

Phytoestrogens as biomarkers of plant raw materials used for fish feed production

Dionysios T. Pavlopoulos ¹, Eleni D. Myrtsi¹, Paschalitsa Tryfinopoulou², Vasilios Iliopoulos¹, Sofia D. Koulocheri¹ * and Serkos A. Haroutounian¹

Supplementary Materials

Table S1. Transition, collision energy, polarity, retention time (RT), calculation of equations, determination coefficients, LOD and LOQ for each phytoestrogen standard

Table S2. Phytoestrogen concentration ranges of soymeal, sunflower meal, rapeseed meal and wheat meal.

Figure S1. Concentration distributions before and after normalization based on the kernel density. Similarly, the bottom plots graphically summarize the concentrations of individual compounds.

Figure S2. Results of the analysis of variance (ANOVA) of phytoestrogens, ranked by their P values ($P < 0.05$)

Figure S3. Chromatograms of phytoestrogens extracted from samples of soybean (a), rapeseed (b), sunflower (c) and wheat (d) meals.

Table S1. Transition, collision energy, polarity, retention time (RT), calculation of equations, determination coefficients, LOD and LOQ for each phytoestrogen standard.

Compound	Parent Mass	Product Mass	Collision Energy (eV)	Polarity	RT (min)	LOD (ng/mL)	LOQ (ng/mL)	Equation	R ²
<i>Phenolic acids</i>									
1 Caffeic Acid	180.0	135.9	19	(-)	3.89	51.4	155.8	$Y = 0.00205272 + 0.800781 * X$	0.9997
2 Chlorogenic Acid	354.2	191.7	22	(-)	2.36	44.6	135.1	$Y = 0.00242681 + 0.0435718 * X$	0.9988
3 Gallic Acid	170.1	126.0	18	(-)	2.05	73.1	221.5	$Y = -0.000734999 + 0.247545 * X$	0.9998
4 Neochlorogenic Acid	354.2	190.9	21	(-)	2.04	12.6	38.2	$Y = 0.00126487 + 0.332059 * X$	0.9992
5 p-Coumaric Acid	164.0	120.0	18	(-)	6.16	46.0	139.5	$Y = 0.00445059 + 0.384097 * X$	0.9998
6 Protocatechuic Acid	154.1	110.0	18	(-)	2.37	16.2	49.1	$Y = 0.00114193 + 0.336255 * X$	0.9996
7 Sinapic Acid	224.1	208.9	16	(-)	6.78	54.9	166.2	$Y = 0.00292367 + 0.00171603 * X$	0.9999
<i>Flavonoids</i>									
8 Apigenin	270.0	118.1	42	(-)	16.98	8.7	26.4	$Y = -0.00105394 + 1.4346 * X$	0.9992
9 Catechin	290.1	204.1	21	(-)	2.9	75.5	228.9	$Y = 0.0467805 + 0.934157 * X$	0.9996
10 Diosmetin	300.3	285.0	20	(-)	17.67	53.9	163.3	$Y = 0.000344824 + 0.00220693 * X$	1.0000
11 Diosmin	608.4	607.6 284.1	13	(-)	8.1	12.5	37.7	$Y = -0.000727451 + 0.146644 * X$	0.9983
12 Epicatechin	290.2	246.3	18	(-)	3.57	18.9	57.3	$Y = 0.0105637 + 0.0205232 * X$	0.9993
13 Epigallocatechin	306.2	125.0	29	(-)	2.47	39.2	118.9	$Y = 0.00338522 + 0.0195243 * X$	0.9996
14 Epigallocatechin Gallate	458.2	168.8	21	(-)	3.44	30.7	93.1	$Y = -0.0193777 + 0.909754 * X$	0.9993
15 Eriodictyol	288.2	151.2	17	(-)	12.67	23.6	71.6	$Y = -0.00580681 + 0.335251 * X$	0.9996
16 Gallocatechin	306.1	125.0	24	(-)	2.23	109.5	331.8	$Y = 0.0110903 + 0.0536722 * X$	0.9993
17 Herperidin	610.1	301.7	26	(-)	7.78	27.2	82.3	$Y = -0.0393733 + 3.85778 * X$	0.9991
18 Hesperetin	302.1	286.9	20	(-)	17.37	13.8	42.0	$Y = 0.000690733 + 0.189778 * X$	0.9991
19 Isoquercetin	464.0	300.7	27	(-)	6.42	46.2	140.0	$Y = -8.85249e-005 + 0.0177665 * X$	0.9996

Compound	Parent Mass	Product Mass	Colision Energy (eV)	Polarity	RT (min)	LOD (ng/mL)	LOQ (ng/mL)	Equation	R ²
20 Isorhamnetin	316.1	301.0	24	(-)	18.08	47.5	144.0	Y = 3.04779e-005+0.0167596*X	0.9998
21 Kaempferol	286.1	240.0	31	(-)	17.46	15.0	45.5	Y = 0.00622424+0.0165355*X	0.9999
22 Liquiritigenin	256.2	135.0	18	(-)	12.64	77.4	234.7	Y = 0.0106288+0.760192*X	0.9989
23 Liquiritin	418.2	256.0	24	(-)	5.96	26.3	79.6	Y = -0.000555141+0.784532*X	0.9997
24 Luteolin	286.1	133.0	36	(-)	13.74	20.6	62.3	Y = 0.000398407+1.0949*X	0.9994
25 Luteolin-4'-o-glucoside	448.3	284.9	21	(-)	8.62	29.8	90.3	Y = -0.000145195+0.0271768*X	0.9992
26 Myricetin	318.1	150.8	26	(-)	9.85	67.1	203.2	Y = -0.00126106+0.200528*X	0.9993
27 Pelargonidin	271.0	121.0	33	(+)	5.20	23.3	70.5	Y = 1.18774+4.54226*X	0.9994
28 Pelargonin	595.2	270.9	33	(+)	2.05	7.1	21.4	Y = -1.33029+242.479*X	0.9995
29 Procyanidin B1	578.3	407.7	25	(-)	2.3	46.9	142.2	Y = -0.0157375+1.99064*X	0.9997
30 Procyanidin B2	578.3	408.3	29	(-)	2.83	55.0	166.8	Y = -0.000121611+0.0297545*X	0.9991
31 Quercetagenin	318.1	139.1	32	(-)	8.49	61.1	185.2	Y = -0.0126383+0.591688*X	0.9995
32 Quercetagenin-7-o-glucoside	480.3	317.7	25	(-)	3.91	29.8	90.2	Y = -0.0195021+1.5577*X	0.9996
33 Quercetin	302.1	151.0	25	(-)	13.74	17.9	54.3	Y = -0.00174477+0.284061*X	0.9993
34 Quercitrin	448.2	300.7	28	(-)	8.3	38.5	116.8	Y = -0.0597938+6.41218*X	0.9986
35 Rhamnetin	316.1	165.5	25	(-)	20.58	13.3	40.3	Y = -0.000444812+0.557033*X	0.9992
36 Rutin	610.3	300.6	36	(-)	5.38	102.2	309.5	Y = 0.0749463+3.00856*X	0.9996
37 Taxifolin	304.1	286.0	15	(-)	7.19	17.2	52.2	Y = -0.000622447+0.252727*X	0.9987
<i>Isoflavonoids</i>									
38 3'.4'.7-trihydroxyisoflavone	270.1	242.1	24	(-)	9.01	21.8	65.9	Y = 0.144262+0.669305*X	0.9998
39 4'.6.7-Trihydroxyisoflavone	270.1	241.0	31	(-)	9.66	1.8	5.5	Y = -0.00907706+1.71037*X	1.0000
40 Biochanin A	284.1	268.8	21	(-)	25.08	11.0	33.2	Y = 0.000563934+3.01299*X	0.9998
41 Calycosin	284.1	268.9	21	(-)	13.29	22.2	67.3	Y = 0.00514076+2.78286*X	0.9995
42 Calycosin-7-O-D-glycoside	446.3	445.6 368.0	14	(+)	446.305	21.8	66.2	Y = -6.88702e-005+0.0820548*X	0.9993
43 Daidzein	254.3	253.6	15	(-)	254.287	51.3	155.3	Y = 0.000239129+2.1368*X	0.9997

Compound	Parent Mass	Product Mass	Colision Energy (eV)	Polarity	RT (min)	LOD (ng/mL)	LOQ (ng/mL)	Equation	R ²
44 Daidzein-7-O-glucuronide	430.3	254.0	32	(-)	4.15	52.0	157.4	Y = 3.55407e-006+0.13471*X	0.9994
45 Daidzin	416.7	254.8	26	(+)	4.02	57.1	173.0	Y = 0.000401047+0.0236351*X	0.9991
46 Equol	242.3	121.5	17	(-)	16.38	1681.4	5095.2	Y = -0.000448591+0.000405631*X	0.9991
47 Formononetin	268.2	252.9	23	(-)	19.69	30.5	92.4	Y = 0.00559216+2.84812*X	0.9989
48 Genistein	270.1	133.8	37	(-)	16.66	18.1	54.8	Y = 0.0507112+3.84183*X	0.9996
49 Genistein-7-O-glucuronide	446.3	268.8	31	(-)	7.32	47.1	142.7	Y = -0.00305471+0.563188*X	0.9993
50 Genistin	432.3	268.9	32	(-)	8.43	12.4	37.6	Y = -0.000508358+0.187553*X	0.9992
51 Glycitein	284.2	268.6	24	(-)	13.42	10.7	32.5	Y = 0.000638602+2.78027*X	0.9994
52 Glycitin	446.8	284.8	26	(+)	5.21	70.5	213.7	Y = -4.05352+1140.78*X	0.9991
53 Ononin	430.7	268.8	21	(+)	10.24	20.8	62.9	Y = -6.33165+830.735*X	0.9993
54 Puerarin	416.2	295.6	24	(-)	2.79	47.5	144.0	Y = 0.000211845+0.00598467*X	0.9989
55 Sissotrin	446.3	283.9	22	(-)	14.06	33.8	102.3	Y = -0.000233527+0.0801326*X	0.9998
56 Sophoricoside	432.3	431.6 270.1	19	(-)	8.3	20.5	62.1	Y = 0.0606486+6.68509*X	0.9993
<i>Chalconoids</i>									
57 Isoliquiritigenin	256.2	120.0	29	(-)	19.67	13.7	41.4	Y = -0.000899955+1.59136*X	0.9995
58 Phloretin	274.1	167.9	19	(-)	16.27	20.6	62.3	Y = -0.000347951+1.54746*X	0.9994
59 Phloridzin	436.2	273.9	19	(-)	9.13	32.4	98.2	Y = -0.00080977+0.23241*X	0.9995
60 Xanthoxumol	354.2	234.0	21	(-)	31.82	81.4	246.6	Y = -0.00111669+0.00335894*X	0.9999
<i>Lignans</i>									
61 Lariciresinol	360.2	330.0	12	(-)	9.98	42.6	129.1	Y = 0.0036846+0.00281203*X	0.9999
62 Matairesinol		343.0 82.9	23	(-)	15.88	72.2	218.9	Y = 0.00327439+0.0181311*X	0.9989
63 Secoisolariciresinol	362.3	361.5 165.8	11	(-)	9.4	98.8	299.4	Y = 0.00211407+0.247818*X	0.9988
<i>Coumestan</i>									
64 Coumestrol	268.1	239.9	26	(-)	17.23	4.0	12.1	Y = 0.0238974+1.11973*X	0.9995

Compound	Parent Mass	Product Mass	Collision Energy (eV)	Polarity	RT (min)	LOD (ng/mL)	LOQ (ng/mL)	Equation	R ²
<i>Phenylethanoil</i>									
65 Hydroxytyrosol	154.1	124.1	17	(-)	2.25	33.8	102.3	Y = 0.000315019+0.114629*X	0.9999
<i>Stilbenoids</i>									
66 Polydatin	390.2	227.7	20	(-)	5.94	39.4	119.4	Y = -0.0178066+4.15442*X	0.9984
67 Resveratrol	228.0	184.1	24	(-)	11.41	120.5	365.1	Y = 0.0146079+0.0367231*X	0.9984

ng/mL: ng of std/mL of extract

Table S2. Phytoestrogen concentration range in soya, sunflower, rapeseed, and wheat meals.

Compound	SOYA (µg/g)	SUNFLOWER (µg/g)	RAPESEED (µg/g)	WHEAT (µg/g)
<i>Phenolic acids</i>				
Caffeic Acid	n.d.	15.6–34.8	0.5–9.4	n.d. –2.1
Chlorogenic Acid	n.d.–1.11	132.1–261.6	0.23–2.1	n.d. –1.2
Neochlorogenic Acid	0.22–3.24	205.3–441.8	0.44–2.6	n.d. –7.8
p-Coumaric Acid	n.d.–1.65	n.d. –0.101	n.d.–3.2	n.d. –1.11
Protocatechuic Acid	n.d.–0.263	0.9–3.9	1.5–13.5	n.d.–0.21
Sinapic Acid	n.d.	n.d.	10.13– >1,000	n.d.
<i>Flavonoids</i>				
Apigenin	1.1–18.0	n.d.	n.d.–0.7	n.d.
Diosmetin	0.03–0.75	0.17–0.66	n.d.–0.70	n.d.–0.782
Eriodictyol	n.d.	0.69–2.8	n.d.–0.14	n.d.
Hesperetin	n.d. –0.05	n.d.	n.d.	n.d.
Isoquercetin	tr	n.d.–0.08	n.d.	n.d.
Isorhamnetin	n.d.	n.d.–0.262	n.d.–2.6	n.d. –0.024
Kaempferol	n.d.–0.6	n.d.	n.d.–1.7	n.d.
Liquiritigenin	n.d.–0.4	n.d.	n.d.	n.d.
Liquiritin	n.d.–0.121	n.d.	n.d.	n.d.
Luteolin	n.d.–0.31	n.d.–0.40	n.d.–0.88	n.d.
Luteolin-4'-O-glucoside	n.d.	n.d.	n.d.–0.04	n.d.
Pelargonidin	n.d.	n.d.	n.d.	n.d.
Quercetagenin-7-O-glucoside	n.d.	n.d.–0.2	n.d.	n.d.
Quercetin	n.d.	3.63–38.5	0.6–19.63	n.d.–0.136
Rhamnetin	n.d.–0.03	n.d.–0.068	n.d.–0.009	n.d.–tr
Rutin	n.d.	n.d. –0.03	n.d.	n.d.
Taxifolin	n.d.	n.d. –0.104	n.d.–1.4	n.d.
<i>Isoflavonoids</i>				
3',4',7-trihydroxyisoflavone	n.d.–2.6	n.d.	n.d.	n.d.–0.115
4',6,7-Trihydroxyisoflavone	n.d.–14.3	n.d.	n.d.	n.d.
Biochanin A	n.d.–0.012	n.d.	n.d.	n.d.
Calycosin	0.04–3.3	n.d.–0.047	n.d.	n.d.
Daidzein	0.63–36.5	n.d.–0.46	n.d.–0.05	n.d.
Daidzin	2.1–11.6	n.d.–0.34	n.d.–0.1	n.d.–0.26
Formononetin	n.d.–0.22	n.d.	n.d.	n.d.
Genistein	36.3–670.4	n.d.–4.5	n.d.–2.27	n.d.
Genistin	n.d.–0.063	n.d.	n.d.	n.d.
Glycitein	0.06–2.2	n.d.	n.d.	n.d.–0.9

Glycitin	0.44–0.63	n.d.–0.016	n.d.–0.003	n.d.–0.15
Sophoricoside	n.d.–0.110	n.d.	n.d.	n.d.
<i>Chalconoids</i>				
Isoliquiritigenin	n.d.–0.63	n.d.	n.d.	n.d.
Phloretin	n.d.	n.d.	n.d.	n.d.
Xanthoxumol	n.d.	n.d.–6.4	n.d.	n.f
<i>Lignans</i>				
Matairesinol	n.d.	n.d.–3.89	n.d.	n.d.
<i>Coumestan</i>				
Coumestrol	7.8–143.4	n.d.–1.74	n.d.–0.627	n.d.–0.33
<i>Phenylethanoil</i>				
Hydroxytyrosol	n.d–18.4	n.d.	n.d.	n.d.

n.d.: not detected; tr: tracers; µg/g: µg/g raw material

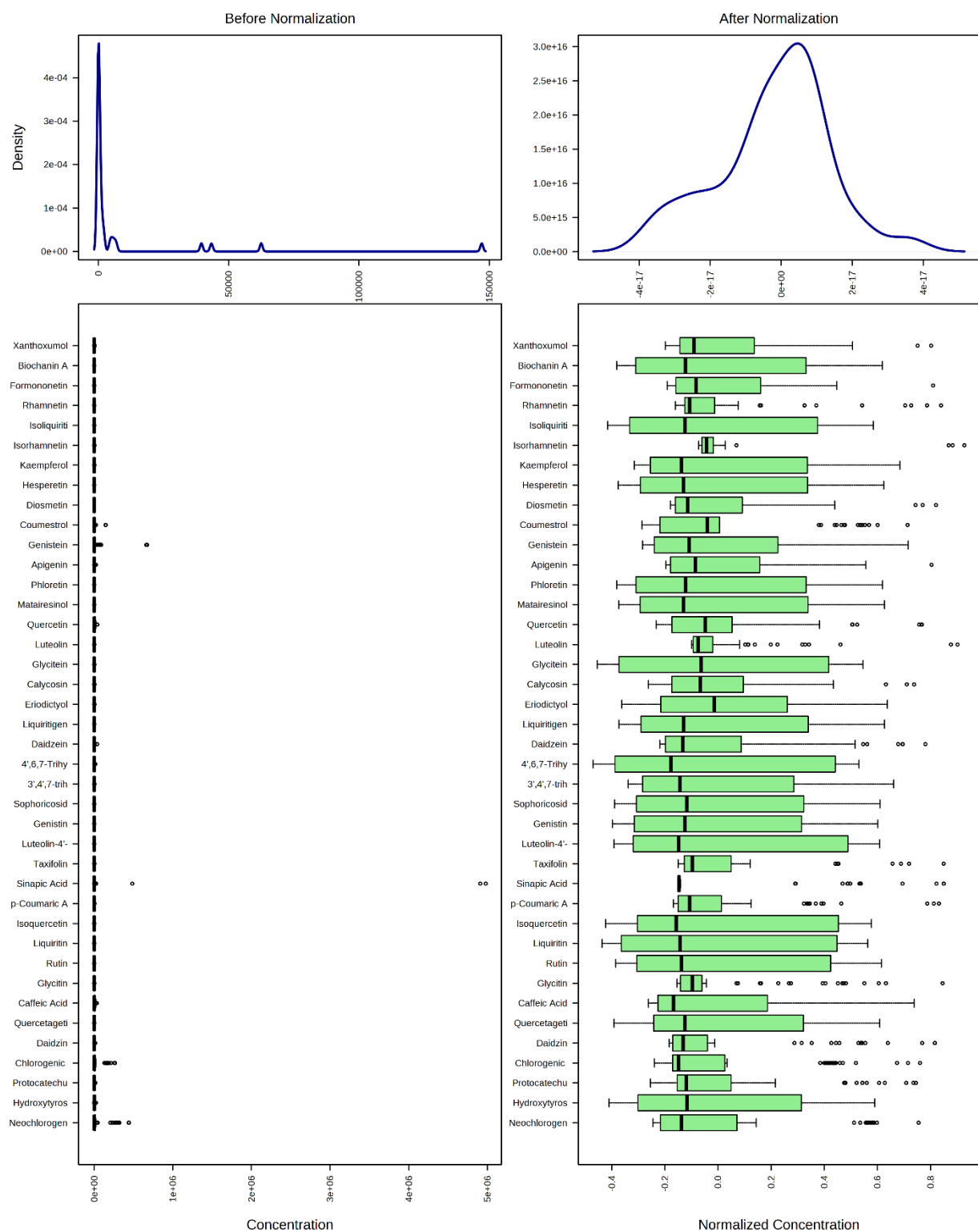


Figure S1. Concentration distributions before and after normalization based on the kernel density. Similarly, the bottom plots graphically summarize the concentrations of individual compounds.

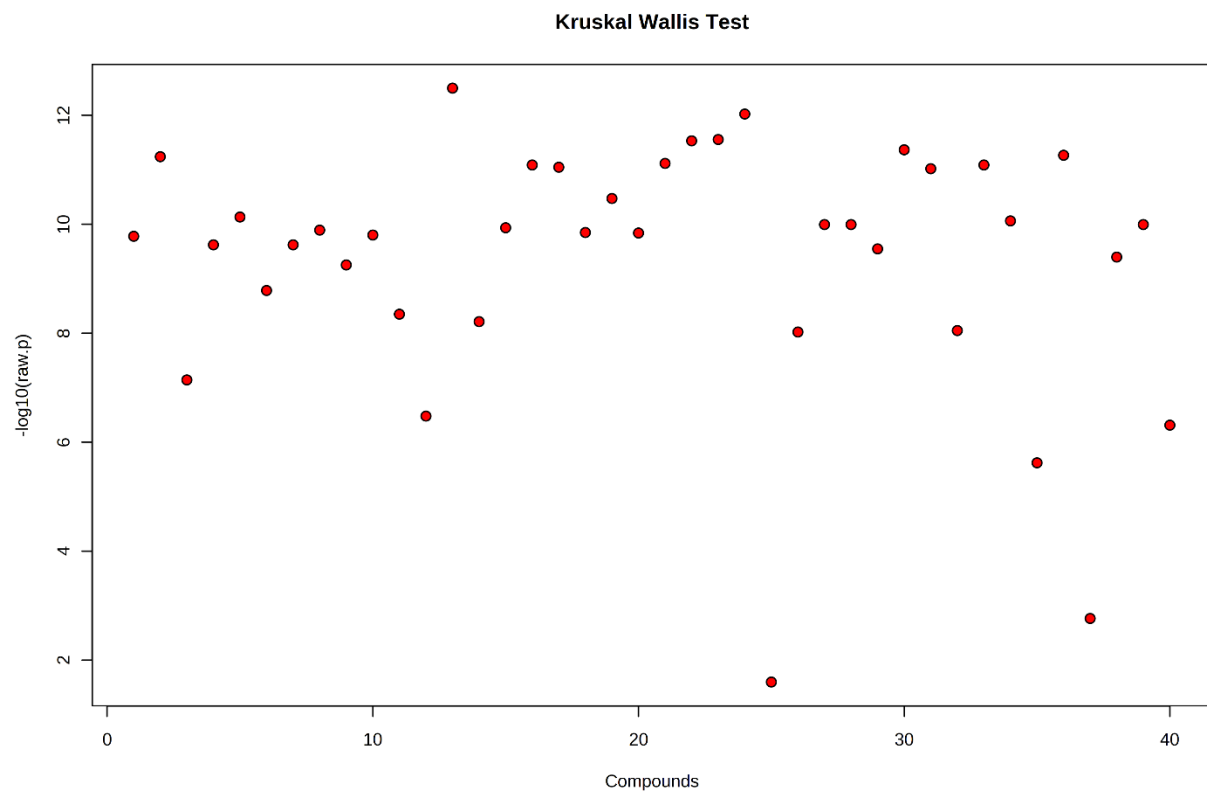


Figure S2. Results of the analysis of variance (ANOVA) of phytoestrogens, ranked by their P values ($P < 0.05$).

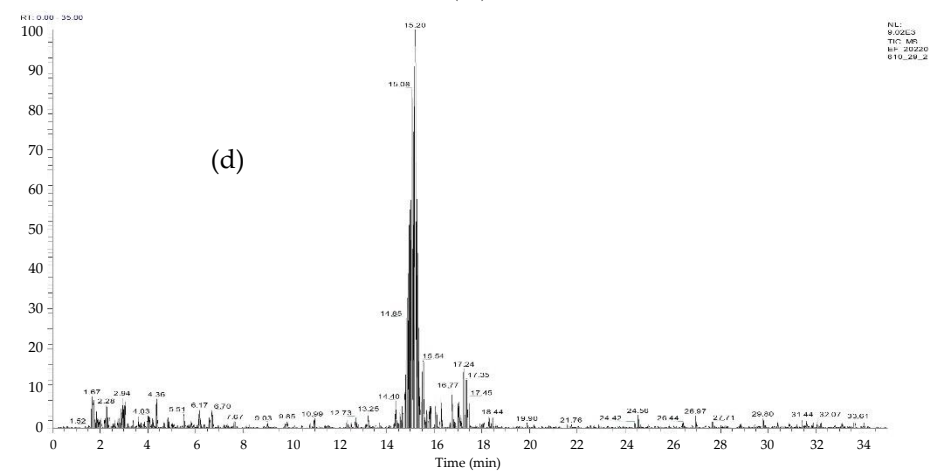
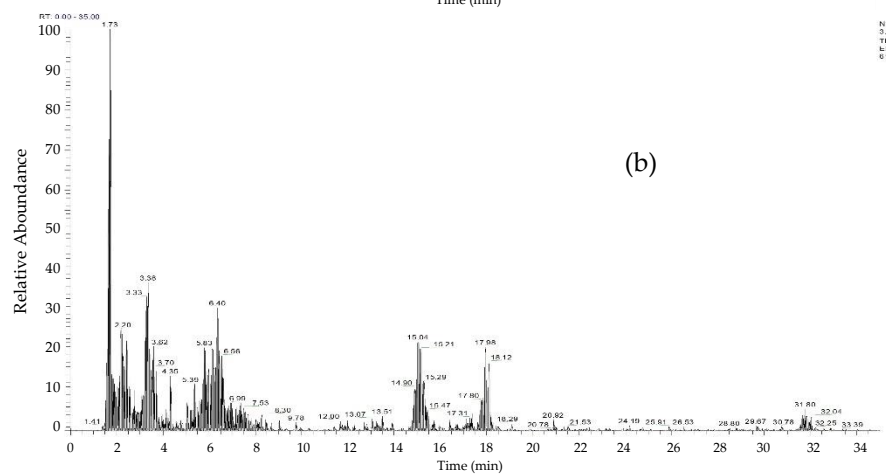
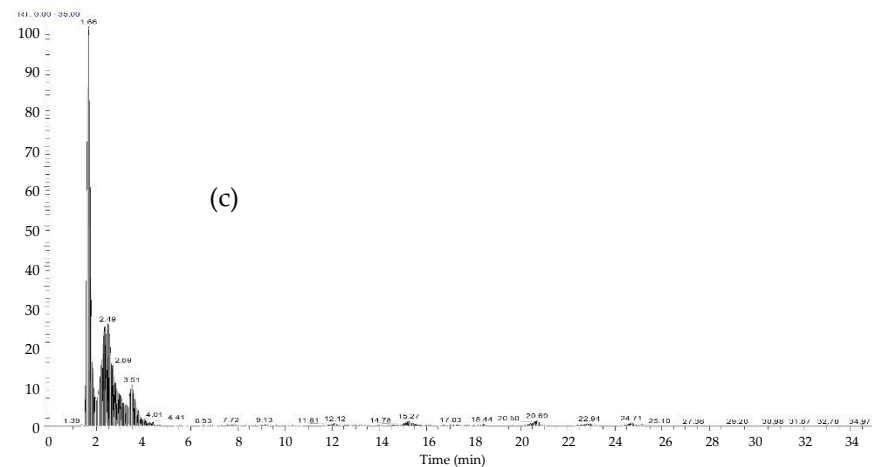
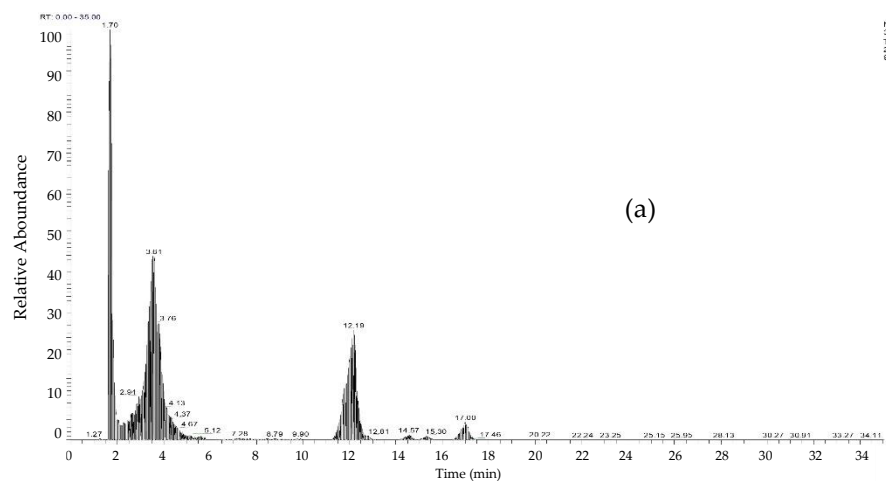


Figure S3. Chromatograms of phytoestrogens extracted from samples of: (a) soybean, (b) rapeseed, (c) sunflower and (d) wheat meals.