

Figure S1 Metabolism of Glucoraphanin via the mercapturic acid pathway.

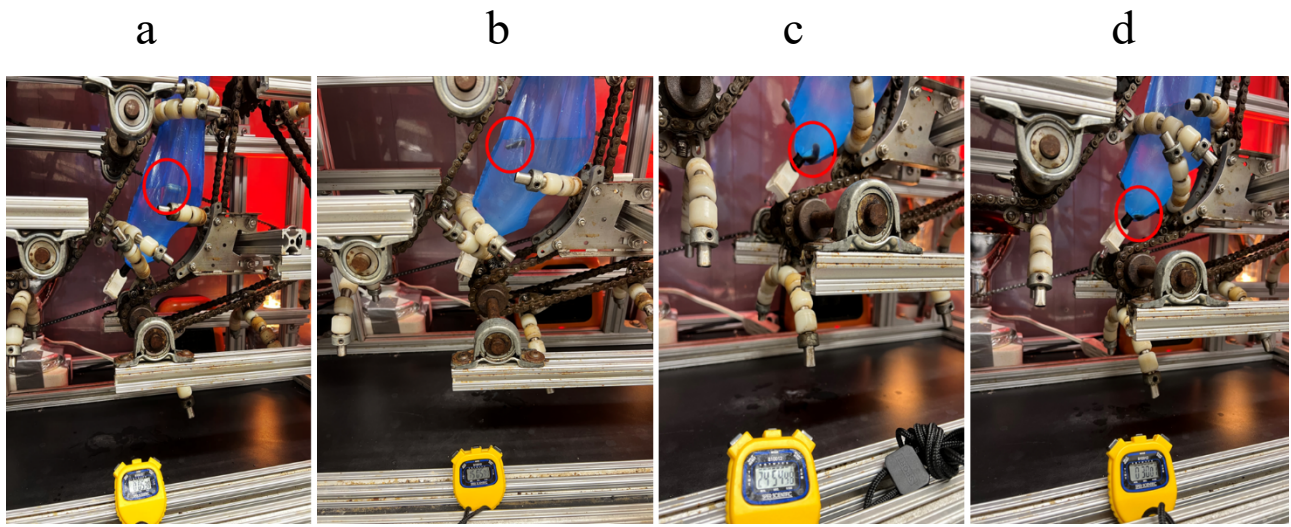


Figure S2 The dynamics of capsule in HGS during gastric digestion. Capsules were loaded into HGS with 250 mL water. Gastric digestion time was recorded immediately when the capsules were loaded to the HGS, and the motor started. (a) 5 secs, capsule was floating on the gastric fluid in the bag; (b) 15 min, capsule was still floating on the gastric fluid in the bag, getting soaked; (c) 24 min, capsule began to breakdown, and the intact capsule went down to the bottom of the bag; (d) 30 min, capsule broke and the whole wet pellet was at the bottom of the bag. The red circle indicates the location of the capsule.

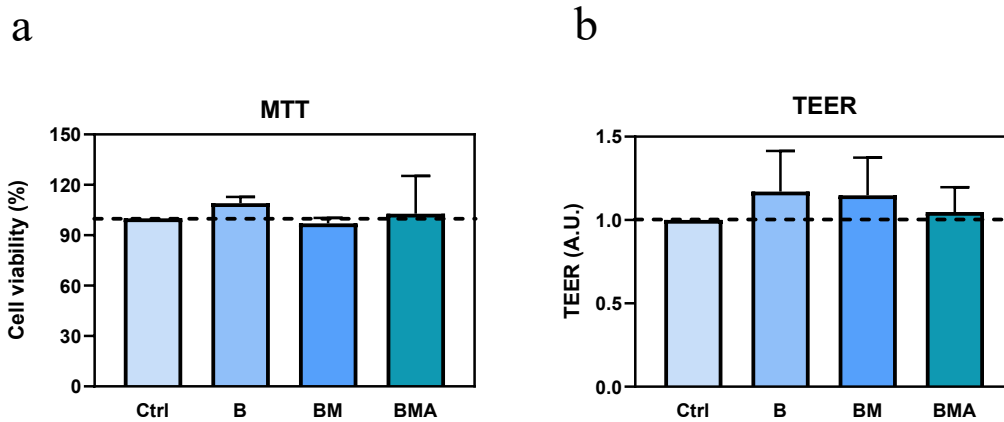


Figure S3 The effect of the small intestinal digesta of capsules on cell viability and TEER of Caco-2 cells. In capsules, the total amount of Broccoli seed extract (BSE) powder was 380 mg (GR content 55 mg), mustard seed powder (MSP) was 95 mg, ascorbic acid (AA) was 154 mg. B represents BSE powder only, BM represents BSE + MSP, BMA represents BSE + MSP + AA. Capsules were first loaded to HGS, then the gastric digesta was collected at 30 min and further incubated in small intestinal fluid for 60 min. (a) Fresh small intestinal digesta samples (100 μ L) were added to Caco-2 cells and incubated for 4 h, MTT was conducted to evaluate cell viability. (b) For Caco-2 monolayers, fresh 250 μ L small intestinal digesta samples were added to the upper chamber and 750 μ L serum-free MEM was added to the lower chamber in transwell inserts. After 4 h, TEER was measured to evaluate the monolayer integrity. Results are shown as mean \pm SD of three independent experiments. No significant differences among groups by One-way ANOVA analysis.

Table S1 *In Vitro* Digestion Formulations

Components	Final Concentration (g/L fluid)		
	Saliva	Gastric Juice	Intestinal Juice
pH	7.0	1.8	7.0
Mucin	1.0	1.5	-
KCl	1.1	0.51	0.51
KH ₂ PO ₄	0.5	0.12	0.11
NaHCO ₃	1.1	2.1	7.14
NaCl	-	2.76	2.25
MgCl ₂ (H ₂ O) ₆	0.03	0.02	0.07
(NH ₄) ₂ CO ₃	0.01	0.05	-
CaCl ₂ (H ₂ O) ₂ **	0.22	0.02	0.09
α -amylase**	1.2	-	-
Pepsin**	-	2000 U	-
Bile Extract	-	-	10
Pancreatin**	-	-	2.4

** Added freshly to the solutions right before digestion. All fluids were pre-warmed to 37 °C before any experiments.

Table S2 Cumulative SF produced (μmol) and conversion efficiency (% CE) during small intestinal digestion compared to total GR added to HGS

Digestion time		BSE-GR: MSP-Myr powder ratio									
Gastric	Intestinal	Ratio 1:0		Ratio 20:1		Ratio 10:1		Ratio 4:1		Ratio 1:1	
		min	min	SF	SF	% CE	SF	% CE	SF	% CE	SF
15	60	ND	5.0 ± 0.7 * 1	1.5 ± 0.2 ^{aA}	20.6 ± 0.8 #	8.4 ± 1.0 ^{bA}	34.2 ± 4.5 ^{\\$ 1}	10.3 ± 1.4 ^{bA}	56.9 ± 0.6 & 1	17.1 ± 0.2 ^{cA}	
30	60	ND	5.8 ± 1.1 * 1	1.8 ± 0.4 ^{aA}	24.3 ± 0.4 #1	9.7 ± 2.0 ^{bA}	47.7 ± 5.3 ^{\\$ 1}	14.8 ± 1.6 ^{cA}	110.8 ± 3.1 & 1	33.4 ± 0.9 ^{dA}	
60	60	ND	5.9 ± 1.0 * 1	1.8 ± 0.4 ^{aA}	29.1 ± 2.8 #1	11.4 ± 1.7 ^{bA}	55.0 ± 4.7 ^{\\$ 1}	16.6 ± 1.4 ^{cA}	121.7 ± 3.5 & 1	36.7 ± 1.1 ^{dA}	
90	60	ND	5.9 ± 1.2 * 1	1.8 ± 0.4 ^{aA}	31.9 ± 3.6 #1	12.4 ± 1.8 ^{bA}	58.9 ± 3.9 ^{\\$ 1}	17.7 ± 1.2 ^{cA}	126.0 ± 3.9 & 1	38.0 ± 1.2 ^{dA}	
120	60	ND	5.9 ± 1.2 * 1	1.8 ± 0.4 ^{aA}	34.2 ± 4.6 #1	13.2 ± 2.6 ^{bA}	60.3 ± 3.2 ^{\\$ 1}	18.2 ± 1.0 ^{cA}	127.8 ± 4.0 &	38.5 ± 1.2 ^d	
15	120	ND	8.5 ± 0.9 * 2	2.6 ± 0.3 ^{aB}	45.4 ± 4.7 #	13.7 ± 2.9 ^{bB}	52.7 ± 6.8 #, \\$ 2	15.9 ± 2.1 ^{bB}	63.4 ± 1.8 ^{\\$ 2}	19.1 ± 0.5 ^{cB}	
30	120	ND	9.8 ± 0.1 * 2	3.0 ± 0.3 ^{aB}	53.3 ± 4.5 #2	16.1 ± 2.9 ^{bB}	82.2 ± 9.3 ^{\\$ 2}	24.8 ± 2.8 ^{cB}	114.3 ± 3.1 & 2	34.4 ± 0.9 ^{dB}	
60	120	ND	10.4 ± 0.2 * 2	3.1 ± 0.5 ^{aB}	61.9 ± 7.2 #2	18.7 ± 2.2 ^{bB}	91.5 ± 8.3 ^{\\$ 2}	27.6 ± 2.5 ^{cB}	125.9 ± 2.8 & 2	37.9 ± 0.8 ^{dB}	
90	120	ND	10.4 ± 0.2 * 2	3.1 ± 0.1 ^{aB}	66.2 ± 6.5 #2	19.9 ± 1.9 ^{bB}	95.6 ± 4.1 ^{\\$ 2}	28.8 ± 1.2 ^{cB}	130.2 ± 2.4 & 2	39.2 ± 0.7 ^{dB}	
120	120	ND	10.4 ± 0.3 * 2	3.1 ± 0.1 ^{aB}	69.1 ± 5.8 #2	20.8 ± 1.8 ^{bB}	97.4 ± 2.5 ^{\\$ 2}	29.3 ± 0.8 ^{cB}	132.1 ± 2.2 &	39.8 ± 0.6 ^d	

The total amount of broccoli seed extract was 1000 mg. GR content in Broccoli seed extract (BSE-GR) was 14.50% (145 mg (332 μmol)). Mustard seed powder (MSP-Myr) was 1000 mg (448 units of Myr activity) (ratio 1:1), 250 mg (ratio 4:1), 100 mg (ratio 10:1)

and 50 mg (ratio 20:1). Results are shown as mean \pm SD of 3 replicates of 3 independent experiments. % CE is based on total produced SF (μmol)/total added GR (μmol) $\times 100$. Two different statistical analyses were done. Firstly, the statistical analysis was performed comparing SF and % CE of each ratio at each time point (rows). Means of SF with different symbols in the same row are significantly different. Means of % CE with different superscript letters in the same row are significantly different. One-way ANOVA ($p < 0.05$) was used for this first statistical analysis. A second analysis was performed comparing SF and % CE 60 min vs 120 min of the intestinal incubation in the same ratio in the same gastric time group (columns) (e.g. ratio 1:0, gastric 15 min intestinal 60 min vs gastric 15 min intestinal 120 min). Means of SF with different numbers in the same column for the same gastric time but different small intestinal incubation (60 min vs 120 min) is significantly different. Means of % CE with different capitalized letters in the same column for the same gastric time but different intestinal incubation (60 min vs 120 min) is significantly different. For this second analysis paired t-test was used ($p < 0.05$).

ND: Not detected.