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Assessing the Fate and Bioavailability of Glucosinolates in Kale (*Brassica oleracea*) Using Simulated Human Digestion and Caco-2 Cell Uptake Models

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ABSTRACT: Glucosinolates and their hydrolysis products were characterized in fresh and in *in vitro* gastric and intestinal digesta of Dinosaur kale (*Brassica oleracea* L var. *palmifolia* DC). In fresh kale, glucoraphanin, sinigrin, gluconapin, gluconasturtiin, glucoerucin, glucobrasscin, and 4-methoxylglucobrassicin were identified. After 120 min of gastric digestion, the levels of glucoraphanin, sinigrin, and gluconapin decreased, and no glucoerucin or glucobrasscin was detected. However, a concomitant increase in the glucosinolate hydrolysis products allyl nitrile, 3-butenyl isothiocyanate, phenylacetonitrile, and sulforaphane was observed. This trend continued through intestinal digestion. After 120 min, the levels of allyl nitrile, 3-butenyl isothiocyanate, phenylacetonitrile, and sulforaphane were 88.19 ± 5.85, 222.15 ± 30.26, 129.17 ± 17.57, and 13.71 ± 0.62 pmol/g fresh weight, respectively. Intestinal digesta were then applied to Caco-2 cell monolayers to assess the bioavailability. After 6 h of incubation, no glucosinolates were detected and the percentage of total cellular uptake of the glucosinolate hydrolysis products ranged from 29.35% (sulforaphane) to 46.60% (allyl nitrile).

KEYWORDS: *kale, glucosinolate, in vitro digestion, caco-2 cell, bioavailability*

INTRODUCTION

Cruciferous vegetables are a popular food crop consumed worldwide and include numerous species and cultivars such as kale, broccoli, cabbage, cauliflower, watercress, and Brussels sprouts.[1] Kale (*Brassica oleracea* var. *acephala* L.), often touted as a superfood, is grown in the United States, Europe, and Asia and is cultivated throughout the year for its leaves and flower buds (i.e., kale raab).[2] The tender leaves, which usually appear 3 months after budding, are consumed either fresh, steamed, stir-fried, or boiled.[3] Kale contains a high content of dietary fiber, essential amino acids, vitamins, flavonoids, and biologically active glucosinolates.[4,5]

Glucosinolates are unique to cruciferous vegetables,[6] and demonstrate protective effects against many types of cancers including gastric, bladder, colorectal, prostate, and breast cancers.[7−9] These exist in plant tissues as glycosides. Glucosinolates have no reported health benefits, but when plant tissue is damaged (e.g., chewed or cut) glucosinolates glycosides are hydrolyzed by the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.1.147). This activity generates biologically active products that include isothiocyanates, thiocyanates, nitriles, and indoles.[10,11] The hydrolysis products of glucosinolates are thought to be primarily responsible for the chemoprotective effects observed with cruciferous vegetables.[11] Particularly, the isothiocyanates (ITCs) act as anticancer agents by inhibiting phase I enzymes responsible for bioactivation of carcinogenic compounds and through the induction of phase II detoxification enzymes that affect xenobiotic transformation.[12,13] Many studies showed that ITCs have also have potent bactericidal, fungistatic, and fungicidal activities.[14,15]

Human digestion is a complex multistage process. It involves the mechanical and chemical breakdown of foods which facilitates the release of embedded nutrients so they can be absorbed into the body through intestinal mucosal cells.[16,17] Food is reduced in size in the mouth and stomach whereas the small intestines are the major site of nutrient absorption. The stomach contains gastric acids, bile salts and digestive enzymes which work to homogenize and transform food.[18] In the intestine, the gastric digesta is further dissolved and nutrients are absorbed through the intestinal walls. Bioaccessibility is defined as the amount or fraction of food that is released from a food into the digestive juices with the potential to be absorbed by the small intestine during digestion.[19] In contrast, bioavailability refers to the fraction of compound absorbed across the gastric and intestinal walls into the bloodstream and therefore has the potential to enter systemic circulation.[20]

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Increasingly, various *in vitro* digestion models are used to estimate bioaccessibility and bioavailability.[21−24] *In vitro* digestion models offer the advantage of cost-efficiency, ease of control, and independence from physiological effects. *In vitro* digestion models also have excellent reproducibility and can provide a good approximation of *in vivo* digestion.[24]

The human epithelial cell line Caco-2 is widely used as an *in vitro* model of the human epithelial barrier in order to help identify the transport, and retention of substances across gastrointestinal tissues.[25] This cell line has similar morphological and biochemical characteristics as enterocytes (e.g., polarity, tight junctions, specific transport systems, and enzymes) after it becomes differentiated in culture.[26] Caco-2 cells are used in numerous studies to estimate the bioavailability of important classes of phytochemicals including carotenoids, polyphenols, and anthocyanins from whole foods including cabbage.[23,27,28] To date, there are no studies assessing the bioaccessibility and bioavailability of glucosinolates from whole kale, and little is known regarding the profile of glucosinolates and their hydrolysis products available in gastrointestinal contents with respect to digestion time.

To address this, the human digestion of fresh kale was simulated using an *in vitro* digestion model.[21] This system was sampled at 30 min intervals during 2 h of gastric digestion and during 2 h of intestinal digestion. The range of glucosinolates and their hydrolysis products in the gastric and intestinal digesta were identified using ultra high-performance liquid chromatography−quadrupole time-of-flight tandem mass spectrometry (UHPLC−QTOF-MS/MS) and quantified using HPLC. The transport of the identified glucosinolates and glucosinolate hydrolysis products in gastric and gastrointestinal digesta was evaluated in Caco-2 cell monolayers differentiated into intestinal epithelial cells. Measuring the glucosinolates and glucosinolate hydrolysis products in gastric and intestinal digesta provides key information on the bioaccessibility of these compounds, while measuring the transport across Caco-2 cell monolayers models provides key information on the bioavailability of these compounds.

MATERIALS AND METHODS

Chemicals and Kale Samples. Glucosinolates standards, including glucoraphanin (≥98%), sinigrin (≥99%), gluconapin (≥98%), and gluconasturtiin (≥98%) were purchased from Extrasynthese (Genay Cedex, France). All solvents used were of HPLC and LC/MS grade and purchased from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Fairlawn, NJ).

Dinosaur kale was grown at the Student Farm at the University of California Davis under agronomic supervision. The kale was grown from seedlings and harvested at ∼110 days from sowing from at least 10 different randomly selected plants in December 2017 and in April 2018 to give two harvest replicates. Fresh samples were stored at 4 °C for <24 h prior to *in vitro* digestion.

Simulated in Vitro Digestion. Fresh kale was digested following previously reported methods.[21,29,30] Fresh kale was cut with a knife approximately 1 cm length and finely chopped with a food processor (Black and Decker, FP2500B) at low speed for 60 s (15 s × 4 times) to simulate breakdown during mastication. A 70 mL aliquot of simulated gastric juice was loaded into a human gastric simulator (HGS) before adding samples.[31] The chopped kale (100 g) was mixed with 20 mL of simulated saliva for 30 s, and then transferred into the HGS. The gastric juice secretion started immediately after the sample was introduced into the HGS and continued at 2.5 mL/min. Every 30 min, 50 mL of digesta fluid was collected in a glass bottle. After 2 h of gastric digestion, the HGS was stopped. The digesta remaining in the HGS was pooled, and 80 g of digesta was mixed with 54 mL of intestinal juice to generate the intestinal digesta. The intestinal digestion was completed by incubation in a shaking water bath (Thermo Scientific Inc., Marietta, OH) at 37 °C and 100 cycles per minute for 2 h. Every 60 min, 30 g of digested sample was collected in a glass bottle. For each sample from the gastric phase, pH, total acidity, particle size, moisture, and glucosinolate and glucosinolate hydrolysis products content were measured. For each sample from the small intestinal phase, glucosinolates and their glucosinolate hydrolysis products were measured.

Moisture Content, pH, Total Acidity of Fresh and Digested Kale. Moisture content of the sample was measured gravimetrically using a vacuum oven (Thermo Scientific Inc., Marietta, OH) at 110 °C for 20 h. The pH was measured using a pH meter (Fisher Scientific Inc.). Titratable acidity (expressed as w/w % citric acid) was measured via potentiometric titration with 0.1 N NaOH until the pH reached 8.2 ± 0.05.

Particle Size and of Fresh and Digested Kale. To measure the particle size, the solid fraction of the gastric digesta samples were separated from the liquid phase by filtration with a double layered cotton cloth (17.3 × 14.2 threads per cm). Aliquots of solid particles (∼1 g) were transferred onto a Petri dish. A volume of ∼10 mL of water was added to disperse the particles, and samples were gently agitated for 5 min on an orbital shaker to allow particles to separate without causing any further particle hydrolysis. Images were taken using a digital camera (Canon Powershot SD 1300IS, Canon USA, Melville, NY). Images were analyzed as previously described to determine the median particle area and the number of particles per gram of sample.[32]

Caco-2 Cells: Uptake and Transport of Glucosinolates and Their Hydrolysis Compounds. Caco-2 cell monolayers were used to evaluate the bioavailability of the glucosinolates and their hydrolysis products in the intestinal digesta. Caco-2 human intestinal cells were cultured at 37 °C and 5% (v/v) CO_2 atmosphere in minimum essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum, antibiotics (50 U/mL penicillin, and 50 μg/mL streptomycin), 1% (v/v) of 100× nonessential amino acids, and 1 mM sodium pyruvate. The medium was replaced every 3 days during cell growth and differentiation. For the experiments, cells were seeded in 6-well plate polyester membrane permeable support inserts (30 mm, 0.4 μm pore size, Corning Inc., Corning, NY) at a density of 1×10^5 cells/well. Monolayers were used 21 days after reaching confluence to allow for full differentiation into intestinal epithelial cells. Cells were used between passages 3 and 15. All the experiments were performed in serum- and phenol red-free MEM. Transepithelial electrical resistance (TEER, Ω cm^2) values were measured before and after adding the sample using a Millicell-ERS Volt-Ohm Meter (Millipore, Bedford, MA) as previously described.[33]

Caco-2 cells were washed twice with MEM medium. An aliquot of 250 μL of freshly prepared gastro-intestinal digesta (obtained after 2 h digestion) was added to the upper chamber (apical side) and 750 μL of MEM was added to the lower chamber (basolateral side). Cells were incubated at 37 °C for 6 h, and the TEER was measured to ensure the integrity of the monolayer. Medium from both sides of the insets was collected, and the cell monolayers were washed twice with ice-cold PBS. Cells were collected in 200 μL of PBS, sonicated, and centrifuged, and the supernatants were collected and stored at −20 °C for a maximum of 2 days until HPLC analysis. Cell studies were performed in triplicate.

Absorption (cellular uptake) and transport (basolateral secretion) efficiency are expressed as the percentage of glucosinolate hydrolysis products detected inside Caco-2 cells and that in the basolateral compartment, with respect to the original glucosinolates and their hydrolysis products originally added to the apical side. The bioavailability of glucosinolate hydrolysis products is defined as the total content of each glucosinolate hydrolysis compound absorbed by the Caco-2 cells (retention and transport) from the intestinal digesta (at 120 min), divided by the total added in the intestinal digesta. Typically, percentage bioavailability is expressed as a percent of the *initial* amount in the food product. However, as glucosinolates are extensively hydrolyzed in the stomach and intestine, the hydrolysis

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Table 1. Moisture Content, pH, and Total Acidity of Fresh Kale Before and During Simulated Gastric and Small Intestinal Digestion[a]

	moisture content (%, wet basis)	pH	total acidity (%) (citric acid)
fresh kale (before digestion)	83.39 ± 0.51 a	6.62 ± 0.12 c	0.23 ± 0.01 a
gastric digestion			
30 min	96.45 ± 1.43 b	5.20 ± 0.29 b	0.62 ± 0.01 c
60 min	96.22 ± 1.40 b	4.96 ± 0.26 ab	0.65 ± 0.00 c
90 min	96.08 ± 1.08 b	4.75 ± 0.11 a	0.69 ± 0.02 c
120 min	96.58 ± 0.72 b	4.47 ± 0.07 a	0.70 ± 0.02 c
intestinal digestion			
60 min	95.62 ± 1.39 b	7.01 ± 0.21 d	0.27 ± 0.00 a
120 min	95.51 ± 1.23 b	7.00 ± 0.16 d	0.35 ± 0.00 b

[a]Data are shown as mean ± SD of triplicate experiment. Means with different superscript letters in the same column are significantly different at $p < 0.05$.

products are reported as a percent of the original sample after intestinal digestion.

$$\text{bioavailability (\%)} = \frac{[\text{compound in basolateral}] + [\text{compound in cell monolayer}]}{[\text{compound in intestinal digesta}]} \times 100$$

Extraction of Glucosinolates. Glucosinolates were extracted from all samples (0.5 g) with 2 mL of boiling 70% (v/v) methanol in a hot water bath (90−92 °C) for 5 min and centrifuged at 20 000*g* for 10 min at 4 °C. The pellet was extracted a second time, and the two supernatants were combined. The glucosinolate extract was applied to a Mini Bio-Spin chromatography column (Bio-Rad Laboratories, Hercules, CA) containing 1 mL of cross-linked dextran gel (type G-25) anion exchange resin, which was preactivated with 20 mM sodium acetate (pH 5.5). Glucosinolates were removed from resins using desulfation carried out by the addition of 75 μL of purified aryl sulfatase (EC 3.1.6.1, type H-1 from *Helix pomatia*). The column was capped and allowed to stand at room temperature for 24 h. The desulfo-glucosinolates were eluted from the column with 1.5 mL distilled water. Eluates were filtered through a 0.2 μm syringe filter prior to injection onto the HPLC.

Authentic standards of glucosinolates were desulfated as described above and used for the identification and quantification of the peaks. Concentrations of individual desulfo-glucosinolates were determined from the experimental peak area using external standard calibration curves for each desulfo-glucosinolate across different ranges (depending upon the glucosinolate) and are expressed as micromoles per gram (μmol/g).

Desulpho-Glucosinolate Analysis Using HPLC. HPLC analysis was performed on an Agilent 1200 HPLC system coupled with a photodiode array (PDA) detector (Agilent Technologies, Memphis, TN). The chromatographic column used was a Waters symmetry 300 C_{18} column (75 mm × 4.6 mm, i.d. with 3.5 μm particle diameter, Waters, Franklin, MA) at 40 °C. A mobile phase composed of A (water) and B (acetonitrile) with a gradient elution of 0 min (0% B), 0−1 min (2% B), 2−35 min (2−35% B), 35−40 min (35−2% B), 41 min (0% B) was used in this study. The sample injection volume was 20 μL, and the flow-rate was set at 0.5 mL/min. Peaks were detected at 229 nm. Briefly, individual glucosinolates were identified and quantified in comparison with the retention time and external standard curve of four glucosinolate standards (glucoraphanin, sinigrin, gluconapin, and gluconasturtiin). Relative quantification of individual glucosinolates with no authentic standard was achieved using standard methods reported by ISO 9167-1.[34]

Identification of Desulpho-Glucosinolates Using UHPLC−QTOF-MS/MS. The identification of glucosinolates present in the HPLC peaks of extracts of fresh kale were confirmed using authentic standards and by collecting high-resolution QTOF-MS/MS spectra on an Agilent 1290 Infinity ultrahigh-pressure liquid chromatography system (UHPLC) interfaced to a 6445 quadrupole time-of-flight-tandem mass spectrometer (QTOF-MS/MS) with electrospray ionization (ESI) via Jet Stream technology (Agilent Technologies, Santa Clara, CA). The UHPLC was equipped with a binary pump with an integrated vacuum degasser (G4220A), an autosampler (G4226A) with thermostat (G1330B), and a thermostated column compartment (G1316C). Positive ESI mode was used for all compounds. The drying gas temperature and flow rate was 320 °C and 8.0 L/min, respectively. The sheath gas temperature and flow rate were 350 °C and 11 L/min, respectively. The nebulizer gas pressure, capillary voltage, and nozzle voltage were 35 psi, 3500 V, and 1000 V, respectively. Analysis was carried out using a scan from m/z 50 to m/z 1000. Mass accuracy was maintained by the use of a second reference nebulizer that continuously introduced purine and Hexakis (1H,1H,3H-tetrafluoropropoxy) phosphazine at a flow rate of 8−9 μL/min. Tandem mass spectrometry experiments were performed with the quadrupole set at medium resolution (m/z 4 amu) and fixed collision energies of 10, 20, and 40 were used to facilitate the identification of the glucosinolates based on the parent and fragmented ions.

Glucosinolate hydrolysis products in gastric and intestinal digesta were also confirmed using high-resolution QTOF MS/MS spectra with MassHunter software B07 (Agilent Technologies, Memphis, TN) and by comparing with authentic standards.

Statistical Analysis. HPLC peak areas were obtained in triplicate for each of the three independent measurements of the sample. HPLC peak areas were averaged, and the standard deviation (SD) was determined from the average of the three measurements. Statistical analysis was performed with the statistical analysis system (SPSS software package, version 22.0). Data were compared using repeated measures analysis of variance (ANOVA); $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Measurement of Moisture Content, pH, and Total Acidity. Moisture content was determined in fresh and

Table 2. Median Particle Area and Number of Particles per Gram of Sample in Fresh Kale and Kale Undergoing Gastric Digestion[a]

	median particle area (mm^2)	no. of particles per gram
fresh kale (no gastric digestion)	5.52 ± 2.75 c	1038 ± 354 d
gastric digestion		
30 min	5.93 ± 4.16 c	939 ± 785 d
60 min	5.58 ± 3.03 c	777 ± 561 d
90 min	4.15 ± 2.22 ab	844 ± 684 d
120 min	3.64 ± 0.23 a	945 ± 139 d

[a]Data are shown as mean ± SD of triplicate experiment. Means with different superscript letters in the same column are significantly different at $p < 0.05$.

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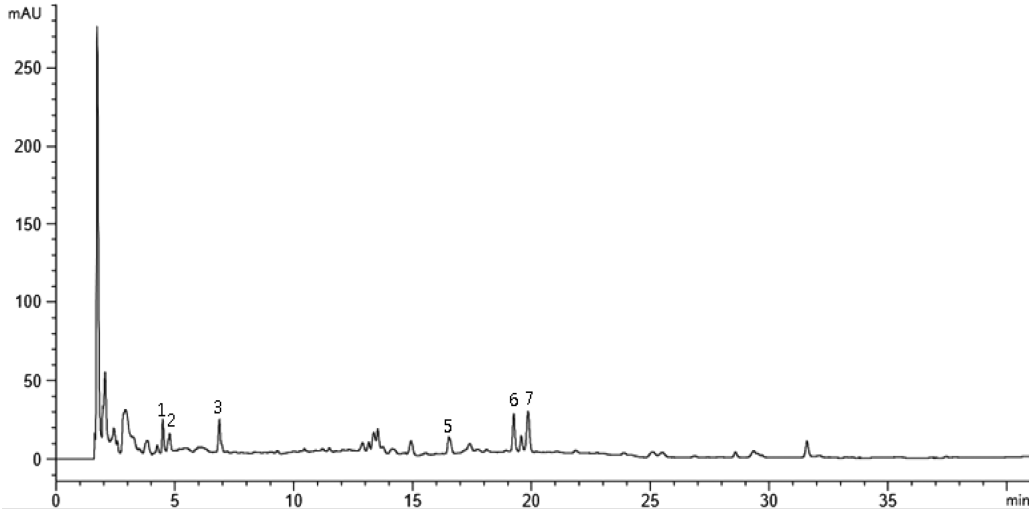

Figure 1. Typical HPLC chromatogram of desulfated glucosinolates isolated from fresh kale: 1, gluconaphanin; 2, sinigrin; 3, gluconapin; 4, gluconasturtiin; 5, glucoerucin; 6, glucobrasscin; 7, 4-methoxyglucobrassicin.

Table 3. UHPLC−QTOF-MS/MS Identification of Desulpho-Glucosinolates in Fresh Kale

no.	RT (min)	trivial name	formula	theoretical mass	measured mass	error (ppm)	response factor
1	4.59	glucoraphanin	$C_{12}H_{23}NO_7S_2$	357.0916	357.0917	0.2800	0.9
2	4.86	sinigrin	$C_{10}H_{17}NO_6S$	279.0776	279.0779	1.0750	1.0
3	7.05	gluconapin	$C_{11}H_{19}NO_6S$	293.0933	293.0937	1.2624	1.11
4	14.92	gluconasturtiin	$C_{15}H_{21}NO_6S$	343.1089	343.1091	0.5829	0.95
5	16.58	glucoerurin	$C_{12}H_{23}NO6S_2$	341.0967	341.0970	0.8795	0.9
6	18.98	glucobrassicin	$C_{16}H_{20}N2O_6S$	368.1042	369.1116	0.2717	0.29
7	20.16	4-methoxyglucobrassicin	$C_{17}H_{22}N_2O_7S$	398.1148	399.1143	1.7583	0.25

Table 4. Glucosinolate Content in Kale (pmol/g Fresh Weight) and in Gastric and Intestinal Digesta[a]

		gastric digestion			intestinal digestion		
glucosinolate	fresh kale	30 min	60 min	90 min	120 min	60 min	120 min
glucoraphanin (std)[b]	13.92 ± 2.30 d	14.31 ± 0.90 d	13.81 ± 1.54 d	9.33 ± 0.90 c	6.68 ± 0.83 b	5.40 ± 0.57 ab	4.76 ± 0.27 a
sinigrin (std)[b]	43.57 ± 6.68 f	34.02 ± 1.89 e	27.76 ± 1.18 d	23.43 ± 3.11 c	17.87 ± 2.65 b	14.70 ± 1.34 ab	12.07 ± 0.61 a
gluconapin (std)[b]	39.78 ± 2.75 d	34.59 ± 2.61 cd	32.22 ± 1.34 cd	33.97 ± 4.29 cd	29.46 ± 3.68 c	22.78 ± 3.63 b	16.86 ± 1.14 a
gluconasturtiin (std)[b]	9.86 ± 0.88 e	8.77 ± 0.90 d	8.26 ± 0.71 d	4.08 ± 0.94 c	2.67 ± 0.83 b	0.30 ± 0.30 a	0.25 ± 0.12 a
glucoerucin[c]	10.05 ± 1.33 c	7.72 ± 0.52 b	5.23 ± 0.65 a	5.49 ± 1.16 a	ND	ND	ND
glucobrasscin[c]	0.87 ± 0.18 c	0.79 ± 0.09 b	0.57 ± 0.21 ab	0.44 ± 0.77 a	ND	ND	ND
4-methoxyglucobrassicin[c]	10.84 ± 1.08 f	7.59 ± 1.88 e	6.70 ± 0.52 d	6.55 ± 0.62 d	5.81 ± 0.18 c	2.13 ± 0.95 b	1.09 ± 0.05 a

[a]Data are shown as the mean ± SD of triplicate experiments. Means with different superscript letters in the same row are significantly different at $p < 0.05$. [b]Absolute quantification against authentic standards. [c]Relative quantification against sinigrin.

digested kale samples (Table 1). Fresh kale contained 83.39% moisture, while gastric and intestinal digested kale contained higher moisture contents ranging from 95.51 to 96.58%. The moisture content was increased in the samples taken during *in vitro* gastric and intestinal digestion relative to the fresh kale. Similar to our results, previous studies have shown that the moisture content of apple or almond increased during *in vitro* gastrointestinal digestion.[35,36] This increase is due to a reduction in the particle size of the kale during in vitro digestion, which may result in damage to the cell structure, leading to water uptake when mixed with gastric secretions.

The pH and total acidity, which represent the amount of citric acid, is shown in Table 1. The pH of fresh kale was 6.62 and decreased with digestion time to 5.20−4.47. During the intestinal digestion, the pH was 7.00−7.01. The total acidity correlated with pH changes. The total acidity of fresh kale was 0.23% and increased during gastric digestion up to 0.62−0.70 but decreased again during the intestinal digestion step by 0.27−0.35%. The change in pH in the *in vitro* digestion model can be a very important factor because the action of digestive enzymes or digestive juice secreted in digestive organs is closely related to pH. The changes of pH and total acidity are considered to be closely related to the pH of the digestive fluids at each digestion stage. The pH of the oral cavity is weakly acidic (pH 5−7), and the pH of the mouth is rapidly decreased from 1 to 3 pH units due to the release of hydrochloric acid in the stomach and restored to a slightly acidic (pH 5−7) due to the high pH of bile acids and bicarbonate.[37]

Median Particle Area and Number of Particles per Gram Samples. The particle size distribution of kale before and during *in vitro* gastric digestion are given in Table 2. The median particle area in fresh kale (5.52 ± 2.75 mm^2) significantly ($p < 0.05$) decreased with digestion time to 3.64

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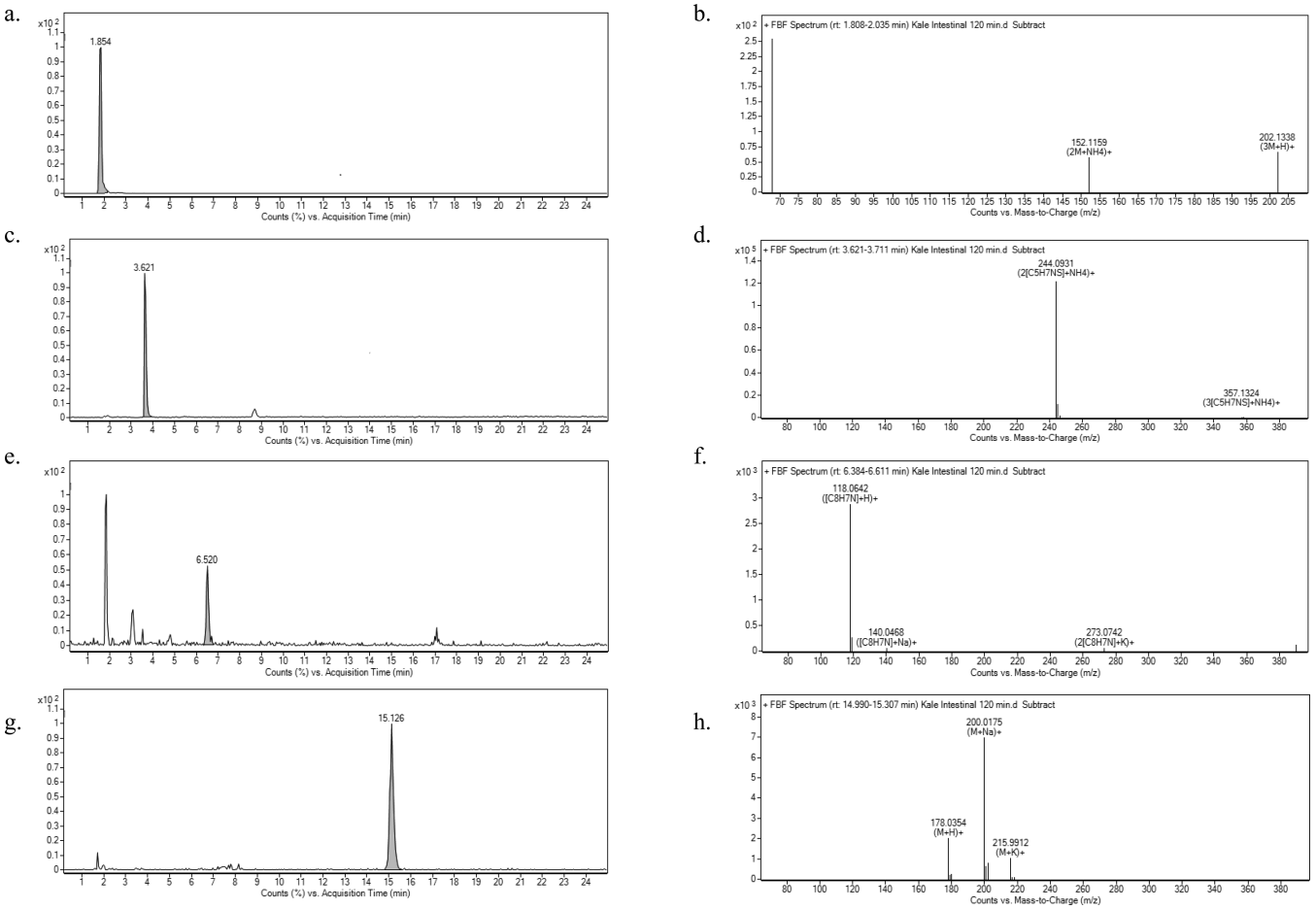

Figure 2. Extracted ion chromatogram of glucosinolate hydrolysis compounds and qTOF spectra obtained in ESI positive mode after *in vitro* digestion of kale. Allyl nitrile (a,b); 3-butenyl isothiocyanate (c,d); phenylacetonitrile (e,f); and sulforophane (g,h).

Table 5. Content of Glucosinolate Hydrolysis Products (pmol/g Fresh Weight) in Fresh Kale and in Gastric and Intestinal Digesta[a]

		gastric digestion				intestinal digestion	
glucosinolate hydrolysis compounds	fresh kale	30 min	60 min	90 min	120 min	60 min	120 min
allyl nitrile[c]	14.90 ± 1.37 a	59.68 ± 3.44 b	103.50 ± 11.45 d	197.98 ± 16.70 f	135.89 ± 5.39 e	88.19 ± 5.85 c	123.26 ± 3.83 e
butenyl isothiocyanate[c]	32.28 ± 8.35 a	61.65 ± 4.74 b	79.65 ± 9.79 c	107.65 ± 22.22 d	102.55 ± 5.05 d	222.15 ± 30.26 e	299.31 ± 25.43 f
phenylacetonitrile[c]	45.59 ± 1.21 a	43.43 ± 6.84 a	45.35 ± 10.91 a	50.55 ± 7.40 ab	46.32 ± 12.63 a	129.17 ± 17.57 b	200.43 ± 17.34 c
sulforaphane[b]	ND	ND	10.09 ± 0.98 a	11.54 ± 0.45 a	13.74 ± 0.45 b	13.71 ± 0.62 b	14.55 ± 1.03 c

[a]Data are shown as mean ± SD of triplicate experiment. Means with different superscript letters in the same row are significantly different at $p < 0.05$. [b]Absolute quantification against authentic standards. [c]Relative quantification against sulforaphane.

± 0.23 mm^2 at 120 min. Physical breakdown during gastric digestion would be expected and has been observed in previous *in vitro* and *in vivo* studies.[31,32]

The number of particles per g sample appeared to increase between 90 and 120 min; however, this increase was not statistically significant. When the median particle area was considered, it was observed that the area of the kale particles decreased to 24.82% and 34.06%, compared to the fresh kale samples after 90 and 120 min of gastric digestion, respectively.

Glucosinolates in Fresh Kale. A typical HPLC chromatogram of the desulfated glucosinolates isolated from fresh kale is given in Figure 1. Seven peaks were identified as glucosinolates (Table 3). Peaks 1, 2, and 4 corresponded to glucoraphanin (t_R 4.56 min), sinigrin (t_R 4.86 min), gluconapin (t_R 7.05 min), and gluconasturtiin (t_R 14.92 min), respectively, as confirmed with authentic standards. High-resolution QTOF-MS/MS allowed for the tentative identification of three additional glucosinolates corresponding to peaks 5 (glucoerucin), 6 (glucobrassicin), and 7 (4-methoxylglucobrassici) at retention times 16.58, 18.98, and 20.16 min, respectively. As commercially available standards are not available for all compounds, concentrations were established using relative quantitation against sinigrin.

Glucosinolates were quantified in undigested kale (Table 4). Sinigrin (43.57 ± 6.68 pmol/g FW) was the predominant glucosinolate, which correlates with previously reported data for kale.[38] Relatively high amounts of gluconapin (39.78 ±

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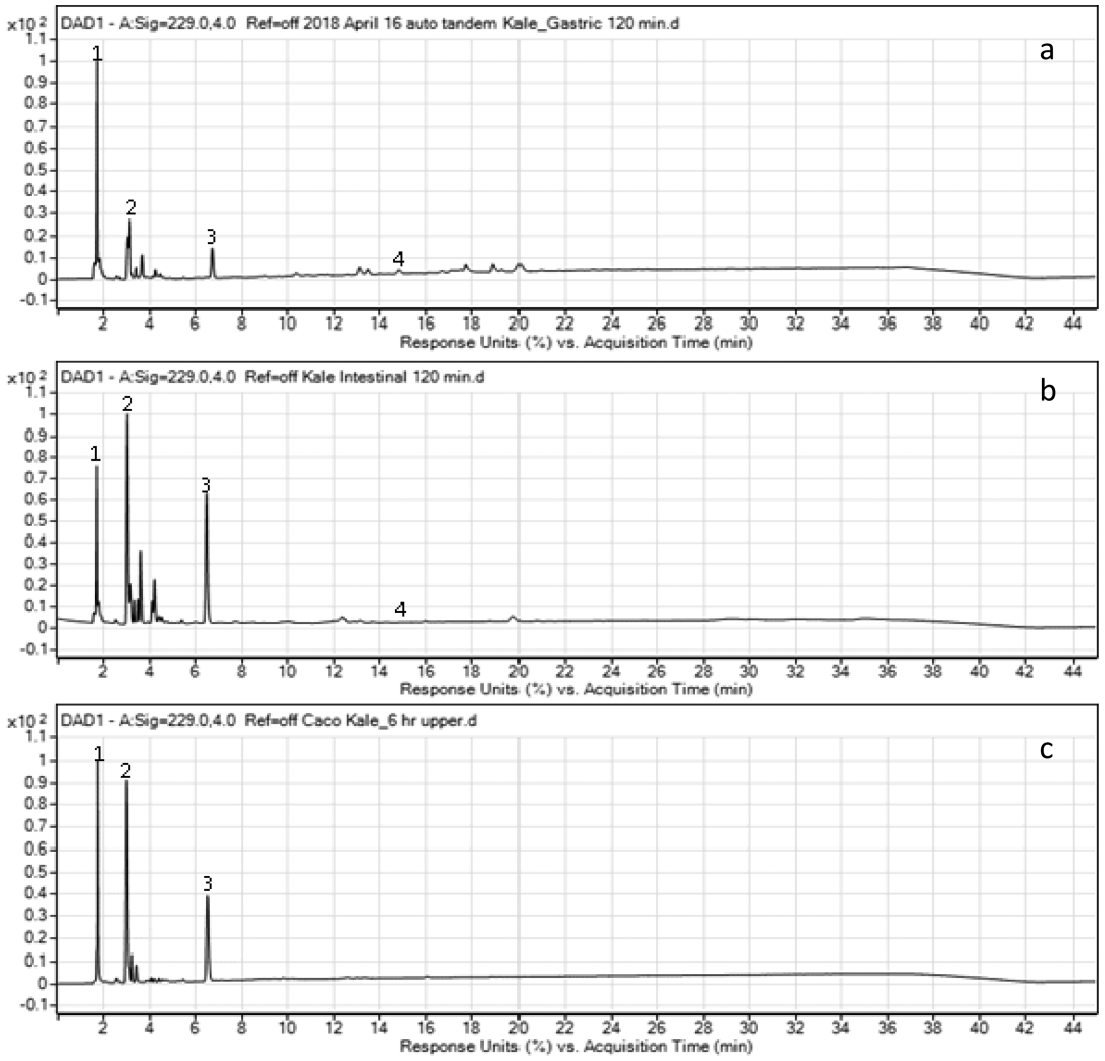

Figure 3. Typical HPLC chromatogram of glucosinolate hydrolysis compounds (a) after 2 h of *in vitro* gastro digestion, (b) after 2 h of *in vitro* intestinal digestion, and (c) after 6 h of incubation with the Caco-2 monolayer. 1, allyl nitrile (RT = 1.947); 2, butenyl isothiocyanate (RT = 3.621); 3, phenylacetonitrile (RT = 6.564); 4, sulforaphane (RT = 15.396).

2.75 pmol/g FW) and glucoraphanin (13.92 ± 2.30 pmol/g FW) were also found in undigested kale.

Influence of Gastric and Intestinal Digestion on Glucosinolates. The gastric digestion of glucosinolates was evaluated at 30, 60, 90, and 120 min in the HGS (Table 4). At 30 and 60 min, the levels of sinigrin, gluconastrurtiin, glucoerucin, glubrasscin, and 4-methoxyglucobrassicin were significantly reduced as compared with the levels in fresh kale. After 90 min of gastric digestion, levels of all glucosinolates were significantly reduced from levels in fresh kale with the exception of gluconapin. At 120 min, the levels of all glucosinolates were reduced and no glucoerucin or glucobrasscin could be detected in digesta. Data demonstrate that the profile of glucosinolates in the gastric digesta will vary based upon gastric digestion time and that gluconapin is the most resistant to hydrolysis during gastric digestion.

To evaluate intestinal digestion, the gastric digesta were allowed to remain in the HGS using conditions that simulate intestinal digestion and were sampled at 60 and 120 min. The levels of glucoraphanin, sinigrin, and gluconapin decreased by 29%, 32%, and 43%, respectively, in intestinal digesta as compared with gastric digesta at 120 min whereas losses of gluconasturtiin (99%) and 4-methoxyglucobrassicin (81%) were even more substantial ($p < 0.05$). Glucoerucin and glucobrasscin were not present in gastric digests after 120 min and were therefore absent from intestinal digesta. In a similar study, Vallejo et al.[39] found that the *in vitro* gastric digestion of broccoli resulted in 69% reduction of total glucosinolates, which were further decreased after intestinal digestion.

Glucosinolate Hydrolysis Products. Glucosinolates are thioglycosides that differ in the structure of the aglycone side chain. Glucosinolates can undergo hydrolysis via the activity of myrosinase to form unstable aglycones that can rearrange to form a wide array of biologically active compounds including epithionitriles, nitriles, thiocyanates, isothiocyantes, and oxazolidine-thiones. Myrosinase is active in fresh vegetables and in the stomach and small intestine.[40] Glucosinolate hydrolytic rearrangement is dependent upon pH. At a low pH ≤ 3 (i.e., the pH of the stomach), hydrolytic rearrangement favors formation of nitriles and at pH ≥ 7 (i.e., the pH of the small intestines) hydrolytic rearrangement favors formation of isothiocyanates.[41] Isothiocyanates are absorbed from the small intestine and in the colon, and metabolites are detectable in

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Table 6. Uptake and Transport of Glucosinolates and Their Hydrolysis Products after 6 h Incubation of Intestinal Digesta with Caco-2 Cells[a]

		apical compartment		basolateral compartment (transport)		cell monolayer (retention)		
cmpd	total added [intestinal digesta] (ng)	(ng)	(%)	(ng)	(%)	(ng)	(%)	bioavailability[d] (total cellular uptake, %)
allyl nitrile[c]	8.51 ± 0.26 b	4.54 ± 0.40 b	53.35 ± 6.30 a	2.61 ± 0.15 b	30.67 ± 0.82 b	1.53 ± 0.38 a	17.98 ± 0.74 b	48.60 ± 1.54 c
butenyl isothiocyanate[c]	46.71 ± 3.97 d	26.97 ± 0.23 d	57.73 ± 4.41 ab	13.15 ± 0.47 d	28.15 ± 1.40 b	5.08 ± 2.21 b	10.88 ± 3.82 a	39.03 ± 2.42 b
phenylacetonitrile[c]	23.46 ± 2.03 c	13.06 ± 1.22 c	55.67 ± 0.37 a	5.88 ± 0.11 c	25.06 ± 2.67 a	2.52 ± 0.92 a	10.74 ± 2.99 a	35.81 ± 0.32 b
sulforaphane[b]	2.58 ± 0.18 a	1.54 ± 0.31 a	59.69 ± 7.95 b	0.76 ± 0.22 a	29.46 ± 1.43 b	ND	0	29.35 ± 1.43 a

[a]The content of glucosinolate hydrolysis products (ng/mL intestinal digesta) was measured in cells and in the apical and basolateral compartments after incubating cell monolayers for 6 h with 250 μL of intestinal digesta obtained after 120 min of digestion added to the apical compartment. Data are shown as mean ± SD of triplicate experiments. Means with different superscript letters in the same column are significantly different at $p < 0.05$. [b]Absolute quantification against authentic standards. [c]Relative quantification against sulforaphane. [d]bioavailability (%) = [(compound in basolateral + compound in cell monolayer)/(compound in intestinal digesta)] × 100.

urine 2−3 h after consumption of Brassica vegetables in humans.[40]

Glucosinolate hydrolysis products are primarily responsible for the anticarcinogenic activity associated with consuming Brassica vegetables. These low-molecular weight compounds are able to rapidly diffuse into the cells of the intestinal epitheilium and modulate the expression of genes important to chemoprevention (e.g., those associated with xenobiotic metabolism, antioxidation, cell cycle regulation, apoptotic pathways, and stress response).[41−48] Understanding the formation and disposition of glucosinolate hydrolysis products in the gut is key for understanding the bioactive potential of Brassica foods. In general, levels of glucosinolate hydrolysis products are lower in undamaged fresh Brassica as compared to Brassica with insect and/or physical damage as damage allows glucosinolates contact with myrosinase. Herein, the hydrolyzed products identified in fresh kale include phenylacetonitrile (45.59 ± 1.21 pmol/g FW), 3-butenyl isothiocyanate (32.28 ± 8.35 pmol/g FW), and allyl nitrile (14.90 ± 1.37 pmol/g FW) (Figure 2 and Table 5).

Glucosinolate Hydrolysis Products in Gastric and Intestinal Digesta. Intact glucosinolates are not absorbed through the intestinal epithelium, and the hydrolysis products, which are absorbed, are the bioactive forms of glucosinolates.[40] Therefore, it is important to understand the disposition of bioactive glucosinolate hydrolysis products available for absorption in the small intestine. Phenylacetonitrile 3-butenyl isothiocyanate, allyl nitrile, and sulforaphane were identified in gastric digesta by 60 min of HGS (Figures 2 and 3 and Table 5). Allyl nitrile, 3-butenyl isothiocyanate, phenylacetonitrile, and sulforophane are the hydrolysis products of sinigrin, gluconapin, glucotropaeolin, and glucoraphanin, respectively.[40] Identities of these compounds were confirmed using high-resolution QTOF-MS/MS (Figure 2). Levels of allyl nitrile and 3-butenyl isothiocyanate increased significantly (228% and 166%, respectively) between 30 and 120 min of gastric digestion whereas levels of phenylacetonitrile did not change significantly. Sulforaphane, which was not detected in fresh kale, was observed only after 60 min of gastric digestion (10.09 ± 0.98 pmol/g FW), and levels were increased significantly at 120 min of gastric digestion (13.74 ± 0.45 pmol/g FW).

During intestinal digestion, the levels of allyl nitrile initially decreased and then increased after 120 min of intestinal digestion (Figure 3 and Table 5). Allyl nitrile is a hydrolysis product of sinigrin.[46] The levels of sinigrin decreased during intestinal digestion and may have contributed to the increase in allyl nitrile as the *in vitro* digestion model is a closed system.

Butenyl isothiocyanate levels were significantly higher in intestinal digesta (299.31 ± 25.43 pmol/g FW at 120 min) as compared with levels in gastric digesta at 120 min (102.55 ± 5.05 pmol/g FW). The levels of phenylacetonitrile, the hydrolysis product of gluconasturtiin, which did not change significantly during gastric digestion, increased significantly during intestinal digestion (280% at 60 min and 656% at 120 min). Sulforaphane levels were relatively low in intestinal digests as compared with the other glucosinolate hydrolysis products, reaching only 14.55 ± 1.03 pmol/g FW after 2 h of intestinal digestion.

In similar studies, Rodriguez-Hernandez et al.[49] performed *in vitro* digestion of different cultivars of raw freeze-dried broccoli and measured corresponding levels of glucoraphanin and sulforaphane. Both glucoraphanin and sulforaphane decreased during digestion of broccoli florets (cv. Naxos)

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whereas levels of glucoraphanin decreased by 7% and sulforaphane increased by 23% in the cultivar Viola. In additional studies in fresh broccoli, Ghawai et al.[47] demonstrated a 1.5−3.0 increase in the levels of sulforaphane after intestinal digestion showing that myrosinase in the intestinal digestion process was still active.

Together these results demonstrate that sinigrin is the most abundant glucosinolate in fresh kale (43.57 ± 6.68 pmol/g FW) and that other glucosinolates identified ranged from 0.87 ± 0.18 to 39.78 ± 2.75 pmol/g FW kale. In general, levels of all identified glucosinolates decreased during gastric and intestinal digestions. Whereas levels of glucosinolate hydrolysis products increased through gastric and intestinal digestions. The primary hydrolysis products formed in gastric digesta are allyl nitrile, butenyl isothiocyanate, and phenylacetonitrile.

Assessing Bioavailabilily. Caco-2 cells are a widely used and accepted model to evaluate the bioavailability of nutrients and drugs after oral ingestion.[48] The intestinal digesta at 120 min, and added to the Caco-2 cells, contained the glucosinolate hydrolysis products allyl nitrile (8.51 ± 0.26 ng), butenyl isothiocyanate (46.71 ± 3.97 ng), phenylacetonitrile (23.46 ± 2.03 ng), and sulforaphane (2.58 ± 0.18 ng) (Table 6). The intestinal digesta also contained the glucosinolates glucoraphanin (3 332.94 ± 0.77 ng), sinigrin (10 813.22 ± 2.18 ng), gluconapin (14 383.63 ± 3.87 ng), gluconasturtiin (182.09 ± 0.39 ng), and 4-methoxyglucobrassicin (684.12 ± 0.13 ng). Although both glucosinolates and the hydrolysis products were present in the intestinal digesta at 120 min, only the hydrolysis products were measured in the Caco-2 cells as no further hydrolysis was observed in intestinal digesta and because only the hydrolysis products are transported across intestinal epithelial cells. Recoveries of hydrolysis products in the Caco-2 cells were 101% allyl nitrile, 97% butenyl isothiocyanate, 91% phenylacetonitrile, and 89% for sulforophane.

After 6 h, the percentage of glucosinolate hydrolysis compounds in the apical compartment varied between 53.44 and 59.47%, whereas 25.19−30.61% of the glucosinolate hydrolysis products were transported into the basolateral compartment and 0−15.68% were retained in the cells in the Caco-2 monolayer. The percentage of total cellular uptake in the intestinal digesta ranged from 29.39% (sulforaphane) to 48.60% (allyl nitrile). These results indicate that buteneyl isothocyanate is significantly higher than other glucosinolate hydrolysis products after the consumption, and digestion of kale although allyl nitrile is the most bioavailable glucosinolate hydrolysis.

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Notes

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REFERENCES

(1) Abdull Razis, A. F.; Noor, N. M. Cruciferous vegetables: dietary phytochemicals for cancer prevention. *Asian Pac. J. Cancer Prev.* **2013**, *14* (3), 1565−1570.

(2) Hahn, C.; Müller, A.; Kuhnert, N.; Albach, D. Diversity of kale (*Brassica oleracea* var. *sabellica*): Glucosinolate content and phylogenetic relationships. *J. Agric. Food Chem.* **2016**, *64* (16), 3215−3225.

(3) Cartea, M. E.; Picoaga, A.; Soengas, P.; Ordás, A. Morphological characterization of kale populations from northwestern Spain. *Euphytica* **2003**, *129* (1), 25−32.

(4) Ayaz, F. A.; Glew, R. H.; Millson, M.; Huang, H.S.; Chuang, L.T.; Sanz, C.; Hayırlıoglu-Ayaz, S. Nutrient contents of kale (*Brassica oleraceae* L. var. *acephala* DC.). *Food Chem.* **2006**, *96* (4), 572−579.

(5) Velasco, P.; Cartea, M. E.; Gonzalez, C.; Vilar, M.; Ordas, A. Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* group). *J. Agric. Food Chem.* **2007**, *55* (3), 955−962.

(6) Holst, B.; Williamson, G. A critical review of the bioavailability of glucosinolates and related compounds. *Nat. Prod. Rep.* **2004**, *21* (3), 425−447.

(7) Atwell, L. L.; Beaver, L. M.; Shannon, J.; Williams, D. E.; Dashwood, R. H.; Ho, E. Epigenetic regulation by sulforaphane: Opportunities for breast and prostate cancer chemoprevention. *Curr. Pharmacol. Rep.* **2015**, *1* (2), 102−111.

(8) Kumar, G.; Tuli, H. S.; Mittal, S.; Shandilya, J. K.; Tiwari, A.; Sandhu, S. S. Isothiocyanates: a class of bioactive metabolites with chemopreventive potential. *Tumor Biol.* **2015**, *36* (6), 4005−4016.

(9) Veeranki, O. L.; Bhattacharya, A.; Tang, L.; Marshall, J. R.; Zhang, Y. Cruciferous vegetables, isothiocyanates, and prevention of bladder cancer. *Curr. Pharmacol. Rep.* **2015**, *1* (4), 272−282.

(10) Halkier, B. A.; Gershenzon, J. Biology and biochemistry of glucosinolates. *Annu. Rev. Plant Biol.* **2006**, *57*, 303−333.

(11) Hossain, M. S.; Ye, W.; Hossain, M. A.; Okuma, E.; Uraji, M.; Nakamura, Y.; Mori, I. C.; Murata, Y. Glucosinolate hydrolysis products, isothiocyanates, nitriles, and thiocyanates, induce stomatal closure accompanied by peroxidase-mediated reactive oxygen species production in Arabidopsis thaliana. *Biosci., Biotechnol., Biochem.* **2013**, *77* (5), 977−983.

(12) James, D.; Devaraj, S.; Bellur, P.; Lakkanna, S.; Vicini, J.; Boddupalli, S. Novel concepts of broccoli sulforaphanes and disease: induction of phase II antioxidant and detoxification enzymes by enhanced-glucoraphanin broccoli. *Nutr. Rev.* **2012**, *70* (11), 654−665.

(13) Lubelska, K.; Wiktorska, K.; Mielczarek, L.; Milczarek, M.; Zbroińska-Bregisz, I.; Chilmonczyk, Z. Sulforaphane regulates NFE2L2/Nrf2-dependent xenobiotic metabolism phase II and phase III enzymes differently in human colorectal cancer and untransformed epithelial colon cells. *Nutr. Cancer* **2016**, *68* (8), 1338−1348.

(14) Dias, C.; Aires, A.; Saavedra, M. J. Antimicrobial activity of isothiocyanates from cruciferous plants against methicillin-resistant *Staphylococcus aureus* (MRSA). *Int. J. Mol. Sci.* **2014**, *15* (11), 19552−19561.

(15) Manyes, L.; Luciano, F. B.; Manes, J.; Meca, G. *In vitro* antifungal activity of allyl isothiocyanate (AITC) against *Aspergillus parasiticus* and *Penicillium expansum* and evaluation of the AITC estimated daily intake. *Food Chem. Toxicol.* **2015**, *83*, 293−299.

(16) Alminger, M.; Aura, A.-M.; Bohn, T.; Dufour, C.; El, S.N.; Gomes, A.; Karakaya, S.; Martinez-Cuesta, M.C.; McDougall, G.J.; Requena, T.; Santos, C.N. *In vitro* models for studying secondary plant metabolite digestion and bioaccessibility. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13* (4), 413−436.

(17) Mennah-Govela, Y. A.; Bornhorst, G. M. Fresh-squeezed orange juice properties before and during *in vitro* digestion as

DOI: 10.1021/acs.jafc.9b03329
J. Agric. Food Chem. 2019, 67, 9492−9500

influenced by orange variety and processing method. *J. Food Sci.* **2017**, *82* (10), 2438−2447.
(18) Kong, F.; Singh, R. P. Disintegration of solid foods in human stomach. *J. Food Sci.* **2008**, *73* (5), R67−R80.
(19) Bornhorst, G. M.; Roman, M. J.; Dreschler, K. C.; Singh, R. Physical property changes in raw and roasted almonds during gastric digestion *in vivo* and *in vitro*. *Food Biophys.* **2014**, *9* (1), 39−48.
(20) Heaney, R. P. Factors influencing the measurement of bioavailability, taking calcium as a model. *J. Nutr.* **2001**, *131* (4), 1344S−1348S.
(21) Bornhorst, G. M.; Singh, R. P. Kinetics of *in vitro* bread bolus digestion with varying oral and gastric digestion parameters. *Food Biophys.* **2013**, *8* (1), 50−9.
(22) Read, A.; Wright, A.; Abdel-Aal, El-S. M. *In vitro* bioaccessibility and monolayer uptake of lutein from wholegrain baked food. *Food Chem.* **2015**, *174*, 263−269.
(23) Kaulmann, A.; André, C. M.; Schneider, Y.; Hoffmann, L.; Bohn, T. Carotenoid and polyphenol bioaccessibility and cellular uptake from plum and cabbage varieties. *Food Chem.* **2016**, *197*, 325−332.
(24) Moreda-Piñeiro, J.; Moreda-Piñeiro, A.; Romarís-Hortas, V.; Moscoso-Pérez, C.; López-Mahía, P.; Muniategui-Lorenzo, S.; Bermejo-Barrera, P.; Prada-Rodríguez, D. *In-vivo* and *in-vitro* testing to assess the bioaccessibility and the bioavailability of arsenic, selenium and mercury species in food samples. *TrAC, Trends Anal. Chem.* **2011**, *30* (2), 324−345.
(25) Liu, D.; Cheng, B.; Li, D.; Li, J.; Wu, Q.; Pan, H. Investigations on the interactions between curcumin loaded vitamin E TPGS coated nanodiamond and Caco-2 cell monolayer. *Int. J. Pharm.* **2018**, *551* (1−2), 177−183.
(26) Primavera, R.; Palumbo, P.; Celia, C.; Cinque, B.; Carata, E.; Carafa, M.; Paolino, D.; Cifone, M. G.; Di Marzio, L. An insight on in vitro transport of PEGylated non-ionic surfactant vesicles (NSVs) across the intestinal polarized enterocyte monolayers. *Eur. J. Pharm. Biopharm.* **2018**, *127*, 432−442.
(27) Liu, C. S.; Glahn, R. P.; Liu, R. H. Assessment of carotenoid bioavailability of whole foods using a Caco-2 cell culture model coupled with an *in vitro* digestion. *J. Agric. Food Chem.* **2004**, *52* (13), 4330−4337.
(28) Sun, D.; Huang, S.; Cai, S.; Cao, J.; Han, P. Digestion property and synergistic effect on biological activity of purple rice (*Oryza sativa* L.) anthocyanins subjected to a simulated gastrointestinal digestion *in vitro*. *Food Res. Int.* **2015**, *78*, 114−123.
(29) Roman, M. J.; Burri, B. J.; Singh, R. P. Release and bioaccessibility of beta-carotene from fortified almond butter during *in vitro* digestion. *J. Agric. Food Chem.* **2012**, *60* (38), 9659−9666.
(30) Phinney, D. M. *Design, Construction, And Evaluation of a Reactor Designed to Mimic Human Gastric Digestion*. Masters Thesis, University of California, Davis, CA, 2013.
(31) Guo, Q.; Ye, A.; Lad, M.; Ferrua, M.; Dalgleish, D.; Singh, H. Disintegration kinetics of food gels during gastric digestion and its role on gastric emptying: an in vitro analysis. *Food Funct.* **2015**, *6* (3), 756−764.
(32) Bornhorst, G. M.; Kostlan, K.; Singh, R. P. Particle Size Distribution of Brown and White Rice during Gastric Digestion Measured by Image Analysis. *J. Food Sci.* **2013**, *78* (9), E1383−E1391.
(33) Cremonini, E.; Mastaloudis, A.; Hester, S. N.; Verstraeten, S. V.; Anderson, M.; Wood, S. M.; Waterhouse, A. L.; Fraga, C. G.; Oteiza, P. I. Anthocyanins inhibit tumor necrosis alpha-induced loss of Caco-2 cell barrier integrity. *Food Funct.* **2017**, *8* (8), 2915−2923.
(34) ISO, International Organization for Standardization. *Method 9167-1 (E)*, 1992.
(35) Bornhorst, G. M.; Singh, R. P. Gastric digestion *in vivo* and *in vitro*: how that structural aspects of food influence the digestion process. *Annu. Rev. Food Sci. Technol.* **2014**, *5*, 111−132.
(36) Dalmau, M. E.; Bornhorst, G. M.; Eim, V.; Rosselló, C.; Simal, S. Effect of freezing, freeze drying and convective drying on *in vitro* gastric digestion of apples. *Food Chem.* **2017**, *215*, 7−16.
(37) Hur, S. J.; Lee, S. K.; Kim, Y. C.; Choi, I. W. Development of *in vitro* human digestion models for health functional food research. *Food Sci. Industry* **2012**, *45*, 40−49.
(38) Jeon, J.; Kim, J. K.; Kim, H.; Kim, Y. J.; Park, Y. J.; Kim, S. J.; Kim, C.; Park, S. U. Transcriptome analysis and metabolic profiling of green and red kale (*Brassica oleracea* var. *acephala*) seedlings. *Food Chem.* **2018**, *241*, 7−13.
(39) Vallejo, F.; Gil-Izquierdo, A.; Pérez-Vicente, A.; García-Viguera, C. *In vitro* gastrointestinal digestion study of broccoli inflorescence phenolic compounds, glucosinolates, and vitamin C. *J. Agric. Food Chem.* **2004**, *52* (1), 135−138.
(40) Johnson, I. T. Glucosinolates: bioavailability and importance to health. *Int. J. Vitam. Nutr. Res.* **2002**, *72* (1), 26−31.
(41) Fenwick, G. R.; Heaney, R. K.; Mullin, W. J.; VanEtten, C. H. Glucosinolates and their breakdown products in food and food plants. *Crit. Rev. Food Sci. Nutr.* **1983**, *18* (2), 123−201.
(42) Al-Gendy, A. A.; Lockwood, G. B. GC-MS analysis of volatile hydrolysis products from glucosinolates in *Farsetia aegyptia* var. *Flavour Fragrance J.* **2003**, *18*, 148−152.
(43) Arora, R.; Kumar, R.; Mahajan, J.; Vig, A. P.; Singh, B.; Singh, B.; Arora, S. 3-Butenyl isothiocyanate: a hydrolytic product of glucosinolate as a potential cytotoxic agent against human cancer cell lines. *J. Food Sci. Technol.* **2016**, *53* (9), 3437−3445.
(44) Núñez-Iglesias, M. J.; Novio, S.; García-Santiago, C.; Cartea, M. E.; Soengas, P.; Velasco, P.; Freire-Garabal, M. Effect of 3-butenyl isothiocyanate on phenotypically different prostate cancer cells. *Int. J. Oncol.* **2018**, *53* (5), 2213−2223.
(45) Matusheski, N. V.; Jeffery, E. H. Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. *J. Agric. Food Chem.* **2001**, *49* (12), 5743−5749.
(46) Tanii, H.; Higashi, T.; Nishimura, F.; Higuchi, Y.; Saijoh, K. Effects of cruciferous allyl nitrile on phase 2 antioxidant and detoxification enzymes. *Med. Sci. Monit.* **2008**, *14* (10), BR189−192.
(47) Rodríguez-Hernández, M.; Medina, S.; Gil-Izquierdo, A.; Martinez-Ballesta, M.; Moreno, D. Broccoli isothiocyanate content and *in vitro* availability according to variety and origin. *Macedonian J. Chem. Chem. Eng.* **2013**, *32* (2), 251−264.
(48) Ghawi, S. K.; Methven, L.; Niranjan, K. The potential to intensify sulforaphane formation in cooked broccoli (*Brassica oleracea var. italica*) using mustard seeds (*Sinapis alba*). *Food Chem.* **2013**, *138* (2−3), 1734−1741.
(49) Chen, X. M.; Dai, Y.; Kitts, D. D. Detection of Maillard reaction product [5-(5, 6-Dihydro-4 H-pyridin-3-ylidenemethyl) furan-2-yl] methanol (F3-A) in breads and demonstration of bioavailability in Caco-2 intestinal cells. *J. Agric. Food Chem.* **2016**, *64* (47), 9072−9077.

DOI: 10.1021/acs.jafc.9b03329
J. Agric. Food Chem. 2019, 67, 9492−9500