Electronic Supplementary Material (ESI) for Food & Function.

Supplementary data.

Composition data of Reinette apple

Reinette apple contained 3.15 g of glucose, 5.85 g of fructose, 1.09 g of saccharose, 0.04 g of citric acid and 1.61 g of malic acid per 100 g of fresh fruit. Dehydroascorbic acid (17.7 mg per 100 g FM) was markedly higher than ascorbic acid (5.9 mg per 100 g).

Table 1. Phenolic compounds identified by UPLC/DAD/ESI-MSn in apple (*Reinette de Flandre*).

No.	t_R (min)	λ_{max} (nm)	$[M\text{-}H]^-$ (*m/z*)	MS^2 fragments (*m/z*)	Proposed structure
1	6.6	280	577	559, 451, **425**, 407, 299, 289, 273, 245	B-Type dimer
2	7.0	280	289	271, **245**, 231, 205, 179	(+)-Catechin
3	7.4	326, 259sh	353	191	5-Caffeoylquinic acid[a] (chlorogenic acid)
4	8.0	316, 290sh	325	265, 235, 205, 187, 163, **145**, 119	Coumaroylhexoside
5	8.25	280	451	**289**, 245, 161	Cinchonain I[b]
6	8.6		865	847, 739, 713, **695**, 577, 575, 451, 407, 287	B-Type trimer
7			353	191, 179, **173**	4-Caffeoylquinic acid[a] (cryptochlorogenic acid)
8	8.85	280	1153	**1027**, 1001, 983, 907, 865, 863, 577, 575, 500, **491**, 451, 449, 425, 413, 407, 288, 286	B-Type tetramer
9			865	847, 739, 713, **695**, 577, 575, 451, 407, 287	B-Type trimer
10	9.05	284, 320sh	465	**303**, 285	Taxifolin hexoside
11	9.85	280	577	559, 451, **425**, 407, 299, 289, 245	B-Type dimer
12			865	847, 739, 713, **695**, 577, 575, 453, 451, 407, 287	B-Type trimer
13	9.95	285sh, 331	355	295, 265, 235, 216, **193**, 175, 160, 135	Feruloylhexoside
14	10.3	290sh, 313	337	**191**, 163	5-*p*-coumaroylquinic acid *(trans)*[a]
15	10.5	280	720[c]	701, 643, **635**, 577, 575, 451, 407, 288, 286	B-Type pentamer
16	10.9	279	289	271, **245**, 231, 205, 179	(-)-Epicatechin
17			1153		B-Type tetramer
18	11.1	312, 285sh	337	**173**, 163	4-*p*-coumaroylquinic acid (*trans*)[a]

No.	t_R (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 fragments (*m/z*)	Proposed structure
19	11.4	280	1153	1135, 1027, 1001, 984, 865, **863**, 739, 701, 577, 575, 425	B-Type tetramer
20	11.5		720[c]	701, **635**, 577, 575, 451, 425, 289, 287	B-Type pentamer
21	12.55	280	864[c]	779, 719, 695, 577, 575, 449, 407, 288, 286	B-Type hexamer
22	13.25	280	720[c]	711, 644, **635**, 577, 575, 559, 451, 407, 289, 287	B-Type pentamer
23	13.45	280	865	847, 739, 713, **695**, 577, 575, 543, 425, 407, 287	B-Type trimer
24	14.35	280	1153	1135, 1027, 1001, 983, **865**, 863, 739, 701, 577, 575, 449, 407	B-Type tetramer
25	14.55	280	1153	1135, 1027, 1001, 983, **865**, 863, 739, 701, 577, 575, 449, 407	B-Type tetramer
26	14.75	280	720[c]	701, **635**, 577, 575, 451, 425, 407, 289, 287	B-Type pentamer
27		280	865	847, 739, 713, **695**, 577, 575, 543, 425, 407, 287	B-Type trimer
28	15.0	280	864[c]	779, 719, 711, 695, 577, **575**, 449, 407, 289, 287	B-Type hexamer
29			720[c]	709, **643**, 634, 577, 575, 558, 449, 288	B-Type pentamer
30	15.15	280	1153	1135, 1027, 1001, 983, 865, **863**, 739, 701, 577, 575	B-Type tetramer
31			864[c]	779, 719, 695, **577**, 575, 449, 413, 287	B-Type hexamer
32	15.3	280	739	721, 629, **587**, 569, 449, 435, 417, 339, 289	Cinchonain II[b]
33	15.45	280	1008[c]	932, **914**, 737, 576,	B-Type heptamer
34	15.55	280	1153	1143, 1067, 1008, **863**, 629, 577, 449	B-Type tetramer
35	15.85	265, 356	463	300	Quercetin-3-O-hexoside
36	15.90		609	**300**, 270	Quercetin-3-O-rhamnoglucoside (rutin)
37	16.1	356	463	300	Quercetin-3-O-hexoside
38	16.4	356	433	300	Quercetin-3-O-pentoside
39	16.6	356	433	300	Quercetin-3-O-pentoside
40	16.7	280	1027	875, 857, **737**, 585, 575, 449, 407	Cinchonain III[b]

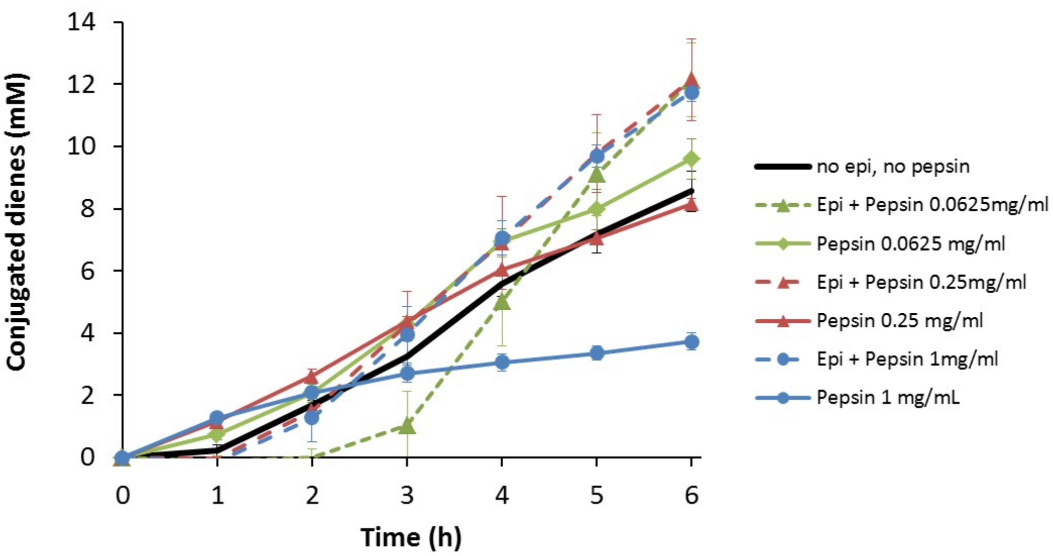
No.	t_R (min)	λ $_{max}$ (nm)	[M-H]$^-$ (*m/z*)	MS^2 fragments (*m/z*)	Proposed structure
41	16.8	354	433	300	Quercetin-3-O-pentoside
42	17.0	354	433	300	Quercetin-3-O-pentoside
43	17.1	354	447	300	Quercetin-3-O-deoxyhexose
44	17.25	284, 300sh	567	273	Phloretin-2-O'-xyloglucoside
45	17.9		447	314	Tamarixetin- or isorhamnetin-3-O-pentoside
46	18.0	284, 300sh	435	273	Phloretin-2-O'-glucoside

NF: Not Fragmented, sh: shoulder. Fragment in bold is major ion. [a]Identified according to Clifford et al. (2003), [b]Hokkanen et al. (2009), [c]doubly-charged ion.

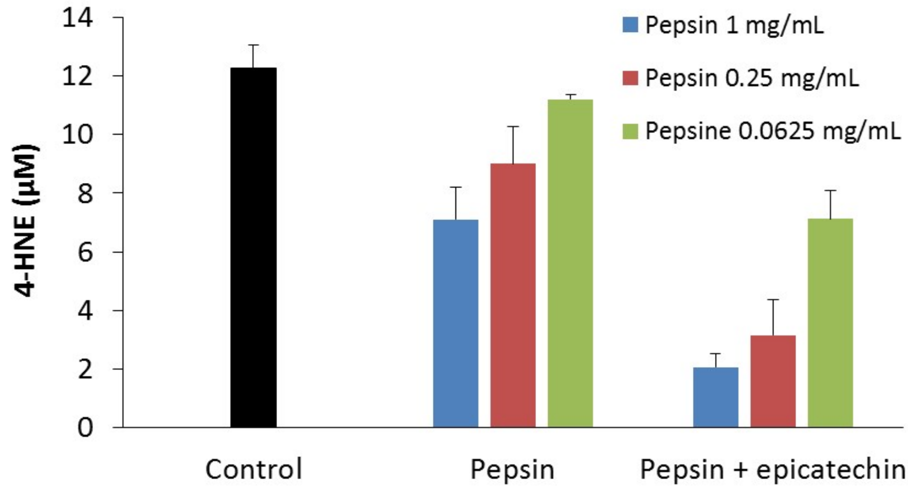
- M. N. Clifford, K. L. Johnston, S. Knight and N. Kuhnert, *Journal of Agricultural and Food Chemistry*, **2003**, 51, 2900-2911.
- J. Hokkanen, S. Mattila, L. Jaakola, A. M. Pirttila and A. Tolonen, *Journal of Agricultural and Food Chemistry*, **2009**, 57, 9437-9447.

Figure 1. Metmyoglobin-initiated lipid oxidation at different pepsin concentrations (1, 0.25, and 0.0625 mg/mL with 2828 U/mg) and inhibition by epicatechin in phospholipid-stabilized emulsions at pH 5. Accumulation of conjugated dienes (A) and 4-HNE at 6 hours (B). Degradation of epicatechin (C) and α-tocopherol without and with epicatechin at different pepsin concentrations (D-E). Epicatechin concentration = 100 μM. Values represent mean ± SD (n = 3).

A

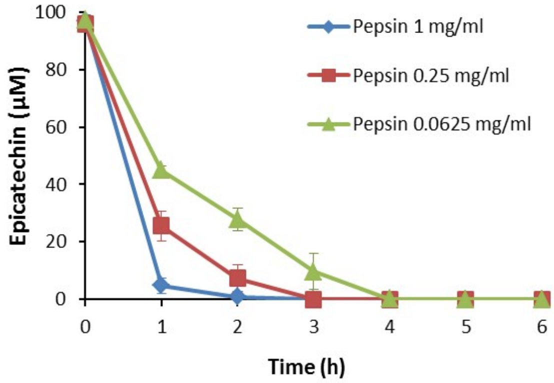


B



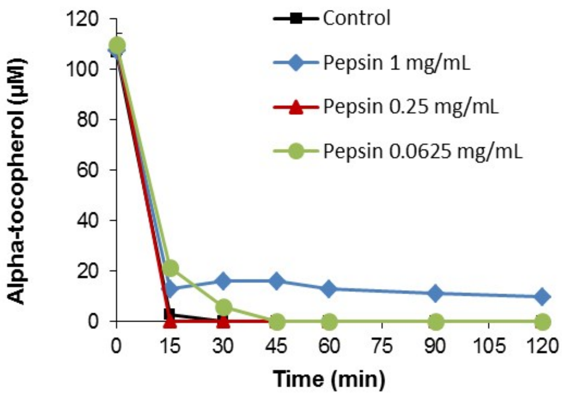
4-HNE (µM)
0
2
4
6
8
10
12
14
Pepsin 1 mg/mL
Pepsin 0.25 mg/mL
Pepsine 0.0625 mg/mL
Control
Pepsin
Pepsin + epicatechin

C



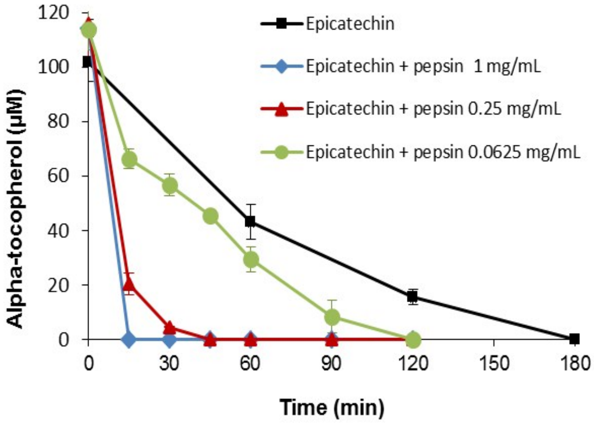
Epicatechin (µM)
0
20
40
60
80
100
Pepsin 1 mg/ml
Pepsin 0.25 mg/ml
Pepsin 0.0625 mg/ml
0
1
2
3
4
5
6
Time (h)

D



Alpha-tocopherol (µM)
0
20
40
60
80
100
120
Control
Pepsin 1 mg/mL
Pepsin 0.25 mg/mL
Pepsin 0.0625 mg/mL
0
15
30
45
60
75
90
105
120
Time (min)

E



Alpha-tocopherol (µM)
0
20
40
60
80
100
120
Epicatechin
Epicatechin + pepsin 1 mg/mL
Epicatechin + pepsin 0.25 mg/mL
Epicatechin + pepsin 0.0625 mg/mL
0
30
60
90
120
150
180
Time (min)

Figure 2. Effect of pepsin on the structure of metmyoglobin (5 µM) in the presence of epicatechin (25 µM). (A) Addition of pepsin (0.0625 mg/mL with 2041 U/mg) onto metmyoglobin at pH 5. (B) Addition of pepsin (0.0625 mg/mL) onto metmyoglobin at pH 4.

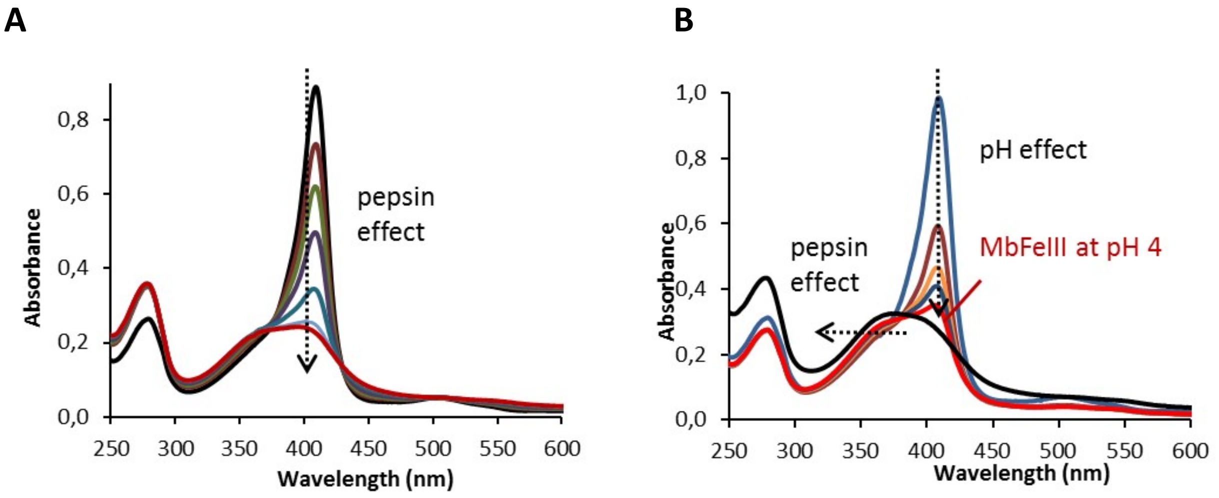
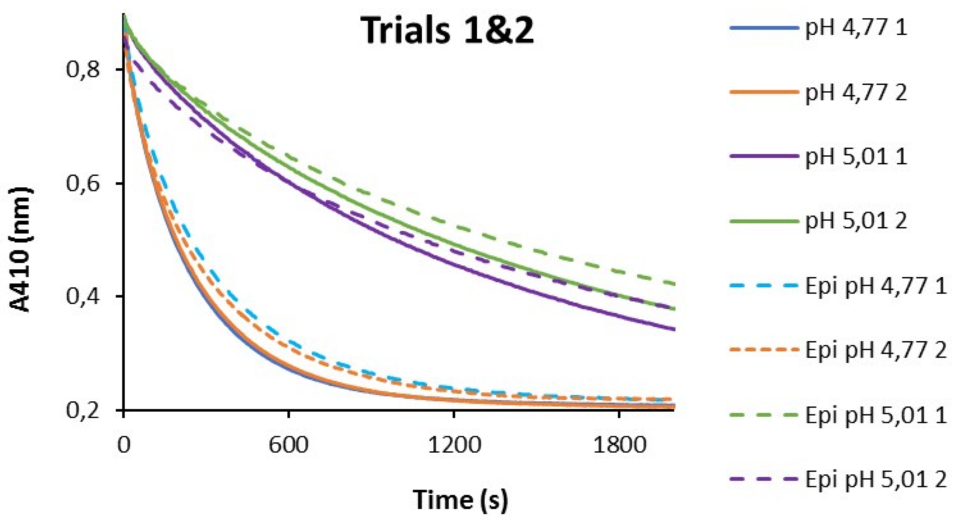


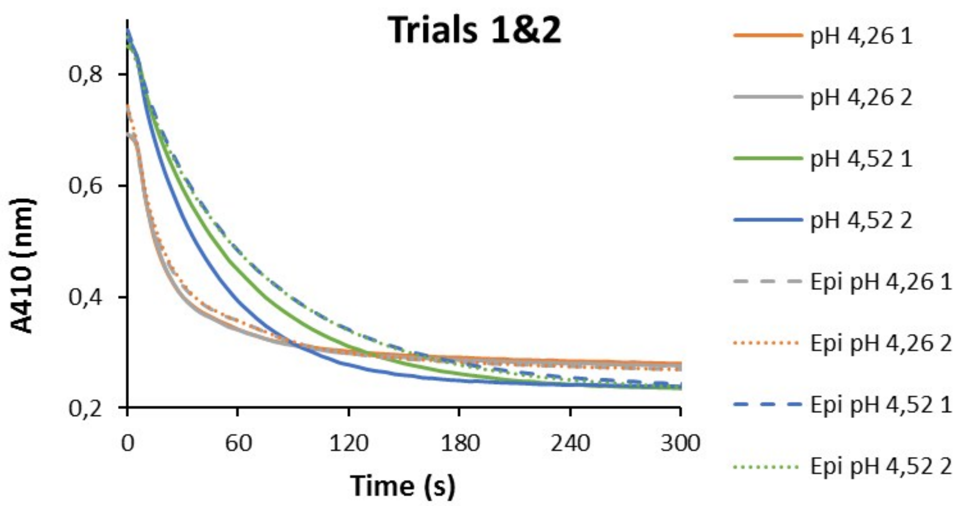
Table 2 & Figure 3. Kinetics for the decay of the Soret band of metmyoglobin (5 µM) at 410 nm upon addition of pepsin (0.0625 mg/mL with 2041 U/mg) at different pH, in the absence or presence of epicatechin (25 µM). Data are reported as first-order rate constants (*k*) and standard deviations between measured absorbances and calculated values.

Three trials with same epicatechin, pepsin, metmyoglobin solutions per trial.

	Without (-)-Epicatechin				With (-)-Epicatechin			
	Trial 1				Trial 2			
	k (s^{-1})	SD (s^{-1})	k (s^{-1})	SD (s^{-1})	k (s^{-1})	SD (s^{-1})	k (s^{-1})	SD (s^{-1})
pH 5.01	9.54×10^{-4}	0.29×10^{-4}	8.79×10^{-4}	0.35×10^{-4}	7.02×10^{-4}	0.17×10^{-4}	7.93×10^{-4}	0.20×10^{-4}
pH 4.77	5.13×10^{-3}	0.10×10^{-3}	4.85×10^{-3}	0.10×10^{-3}	3.92×10^{-3}	0.09×10^{-3}	4.14×10^{-3}	0.08×10^{-3}
pH 4.52	1.69×10^{-2}	0.10×10^{-2}	2.29×10^{-2}	0.11×10^{-2}	1.66×10^{-2}	0.03×10^{-2}	1.61×10^{-2}	0.03×10^{-2}
pH 4.26	3.92×10^{-2}	0.48×10^{-2}	3.75×10^{-2}	0.49×10^{-2}	4.38×10^{-2}	0.20×10^{-2}	4.13×10^{-2}	0.23×10^{-2}

	Without (-)-Epicatechin				With (-)-Epicatechin	
	Trial 3				Trial 3	
	k (s^{-1})	SD (s^{-1})	$(k_{epi}-k_{no\ epi}) / k_{no\ epi}$	$k_{pH\ low} / k_{pH\ high}$	k (s^{-1})	SD (s^{-1})
pH 5.01	6.45×10^{-4}	0.22×10^{-4}	10%	7.0	5.81×10^{-4}	0.14×10^{-4}
pH 4.77	4.50×10^{-3}	0.10×10^{-3}	25%	2.6	3.37×10^{-3}	0.06×10^{-3}
pH 4.52	1.18×10^{-2}	0.02×10^{-2}	7%	3.7	1.10×10^{-2}	0.02×10^{-2}
pH 4.26	4.31×10^{-2}	0.07×10^{-2}			4.92×10^{-2}	0.10×10^{-2}


Trials 1&2
A410 (nm)
0,8
0,6
0,4
0,2
0
600
1200
1800
Time (s)
pH 4,77 1
pH 4,77 2
pH 5,01 1
pH 5,01 2
Epi pH 4,77 1
Epi pH 4,77 2
Epi pH 5,01 1
Epi pH 5,01 2


Trials 1&2
A410 (nm)
0,8
0,6
0,4
0,2
0
60
120
180
240
300
Time (s)
pH 4,26 1
pH 4,26 2
pH 4,52 1
pH 4,52 2
Epi pH 4,26 1
Epi pH 4,26 2
Epi pH 4,52 1
Epi pH 4,52 2