalanced by higher cellular uptake. The potential positive role of the mucus layer for cellular uptake and the fate of the colonic digesta deserve further attention in the future.

1. Introduction

Diets rich in fruits and vegetables are recommended by many health organizations, and epidemiological studies have shown that their consumption can decrease the incidence of several chronic diseases such as type II diabetes (Carter, Gray, Troughton, Khunti, & Davies, 2010), cancer, and cardiovascular diseases (Dauchet, Hercberg, & Dallongeville, 2006). Fruits and vegetables contain an array of phytochemicals, and until today it is unknown which constituents are the most bioactive with respect to beneficial health effects.

Carotenoids and polyphenols represent the most abundant lipid and water soluble phytochemicals, respectively. Cabbage and plum varieties contain reasonable high concentrations of carotenoids/polyphenols, up to ca. 60/40 mg per 100 g for cabbages and 2/125 mg per 100 g for plums, respectively (Kaulmann, Jonville, Schneider, Hoffmann, & Bohn, 2014), and are thus valuable sources of secondary plant compounds. In Luxembourg, for example, Brassica oleracea and Prunus consumption amounts to 14 kg/year and 16 kg/year, respectively.

Upon ingestion, the bioaccessibility of carotenoids and polyphenols depends on several factors (Bohn, 2008; Bohn, 2014). Carotenoids, with lutein and beta-carotene being the most prominent ones in plums and cabbages, need to be released mechanically and/or enzymatically from the food matrix and (due to their hydrophobicity) require, for solubilisation, incorporation into mixed micelles before they can be taken up by small intestinal enterocytes (Sy et al., 2012). As carotenoids in fruits and vegetables are often located in chromoplast (or chloroplast) organelles, their sub-structure and the cell wall are the main barriers for carotenoid bioaccessibility (Palmero, Panozzo, Simatupang, Hendrickx, & Van Loey, 2014). Thermal processes such as boiling or steaming can have positive effects on bioaccessibility, by helping to disrupt the food matrix, though negative influences due to degradation of carotenoids, especially once released from the matrix, have also been reported (Palmero et al., 2014). Following matrix release and transfer into mixed micelles, the next barrier for carotenoid absorption represents the mucus layer in the intestine. A study by Meaney and O’Driscoll (1999) showed that the mucus layer can have a negative impact on the availability of apolar compounds such as of carotenoids.
The bioaccessibility of polyphenols appears more complex, due to the many different polyphenols existing, their changes during digestion and metabolism, depending largely on their polarity, molecular weight, glycosylation and esterification. Polyphenols first need to be released from the food matrix, taking place during the gastric phase (Bouayed, Hoffmann, & Bohn, 2012), and the majority of rather polar polyphenols are available for absorption without micelle incorporation; however, further cleavage into the respective aglycon may be required. Apparent polyphenol concentrations may be increased following digestion, possibly as non-extractable polyphenols (NEPP) are released (Saura-Calixto, Serrano, & Goni, 2007). The same may be true for kitchen preparation procedures, although many household procedures may activate enzymes such as phenol-oxidase, thereby degrading polyphenols (Terefe, Delon, Buckow, & Versteeg, 2015). However, most cabbage varieties are not consumed raw but cooked, and treatments including chopping, freezing, thawing, in combination with different heat treatments, can be expected to influence the concentration of bioactive compounds.

The aim of the present study was to study the bioaccessibility of carotenoids and polyphenols from selected plum and cabbage varieties following simulated gastrointestinal digestion. Furthermore, cellular uptake of carotenoids and polyphenols post-digestion was investigated by Caco-2 and Caco-2/HT-29-MTX cell models, to examine the influence of the mucus layer. Finally, a colonic fermentation step was included to study further degradation pathways of carotenoids. Common kitchen preparation procedures were additionally employed to exemplary determine their influence on carotenoid and polyphenol content of cabbage.

2. Material and methods

2.1. Chemicals and standards

All products were of analytical grade or higher. MilliQ water was prepared with a purification system from Millipore (Brussels, Belgium) and was used for all experiments. Unless otherwise stated, all chemicals including methyl-tert-butyl ether (MTBE), porcine pepsin (≥ 250 U/mg solid, measured as TCA-soluble products using hemoglobin as substrate), porcine bile mixture, and porcine pancreatic (4X USP specifications) were procured from Sigma–Aldrich (St. Louis, MO, USA). Acetone and sodium chloride were purchased from VWR (Haasrode, Belgium). Ammonium acetate, methanol, hexane, acetonitrile and dichloromethane were obtained from BioSolve ( Valkerswaard, the Netherlands). Phosphate buffer solution pH 7 was procured from Chemlab (Zedelgem, Belgium). Carotenoid standards, i.e. β-carotene, β-cryptoxanthin and lutein were from Extrasynthèse ( Lyon, France), while α-carotene, violaxanthin, neoxanthin and phytoene were obtained from CaroteNature (Lupsingen, Switzerland), and trans-β-apo-8′-c- arotenol (internal standard (IS) to check for chromatographic recovery of carotenoids) was from Sigma–Aldrich. Certified purity of all standards was above 95%. Polyphenol standards, i.e. (+)-catechin, caffeic acid, p-coumaric acid, cryptochlorogenic acid (4-cafeoylquinic acid), neochlorogenic acid (3-cafeoylquinic acid), quercetin, quercetin 3-O-galactoside, kaempferol, kaempferol 3-O-glucoside, ferulic acid, syringic acid, sinapic acid, gallic acid and vanillic acid were obtained from Sigma–Aldrich. Chlorogenic acid and cinnamic acid were purchased from Merck (Darmstadt, Germany). Kaempferol-3-O-rutinoside was obtained from Extrasynthèse and 4-hydroxybenzoic acid was purchased from Thermo Fisher Scientific (Geel, Belgium). Cell media (DMEM +GlutaMAX), PBS, and FBS were obtained from Gibco (Karlsruhe, Germany).

2.2. Plum and cabbage varieties

Plums and cabbages were procured and characterized for micro-and macro-constituents as described previously (Kaulmann et al., 2014). In brief, 27 cabbage and 17 plum varieties were obtained from Luxembourgish supermarkets or farmers. Varieties rich in either carotenoids or polyphenols, both, or being low in carotenoids or polyphenols or both were finally retained for further analyses: Plums (Cherry Plum, Plum 620, Ersinger, Italian Plum) and Brassicaceae varieties (Duchy, Scots Kale, Kale, Kalorama), chosen due to their contrasting content of carotenoids and polyphenols (Table 1).

All plum and Brassicaceae varieties were lyophilized (Christ freeze dryer, Thermo Fisher Scientific, Geel, Belgium) during 24 h, homogenized with a grinder (Motor Grinder RZ 200, Retsch, Aarteselaar, Belgium) and stored in 50 mL plastic centrifuge tubes at −20 °C until analysis.

2.3. Kitchen procedures

As most vegetables are typically not consumed in raw form, different cooking procedures were investigated. Kale was used as a reference model to investigate the effect of freezing and thawing alone as well as the effect of cooking combined with boiling or steaming or freezing/thawing. To evaluate the effect of freezing and thawing, 50 g of kale leaves were divided into two equal parts (25 g). One part (denoted as control) was directly crushed under liquid nitrogen to powder, transferred into a 50 mL falcon tube and stored at −80 °C. The other part was first frozen for 48 h at −20 °C and then thawed for 4 h (denoted as freeze–thaw) before it was crushed under liquid nitrogen to powder and stored at −80 °C until analysis.

To evaluate the effect of cooking and boiling or steaming, 360 g of kale leaves were divided into four equal parts. One part (denoted as control) was first chopped into equal pieces of ca. 50 cm² with a kitchen knife, and then directly crushed under liquid nitrogen to powder and stored at −80 °C. A second aliquot (denoted as chop/boiling) was first chopped into equal pieces of 50 cm² and then boiled for 7 min in 500 mL hot tap water before the leaves were then wiped with paper towels, and then crushed under liquid nitrogen to a powder and stored at −80 °C. A third part (denoted as chop/steaming) was first chopped into equal pieces of 50 cm² and then steamed for 7 min in a household steamer (Tefal VS4003, Offenbach, Germany), before the leaves were then wiped with paper towels and crushed under liquid nitrogen to a powder and stored at −80 °C. A fourth part (described as chop/freeze–thaw) was first chopped into equal pieces of 50 cm², frozen for 48 h at −20 °C, and then thawed for 4 h, before it was crushed under liquid nitrogen to a powder and stored at −80 °C. Carotenoid extraction was described elsewhere (Kaulmann et al., 2014). All experiments were done in triplicates.

2.4. Simulation of gastro-intestinal passage

The in vitro digestion protocol was adapted from an earlier study (Biehler, Kaulmann, Hoffmann, Krause, & Bohn, 2011). Briefly, 500 mg of dried cabbage or dried plum were weighted into a 50 mL tube and mixed with 3 g of cream containing 12% fat, also as described earlier, in order to simulate a complete test meal containing fat, and to assure sufficiently high micellarization of carotenoids requiring the presence of some lipids. Milk was chosen as some cabbage and plum dishes (e.g. frozen kale containing cream, plum-cake with cream) do occasionally contain dairy products. Then, 10 mL of 0.15 M NaCl was added, and the mixture was
homogenized for 2 min with an Ultra-Turrax (T25 basic, IKA Werke, Staufen, Germany). Fifteen mL of 0.15 M NaCl were further added and the whole test meal was acidified with 0.2–0.5 mL of 1 M HCl to a pH of 3. Two mL of porcine pepsin (40 mg/mL in 0.1 M HCl) were then added and the samples were sealed with Parafilm and incubated in a shaking water bath (100 rpm, 37 °C). The pH of the post-gastric meal was then increased to 5.5 with 0.5–0.6 mL of 0.9 M NaHCO₃. In order to mimic the duodenal digestion, 9 mL of a pancreatin (4 mg/mL in 0.1 M NaHCO₃) and porcine bile extract (24 mg/mL in 0.1 M NaHCO₃) mixture was added to the samples. The pH of the samples was further increased to 7 with 0.1–0.2 mL of 1 M NaOH. The 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. Following centrifugation (2 min, 1000 × g), the digesta were then stored at −80 °C until further experiments.

### 2.5. Cell culture and uptake experiments

The TC-7 subclone (ATCC Number: HTB-37) of the Caco-2 parental cell line was derived from a tumor isolated by J. Fogh (Chantret et al., 1994) and was a generous gift from Monique Rousset (Nancy University, France). The HT-29-MTX cell line (ATCC Number: HTB-38) was a kind gift from Dr. Técla Lesufreuf (Institut National de la Santé et de la Recherche Médicale, INSERM UMR S 938, Paris, France). Cultures were routinely maintained in 75 cm² plastic flasks (Nunc®, Nunc, Denmark) at 37 °C and 10% CO₂ in a CB-210 CO₂ incubator (Binder GmbH, Tuttingen, Germany). Cells were grown in Dulbecco’s modified eagle medium (DMEM + GlutaMAX™, Gibco, Karlsruhe, Germany), supplemented with 1% nonessential amino acids, 10% heat-inactivated fetal bovine serum (Gibco, Karlsruhe, Germany), 1% of a mixture of penicillin and streptomycin (10,000 units and 10 mg per mL, respectively) and subcultured weekly after reaching ca. 80% confluence.

For the monoculture (Caco-2 cells) uptake experiments, cells at passage numbers 80 to 85 were seeded into 6 well-plates (BD Bioscience, Erembodegem, Belgium) at a cell density of 5 × 10⁴ cells/cm², 15 days prior to experiments. For the co-culture (Caco-2/HT29-MTX cells) uptake experiments, cells (passage numbers of 86 to 93 for Caco-2 cells and 19 to 26 for HT29-MTX cells) were seeded into 6 well plates at a cell density of 5 × 10⁴ cells/cm², and at a cell ratio of 90:10 (Caco-2/HT-29-MTX). Repeated measurements over several days did not show any significant differences in TEER (transepithelial electrical resistance mΩ/cm²) values. For the uptake experiments, medium was removed and cells were washed with 2 mL of PBS (Gibco, Karlsruhe, Germany) before they were incubated for 4 h with 2 mL of medium diluted complete digesta (obtained after small intestinal digestion, centrifuged for 2 min at 1000 g) (1:8 digesta:DMEM, v:v) at 37 °C and 10% CO₂ as described earlier (Biehler & Kaulmann, et al., 2011). Cell studies were performed in triplicate and on three different days (n = 3; N = 3).

### 2.6. Colonic fermentation

For the preparation of inocula, the protocol was adapted from Bindelle et al. (2007). In short, mixed inocula were prepared from faeces of three different pigs, in order to reduce variation. These were placed in a plastic sealed syringe in a water-bath at 39 °C. Then, 17 g of each sample was placed into a plastic bag containing saturated CO₂ and 180 mL of buffer solution (composed of salt and minerals) and subjected for 60 s to a mechanical shaking with a Stomacher Lab Blender 400 (Seward Medical, Norfolk, UK). The solution was then filtered through a 250 μM mesh screen and the volume was adjusted to 820 mL with buffer solution to reach a concentration of 5% (w/v) faeces. For in vitro fermentation, 10 mL of the previously prepared complete digesta (obtained after centrifugation of the small intestinal digesta at 2 min and 1000 g) was then mixed with 20 mL of freshly prepared inoculum in a 100 mL glass bottle and incubated for 24 h at 39 °C.

### 2.7. Carotenoid and polyphenol extractions

Carotenoid extraction details from digesta and the cell culture are described elsewhere (Biehler & Kaulmann, et al., 2011). For the colonic fermented samples, 5 mL of sample was transferred into a 50 mL centrifuge tube and 2.5 mL of saturated NaCl and 7 mL of hexane:acetone (1:1, v/v) were added. The mixture was shaken for 1 min and centrifuged for 2 min at 4000 g. The supernatant was transferred into a 15 mL centrifuge tube. Then, 4 mL of pure hexane was added to the 50 mL centrifuge tube, shaken for 1 min and centrifuged for 2 min at 4000 g. Again, the supernatant was transferred to the 15 mL centrifuge tube. The extraction process with hexane was repeated once. The combined supernatants were dried under a stream of nitrogen at 30 °C.

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**Table 1**

<table>
<thead>
<tr>
<th>Native content in mg/100 g FW</th>
<th>Bioaccessibility in % of native content</th>
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</thead>
<tbody>
<tr>
<td>Total carotenoids</td>
<td>Total xanthophylls</td>
</tr>
<tr>
<td>± (SEM)</td>
<td>± (SEM)</td>
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<td>---</td>
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</tr>
<tr>
<td>Duchy</td>
<td>8.1</td>
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<tr>
<td>Kale</td>
<td>13</td>
</tr>
<tr>
<td>Scots Kale</td>
<td>1.0</td>
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<tr>
<td>Kalorama</td>
<td>0.1</td>
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<tr>
<td>Mean cabbage</td>
<td>5.5</td>
</tr>
<tr>
<td>Cherry Plum</td>
<td>2.0</td>
</tr>
<tr>
<td>Plum 620</td>
<td>0.6</td>
</tr>
<tr>
<td>Ersinger</td>
<td>0.5</td>
</tr>
<tr>
<td>Italian Plum</td>
<td>2.0</td>
</tr>
<tr>
<td>Mean plum</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Each value presents the mean ± SEM of three independent replications. Mean values in a column sharing the same capital letter symbol are not significantly different. Mean total carotenoids, xanthophylls and carotene values in a row sharing the same small letter are not significantly different (p < 0.05). FW = fresh weight. Nd = not detectable. ° Total polyphenols as determined by UPLC were the sum of: chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, kaempferol-3-glucoside, quercetin-3-O-galactoside, catechin, gallic acid, sinapic acid, p-coumaric acid, ferulic acid, 4-hydroxybenzoic acid.
For polyphenol detection, digested samples were not extracted in order to increase detectability. Prior to UPLC injection, 1 mL of sample was filtered through a 0.2 μm filter (PALL Life Sciences, Ann Arbor, MI, USA) into a UPLC vial. After the incubation of the cells with the diluted digesta, cells were washed with 2 mL of cold PBS, and then 500 μL of cold water:MeOH (1:1, v/v) mixture was added and cells were incubated during 20 min at room temperature. Cells of 6 wells were pooled to improve detection sensitivity. Samples were then sonicated for 5 min and centrifuged at 2000g for 10 min. The supernatant was then transferred into a new 15 mL centrifuge tube and the pellet was then re-extracted with 1 mL of 100% methanol, including a second sonication (5 min) and centrifugation step (10 min at 2000g). The supernatants were combined and dried under a stream of nitrogen at 40 °C. If the samples were not used directly, they were overlaid with a blanket of argon and stored at −80 °C.

2.8. Quantification of carotenoids and polyphenols

For the UPLC analyses of carotenoids, dried extracts were spiked with appropriate amounts of the IS, dissolved in 600 μL cold MTBE: methanol (1:1, v/v), and filtered through a 0.2 μm filter (PALL Life Sciences, Ann Arbor, MI, USA) into cold UPLC amber vials. For the UPLC analysis of polyphenols, extracts were filtered through a 0.2 μm filter (PALL Life Sciences, Ann Arbor, MI, USA) into a UPLC vial.

For carotenoid quantification, an Acquity UPLC BEH C18 column (Waters Inc., Zellik, Belgium, 2.1 × 100 mm, 1.7 μm particle size, set at 40 °C) was used in combination with a Waters Acquity UPLC® system (Milford, MA) equipped with a photodiode array detector. The eluents were (A) ammonium acetate (50 mM) in water: methanol (60:40, v/v) and (B) acetonitrile: dichloromethane (80:20, v/v) and the gradient was as follows: 0 min, 42% B, flow rate 0.35 mL/min; 4 min, 47% B; 13 min, 80% B, flow rate 0.40 mL/min; 18 min, 85% B; 29 min, 85% B, flow rate 0.35 mL/min; 30 min 42% B. The injection volume was 2.5 μL. Carotenoids were detected at 286 nm (phytoene), 440 nm (neoxanthin, violaxanthin), 450 nm (lutein, β-carotene and α-carotene) or 455 nm (zeaxanthin and β-cryptoxanthin) according to their absorption maxima. For quantification, 7 point – linear calibration curves were prepared with external standards for each compound, with concentrations ranging from 0.01 to 25 μg/mL. In addition, the internal standard was measured as an additional chromatographic control.

The detection of polyphenols was adapted from Deussser, Guignard, Hoffmann, and Evers (2012). For the quantification of polyphenols, a Waters Acquity UPLC® system equipped with a photodiode array detector was employed. For separation, an Acquity UPLC® HSS T3 column (1.8-μm particle size, 2.1 × 100 mm) with a flow rate of 0.75 mL/min at 50 °C was used. The eluents were 0.1% (v/v) formic acid in water (A) and 0.1% formic acid in acetonitrile (B), and the gradient was as follows: 0 min, 5% B; 9.27 min, 5% B; 13.53 min, 14% B; 22.60 min, 35% B; 23 min, 95% B; 25 min, 95% B; 26 min, 5% B. The injection volume was 10 μL. Polyphenols were detected at 254 nm, 280 nm, 300 nm or 320 nm, according to their absorption maxima. For quantification, 7 point – linear calibration curves were prepared with external standards for each compound, with concentrations ranging from 0.01 to 50 mg/L. Samples with higher concentrations were appropriately diluted with water/methanol (90:10, v/v). The sum of polyphenols (UPLC) was detected as the sum of the 18 individual polyphenols.

Total phenolic content following kitchen preparation procedures was determined with Folin–Ciocalteu’s phenol reagent as described by Kaulmann et al. (2014), based on spectrophotometric analysis (DU800 UV/Visible spectrophotometer, Beckman Coulter, Palo Alto, CA, USA).

2.9. Data interpretation

Unless otherwise stated, all data represent mean ± standard error of mean (SEM). All data was evaluated by SPSS vs 19.0 (IBM, Chicago, IL, USA). Normal distribution of results and equality of variance were verified by Q–Q plots and box plots, respectively. Data was log-transformed to achieve normal distribution. For predicting differences in carotenoid and polyphenol profiles following selected kitchen preparation procedures, differences in bioaccessibility of carotenoids and polyphenols after in vitro digestion, differences in the cellular uptake and following colonic fermentation of carotenoids and polyphenols between the different cell models, linear mixed models were developed with carotenoid or polyphenol content as the dependent variables and kitchen procedures, carotenoid type, family (cabbage, plum) and cabbage and plum varieties (nested within family as random factor) as independent variables. P-values below 0.05 (2-sided) were considered statistically significant. Significant Fisher–F-values were followed by either Bonferroni or LSD tests (for comparison of up to 3 groups). All recoveries given represent percentages of the respective native content.

3. Results

No significant differences were detected for carotenoids following different kitchen treatment procedures (Fig. 1). For polyphenols, freezing followed by thawing did not alter total phenolic content significantly, while chopping followed by boiling and chopping followed by steaming significantly decreased total polyphenol content by 8% and 51%, respectively (p < 0.05, Fig. 2).

The bioaccessibility of total carotenoids from cabbage and plum varieties differed significantly, and was highest in Kale and lowest in Scots Kale (Table 1). Similarly, Plum 620 showed highest and Italian Plum lowest bioaccessibility. Bioaccessibility of total carotenoids from plum varieties vs. cabbage varieties did not differ significantly. Xanthophylls (the sum of lutein and beta-cryptoxanthin, when present) showed generally a significantly higher bioaccessibility than carotenones (p < 0.001) in the different kitchen treatment procedures.
Fig. 2. Effect of different kitchen procedures on total polyphenol content, as determined via the Folin–Ciocalteu assay. The effect of freezing (overnight, −20 °C) combined with thawing (freeze–thaw) was compared to kale crushed under liquid nitrogen and stored until analysis (−80 °C, ctrl freeze–thaw), and the effect of and chopping and boiling (chop/boil), chopping and steaming (chop/steam) as well as chopping and freezing and thawing (chop/freeze–thaw) vs. chopping only (kale cut into ca. 50 cm² pieces, than crushed under liquid nitrogen and stored at −80 °C until analysis, ctrl chop) on total phenolics was evaluated. Each bar represents the mean ± SD of three independent replicates. For all trials, kale was used. Values sharing a common letter are not significant different.

4. Discussion

In the present study, we investigated bioavailability aspects of carotenoids and polyphenols from selected plum and cabbage varieties, together with the impact of typical kitchen procedures and colonic fermentation. The findings emphasize the inter-variety variability of bioavailability and cellular uptake of phytochemicals, and that cellular uptake (of carotenoids) was not compromised by the protection of an additional mucus-layer, which rather increased availability. Better xanthophyll bioaccessibility was counterbalanced by lower cellular uptake, compared to carotenones. A high recovery of carotenoids following colonic recovery was also demonstrated. In addition, carotenoids in cabbage appeared not to be significantly influenced by the selected kitchen procedures, in contrast to total phenolics, which were especially negatively influenced by boiling and steaming.

Kitchen preparations, similar to food processing, may be a double-edged sword with respect to phytochemical bioavailability. While it has been highlighted that e.g. maceration and heat application can be beneficial for both carotenoid (Murador, da Cunha, & de Rosso, 2014; Unlu et al., 2007) and polyphenol availability (Bohn, 2014; Bugianesi et al., 2004; Kamiloglu et al., 2014), due to improved release from the matrix and solubility during digestion, it also poses the risk for losses through thermal degradation (Fratianni, Cinquanta, & Panfili, 2010; Jaiswal & Abu-Ghanman, 2013). For instance, Fratianni et al. (2010) showed that heating orange juice to 85 °C decreased carotenoid content by 50%. In the present study, the decrease of carotenoid content was insignificant, about 4–12%, possibly due to the protection by the cell walls or other matrix components. Polyphenol content however appears more prone to degradation. For example, fennel total polyphenol content decreased by 85–90% after microwave heating (Jaiswal & Abu-Ghanman, 2013), which is in line with our results (51–89%). Freezing or thawing may likewise result in cell wall maceration, liberating bioactive compounds. In a study done on summer fruits, freezing appeared to increase the detectable total carotenoid content of apricots by 21% and the anthocyanin content of plums by 55% (Leong & Oey, 2012). In the present study, freezing also appeared, though not significantly, to increase the total carotenoid content of Kale, while the total polyphenol content was significantly decreased.

The observation that only polyphenols but not carotenoids were negatively influenced by boiling/steaming, though observed only
The decrease of polyphenol content due to heating processes was polyphenol-oxidase (Schinella et al., 2010; Terefe et al., 2015). Degradation by enzymes prior to their deactivation, such as carotene did not differ significantly.

Carotenoids between the varieties, while the type of carotenoid (total, xanthophyll, isomers) varied. Different letters indicate significant statistical differences in total xanthophylls lutein, beta-cryptoxanthin and neoxanthin/violaxanthin and their isomers. Total carotenes include alpha-and beta-carotene and isomers, total xanthophylls lutein, beta-cryptoxanthin and neoxanthin/violaxanthin and their isomers. Different letters indicate significant statistical differences in total carotenoids between the varieties, while the type of carotenoid (total, xanthophyll, carotene) did not differ significantly.

Within a single matrix, may be associated with their additional degradation by enzymes prior to their deactivation, such as polyphenol-oxidase (Schinella et al., 2010; Terefe et al., 2015). The decrease of polyphenol content due to heating processes was already observed in another study (Xu et al., 2014). The degradation was significantly lower (38%) in the case of steaming, emphasizing, as pointed out earlier, that direct avoidance of hot water may be superior to preserve polyphenol content (Faller & Fialho, 2009). Indeed, phenolic compounds are highly hydrophilic molecules and losses through leaching might also occur (Rawson, Hossain, Patras, Tuohy, & Brunton, 2013).

Bioaccessibility is a pre-requisite for later absorption and is a crucial step for bioavailability (Lemmens et al., 2014). The present results indicate that carotenoids from plum varieties may be slightly better micellarized than carotenoids from cabbage varieties, although results did not reach significance in the chosen model with varieties as random factor, and their total amount in plums (Table 1) and therefore also their micellarized amounts were lower as opposed to cabbages. Schweiggert and Carle (in press) showed that carotenoids in carrots and tomatoes were present in chromoplast in large crystalloid form, while in mango and papaya they occurred in globular and tubular substructures in liquid-crystallize form, with the latter form showing higher bioaccessibility. It can only be speculated which form of carotenoids is present in cabbage and plum, but due to their association with chloroplasts and chromoplasts, respectively, their presence as crystalloids and in liquid-crystallized form, respectively, can be hypothesized (Schweiggert & Carle, in press). The presence of carotenoids in crystalloid form such as from cabbage would limit the transfer to the aqueous phase during extraction (Palmero et al., 2014). The fact that in the present study, we did not find large, significant differences between the bioaccessibility may be in part due to the relatively large intra-individual variation of carotenoids within both cabbages and plums. The difference may also in part be explained by the varying carotenoid profile encountered, as xanthophylls are typically better micellarized than carotenes (Furr & Clark, 1997), which was also found in the present study, and higher amounts of xanthophylls were encountered in the plum varieties.

Polyphenol bioaccessibility was quite low, and most likely compromised due to our in vitro digestion model, employing cream as a micellarization enhancer, so that only some polyphenols were detected following digestion. Earlier reports have stated that the addition of milk can indeed reduce polyphenol content, due to the formation of complexes with proteins (Serafini et al., 2009). However, some studies have shown that the addition of dietary fat may also increase the bioaccessibility of polyphenols (Ortega, Reguant, Romero, Macia, & Motilva, 2009), especially of the more apolar ones such as aglycons, which likewise may require micellarization. In this respect, other lipid choices such as oils may have resulted in superior bioaccessibility of both carotenoids and polyphenols, given that they can be sufficiently emulsified during digestion. Nonetheless, some studies have shown that polyphenol content decreased by 40–70% after intestinal digestion, and that the remaining polyphenols were mostly found in the non-absorbable fraction (Bouayed, Deusser, Hoffmann, & Bohn, 2012; Correa-Betanzo et al., 2014). This non-absorbable fraction is then, together with the non-absorbed or re-excreted polyphenols, under physiological conditions, undergoing further colonic metabolism, leading to the production of additional metabolites (Mosele,
This colonic metabolism is an important step in polyphenol bioavailability and normal in vitro digestion without colonic fermentation would likely underestimate polyphenol bioaccessibility.

The cellular uptake of total carotenoids was relatively low compared to previous studies (Biehler, Hoffmann, Krause, & Bohn, 2011) and varied between 0.3% and 4.1%. Contrary to bioaccessibility, carotenones were taken up significantly better than xanthophylls, despite the fact that xanthophylls may reside at the surface of the mixed micelles (while carotenes are located in the core), making them potentially more available for cellular uptake (Sy et al., 2012). However, as transporters may be involved in carotenoid absorption (CD36, SRB-1, NPL-1P), it cannot be excluded that certain carotenones may be taken up preferably (Biehler, 2014). Nevertheless, data on colonic fermentation of our digesta showed that the mucus layer was not influencing the absorption of curcumin (Guri, Gulsener, & Corredig, 2013), a rather apolar polyphenol. Whether the colostrum may even foster a better uptake than the monoculture remains speculative – it cannot be excluded that the mucus produced by the HT-29-MTX cells aid to a superior binding of the mixed micelles to the cells, facilitating the uptake of carotenones, e.g. via increasing the unstirred water-layer area.

With respect to colonic fermentation, blank samples contained relatively high carotenoid concentrations, especially zeaxanthin, resulting from the faeces of pigs, possibly originating from corn containing feedingstuffs. However, carotenones were quantifiable after colonic fermentation of our digesta and recovery was between 4% and 25% for total carotenones. Colonic fermentation of the digesta did not considerably affect total carotenoid content or profile, although the overall recovery was low. Similar results were observed in another study with green leafy vegetables, were the colonic recovery of beta-carotene and lutein varied between 2% and 11% (Serrano, Goni, & Saura-Calixto, 2005). Care should be taken not to over-interpret results, as pig microflora certainly shows some differences to human microflora, though the present aim was to find relative differences between the matrices, and earlier studies on bioactive compounds have employed pig faeces as a model for human fermentation (Labib, Hummel, Richling, Humpf, & Schreier, 2006). Nevertheless, data on colonic fermentation of both carotenones and polyphenols, their resulting structures and their bioactivity are perceived as a gap of knowledge (Bohn et al., 2015). To our knowledge, no reports on carotenone degradation following colonic fermentation of cabbage and plums exist, and future studies in this domain would be much warranted, as also colonic uptake of carotenones cannot be excluded.

5. Conclusions

When employing kitchen heating procedures, those requiring less liquid such as steaming were superior in preserving bioactive constituents. Following simulated digestion, the bioaccessible fraction varied considerably from the native profile and from variety to variety, with a similar fractional bioaccessibility and cellular uptake for plums and cabbages. While xanthophylls showed a higher bioaccessibility, cellular uptake of carotenones was higher, which may be related to specific uptake transporters. The use of cream as a fat source to increase carotenone bioaccessibility compromised polyphenol bioaccessibility. Finally, it appeared that an additional layer of mucus as produced in the present study by mucus producing cells (HT-29-MTX) did not compromise carotenone uptake, and a large proportion of carotenones remained intact following colonic fermentation, which warrants further studies to investigate potential colonic availability.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.10.049.

References


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