

Investigation of Pharmacologically Important Polyphenolic Secondary Metabolites in Plant-based Food Samples Using HPLC-DAD

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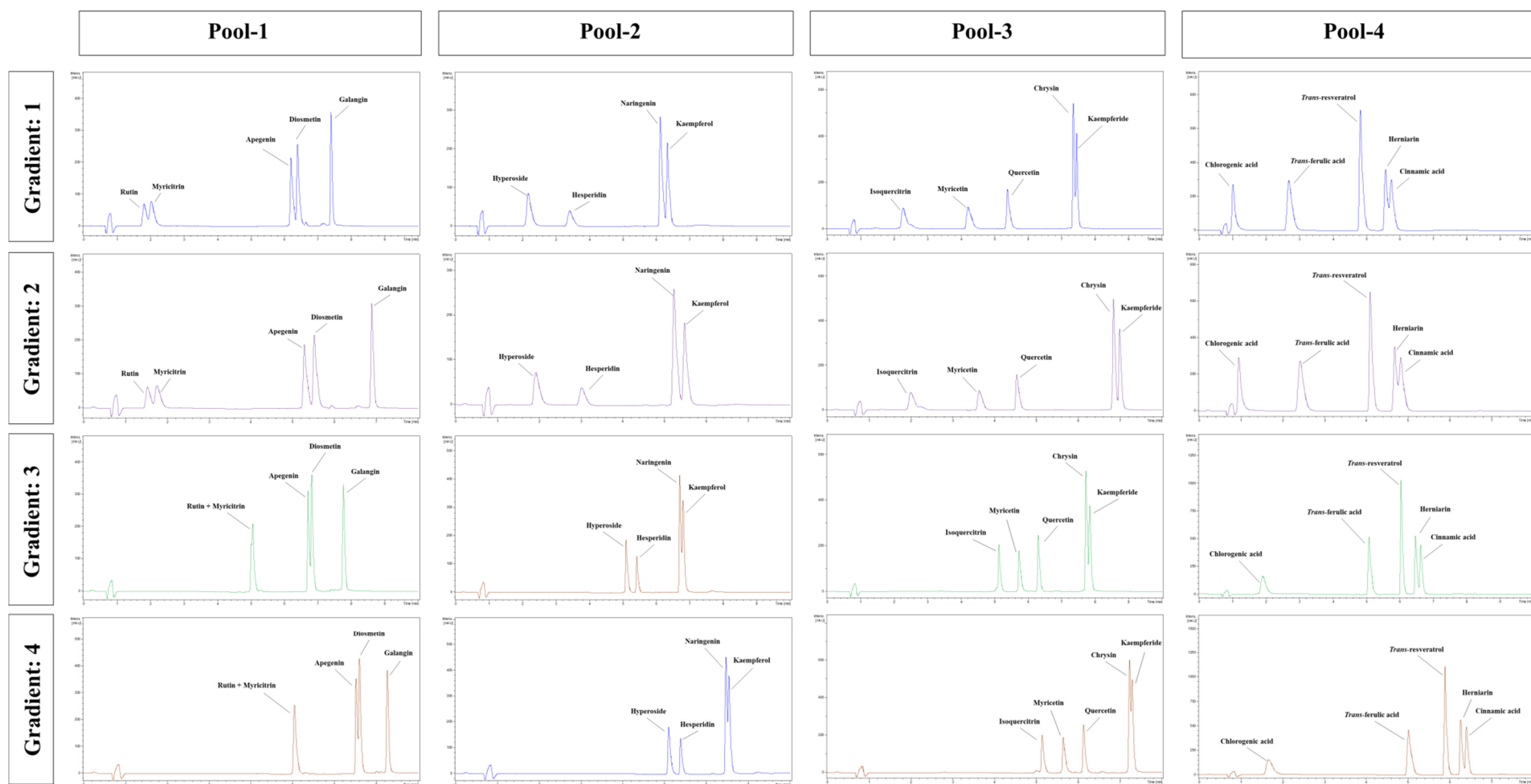
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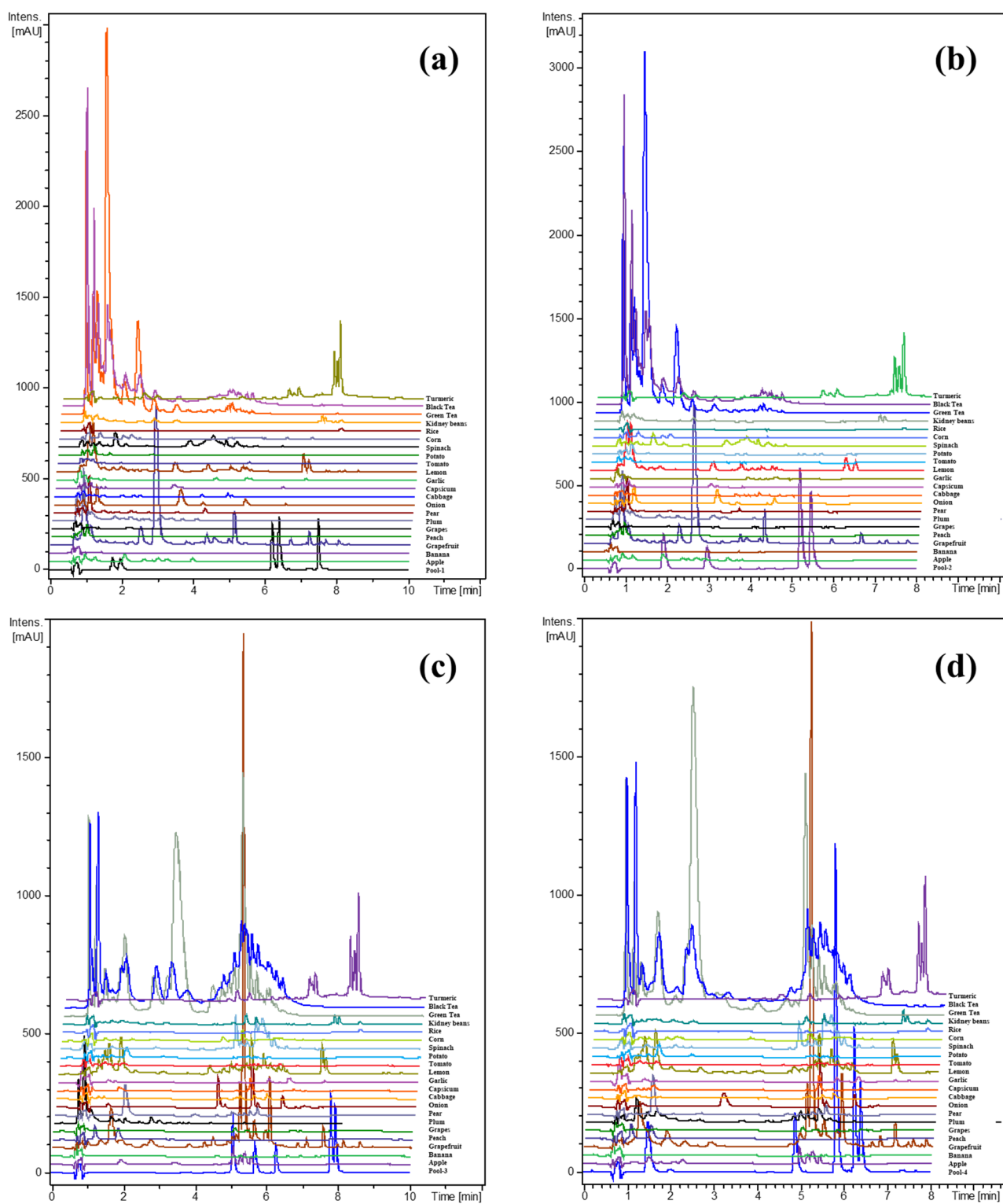
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 Pool-4: Chlorogenic acid, *Trans*-ferulic acid, *Trans*-resveratrol, Herniarin and Cinnamic acid

Supplementary Fig. 1. The comparison of pools 1-4 on different gradient systems.



Supplementary Fig. 2. The comparison of standard compounds of each pool (a) pool-1, (b) pool-2, (c) pool-3, (d) pool-4 with analyzed real samples.

Supplementary Table S1. The %accuracy and %RSD of all compounds.

POOL-1							
Compound Name	Concentrations in $\mu\text{g/ml}$	Intra-day	%RSD	%Accuracy	Inter-day	%RSD	%Accuracy
		Found concentration in			Found concentration in		
		$\mu\text{g/mL}$			$\mu\text{g/mL}$		
Rutin	100	99.2 ± 0.9	0.91	99.21	96 ± 3	3.59	95.93
	400	399 ± 5	1.24	99.57	401 ± 3	0.69	100.15
	800	805 ± 5	0.60	100.60	806 ± 5	0.58	100.78
Myricitrin	100	103 ± 3	3.04	103.17	107 ± 2	1.56	107.26
	400	402.2 ± 0.2	0.04	100.53	405 ± 5	1.10	101.37
	800	793 ± 1	0.15	99.09	790 ± 2	0.21	98.77
Apigenin	100	102.5 ± 0.2	0.15	102.53	105 ± 4	4.01	105.54
	400	399 ± 2	0.60	99.63	401 ± 4	0.97	100.18
	800	801 ± 8	1.02	100.09	796 ± 7	0.86	99.50
Diosmetin	100	101.5 ± 0.2	0.21	101.47	104 ± 4	3.85	104.26
	400	402 ± 3	0.70	100.42	403 ± 2	0.50	100.69
	800	800 ± 3	0.40	99.97	796 ± 3	0.42	99.56
Galangin	100	99 ± 2	2.10	99.30	100 ± 2	2.01	99.59
	400	400 ± 1	0.37	100.06	402 ± 4	0.98	100.45

	800	797 ± 4	0.51	99.65	798 ± 1	0.16	99.79
POOL-2							
Compound Name	Concentrat ions in µg/ml	Intra-day	%RSD	%Accuracy	Inter-day	%RSD	%Accuracy
		Found concentration in			Found concentration in		
		µg/mL			µg/mL		
Hyperoside	100	103 ± 5	4.34	103.03	101 ± 4	3.92	101.39
	400	396 ± 1	0.25	99.02	412 ± 3	0.62	102.90
	800	798 ± 1	0.18	99.70	798 ± 2	0.23	99.74
Hesperidin	100	104 ± 3	2.67	104.14	103 ± 3	2.53	103.43
	400	400 ± 2	0.41	99.92	403.0 ± 0.4	0.09	100.76
	800	800 ± 2	0.27	100.06	808 ± 9	1.16	100.95
Naringenin	100	102 ± 1	1.41	101.56	102 ± 4	3.55	102.84
	400	400 ± 4	0.92	99.74	408 ± 1	0.23	102.01
	800	803± 4	0.45	100.43	804 ± 5	0.67	100.55
Kaempferol	100	98 ± 3	2.61	97.55	101 ± 3	2.80	100.62
	400	398 ± 3	0.73	99.59	399.3 ± 0.9	0.22	99.84
	800	805 ± 3	0.36	100.58	801 ± 3	0.34	100.17
POOL-3							
Compound Name	Concentrat ions in	Intra-day	%RSD	%Accuracy	Inter-day	%RSD	%Accuracy
		Found			Found		

	$\mu\text{g/ml}$	concentration in			concentration in		
		$\mu\text{g/mL}$			$\mu\text{g/mL}$		
Isoquercitrin	100	100 ± 2	2.29	99.83	101 ± 5	4.66	100.73
	400	402 ± 2	0.43	100.52	409 ± 8	1.90	102.27
	800	804 ± 7	0.85	100.50	820 ± 10	1.48	102.54
Myricetin	100	100 ± 1	1.16	100.24	104 ± 5	4.68	103.87
	400	406 ± 5	1.18	101.41	408.5 ± 0.2	0.06	102.13
	800	799 ± 3	0.40	99.91	795 ± 7	0.88	99.48
Quercetin	100	101 ± 1	1.24	100.89	104 ± 4	4.37	104.11
	400	397 ± 2	0.43	99.19	395 ± 2	0.50	98.88
	800	800 ± 2	0.23	99.98	799 ± 4	0.46	99.87
Chrysin	100	100 ± 1	1.22	99.56	99 ± 3	2.67	98.76
	400	398.2 ± 0.8	0.20	99.54	392 ± 8	1.91	97.99
	800	804 ± 1	0.13	100.46	807 ± 5	0.59	100.88
Kaempferide	100	103 ± 1	0.98	103.21	107 ± 4	4.04	107.12
	400	395 ± 2	0.42	98.67	398 ± 7	1.66	99.51
	800	800 ± 1	0.16	100.02	808 ± 10	1.20	101.02
POOL-4							
Compound Name	Concentrat ions in $\mu\text{g/ml}$	Intra-day	%RSD	%Accuracy	Inter-day	%RSD	%Accuracy
		Found concentration in			Found concentration in		

		$\mu\text{g/mL}$			$\mu\text{g/mL}$		
Chlorogenic Acid	100	101.6 ± 0.5	0.55	101.58	102 ± 2	2.02	102.41
	400	397.1 ± 0.8	0.20	99.28	394 ± 4	1.07	98.42
	800	805 ± 2	0.23	100.59	810 ± 5	0.65	101.29
<i>Trans</i>-Ferulic Acid	100	103 ± 2	1.71	103.39	104 ± 2	2.03	103.55
	400	401 ± 1	0.36	100.16	393 ± 9	2.41	98.33
	800	798 ± 5	0.64	99.75	795 ± 5	0.66	99.32
<i>Trans</i>-Resveratrol	100	98 ± 1	1.05	98.45	103 ± 4	4.16	102.60
	400	400 ± 2	0.47	100.09	400 ± 3	0.81	100.12
	800	800 ± 5	0.58	100.07	798 ± 4	0.47	99.75
Herniarin	100	102 ± 2	2.17	101.87	102 ± 2	2.07	101.80
	400	397 ± 2	0.64	99.12	397 ± 1	0.38	99.29
	800	802 ± 1	0.15	100.25	801 ± 3	0.44	100.09
Cinnamic Acid	100	99 ± 0.8	0.90	98.77	100 ± 2	2.21	100.14
	400	403 ± 3	0.86	100.72	402 ± 3	0.79	100.52
	800	804 ± 2	0.29	100.50	801.2 ± 0.6	0.07	100.16

Supplementary Table S2. shows the Results of the percent recovery study.

POOL-1							
Sample Category	Sample Name	Spiking Level	% Recovery				
			Rutin	Myricitrin	Apigenin	Diosmetin	Galangin
Fruit	Grapefruit	S1	94.18	102.94	96.34	96.12	92.80
		S2	98.16	98.23	92.78	93.17	106.59
		S3	104.54	101.69	95.11	97.04	103.01
Vegetable	Spinach	S1	92.98	97.59	96.42	101.52	95.42
		S2	98.55	106.96	104.66	98.06	93.69
		S3	96.61	103.58	102.62	91.51	91.66
Other	Corn	S1	94.18	102.94	96.34	96.12	92.80
		S2	98.16	98.23	92.78	93.17	106.59
		S3	104.54	101.69	95.11	97.04	103.01
POOL-2							
Sample Category	Sample Name	Spiking Level	% Recovery				
			Hyperoside	Hesperidin	Naringenin	Kaempferol	
Fruit	Grapefruit	S1	93.48	91.01	105.35	101.64	
		S2	103.69	102.96	98.11	93.48	
		S3	97.68	97.68	101.60	103.46	
Vegetable	Spinach	S1	104.21	96.01	87.34	98.38	
		S2	97.43	104.92	93.00	93.83	

		S3	96.11	95.53	90.57	95.38	
Other	Corn	S1	93.48	91.01	105.35	101.64	
		S2	103.69	102.96	98.11	93.48	
		S3	97.68	97.68	101.60	103.46	
POOL-3							
Sample Category	Sample Name	Spiking Level	% Recovery				
			Isoquercitrin	Myricetin	Quercetin	Chrysin	Kaempferide
Fruit	Grapefruit	S1	97.51	100.02	106.64	94.54	103.41
		S2	106.88	98.24	98.20	97.94	97.16
		S3	96.70	103.85	97.00	90.70	100.28
Vegetable	Spinach	S1	103.51	101.82	95.76	92.22	95.31
		S2	92.30	99.56	100.41	105.40	103.67
		S3	97.34	97.09	91.35	91.86	97.72
Other	Corn	S1	97.51	100.02	106.64	94.54	103.41
		S2	106.88	98.24	98.20	97.94	97.16
		S3	96.70	103.85	97.00	90.70	100.28
POOL-4							
Sample Category	Sample Name	Spiking Level	% Recovery				
			Chlorogenic Acid	Trans-Ferulic Acid	Trans-Resveratrol	Herniarin	Cinnamic Acid
Fruit	Grapefruit	S1	101.29	97.66	96.68	94.67	97.39

		S2	98.63	96.63	103.61	99.12	94.97
		S3	103.30	101.12	98.45	97.50	91.62
Vegetable	Spinach	S1	99.64	90.20	101.56	89.84	101.47
		S2	91.49	103.18	98.18	91.62	99.28
		S3	92.24	92.87	95.72	98.22	89.83
Other	Corn	S1	101.29	97.66	96.68	94.67	97.39
		S2	98.63	96.63	103.61	99.12	94.97
		S3	103.30	101.12	98.45	97.50	91.62

Supplementary Table S3. The quantified results of food samples in mg/Kg.

POOL-1						
Analyte Conc. mg/kg of Food Samples						
Sample Category	Sample Name	Rutin	Myricitrin	Apigenin	Diosmetin	Galangin
Fruits	Apple	ND	228.02 ± 1.5	ND	ND	ND
	Banana	ND	ND	63.19 ± 5.5	73.48 ± 1.5	ND
	Grapefruit	ND	ND	124.22 ± 0.9	39.00 ± 0.4	122.46 ± 0.8
	Peach	ND	ND	ND	ND	ND
	Grapes	ND	ND	31.87 ± 0.4	25.81 ± 1.3	ND
	Plum	277.45 ± 3.6	ND	40.04 ± 1.4	ND	ND
	Pear	124.89 ± 5.3	ND	ND	ND	ND
Vegetables	Onion	ND	240.02 ± 1.1	ND	ND	ND
	Cabbage	49.62 ± 1.8	ND	ND	ND	ND
	Capsicum	ND	ND	ND	ND	ND
	Garlic	ND	ND	ND	ND	ND
	Lemon	ND	ND	ND	ND	ND
	Tomato	93.36 ± 3.6	ND	ND	ND	ND
	Potato	55.00 ± 3.3	75.36 ± 0.5	ND	ND	ND
	Spinach	166.07 ± 0.4	398.18 ± 5.0	ND	ND	ND
Others	Corn	37.82 ± 0.7	148.24 ± 1.0	ND	23.51 ± 1.0	ND

	Rice	ND	ND	ND	ND	ND
	Kidney beans	ND	ND	ND	ND	75.56 ± 0.6
	Green Tea	337.35 ± 0.2	ND	ND	ND	ND
	Black Tea	224.12 ± 0.2	121.08 ± 0.6	ND	ND	ND
	Turmeric	62.69 ± 2.1	ND	ND	137.59 ± 1.3	100.20 ± 0.1
POOL-2						
Analyte Conc. mg/kg of Food Samples						
Sample Category	Sample Name	Hyperoside	Hesperidin	Naringenin	Kaempferol	
Fruits	Apple	318.02 ± 2.3	162.62 ± 0.4	ND	ND	
	Banana	ND	245.66 ± 0.9	ND	35.64 ± 0.5	
	Grapefruit	ND	2483.16 ± 0.5	108.92 ± 2.0	ND	
	Peach	ND	ND	ND	ND	
	Grapes	ND	102.32 ± 1.4	52.77 ± 1.7	55.95 ± 1.2	
	Plum	134.23 ± 1.6	165.16 ± 0.5	ND	63.94 ± 1.7	
	Pear	110.28 ± 2.1	ND	ND	ND	
Vegetables	Onion	332.78 ± 1.5	37.82 ± 0.9	ND	35.64 ± 5.8	
	Cabbage	118.85 ± 1.4	ND	ND	ND	
	Capsicum	44.93 ± 4.1	62.25 ± 0.4	ND	64.53 ± 1.0	
	Garlic	ND	ND	ND	ND	
	Lemon	ND	303.18 ± 3.1	ND	ND	

	Tomato	ND	69.98 ± 4.8	39.34 ± 2.8	ND
	Potato	ND	ND	ND	ND
	Spinach	ND	ND	ND	69.30 ± 2.0
Others	Corn	45.57 ± 0.2	ND	ND	ND
	Rice	ND	ND	ND	ND
	Kidney beans	ND	168.96 ± 2.7	ND	ND
	Green Tea	1098.34 ± 0.3	158.08 ± 0.5	ND	ND
	Black Tea	273.86 ± 0.3	103.04 ± 0.5	ND	ND
	Turmeric	49.17 ± 1.6	ND	ND	30.93 ± 0.1

POOL-3						
Analyte Conc. mg/kg of Food Samples						
Sample Category	Sample Name	Isoquercitrin	Myricetin	Quercetin	Chrysin	Kaempferide
Fruits	Apple	319.33 ± 0.1	199.83 ± 1.9	ND	ND	ND
	Banana	ND	68.21 ± 2.7	160.89 ± 1.4	ND	ND
	Grapefruit	80.40 ± 1.3	301.74 ± 2.1	ND	32.97 ± 3.7	48.94 ± 0.6
	Peach	ND	ND	ND	ND	ND
	Grapes	71.82 ± 1.2	ND	ND	ND	ND
	Plum	168.43 ± 2.2	42.42 ± 1.4	30.96 ± 3.3	ND	ND

	Pear	38.92 ± 2.6	131.19 ± 1.5	ND	ND	ND
Vegetables	Onion	ND	68.21 ± 1.1	160.89 ± 0.9	ND	ND
	Cabbage	43.02 ± 1.8	51.35 ± 2.2	ND	ND	ND
	Capsicum	ND	ND	ND	ND	ND
	Garlic	ND	80.14 ± 1.0	190.31 ± 1.9	ND	ND
	Lemon	96.17 ± 0.1	215.15 ± 2.1	172.25 ± 1.9	ND	ND
	Tomato	ND	ND	62.53 ± 1.9	ND	ND
	Potato	41.64 ± 1.3	51.95 ± 3.3	ND	ND	ND
	Spinach	122.11 ± 1.0	245.75 ± 0.2	70.68 ± 2.0	ND	ND
Others	Corn	ND	37.46 ± 0.1	ND	ND	ND
	Rice	49.14 ± 0.3	ND	ND	ND	ND
	Kidney beans	201.10 ± 0.2	ND	ND	112.65 ± 0.2	ND
	Green Tea	227.75 ± 0.1	182.15 ± 1.8	72.18 ± 0.1	ND	ND
	Black Tea	130.48 ± 0.4	140.87 ± 0.1	54.14 ± 0.7	ND	ND
	Turmeric	29.84 ± 0.1	ND	ND	ND	118.31 ± 0.1
POOL-4						
Analyte Conc. mg/kg of Food Samples						
Sample Category	Sample Name	Chlorogenic Acid	<i>Trans</i>-Ferulic Acid	<i>Trans</i>-Resveratrol	Herniarin	Cinnamic Acid
Fruits	Apple	216.39 ± 0.8	81.03 ± 0.3	ND	ND	ND
	Banana	73.74 ± 1.5	22.17 ± 3.6	ND	ND	48.38 ± 1.3

	Grapefruit	ND	156.79 ± 2.3	80.31 ± 0.4	36.00 ± 1.3	ND
	Peach	200.62 ± 0.4	ND	ND	ND	ND
	Grapes	34.75 ± 2.5	64.40 ± 1.3	38.24 ± 3.6	ND	23.00 ± 3.1
	Plum	54.27 ± 1.4	98.53 ± 1.3	42.69 ± 2.0	ND	35.24 ± 3.6
	Pear	99.20 ± 0.2	ND	ND	ND	ND
Vegetables	Onion	ND	ND	ND	ND	ND
	Cabbage	ND	ND	ND	ND	ND
	Capsicum	ND	ND	ND	ND	ND
	Garlic	ND	ND	ND	ND	ND
	Lemon	81.21 ± 0.2	108.62 ± 1.0	49.79 ± 0.8	ND	ND
	Tomato	57.51 ± 0.3	28.27 ± 0.2	ND	ND	ND
	Potato	153.15 ± 0.5	ND	ND	ND	ND
	Spinach	45.63 ± 1.3	142.48 ± 0.7	ND	ND	ND
Others	Corn	ND	157.01 ± 0.1	BQL	ND	20.79 ± 0.1
	Rice	ND	60.07 ± 2.1	ND	ND	ND
	Kidney beans	ND	132.78 ± 1.0	BQL	ND	ND
	Green Tea	ND	269.20 ± 0.1	54.61 ± 0.9	ND	ND
	Black Tea	ND	105.42 ± 0.1	145.10 ± 1.0	ND	ND
	Turmeric	ND	67.44 ± 0.7	ND	ND	ND

ND: Not detected

BQL: Below quantification limit

Supplementary Table S4. Tested parameters for the final optimization of HPLC method.

Pool No.	Flow Rate (mL/min)	Injection Volume (μL)	Mobile Phase Gradient
Pool-1	0.3	3	10-30% B, 0–1 min; 30-40% B, 1–2 min; 40-80% B, 2–5 min; 80-40% B, 5–7 min; 40-30% B, 7–8 min; 30-10% B, 8–10 min
	0.35	2.5	10-20% B, 0–1 min; 20-40% B, 1–3 min; 40-80% B, 3–6 min; 80-40% B, 6–7 min; 40-20% B, 7–8 min; 20-10% B, 8–10 min
	0.35	2	20-30% B, 0–1 min; 30-40% B, 1–3 min; 40-80% B, 3–5 min; 80-40% B, 5–7 min; 40-30% B, 7–8 min; 30-20% B, 8–10 min
Pool-2	0.3	3	10% B, 0–1 min; 10-90% B, 1–7 min; 90% B, 7-8 min; 90-10% B, 8-9 min; 10% B, 9-10 min
	0.3 mL/min, (0-5) min, 0.2 mL/min, (5-10) min, 0.3 mL/min, (10-13) min	2.5	20-30% B, 0–1 min; 30-40% B, 1–4 min; 40-70% B, 4–5 min; 70-80% B, 5–10 min; 80-30% B, 10–11 min; 30-20% B, 11-13 min
	0.35 mL/min, (0-3) min, 0.5 mL/min, (4-5) min, 0.35 mL/min, (5-8) min	2	20-30% B, 0–1 min; 30-40% B, 1–3 min; 40-70% B, 3–4.5 min; 70-40% B, 4.5–6.5 min; 40-30% B, 6.5–7 min; 30-20% B, 7–8 min

Pool-3	0.3	3	10-20% B, 0–1 min; 20-70% B, 1–6 min; 70% B, 6–8 min; 70-20% B, 8–9 min; 20-10% B, 9–10 min
	0.35	2.5	20% B, 0–1 min; 20-70% B, 1–6 min; 80% B, 6–8 min; 80-20% B, 8–9 min; 20% B, 9–10 min
	0.35	2	10-20% B, 0–1 min; 20-70% B, 1–5 min; 70% B, 5–7.5 min; 70-20% B, 7.5–8 min; 20-10% B, 8–10 min
Pool-4	0.3	3	10% B, 0–1 min; 10-100% B, 1–8 min; 100-10% B, 8–9 min; 10% B, 9–10 min
	0.35	2.5	20% B, 0–1 min; 20-80% B, 1–8 min; 80-20% B, 8–9 min; 20% B, 9–10 min
	0.35	2	10% B, 0–1 min; 10-100% B, 1–6 min; 100-10% B, 6–7 min; 10% B, 7–8 min