Alterations in Nonvolatile Components of Tea (*Camellia sinensis*) Induced by Insect Feeding under Field Conditions

Ayumi Ito [1], Jin Kamiya [2], Nakako Katsuno [1,3] and Emiko Yanase [1,3,*]

[1] The United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu City 501-1193, Japan
[2] Gifu Prefectural Agricultural Technology Center, 729-1 Matamaru, Gifu City 501-1152, Japan
[3] Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu City 501-1193, Japan
* Correspondence: yanase.emiko.h2@f.gifu-u.ac.jp; Tel./Fax: +81-58-293-2914

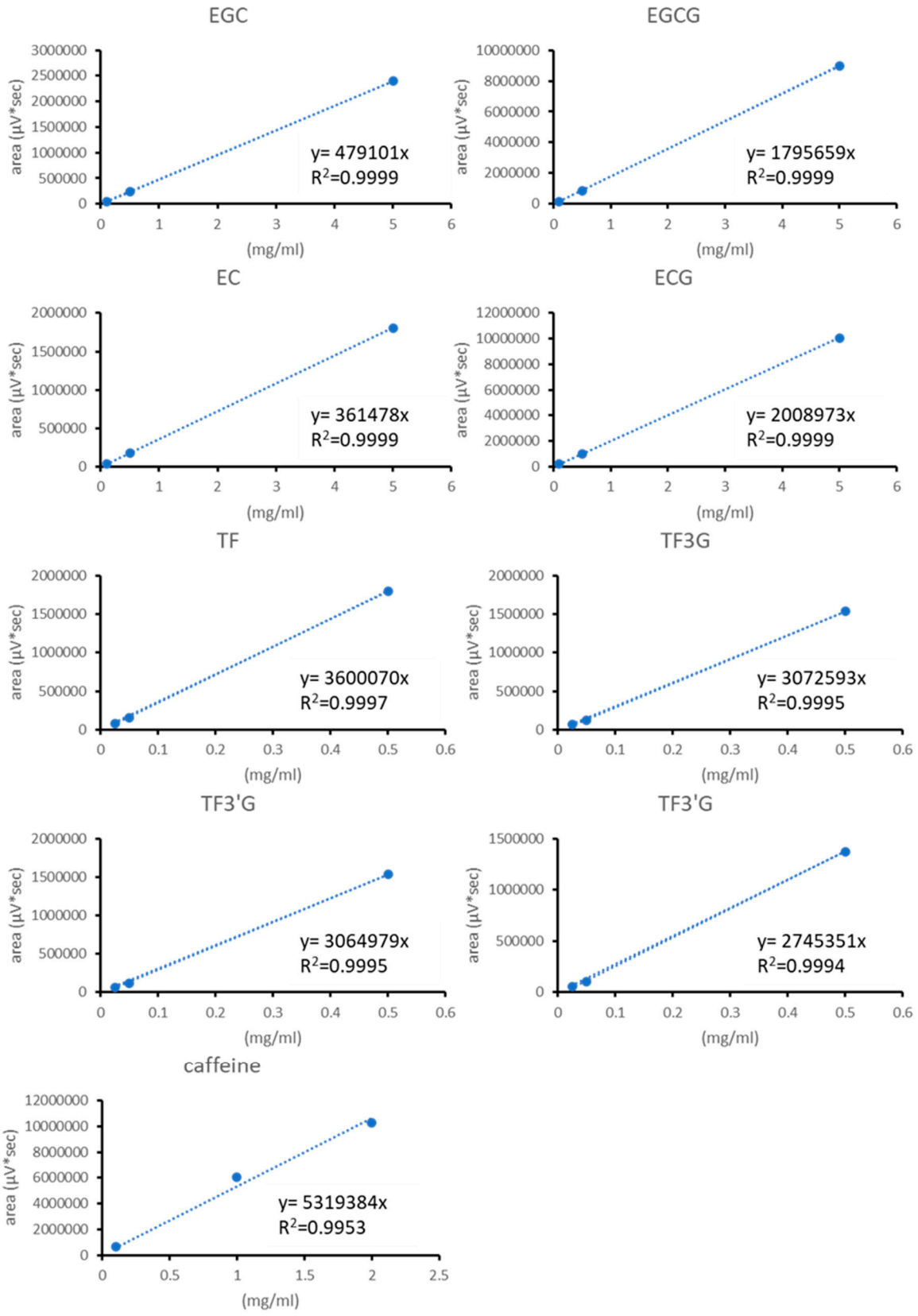


Figure S1. Calibration curves for catechins and theaflavins, caffeine

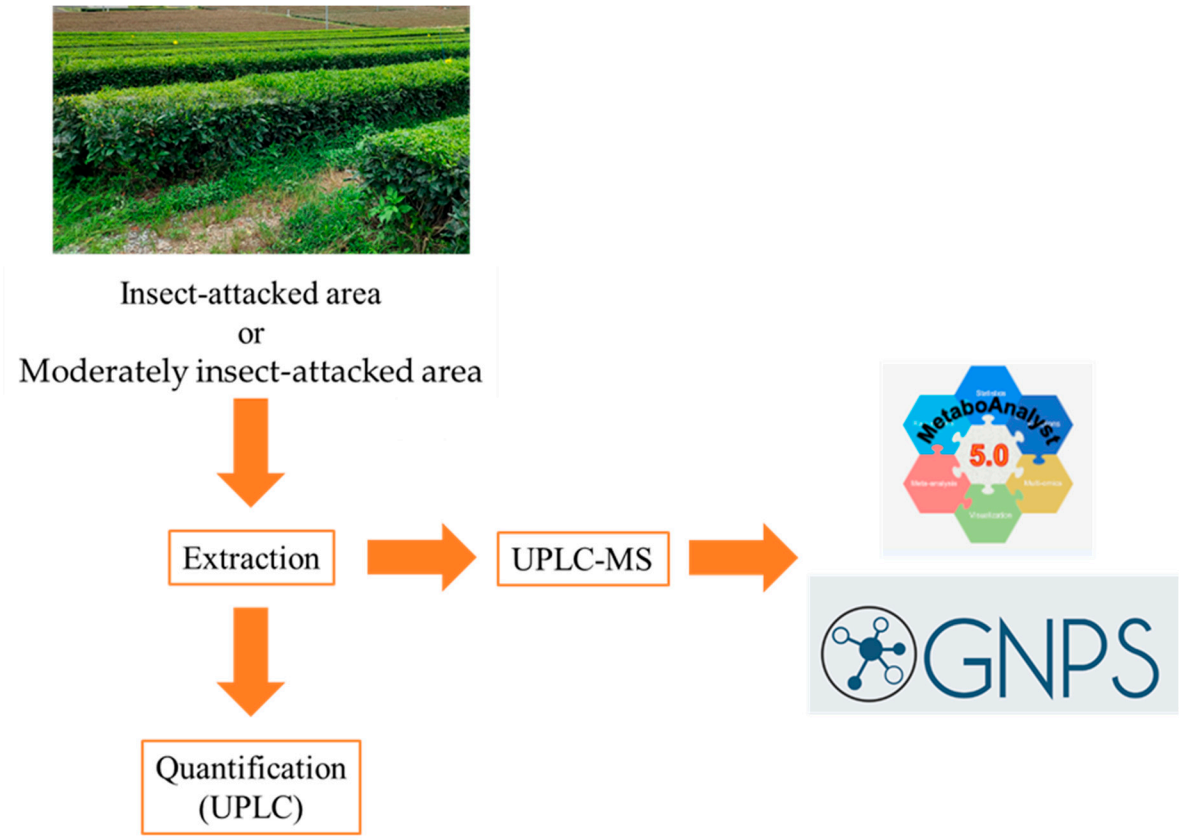


Figure S2. The process of the whole methodology in this study

Table S1 IDs list detected by MZmine.

ID	RT	*m/z*	ID	RT	*m/z*	ID	RT	*m/z*
1	7.97	195.0855	36	12.83	319.0455	71	11.06	268.0739
2	0.89	175.1046	37	5.12	188.0671	72	0.83	402.1194
3	9.13	459.0938	38	14.30	487.0843	73	12.29	439.1559
4	5.75	307.0831	39	1.64	367.0654	74	11.82	595.1639
5	9.86	291.0884	40	0.60	543.1337	75	6.00	601.1305
6	0.89	158.0778	41	11.60	469.1302	76	1.26	165.0521
7	12.23	443.0981	42	4.11	612.1469	77	5.20	205.0938
8	12.23	465.0813	43	0.61	258.1098	78	9.78	261.1708
9	9.13	289.0716	44	7.44	764.1590	79	12.42	285.1298
10	9.85	313.0695	45	7.89	579.1486	80	15.97	355.1733
11	3.46	181.0687	46	13.12	565.1539	81	8.06	293.0997
12	14.98	779.2021	47	13.97	487.2147	82	5.79	666.0643
13	13.96	795.1959	48	5.70	361.0894	83	13.01	587.1359
14	9.13	481.0766	49	15.89	287.0553	84	12.75	481.0946
15	0.61	381.0809	50	1.34	182.0781	85	1.22	178.0888
16	5.75	329.0653	51	10.64	425.1399	86	9.36	373.1808
17	0.64	351.0716	52	13.57	401.1554	87	13.14	587.1352
18	14.11	795.1973	53	0.68	365.1054	88	13.04	563.2078
19	1.15	268.1053	54	12.90	503.0789	89	14.97	398.0793
20	2.52	307.0818	55	12.79	261.1690	90	0.62	513.1243
21	1.70	345.0834	56	8.62	361.0894	91	13.95	406.0774
22	12.23	273.0759	57	16.27	487.2138	92	1.79	167.0534
23	10.38	441.1727	58	4.67	763.1470	93	4.60	298.0936
24	13.84	303.0507	59	0.80	175.1064	94	1.15	307.0830
25	10.40	361.0887	60	7.99	217.0734	95	14.67	633.1407
26	15.78	779.1982	61	5.48	185.0414	96	12.56	561.1920
27	5.67	635.1381	62	1.48	171.0248	97	11.71	275.0901
28	5.75	289.0724	63	12.54	439.1561	98	1.03	227.0153
29	12.81	503.0793	64	9.82	634.0754	99	13.72	449.0848
30	15.04	287.0556	65	5.66	361.0882	100	11.72	297.0815
31	6.04	579.1499	66	10.41	605.1470	101	0.97	167.0537
32	14.16	303.0492	67	1.62	153.0135	102	2.48	306.0939
33	9.87	603.1477	68	2.74	166.0822	103	12.25	405.1725
34	9.05	939.1559	69	15.72	471.0896	104	8.00	747.1542
35	12.84	565.1560	70	12.87	319.0446	105	14.17	406.0778

ID	RT	*m/z*
106	10.96	373.1820
107	14.51	487.0836
108	0.87	299.0965
109	9.90	731.1571
110	1.40	152.0501
111	13.40	417.1710
112	1.90	171.0232
113	1.44	153.0137
114	1.39	264.9661
115	15.95	398.0814
116	15.55	287.0558
117	13.94	475.1011
118	15.42	545.1983
119	2.51	289.0715
120	7.34	507.0748
121	2.43	329.0652
122	10.47	565.1543
123	14.12	355.1726
124	14.91	795.1768
125	11.90	633.1067
126	10.29	441.1722
127	10.79	337.0909
128	9.96	439.1610
129	1.06	215.0148
130	14.57	535.1442
131	11.92	659.0836
132	1.44	284.0992
133	24.31	471.2204
134	0.99	169.0102
135	14.46	303.0487
136	14.03	465.1027
137	11.81	655.0887
138	9.96	361.0878
139	12.03	361.0909
140	9.86	439.1579
141	13.69	475.0996
142	5.48	278.9821
143	14.63	457.2396
144	16.23	471.0897
145	13.77	427.1021
146	5.92	447.0558
147	13.60	433.1136
148	1.18	313.0579
149	5.94	657.0703
150	4.98	633.1081
151	8.04	326.0340
152	13.71	355.1739
153	16.63	617.1470
154	24.56	471.2206
155	10.95	320.1120
156	13.73	185.1122
157	10.44	547.1460
158	10.85	715.1650
159	15.78	449.1084
160	15.20	487.2133
161	7.29	321.0614
162	13.04	649.1352
163	7.39	747.1565
164	13.52	275.1858
165	1.27	355.0652
166	11.04	411.1642
167	7.00	349.0910
168	2.54	401.0209
169	1.16	304.1542
170	11.03	473.1040
171	13.79	257.0815
172	14.02	453.1192
173	7.93	601.1316
174	10.31	867.2102
175	4.83	458.1093
176	14.03	289.0703
177	10.33	596.1553
178	5.00	579.1492
179	14.54	611.1597
180	16.30	287.0557
181	4.63	333.0607
182	0.94	593.1285
183	5.80	401.0245
184	8.64	395.1314
185	4.24	579.1502
186	12.26	411.1632
187	11.02	433.1156
188	6.56	323.0739
189	11.18	411.1976
190	13.37	355.1728
191	8.10	349.0910
192	12.83	593.2211
193	21.75	617.1246
194	17.68	457.2407
195	13.12	579.1499
196	14.79	621.1798
197	6.07	427.1036
198	14.75	325.0550
199	7.32	315.0720
200	1.66	275.1123
201	5.38	153.0133
202	13.04	399.1439
203	10.93	275.1870
204	16.51	287.0557
205	3.38	312.0172
206	15.69	449.1102
207	14.68	185.1129
208	10.13	371.1700
209	14.48	597.2308
210	12.95	565.1548

ID	RT	*m/z*
211	2.66	259.0443
212	15.12	431.1699
213	3.32	203.0540
214	15.66	487.2149
215	3.44	763.1505
216	26.51	315.2145
217	14.48	597.2311
218	14.60	633.1422
219	27.57	391.2117
220	4.68	785.1295
221	5.03	655.0926
222	7.25	287.0557
223	14.17	611.1619
224	1.33	171.0258
225	5.36	243.0473
226	2.37	611.1401
227	6.46	867.2124
228	1.17	503.0789
229	13.39	617.1108
230	16.43	821.2075
231	11.90	449.0858
232	12.09	633.1144
233	1.45	355.0642
234	12.41	437.2374
235	0.74	203.0518
236	8.64	165.0527
237	10.35	579.1518
238	10.91	761.1361
239	9.69	921.1475
240	0.93	304.1549
241	13.39	639.0939
242	9.54	899.1673
243	7.20	915.1590
244	7.76	286.1283
245	13.78	289.0706
246	2.86	259.0421
247	19.08	153.1229
248	11.41	505.2246
249	12.86	491.0961
250	9.54	287.0549
251	9.59	289.0726
252	14.54	597.2313
253	13.80	525.2888
254	1.96	203.0783
255	10.77	459.0929
256	14.80	355.1735
257	5.86	291.0885
258	0.66	353.0790
259	0.84	175.1069
260	14.62	535.1435
261	9.93	310.0586
262	10.61	745.1391
263	26.35	427.2297
264	12.36	593.1299
265	10.60	785.1324
266	9.85	597.1235
267	5.54	166.0822
268	13.18	785.1309
269	13.79	401.1579
270	5.15	259.1165
271	14.31	465.1024
272	12.62	811.1902
273	13.12	745.1383
274	0.70	203.0524
275	13.44	617.1154
276	13.10	665.1240
277	12.60	745.1377
278	11.72	745.1390
279	8.48	593.1305
280	12.54	455.1345
281	11.30	645.1915
282	13.25	417.1718
283	14.11	487.2159
284	16.90	455.1740
285	11.60	563.2086
286	3.26	220.1183
287	9.60	493.1833
288	11.91	413.1792
289	4.04	384.1159
290	26.71	315.1214
291	16.87	495.1638
292	7.03	377.0852
293	11.61	563.2072
294	8.60	392.0209
295	8.55	438.0260
296	10.23	425.1437
297	23.43	245.0793
298	16.15	487.2150
299	13.95	487.2158
300	0.63	194.0827
301	0.63	194.0827
302	27.86	227.1427
303	1.24	233.1482
304	1.22	385.9950
305	15.57	193.1232
306	8.13	391.1583
307	1.25	289.9755
308	26.53	269.0689
309	1.03	314.0954
310	2.16	383.1467
311	5.82	289.0704
312	1.99	221.0930
313	8.33	318.0973
314	7.47	153.0135
315	5.18	318.0957

ID	RT	*m/z*
316	15.07	487.2172
317	15.35	355.1739
318	10.15	411.1642
319	11.49	633.0878
320	13.00	563.2069
321	10.63	441.1234
322	11.29	905.1550
323	2.59	287.0563
324	11.34	905.1540
325	16.39	459.1067
326	13.94	487.2165
327	11.28	441.0821
328	4.86	334.0892
329	7.19	937.1436
330	4.58	284.1115
331	3.73	595.1453
332	12.13	371.1701
333	9.40	418.1692
334	12.09	867.1735
335	15.68	579.2195
336	16.71	397.2196
337	10.56	420.1871
338	0.55	182.9625
339	6.90	365.1185
340	0.65	402.1633
341	12.24	317.0687
342	15.35	261.9097
343	14.39	261.9097
344	14.32	406.0766
345	13.97	261.9100
346	20.22	163.9744
347	18.32	163.9741
348	1.77	261.1109
349	14.97	487.2166
350	12.11	889.1557

ID	RT	*m/z*
351	12.59	455.1316
352	14.87	355.1730
353	12.49	937.1404
354	12.52	897.1462
355	0.69	465.2082
356	16.09	153.1235
357	16.32	487.2144
358	13.50	617.1221
359	14.31	487.2170
360	10.66	481.0753
361	6.06	915.1629
362	4.15	187.0943
363	10.71	737.1490
364	12.56	369.1523
365	14.88	373.1842
366	16.14	252.0912
367	11.03	373.1841
368	11.28	469.1325
369	11.43	307.1147
370	11.29	883.1720
371	11.74	425.0876
372	1.08	290.1363
373	15.23	457.2390
374	25.30	277.0864
375	23.54	245.0782
376	14.21	303.0489
377	15.93	779.1973
378	16.05	355.1725
379	12.99	565.1538
380	12.60	439.1562
381	17.79	457.2403
382	22.60	257.1716
383	8.16	747.1564
384	22.68	257.1716
385	14.76	303.0466

ID	RT	*m/z*
386	13.99	475.1001
387	24.61	471.2206
388	14.64	487.0843
389	23.54	177.0524
390	26.51	315.2144
391	14.07	453.1180
392	22.70	617.1288
393	5.98	289.0721
394	16.44	471.0894
395	16.15	153.1231
396	21.48	241.2023
397	17.15	455.1742
398	21.48	213.1425
399	14.78	611.1590
400	22.09	264.2340
401	17.06	495.1644
402	16.77	287.0557
403	11.22	153.1227
404	14.79	611.1591

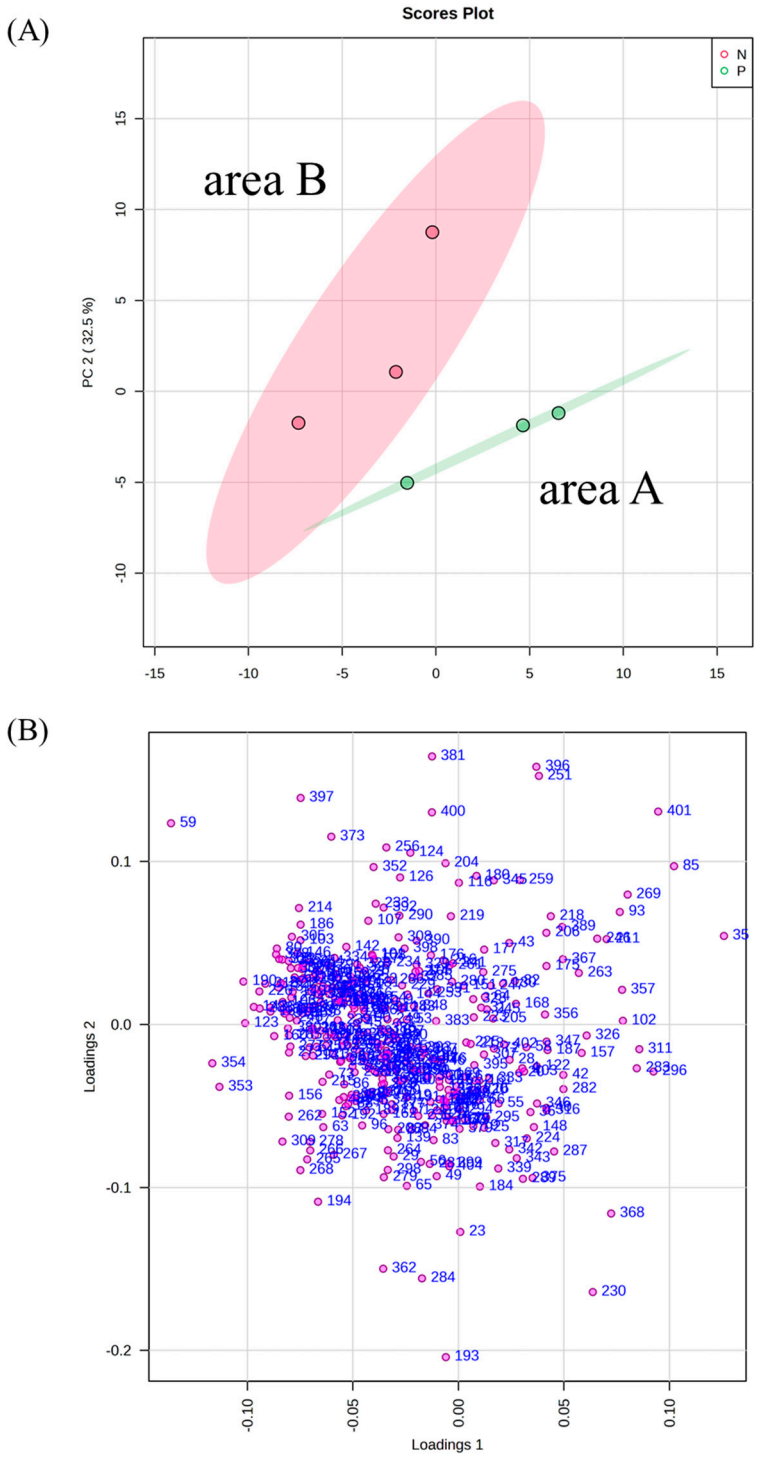


Figure S3. PCA based on the UPLC−MS spectra of the second−flush fresh tea extracts (A) The PCA score plot, and (B) the loading plot.

Table S2 Components abundance in area B (second−flush fresh tea)

ID	RT	*m/z*	area B/ area A ratio[a]
1	7.97	195.0855	1.20
7	12.23	443.0981	1.37
21	1.70	345.0834	1.58
22	12.23	273.0759	1.36
37	5.12	188.0671	1.54
39	1.64	367.0654	1.56
50	1.34	182.0781	1.25
61	5.48	185.0414	2.06
67	1.62	153.0135	1.37
77	5.20	205.0938	1.58
80	15.97	355.1733	2.63
99	13.72	449.0848	1.65
101	0.97	167.0537	1.45
103	12.25	405.1725	2.36
106	10.96	373.1820	1.84
108	0.87	299.0965	1.43
109	9.90	731.1571	1.80
123	14.12	355.1726	2.90
131	11.92	659.0836	2.27
136	14.03	465.1027	1.40
142	5.48	278.9821	1.78
143	14.63	457.2396	2.25
145	13.77	427.1021	1.89
146	5.92	447.0558	2.39
149	5.94	657.0703	2.06
154	24.56	471.2206	1.60
155	10.95	320.1120	1.40
158	10.85	715.1650	1.42
163	7.39	747.1565	1.31
166	11.04	411.1642	2.22
171	13.79	257.0815	1.78
174	10.31	867.2102	1.31
176	14.03	289.0703	1.25
181	4.63	333.0607	1.29

186	12.26	411.1632	2.86
188	6.56	323.0739	1.58
190	13.37	355.1728	2.69
195	13.12	579.1499	1.35
199	7.32	315.0720	1.75
201	5.38	153.0133	1.99
214	15.66	487.2149	2.76
222	7.25	287.0557	1.44
226	2.37	611.1401	1.96
233	1.45	355.0642	1.87
234	12.41	437.2374	1.32
239	9.69	921.1475	1.52
242	9.54	899.1673	1.54
243	7.20	915.1590	1.44
244	7.76	286.1283	2.67
248	11.41	505.2246	1.61
250	9.54	287.0549	1.62
254	1.96	203.0783	2.08
270	5.15	259.1165	1.97
271	14.31	465.1024	3.08
288	11.91	413.1792	2.83
290	26.71	315.1214	1.54
291	16.87	495.1638	1.60
302	27.86	227.1427	1.99
305	15.57	193.1232	3.21
306	8.13	391.1583	2.11
310	2.16	383.1467	1.51
312	1.99	221.0930	1.67
313	8.33	318.0973	1.68
314	7.47	153.0135	1.43
315	5.18	318.0957	1.83
318	10.15	411.1642	2.36
322	11.29	905.1550	2.00
324	11.34	905.1540	1.74
325	16.39	459.1067	1.40
327	11.28	441.0821	2.46

328	4.86	334.0892	1.49
330	4.58	284.1115	1.64
332	12.13	371.1701	2.23
334	12.09	867.1735	1.67
337	10.56	420.1871	1.40
350	12.11	889.1557	1.87
355	0.69	465.2082	2.73
359	14.31	487.2170	2.07
361	6.06	915.1629	1.95
365	14.88	373.1842	2.31
370	11.29	883.1720	1.98
371	11.74	425.0876	1.61
373	15.23	457.2390	4.88
380	12.60	439.1562	1.72

[a]: Peak area in area B was divided by peak area in area A.

The components with VIP greater than 1 and p (corr) ≤ −0.7 were extracted based on the OPLS−DA.

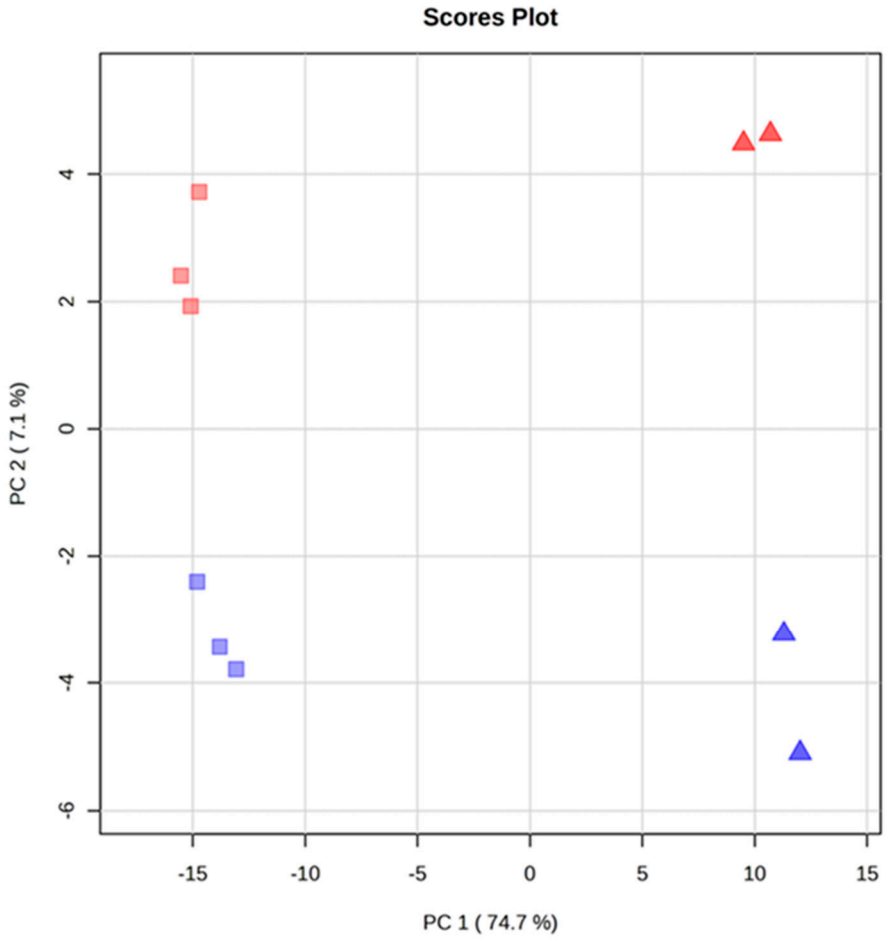


Figure S4. PCA based on the UPLC−MS spectra of the tea leaf extracts second−flush tea.

Blue: area A, Red: area B, ▲: Fresh, ■: Black tea product.

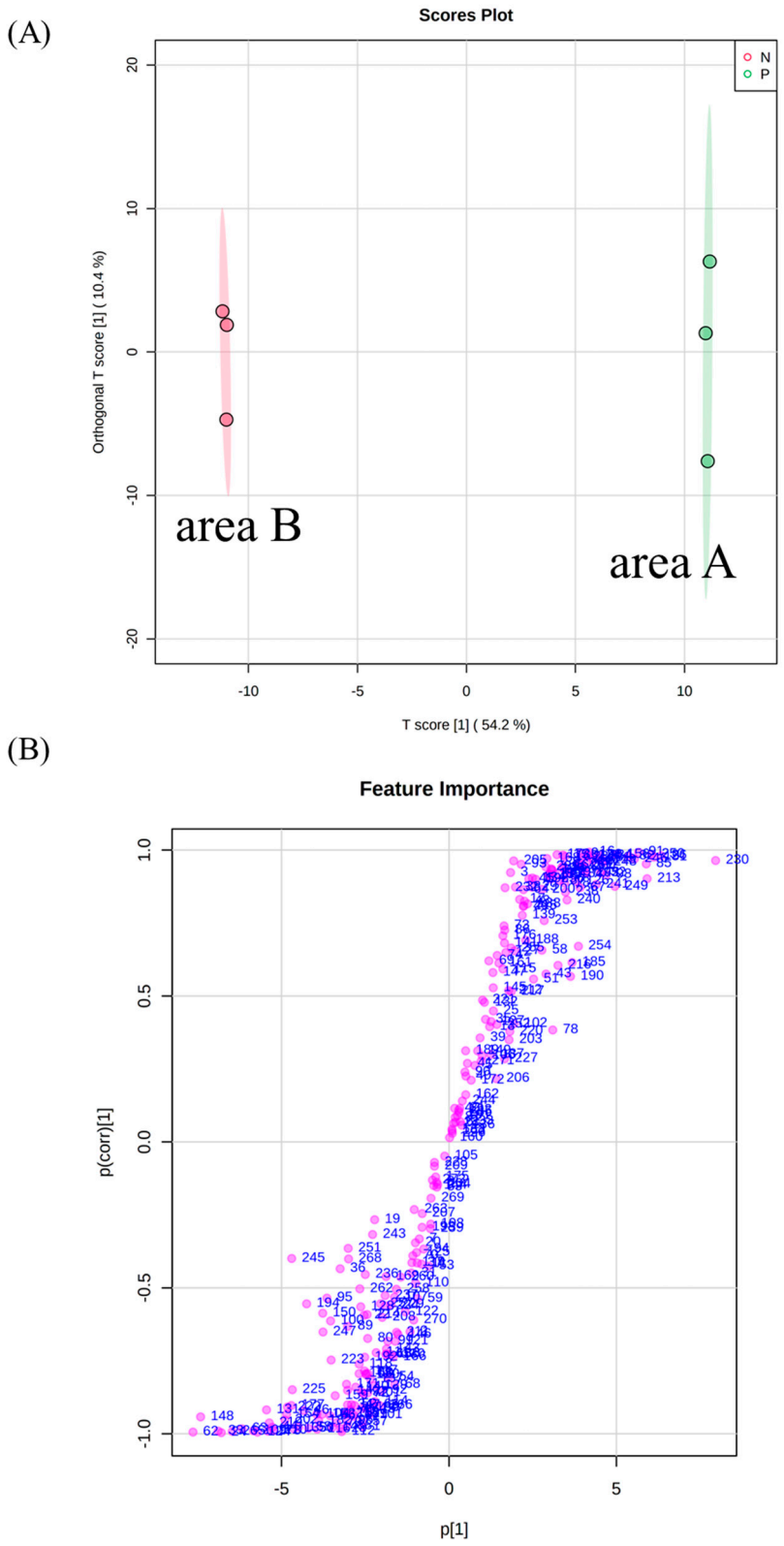


Figure S5. OPLS−DA based on the UPLC−MS spectra of the black tea leaf extracts. (A) The OPLS−DA score plot, and (B) the S−plot.

Table S3 Components abundance in area B (second−flush black tea)

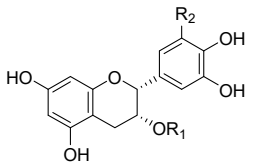
m/z	RT	annotation
297.0791	11.81	
597.2322	14.53	
279.0435	8.08	
717.1462	22.76	theaflavin−3'−gallate
575.1737	16.15	
441.0831	11.36	
899.1656	8.23	
284.1124	4.63	
545.1998	15.54	
195.0853	8.08	Caffeine
217.0741	7.99	
579.1124	22.19	
411.1627	11.03	
739.1251	22.10	theaflavin−3−gallate[a]
553.0946	18.34	
429.0825	19.16	
717.1421	22.14	theaflavin−3−gallate
699.1330	22.18	
337.0901	10.89	
401.1569	13.70	
575.0776	18.35	
333.0618	9.52	
427.1021	13.80	ID 145
333.0609	3.32	
320.1108	10.91	
257.0817	13.79	
205.0928	5.16	
454.0501	22.90	
505.2261	11.39	
188.0678	5.19	
891.1367	22.92	theaflavin−3,3'−gallate[a]
318.0945	8.37	
307.1158	11.54	
333.0612	12.07	

333.0609	13.81	
439.1559	12.37	
869.1557	22.97	theaflavin−3,3'−gallate
411.1638	10.18	
383.1453	2.28	
487.2162	15.07	
413.1790	11.95	
193.1243	15.62	
318.0949	5.15	
334.0878	4.95	
373.1826	11.02	
259.1150	5.05	
439.2307	21.01	
487.2148	15.52	
439.2306	21.60	
457.2422	13.81	
457.2404	14.63	
487.2162	15.39	
355.1729	16.01	ID 80
355.1715	14.26	
457.2422	15.48	
457.2407	17.72	
275.0921	11.82	
333.0614	6.87	
293.0992	8.17	
378.0456	22.10	
275.1864	10.95	
935.1233	9.46	
326.0331	8.04	
373.1838	9.37	
220.1171	3.32	
913.1441	13.86	
411.1979	11.10	
731.1596	10.00	ID109
665.0463	9.43	
597.2299	14.57	

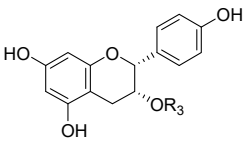
286.1271	7.85
487.2150	15.77

[a]: Annotated as a Na adduct

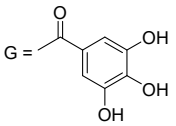
The components with VIP greater than 1 and p (corr) ≤ −0.7 were extracted based on the OPLS−DA.

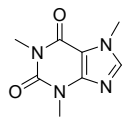


R_1 = H, R_2 = H : (-)-epicatechin
R_1 = H, R_2 = OH : (-)-epigallocatechin
R_1 = G, R_2 = H : (-)-epicatechin gallate
R_1 = G, R_2 = OH : (-)-epigallocatechin gallate

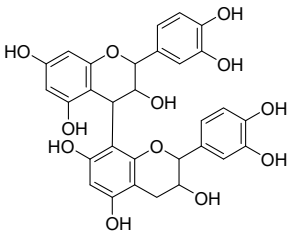


R_3 = H : (-)-epiafzelechin
R_3 = G : (-)-epiafzelechin 3-O-gallate





Caffeine



B-type procyanidin

Figure S6. The structures of catechins, caffeine and procyanidin