

Electronic Supplementary Information (ESI)

Table S1: List of food products and Ti concentrations determined by ICP-MS

Sample	Ti concentration (ppb)	%Ti concentration (w/w)
Coles Garlic Aioli	1157.58	0.000115
Queen Designer Icing (White)	607.12	0.000061
Mars Chocolate M&M's	12489.94	0.001249

Table S2: List of titanium dioxide samples used in this work.

	Sample Name	Sample treatment
1	A	TiO ₂ extracted from Aioli food matrix
2	D1-A	TiO ₂ extracted from Aioli food matrix and taken through phase 1 of GIT simulator
3	D2-A	TiO ₂ extracted from Aioli food matrix and taken through phase 1 and 2 of GIT simulator
4	D3-A	TiO ₂ extracted from Aioli food matrix and taken through phase 1, 2, and 3 of GIT simulator
5	I	TiO ₂ extracted from Icing food matrix
6	D1-I	TiO ₂ extracted from Icing food matrix and taken through phase 1 of GIT simulator
7	D2-I	TiO ₂ extracted from Icing food matrix and taken through phase 1 and 2 of GIT simulator
8	D3-I	TiO ₂ extracted from Icing food matrix and taken through phase 1, 2, and 3 of GIT simulator
9	M	TiO ₂ extracted from M&M food matrix
10	D1-M	TiO ₂ extracted from M&M food matrix and taken through phase 1 of GIT simulator
11	D2-M	TiO ₂ extracted from M&M food matrix and taken through phase 1 and 2 of GIT simulator
12	D3-M	TiO ₂ extracted from M&M food matrix and taken through phase 1, 2, and 3 of GIT simulator
13	E	Commercial food grade E171 (control)
14	D1-E	Commercial food grade E171 (control) taken through phase 1 of GIT simulator
15	D2-E	Commercial food grade E171 (control) taken through phase 1 and 2 of GIT simulator
16	D3-E	Commercial food grade E171 (control) taken through phase 1, 2, and 3 of GIT simulator

GIT simulator for the digestion of the extracted TiO₂

Phase 1

Artificial saliva stock solution (ASSS) was prepared by adding sodium chloride (1.594 g), ammonium nitrate (0.328 g), potassium phosphate (90.636 g), potassium chloride (0.202 g), potassium citrate (monohydrate) (0.308 g), uric acid sodium salt (0.021 g), urea (90.198 g), and sodium DL-lactate (0.146 g) to Milli-Q H₂O (1 L). Porcine gastric mucin type II (0.6 g) was then added to the ASSS solution (20 mL) and stirred overnight at rt to produce an Artificial Saliva Working Solution (ASWS). Titanium dioxide obtained from processed food samples (20 mg) was suspended in Milli-Q H₂O (1 mL) and added to ASWS (10 mL). The suspension (pH 6.8) was then stirred for 5 min at 37 °C. A sample of the suspension (5 mL) was collected to be carried onto Phase 2 of the GIT simulation. The remainder of the suspension was centrifugated, washed thrice in Milli-Q H₂O (3×20 mL), and lyophilised for further characterisation. This process was repeated for each of the four titanium dioxide samples. This afforded titanium dioxide (~10 mg per food sample) as a white powder (Table S1).

Phase 2

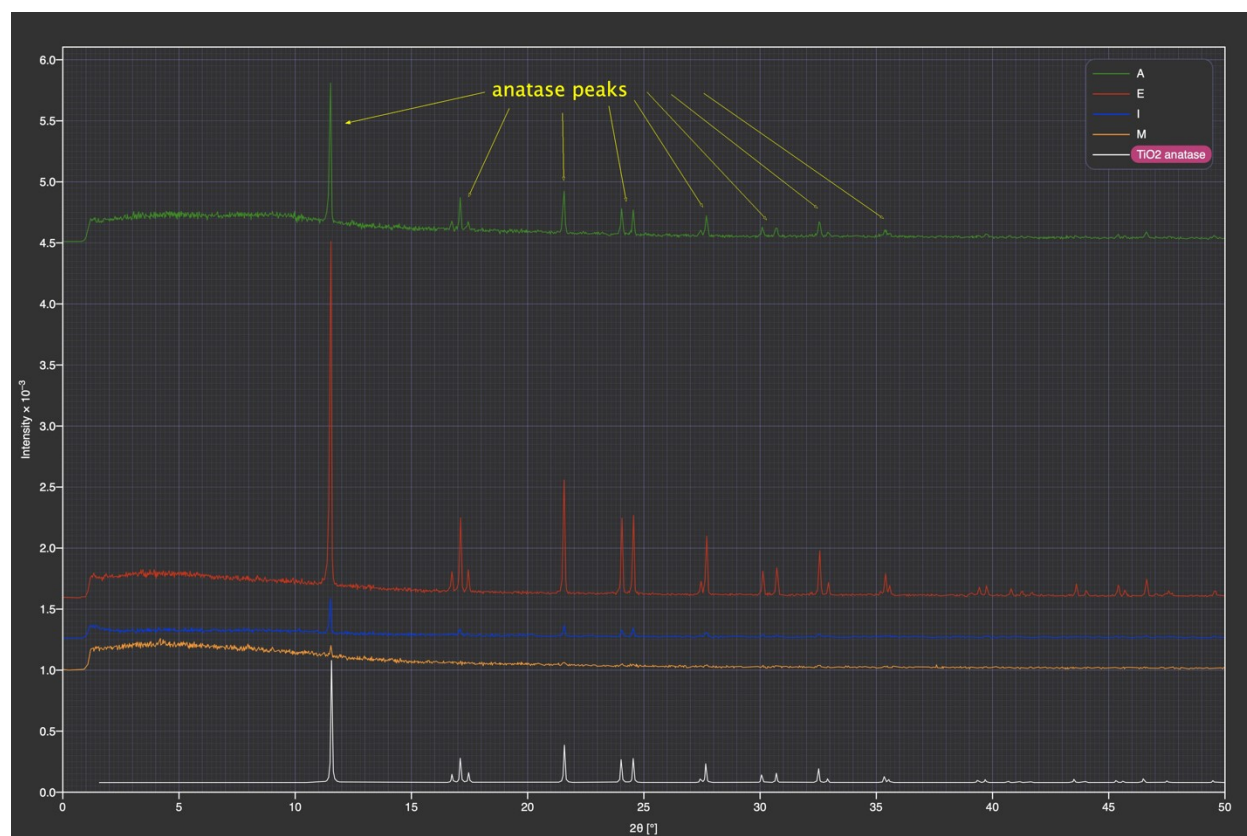
Phosphate buffer (5 mM) was prepared by dissolving potassium phosphate (0.6 g) in Milli-Q H₂O (150 mL). The pH of the solution was adjusted to pH 7 with HCl (<1 mL), before being diluted to 1 L with Milli-Q H₂O. Sodium chloride (2 g) was dissolved in Milli-Q H₂O (150 mL) and hydrochloric acid (7 mL) added to afford a Simulated Gastric Fluid Stock Solution (SGFSS). Porcine pepsin (0.064 g) was then added to the SGFSS solution (20 mL) to afford a Simulated Gastric Fluid Working Solution (SGFWS). Phase 1 suspensions (5 mL) were warmed to 37 °C and added separately to SGFWS solutions (~5 mL per titanium dioxide sample, 37 °C). These suspensions were then stirred for 2 h at 37 °C. Following this, phosphate buffer (10 mL) was added to each of the suspensions. A sample of each suspension (10 mL) was collected to be carried onto Phase 3 of the GIT simulation. The remainder of each suspension was centrifugated, washed thrice in Milli-Q H₂O (3×20 mL), and lyophilised for further characterisation. This process was repeated for each of the four titanium dioxide samples. This afforded titanium dioxide (~5 mg per food sample) as a white powder (Table S1).

Phase 3

Lipase (porcine) (0.06g) was added to phosphate buffer (5mM, 2.5 mL) and stirred for 30 min to afford a Simulated Intestinal Fluid Lipase Solution (SIFLS). Bovine bile extract (0.1875 g) was then added to the phosphate buffer (5 mM, 3.5 mL) and stirred overnight at rt to afford a Simulated Intestinal Fluid Bile Salt Solution (SIFBSS). Sodium chloride (15 g) and calcium chloride (dihydrate) (2.5 g) was added to Milli-Q H₂O (75 mL) to afford Simulated Intestinal Fluid Stock Salt Solution (SIFSSS). Phase 2 suspensions (10 mL) were warmed to 37 °C and the pH adjusted to 6.9 with aqueous sodium hydroxide (0.25 M). SIFLS (2.5 mL), SIFBSS (3.5 mL), and SIFSSS (1.5 mL) were then combined and added, separately, to the Phase 2 suspensions (~1.5 mL per suspension). The suspensions (pH 7) were then stirred at 37 °C for 2 h. Each suspension was then centrifugated, washed thrice in Milli-Q H₂O (3×20 mL), and lyophilised for further characterisation. This process was repeated for each of the four titanium dioxide samples. This afforded titanium dioxide (~5 mg per food sample) as a white powder (Table S1).

Table S3: Mean size of TiO₂ nanoparticles from food samples (n=50).

Sample	Mean particle size (nm)
E	146.56 ± 72.6
D3-E	153.79 ± 61.3
M	128.22 ± 32.96
D3-M	117.19 ± 42.6
I	117.60 ± 43.2
D3-I	119.00 ± 41.9
A	158.62 ± 37.5
D3-A	153.17 ± 48.2

**Figure S1:** Comparison of XRD spectra of unprocessed TiO₂ samples (**A**, **E**, **I**, **M**) to calculated spectra of anatase TiO₂. **A**: NP from Aioli. **I**: NP from Icing food matrix. **M**: NP from M&M. **E**: Commercial food grade E171 (control).**Table S4:** Size distribution of TiO₂ nanoparticles from food samples (n=50).

Sample	% particles < 200 nm
E	78
D3-E	82
M	96
D3-M	98
I	96
D3-I	92
A	94
D3-A	84

Table S5: Crystallinity of TiO₂ samples by XRD. ‘Y’ indicates presence of the phase.

	Anatase-TiO ₂	KCl	NaCl
D1-A	Y-trace	-	-
D2-A	Y	-	-
D3-A	-	Y	Y-trace
D1-E	Y	-	-
D2-E	Y	Y	Y
D3-E	Y - trace	Y - trace	Y
D1-I	Y	-	-
D2-I	Y - trace	Y	Y
D3-I	-	Y - trace	Y
D1-M	Y - trace	-	-
D2-M	Y	Y	Y
D3-M	-	Y - trace	Y



Figure S2: Comparison of XRD spectra of Aioli TiO₂ samples to calculated spectra of common salts KCl and NaCl. **A:** NP from Aioli. **D1-A:** NP underwent digestion phase 1. **D2-A:** NP underwent digestion phase 1 and 2. **D3-A:** NP underwent digestion phase 1, 2, and 3.

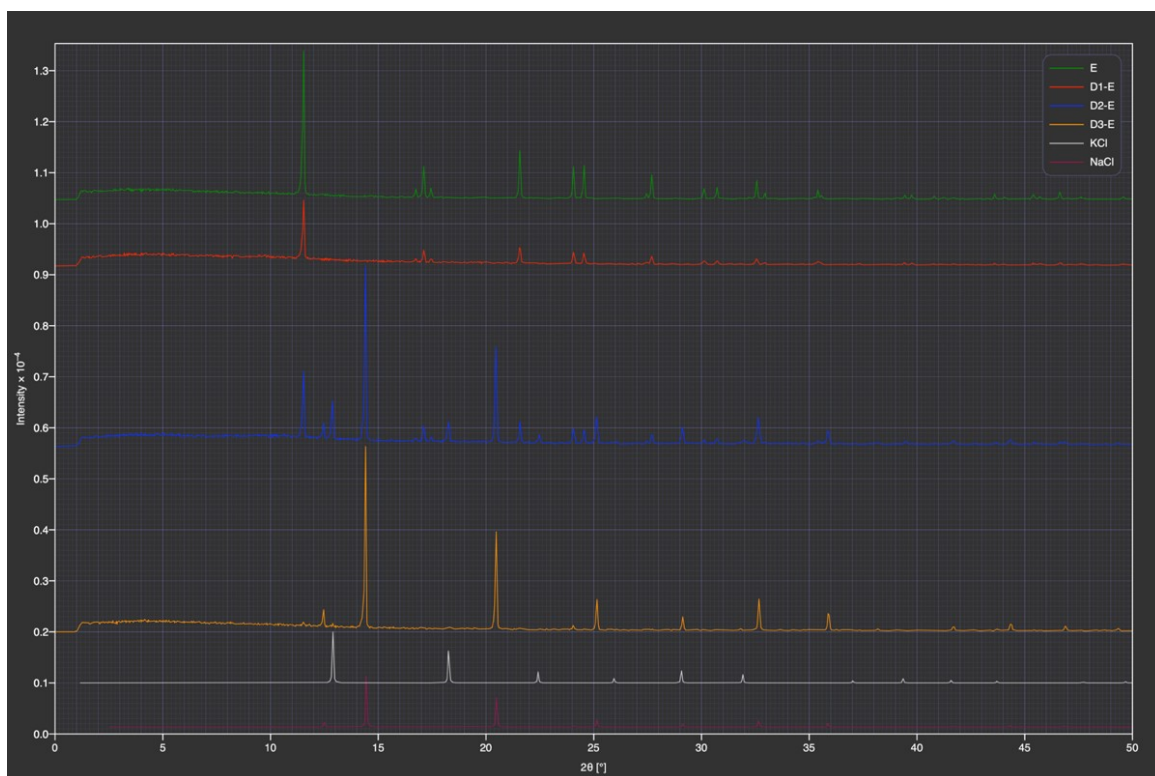


Figure S3: Comparison of XRD spectra of food grade E171 to calculated spectra of common salts KCl and NaCl. **E:** NP from E171. **D1-E:** NP underwent digestion phase 1. **D2-E:** NP underwent digestion phase 1 and 2. **D3-E:** NP underwent digestion phase 1, 2, and 3.



Figure S4: Comparison of XRD spectra of Icing TiO_2 samples to calculated spectra of common salts KCl and NaCl. **I:** NP from Icing. **D1-I:** NP underwent digestion phase 1. **D2-I:** NP underwent digestion phase 1 and 2. **D3-I:** NP underwent digestion phase 1, 2, and 3.

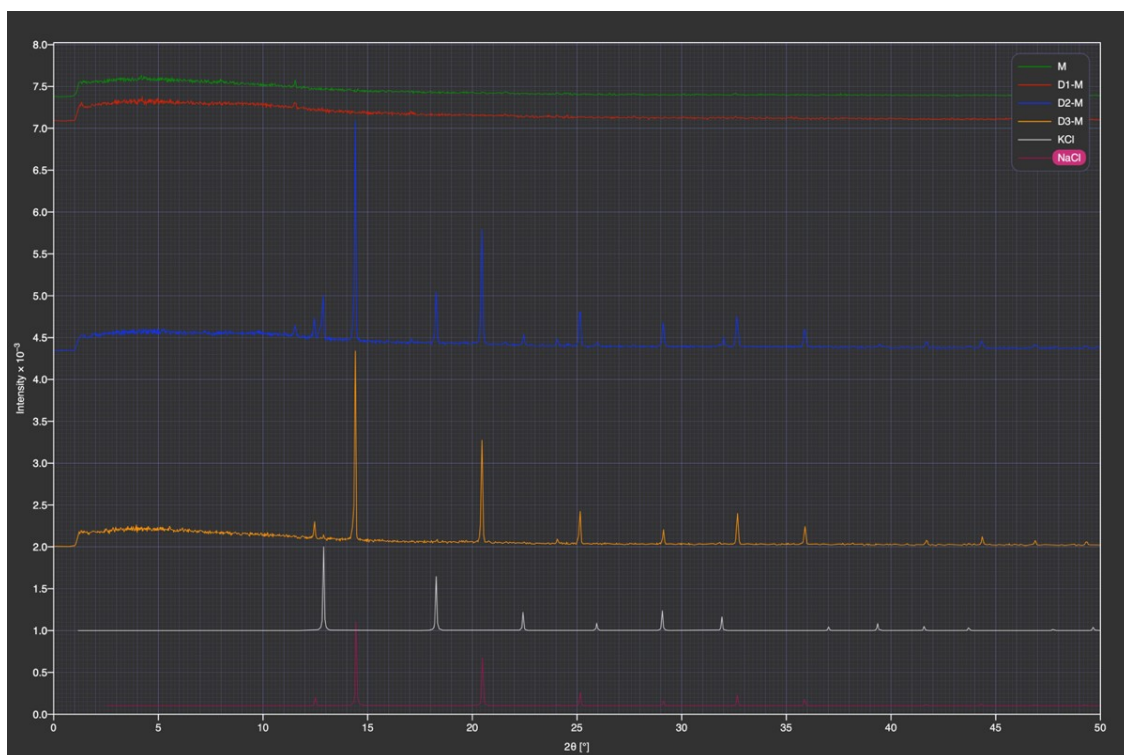


Figure S5: Comparison of XRD spectra of M&M TiO₂ samples to calculated spectra of common salts KCl and NaCl. **M:** NP from M&M. **D1-M:** NP underwent digestion phase 1. **D2-M:** NP underwent digestion phase 1 and 2. **D3-M:** NP underwent digestion phase 1, 2, and 3.

Table S6: % Ti uptake in Caco-2 cells after treatment with TiO₂ NP samples (20 µg/mL, 72 h) from ICP- MS analysis, n=3.

TiO ₂ sample	% Ti uptake in cells	Mean Ti concentration in cell lysate (ppb)
D3-A	49%	11.30 ± 4.7
D3-E	59%	49.21 ± 32.2
D3-I	21%	10.18 ± 2.7
D3-M	46%	17.44 ± 11.56

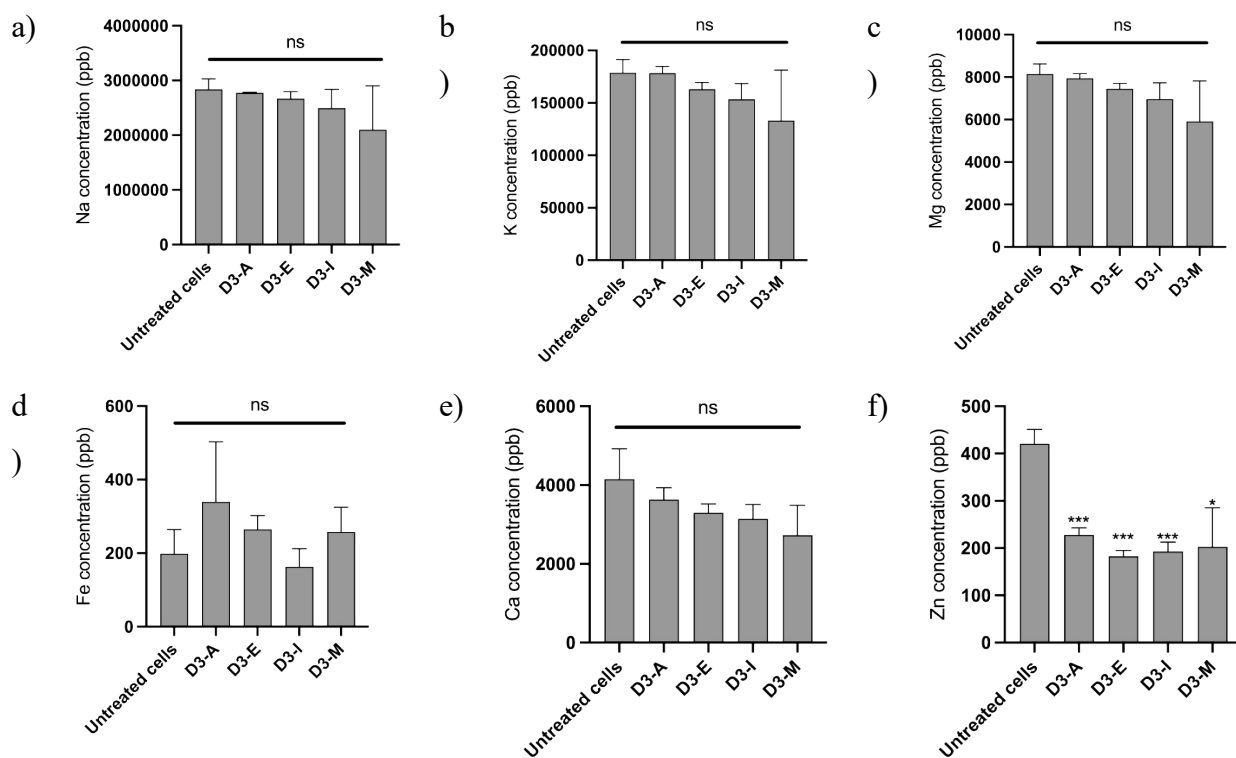


Figure S6: Cellular concentrations of essential metals in Caco-2 cells after treatment with TiO₂ samples (20 µg/mL, 72 h), as analysed by ICP-MS (n=3). Unpaired, 2-sided t-test. Asterisk denotes significant difference compared to the control untreated cells. Statistical significance was defined as $P < 0.05$, with * = $P < 0.05$, *** = $P < 0.001$.