Electronic Supplementary Material (ESI) for Food & Function.

Supplementary Data

In vitro digestion of galactolipids from chloroplast-rich fraction (CRF) of postharvest, pea vine field residue (haulm) and spinach leaves

Jutarat Wattanakul[a,b], Moulay Sahaka[c], Sawsan Amara[c,d], Mansor Syamila[a], Brigitte Gontero[c], Frédéric Carrière[c], David Gray[a*]

[a] Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, United Kingdom.

[b] Department of Food Sciences and Technology, Faculty of Home Economics Technology, Rajamangala University of Technology Krungthep, Bangkok, 10120, Thailand.

[c] Aix Marseille Univ, CNRS, UMR7281 Bioénergétique et Ingénierie des Protéines, 31 Chemin Joseph Aiguier, 13402 Marseille, Cedex 09, France.

[d] Lipolytech, Zone Luminy Biotech, 163 avenue de Luminy, 13288 Marseille Cedex 09, France

*Corresponding Author

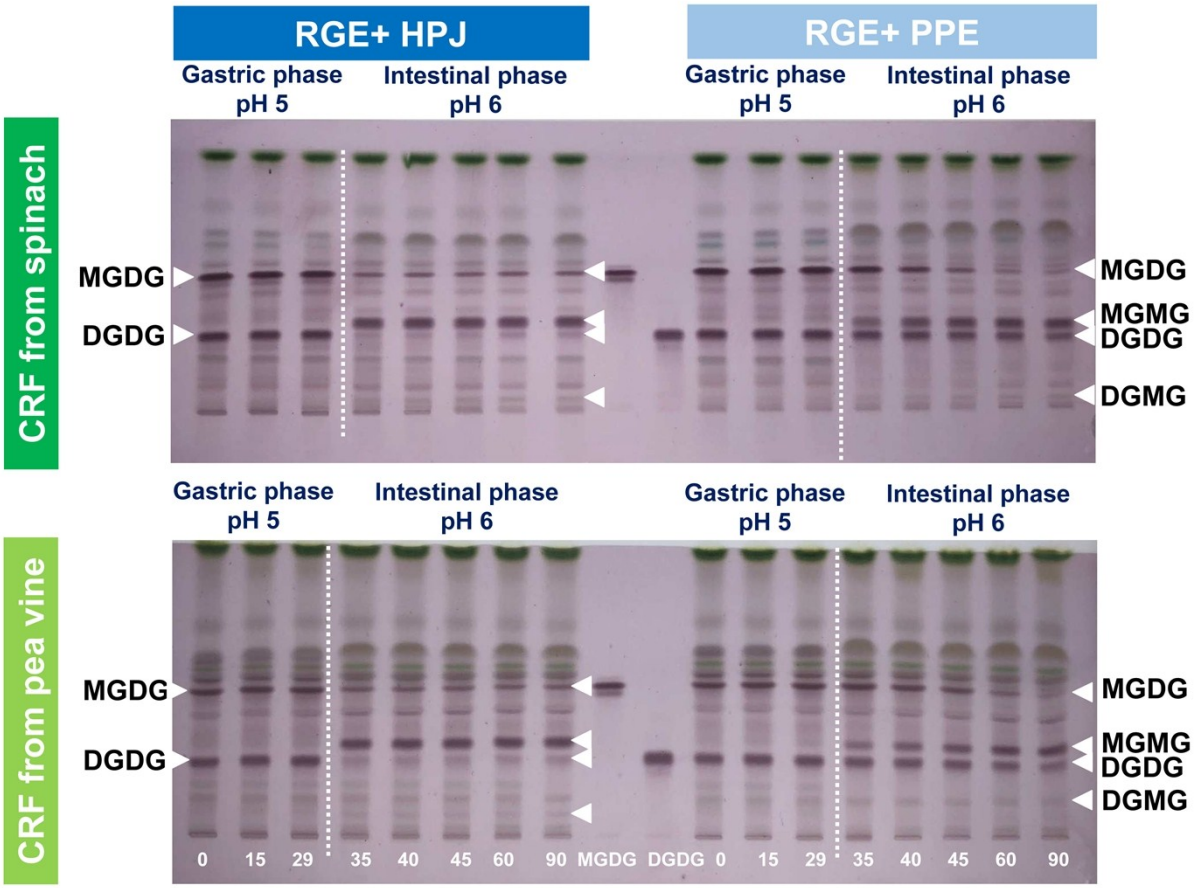

Figure S1: *Representative thin-layer chromatography analysis of polar lipids in the course of two-step static digestion of CRFs*. CRF from blanched spinach leaves were digested using a combination of RGE and HPJ as sources of gastric and pancreatic enzymes respectively or a combination of RGE and PPE as an alternative source of pancreatic enzymes (upper TLC plate). Similarly, CRF from steam sterilised PVH were digested using a combination of RGE and HPJ or a combination of RGE and PPE (lower TLC plate). Samples were collected at times 0, 15 and 29 min during the gastric phase and at times 35, 40, 45, 60 and 90 min during the intestinal phase. Lipids were extracted with methanol-chloroform as described in the main text. Polar lipids were separated using a chloroform/methanol/water (47.5:10:1.25, v/v/v) elution mixture and were stained with a thymol solution, especially for revealing galactolipids. Pure monogalactosyldiglycerides (MGDG) and digalactosyldiglycerides (DGDG) were uses as reference standards for the identification and quantitation of these lipids in CRFs. The monogalactosylmonoglycerides (MGMG) and digalactosylmonoglycerides (DGMG) generated upon the hydrolysis of MGDG and DGDG are also shown.

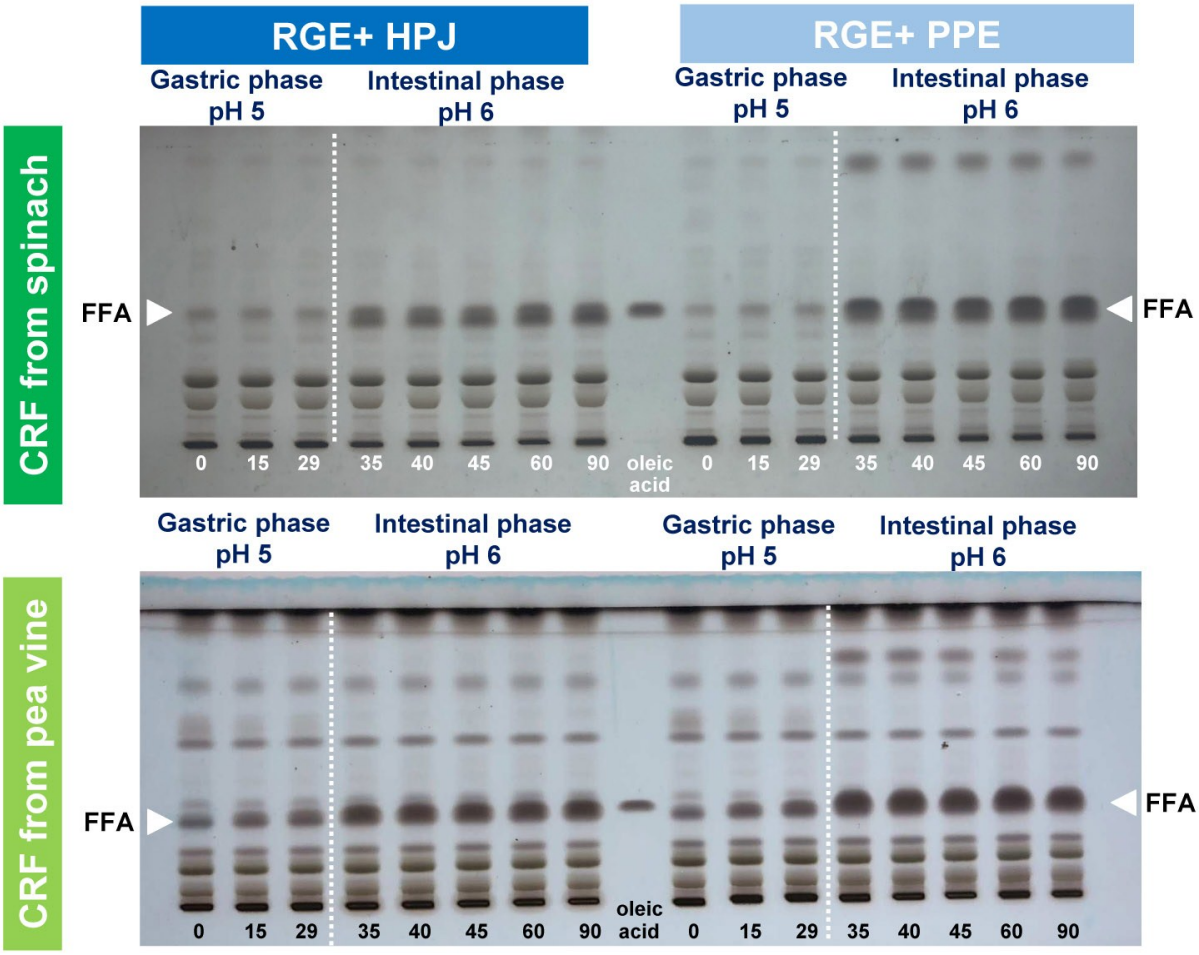

Figure S2: *Representative thin-layer chromatography analysis of neutral lipids in the course of two-step static digestion of CRFs*. CRF from blanched spinach leaves were digested using a combination of RGE and HPJ as sources of gastric and pancreatic enzymes respectively or a combination of RGE and PPE as an alternative source of pancreatic enzymes (upper TLC plate). Similarly, CRF from steam sterilised PVH were digested using a combination of RGE and HPJ or a combination of RGE and PPE (lower TLC plate). Samples were collected at times 0, 15 and 29 min during the gastric phase and at times 35, 40, 45, 60 and 90 min during the intestinal phase. Lipids were extracted with methanol-chloroform as described in the main text. Neutral lipids were separated using a mobile phase consisting of heptane: diethyl ether: formic acid (55:45:1, v/v/v) solvent mixture and they were revealed, especially free fatty acids (FFA), with a copper acetate-phosphoric acid solution. Pure oleic acid was used as reference standards.

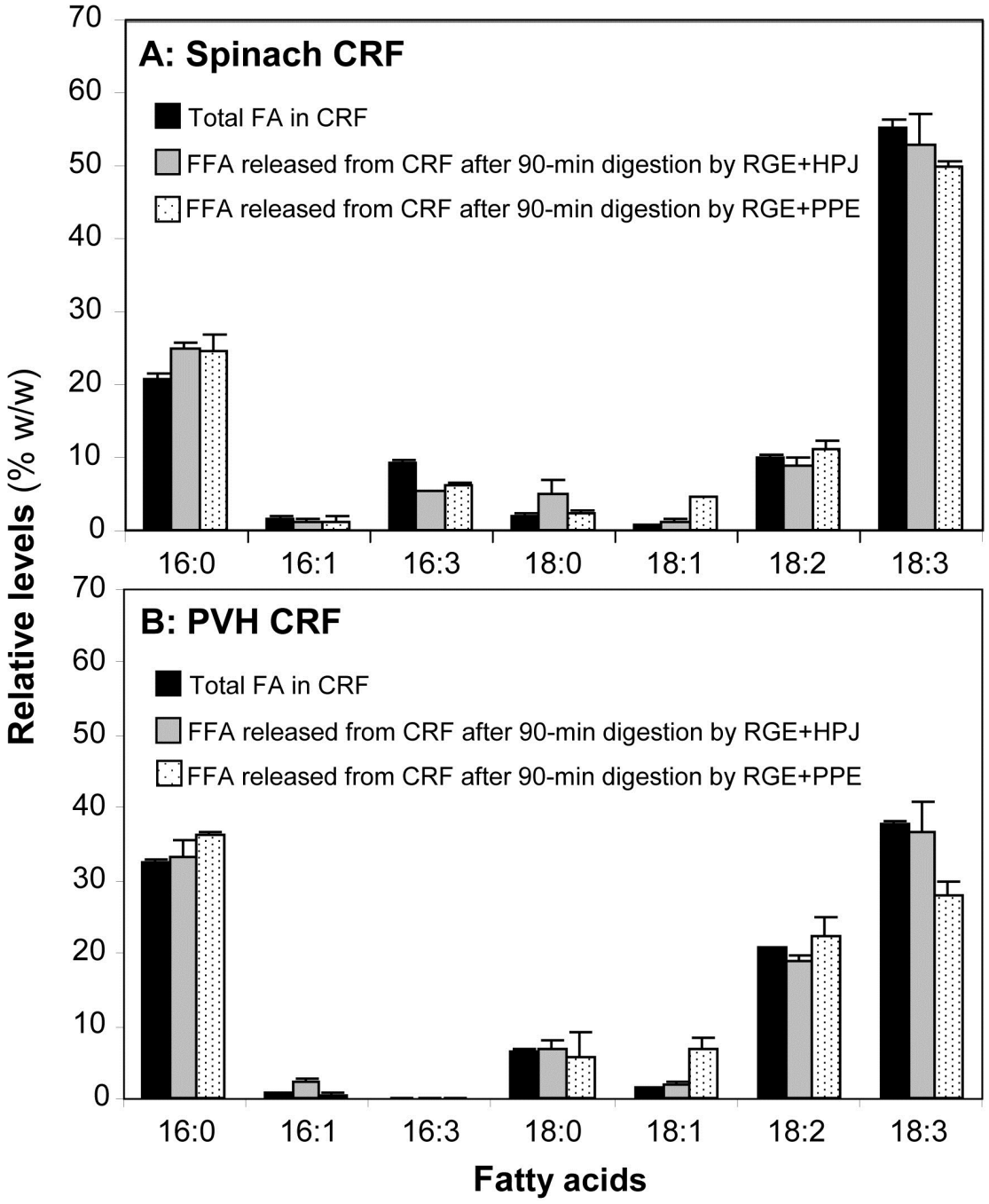

Figure S3: GC-MS analysis of total fatty acids initially present in blanched spinach (panel A) and steam sterilised pea vine haulm (panel B) CRFs and corresponding free fatty acids released after 90-min digestion. CRFs were digested with either a combination of RGE and HPJ as sources of gastric and pancreatic enzymes or a combination of RGE and PPE as an alternative source of pancreatic enzymes. Data (see Table S1) are expressed in relative mass levels (% w/w) and presented as mean ± SD (n=3).

Table S1: *Composition of total fatty acids in CRFs and free fatty acids released from CRFs after 90 minutes of in vitro digestion*. Fatty acid levels are expressed in mg/g of CRF dry weight, in % w/w, in µmoles/g of CRF dry weight and mole %. Values are means ± SD (n=3)

CRF	Sample	Fatty acid composition (mg/g CRF)															
		16:0		16:1		16:3		18:0		18:1		18:2		18:3		Total FA	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Spinach	Total FA in CRF	13.31	0.24	1.08	0.12	5.98	0.12	1.33	0.05	0.54	0.03	6.35	0.17	35.56	2.56	64.16	3.16
	FFA released at 90 min / RGE+HPJ	19.00	1.96	1.02	0.36	4.20	0.31	3.92	1.64	0.88	0.35	6.75	1.31	40.14	0.02	75.92	5.90
	FFA released at 90 min / RGE+PPE	16.39	1.88	0.90	0.36	4.15	0.19	1.56	0.35	3.08	0.03	7.42	0.70	33.52	0.18	67.02	0.77
Pea vine haulm	Total FA in CRF	12.35	1.86	0.33	0.05	0.00	0.00	2.45	0.45	0.51	0.07	7.88	1.18	14.29	2.06	37.81	5.67
	FFA released at 90 min / RGE+HPJ	17.37	2.52	1.14	0.40	0.00	0.00	3.65	0.84	0.90	0.10	9.81	1.21	18.95	0.64	51.81	4.23
	FFA released at 90 min / RGE+PPE	17.01	2.46	0.20	0.06	0.00	0.00	2.79	1.88	3.28	1.05	10.41	0.26	13.11	0.94	46.80	6.52
CRF	**Sample**	**Fatty acid composition (% w/w total FA)**															
		16:0		**16:1**		**16:3**		**18:0**		**18:1**		**18:2**		**18:3**		**Total FA**	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Spinach	Total FA in CRF	20.77	0.76	1.68	0.11	9.34	0.29	2.08	0.10	0.85	0.05	9.91	0.32	55.38	1.33	100.00	0.00
	FFA released at 90 min / RGE+HPJ	25.00	0.64	1.33	0.37	5.54	0.03	5.10	1.76	1.14	0.37	8.86	1.04	53.03	4.16	100.00	0.00
	FFA released at 90 min / RGE+PPE	24.44	2.52	1.34	0.56	6.19	0.35	2.33	0.49	4.60	0.10	11.08	1.16	50.02	0.84	100.00	0.00
Pea vine haulm	Total FA in CRF	32.65	0.12	0.88	0.01	0.00	0.00	6.45	0.21	1.35	0.01	20.85	0.14	37.82	0.31	100.00	0.00
	FFA released at 90 min / RGE+HPJ	33.43	2.13	2.18	0.60	0.00	0.00	7.00	1.05	1.76	0.34	18.89	0.79	36.74	4.24	100.00	0.00
	FFA released at 90 min / RGE+PPE	36.32	0.19	0.43	0.19	0.00	0.00	5.74	3.21	6.92	1.28	22.43	2.58	28.15	1.91	100.00	0.00

Table S1 (continued): *Composition of total fatty acids in CRFs and free fatty acids released from CRFs after 90 minutes of in vitro digestion*. Fatty acid levels are expressed in mg/g of CRF dry weight, in % w/w, in µmoles/g of CRF dry weight and mole %. Values are means ± SD (n=3)

CRF	Sample	Fatty acid composition (µmol/g CRF)															
		16:0		16:1		16:3		18:0		18:1		18:2		18:3		Total FA	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Spinach	Total FA in CRF	51.91	0.94	4.43	0.49	23.90	0.48	4.68	0.17	1.92	0.09	22.64	0.61	127.71	9.20	237.18	11.51
	FFA released at 90 min / RGE+HPJ	74.09	7.66	4.18	1.48	16.79	1.22	13.79	5.75	3.10	1.23	24.09	4.68	144.16	0.09	280.20	21.93
	FFA released at 90 min / RGE+PPE	63.91	7.33	3.67	1.49	16.57	0.74	5.50	1.21	10.91	0.11	26.46	2.48	120.39	0.65	247.41	3.08
Pea vine haulm	Total FA in CRF	48.15	7.27	1.36	0.20	0.00	0.00	8.60	1.58	1.80	0.25	28.11	4.22	51.33	7.39	139.35	20.90
	FFA released at 90 min / RGE+HPJ	67.73	9.82	4.67	1.65	0.00	0.00	12.82	2.96	3.20	0.35	34.96	4.31	68.05	2.30	191.43	16.09
	FFA released at 90 min / RGE+PPE	66.32	9.59	0.80	0.25	0.00	0.00	9.82	6.60	11.62	3.72	37.13	0.91	47.09	3.39	172.77	23.96
CRF	**Sample**	**Fatty acid composition (mole % total FA)**															
		16:0		**16:1**		**16:3**		**18:0**		**18:1**		**18:2**		**18:3**		**Total FA**	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Spinach	Total FA in CRF	21.91	0.79	1.86	0.12	10.09	0.31	1.98	0.09	0.81	0.04	9.55	0.30	53.80	1.33	100.00	0.00
	FFA released at 90 min / RGE+HPJ	26.42	0.66	1.48	0.41	5.99	0.03	4.86	1.67	1.09	0.35	8.56	1.00	51.61	4.07	100.00	0.00
	FFA released at 90 min / RGE+PPE	25.82	2.64	1.49	0.62	6.70	0.38	2.22	0.46	4.41	0.10	10.70	1.14	48.67	0.87	100.00	0.00
Pea vine haulm	Total FA in CRF	34.55	0.13	0.98	0.01	0.00	0.00	6.15	0.20	1.29	0.01	20.17	0.14	36.85	0.30	100.00	0.00
	FFA released at 90 min / RGE+HPJ	35.29	2.16	2.41	0.66	0.00	0.00	6.65	0.99	1.68	0.33	18.23	0.72	35.73	4.20	100.00	0.00
	FFA released at 90 min / RGE+PPE	38.37	0.23	0.48	0.21	0.00	0.00	5.47	3.06	6.64	1.23	21.66	2.48	27.38	1.84	100.00	0.00