Electronic Supplementary Material (ESI) for Food & Function.

Table S1. Concentration (mg/mL) of the folate calibrant stock solutions (before dilution)

Folates	Nominal concentration (mg/ml)	Spectrophotometrically confirmed concentration (mg/ml)
PGA	1.018	0.908
10-HCO-H_2folate	0.421	0.281
10-HCO-PGA	1.004	1.101
H_4folate	1.008	0.675
5-CH_3-H_4folate	1.037	0.675
5-HCO-H_4folate	1.048	0.694
5,10-CH^+-H_4folate	1.114	0.909

Table S2. The tested activities of the enzymes and the concentration of the bile acid in the bile extract.

Enzymes or bile	Company	Lot	Activity or concentration
α-Amylase from *Aspergillus oryzae*	Sigma Aldrich	SLBZ1496	104.4 U/mg
Pepsin from porcine gastric mucosa	Sigma Aldrich	BCCB2294	574.9 U/mg
Trypsin from porcine pancreas	Sigma Aldrich	SLBT2691	137.5 U/mg
α-Chymotrypsin from bovine pancreas	Sigma Aldrich	SLBV2540	42.3 U/mg
Bile from bovine and ovine	Sigma Aldrich	B8381	1220.9 g/mol

Note: the protocols used for the activity tests were referred to Minekus et al., 2014

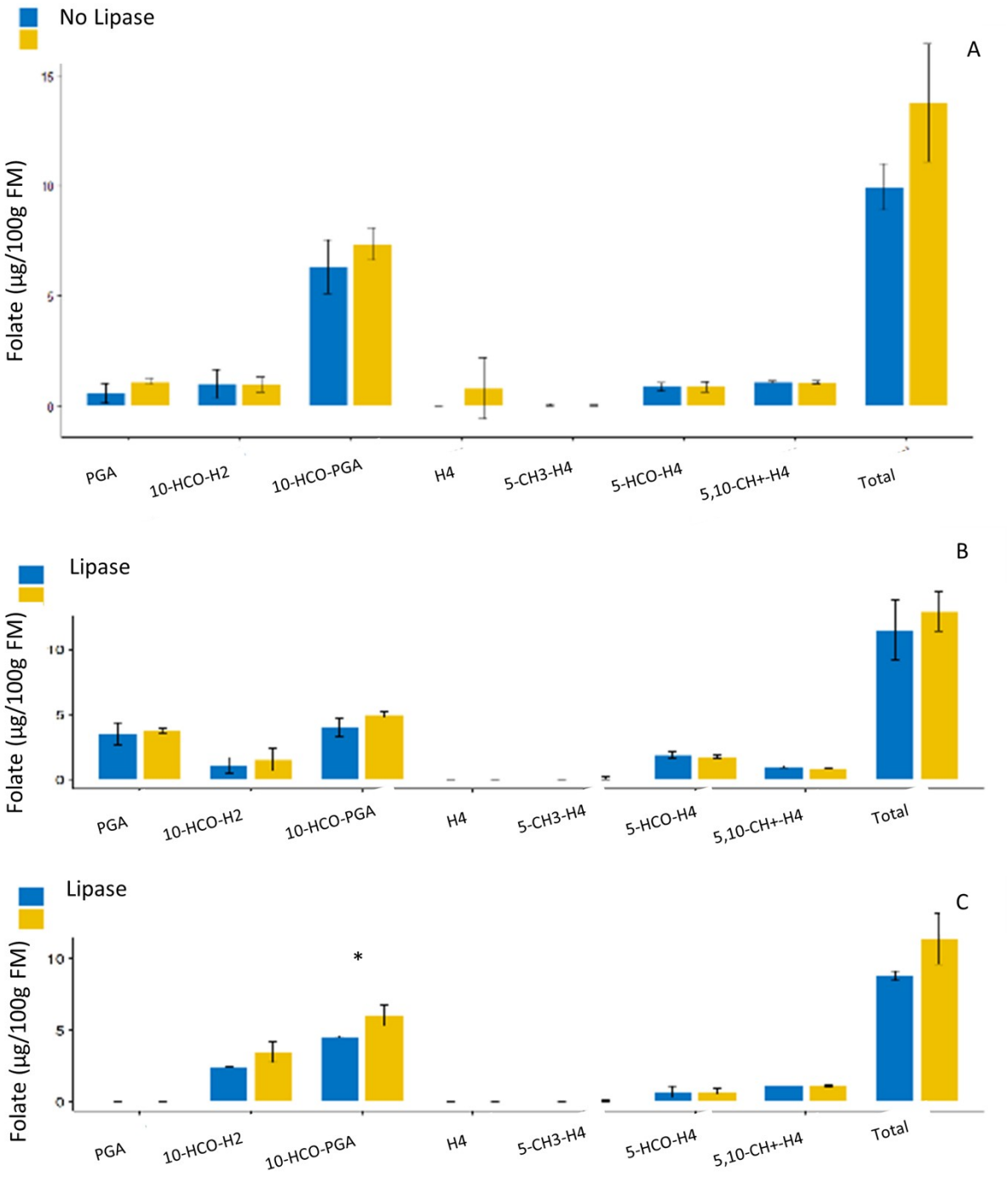

Figure S1. The effect of lipase on the folate level of the selected bread digesta. A, whole-grain oat toast; B, whole-grain oat flat portion bread; C, whole-grain rye toast. "No Lipase" means that bile extract, α-amylase, trypsin, and chymotrypsin, but not lipase, were used during the intestinal phase; "Pancreatin" means that pancreatin (including lipase) was used during the intestinal phase; "Lipase" means that individual enzymes – bile extract, α-amylase, trypsin, chymotrypsin as well as lipase from *Candida rugosa* (L1754, Sigma, St Louis, MO, USA) – were used during the intestinal phase. The amount of pancreatin added was based on the tested activity of trypsin (4.1 U/mg) according to the INFOGEST protocol (Minekus et al., 2014), and the amount of lipase from *Candida rugosa* added was based on the labelled activity (≥ 700 U/mg) and the recommended targeted lipase activity (2000 U/mL) described by the INFOGEST protocol. Two sample *t*-tests were performed to study the

differences between folate contents (mean ± standard, n = 3) of the two different treatments, and * indicates a significant difference in vitamer contents between different treatments at a level of $p < 0.05$.

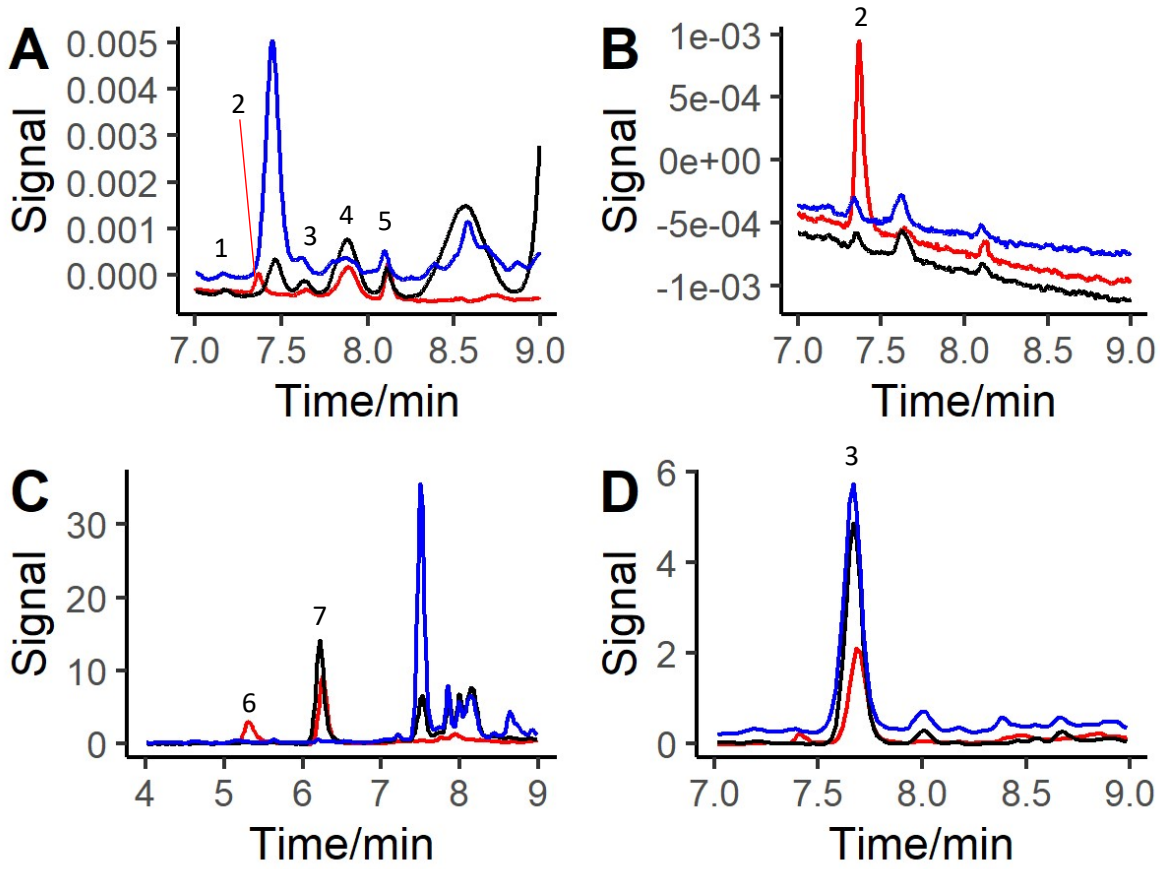

Figure S2. UHPLC chromatograms from different detectors (red: calibrants, black: whole-grain oat toast, blue: whole-grain oat toast digesta). A, photodiode array detector (290 nm); B, photodiode array detector (360 nm); C, fluorescence detector (excitation wavelength: 290 nm; emission wavelength: 356 nm); D, fluorescence detector (excitation wavelength: 360 nm; emission wavelength: 465 nm); vitamer peaks were labelled with numbers: 1, 10-formyldihydrofolate; 2, 5,10-methenyltetrahydrofolate; 3, 10-formylfolic acid; 4, 5-formyltetrahydrofolate; 5, folic acid; 6, tetrahydrofolate; 7, 5-methyltetrahydrofolate.