SUPPORTING INFORMATION

Rapid Identification Method for Gamma-irradiated Soybeans Using Gas Chromatography–Mass Spectrometry Coupled with a Headspace Solid-phase Microextraction Technique

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SUPPORTING FIGURE AND TABLE CAPTIONS

Table S1. Experimental design using a central composite design and their responses for optimization of extraction time and temperature in headspace solid-phase microextraction gas chromatography-mass spectrometry analysis.

Table S2. Analytical conditions of the gas chromatography-mass spectrometry analysis.

Table S3. Retention time and peak area of volatile compounds in the non-irradiated and irradiated soybeans analyzed using headspace solid-phase microextraction gas chromatography-mass spectrometry.

Table S4. Analysis of variance (ANOVA) analysis of the RSM experiments for optimization of headspace solid-phase microextraction of 1,7-hexadecadiene.

Table S5. Analysis of variance (ANOVA) and regression analyses for the selected quadratic model.

Figure S1. Thermoluminescence glow curves of the gamma-irradiated soybeans.

Table S6. Fatty acid compositions of non-irradiated soybeans.

Figure S2. The mass spectrum of 1,7-hexadecadiene standard for the 67, 82, and 96 m/z ions.

Table S1.

Order	Run	Extraction temp (°C)	Extraction time (min)	Peak area (a.u.*)
6	1	60 (-1)	80 (+1)	1,405,995
3	2	140 (+1)	20 (-1)	957,638
14	3	100 (0)	7.6 (-α)	1,637,988
1	4	60 (-1)	20 (-1)	342,560
17	5	100 (0)	50 (0)	2,278,251
10	6	43.4 (-α)	50 (0)	193,860
16	7	100 (0)	92.4 (+α)	1,730,616
2	8	60 (-1)	20 (-1)	324,137
8	9	140 (+1)	80 (+1)	516,674
19	10	100 (0)	50 (0)	2,273,516
21	11	100 (0)	50 (0)	2,188,527
20	12	100 (0)	50 (0)	2,525,738
13	13	100 (0)	7.6 (-α)	1,560,412
5	14	60 (-1)	80 (+1)	1,488,731
12	15	156.6 (+α)	50 (0)	294,681
4	16	140 (+1)	20 (-1)	893,455
11	17	156.6 (+α)	50 (0)	327,121
9	18	43.4 (-α)	50 (0)	247,480
15	19	100 (0)	92.4 (+α)	1,981,400
18	20	100 (0)	50 (0)	2,223,525
7	21	140 (+1)	80 (+1)	406,967

Values in parenthesis are the coded values (α = 1.414)
* arbitrary units

Table S2.

Device	Analytical conditions	Hydrocarbons	2-Alkylcyclobutanones
Gas chromatography	Column	DB-5MS Ultra Inert capillary column (0.25 mm i.d. × 30 m and 0.25 μm film thickness)	DB-5MS Ultra Inert capillary column (0.25 mm i.d. × 30 m and 0.25 μm film thickness)
	Column flow	1.0 mL/min	1.0 mL/min
	Carrier gas	Helium (99.999%)	Helium (99.999%)
	Injection volume	1 μL	1 μL
	Injection mode	Split mode (1:20)	Splitless mode
	Injection Temperature	250 °C	250 °C
	Oven	25 °C/min up to 170 °C 2 °C/min up to 205 °C 10 °C/min up to 270 °C	15 °C/min up to 300 °C and held it for 10 min
Mass spectrometry	Interface temperature	280 °C	280 °C
	Ion source	EI + 70 eV	EI + 70 eV
	Ion source temperature	230 °C	230 °C
	Scan range	40–350 m/z	40–350 m/z
	Detection mode	Full scan	Selected ion monitoring (SIM) monitoring 98 m/z and 112 m/z ions

Table S3.

No.	Compound	RT (min)	Identification Behaviour[1)]	Absorbed dose (kGy)				
				0	0.5	1	3	5
1	Methyl Alcohol	1.06	IS	40401669 ± 11314027	42988500 ± 7510020	41352837 ± 5881584	36132071 ± 4606567	46108110 ± 2199880
2	Ethanol	1.10	IS	4984117 ± 787047	5955463 ± 1413259	5613306 ± 1130665	9006279 ± 1307022	7448745 ± 904012
3	Acetone	1.12	IS	7960462 ± 3000746	12468650 ± 2317077	11645234 ± 1209829	12653317 ± 1219174	11963490 ± 1820187
4	n-Hexane	1.32	IS	5482599 ± 1091065	3063176 ± 672552	3850119 ± 1067161	3892822 ± 861489	4012866 ± 1353982
5	Butanal, 3-methyl-	1.45	TI	525608 ± 135076	649083 ± 176781	576576 ± 162257	575150 ± 113668	514143 ± 99418
6	Butanal, 2-methyl	1.48	TI	1283304 ± 421311	1601126 ± 254365	1072374 ± 184529	1255837 ± 328570	1047904 ± 119347
7	Hexanal	2.03	IS	2309551 ± 161627	2619720 ± 1463780	2817355 ± 575062	3299267 ± 842222	4596701 ± 1092189
8	Acetic acid	2.23	IS	22401280 ± 3530563	25244761 ± 3650987	19189290 ± 3953729	21716384 ± 6363506	30560545 ± 10758627
9	1-Hexanol	2.43	IS	4817431 ± 835989	5584251 ± 411373	6526303 ± 872016	7009325 ± 537879	6005704 ± 676223
10	2-Heptanone	2.59	IS	656569 ± 100096	606341 ± 32737	850171 ± 151063	1938861 ± 2861198	952725 ± 70502
11	Styrene	2.63	TI	802190 ± 153923	884734 ± 82813	1517245 ± 337935	990405 ± 426559	1316020 ± 409328
12	Heptanal	2.65	IS	189495 ± 56626	234338 ± 64921	273206 ± 60365	331337 ± 81362	358012 ± 31644
13	Benzaldehyde	3.20	TI	7910129 ± 1367264	8387753 ± 1360107	8866443 ± 1102078	9858472 ± 1470755	8496762±669582
14	1-Octen-3-ol	3.31	IS	1998792 ± 320943	1314140 ± 227096	1275085 ± 150367	1159545 ± 210251	1628843 ± 231659
15	Benzene, 1,4-dichloro-	3.75	TI	4186383 ± 1338321	3341978 ± 1208715	3462028 ± 1034184	2465945±446900	2748983±599019
16	2-Pyrrolidinone, 1-methyl-	4.32	TI	6204472 ± 488140	2799315 ± 567222	2977077 ± 730514	2346511 ± 1113029	3657950 ± 603712
17	Nonanal	5.02	IS	1959947 ± 562726	1166319 ± 140698	1194563 ± 176195	1072353 ± 202945	1278822 ± 79653

18	Maltol	5.48	TI	116464142 ± 10363261	106556225 ± 10048508	106251826 ± 17717433	105078754 ± 12588272	109220787 ± 7977989
19	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	5.56	TI	1449283 ± 561212	874244 ± 169804	847369 ± 212135	708933 ± 237421	978460 ± 229235
20	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.00	TI	3757269 ± 286302	7172482 ± 1949450	7171601 ± 1535658	4952432 ± 1775165	8406617 ±1824781
21	1-Nonanol	6.39	TI	2409703 ± 386167	1606143 ± 345908	1861988 ± 592961	1677959 ± 398848	2460714 ± 172511
22	2-Decanone	6.95	IS	504997 ± 121869	341165 ± 32501	461583 ± 194417	500851 ± 176410	419531 ± 82782
23	Dodecane	7.12	IS	1153060 ± 232679	783541 ± 78684	870498 ± 317430	779494 ± 313527	990589 ± 197474
24	Decanal	7.30	IS	728000 ± 138033	756047 ± 69987	751269 ± 86290	742551 ± 148664	785170 ± 58655
25	Benzofuran, 2,3-dihydro-	7.68	TI	12316657 ± 2711607	7883531 ± 5175210	13065431 ± 2766416	12862509 ± 1901954	12605602 ± 2434554
26	Nonanoic acid	9.23	IS	1535432 ± 117370	690096 ± 159369	855662 ± 87320	836662 ± 416590	1038687 ± 151569
27	2-Methoxy-4-vinylphenol	10.85	TI	42896782 ± 2035618	56616708 ± 9070623	58479210 ± 8444432	61193533 ± 5928218	53937923 ± 7008041
28	Phenol, 2,6-dimethoxy-	12.30	TI	3442769 ± 1608807	3122930 ± 240556	3500742 ± 604516	3111403 ± 761433	3291452 ± 381166
29	2(3H)-Furanone, dihydro-5-pentyl-	12.71	TI	1701166 ± 269378	2309850 ± 109243	2650701 ± 356471	2410294 ± 458182	2405678 ± 210122
30	Biphenyl	13.29	TI	2150916 ± 843277	2173711 ± 284576	2522108 ± 239352	1788691 ± 316804	2334206 ± 342804
31	Unknown 1	14.00	TI	557536 ± 182303	919411 ± 221336	1070885 ± 49018	2205384 ± 337967	2623425 ± 399800
32	Diphenyl ether	14.30	TI	4209340 ± 869653	4476147 ± 391161	5203142 ± 453690	3832712 ± 634192	5907663 ± 433621
33	Pentadecane	19.03	IS	1465934 ± 740236	578059 ± 68517	956906 ± 138214	1893612 ± 360847	2866393 ± 225893
34	Butylated Hydroxytoluene	19.58	TI	24971327 ± 4335509	22393151 ± 2144266	23857054 ± 1664428	20058169 ± 3388721	29426649 ± 1268034
35	1,7-Hexadecadiene	22.52	IS	ND*	3781181 ± 469215	7384218 ± 566830	16130192 ± 1989949	22995157 ± 2930653

36	Dodecanoic acid	22.72	IS	2547331 ± 315571	3558553 ± 1501496	3799967 ± 920296	4536579 ± 852593	5106613 ± 1070838
37	6,9-Heptadecadiene	27.47	TI	ND	2588198 ± 161535	2314895 ± 570154	11981314 ± 2551398	17497713 ± 2194362
38	Unknown 2	27.69	TI	ND	409327 ± 83853	411086 ± 145536	988659 ± 334824	1175103 ± 154597
39	8-Heptadecene	27.85	IS	ND	1333333 ± 75234	2936061 ± 266943	3145922 ± 521394	4867479 ± 526644
40	Unknown 3	34.77	TI	ND	ND	ND	1333814 ± 506767	2151720 ± 507605
41	2-Dodecylcyclobutanone	36.05	IS	ND	ND	ND	971058 ± 114344	1543754 ± 123703
42	2-Pentadecanone, 6,10,14-trimethyl-	36.31	TI	2815230 ± 1019026	1222693 ± 115567	1256465 ± 145394	1282521 ± 206281	1476524 ± 89349
43	9-Heptadecanone	37.98	TI	1405617 ± 938440	375620 ± 174351	1568261 ± 1483279	558592 ± 125232	1353964 ± 231410
44	Hexadecanoic acid, methyl ester	41.08	TI	4168952 ± 1519101	3363043 ± 359026	3788264 ± 604139	2732767 ± 131804	6182573 ± 171830
45	n-Hexadecanoic acid	42.39	TI	11449106 ± 3351805	7978664 ± 1906225	15028142 ± 8776869	10813797 ± 4864005	8966963 ± 882917
46	Unknown 4	42.86	TI	ND	ND	ND	689996 ± 198619	1191833 ± 173350
47	Unknown 5	43.04	TI	ND	ND	ND	716150 ± 433560	714549 ± 266215
48	Unknown 6	43.42	TI	ND	ND	ND	965959 ± 192428	1843957 ± 125660
49	Unknown 7	43.60	TI	ND	ND	ND	627503 ± 115595	1007970 ± 106342
50	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	44.97	TI	3628054 ± 994678	5090536 ± 969623	5692501 ± 498522	5091827 ± 349983	8716726 ± 347451
51	Unknown 8	45.07	TI	1554521 ± 342567	1432192 ± 222140	1615999 ± 218660	1266342 ± 119442	2217715 ± 150493
52	Dodecanoic acid, isooctyl ester	45.15	TI	564800 ± 322844	2010942 ± 3029731	1885364 ± 2660631	728870 ± 262744	524213 ± 118641
53	9,12-Octadecadienoic acid	45.58	TI	537174 ± 203622	949475 ± 333922	2579732 ± 2839326	680157 ± 224632	1051149 ± 209535

	(Z,Z)-							
54	Unknown 9	45.97	TI	2651373 ± 913203	390405 ± 47817	600964 ± 394769	529471 ± 167082	817188 ± 88390

Means ± standard deviation (*n*=6)

* Not detected

1) TI: Tentatively identified using mass spectrum in NIST library, IS: Identified by comparison with standard.

Table S4.

Model	Sequential model sum of squares		Lack-of-fit test		R^2	Adjusted R^2
	Sum of squares	p-value	Sum of squares	p-value		
Linear	2.744E+011	0.8332	1.328E+013	< 0.0001	0.0201	-0.0888
2-factor interaction	1.245E+012	0.2045	1.203E+013	< 0.0001	0.1111	-0.0458
Quadratic	1.178E+013	< 0.0001	2.560E+011	0.0025	0.9726	0.9635
Cubic	8.845E+010	0.1735	1.676E+011	0.0014	0.9791	0.9678

Table S5.

Term	Coefficient	Sum of squares	Degree of freedom	Mean square	F-value	p-value
Model		1.330E+013	5	2.660E+012	106.52	< 0.0001
Intercept	2.298E+006					
X_1	-33217.32	1.765E+010	1	1.765E+010	0.71	0.4136
X_2	1.267E+005	2.568E+011	1	2.568E+011	10.29	0.0059
X_1X_2	-3.944E+005	1.245E+012	1	1.245E+012	49.85	< 0.0001
X_1^2	-1.067E+006	1.176E+013	1	1.176E+013	470.92	< 0.0001
X_2^2	-3.363E+005	1.168E+012	1	1.168E+012	46.77	< 0.0001
Residual		3.745E+011	15	2.497E+010		
Lack of fit		2.560E+011	3	8.534E+010	8.64	0.0025
Pure error		1.185E+011	12	9.873E+009		
Total		1.367E+013	20			
R^2	0.9726					
Adjusted R^2	0.9635					
Quadratic polynomial equation	$Y = -7078090 + 149008X_1 + 74461X_2 - 329X_1X_2 - 667X_1^2 - 374X_2^2$					

X_1: extraction temperature (°C); X_2: extraction time (min)

The ANOVA results indicate that the X_1 term is the only no significant factor (0.05 of probability level according to the Duncan test) affecting the extraction of 1,7-hexadecadiene.

Figure S1.

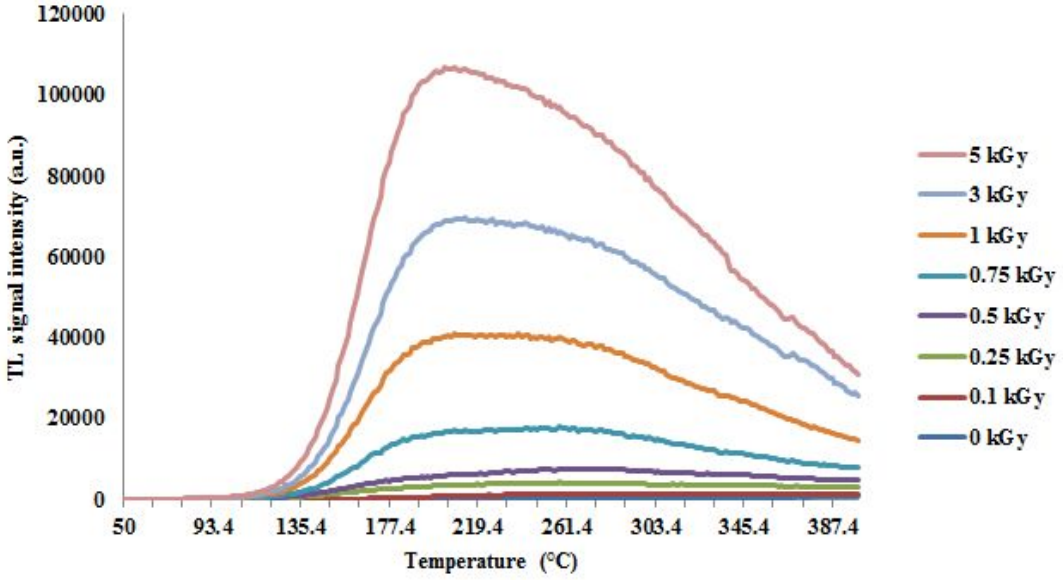
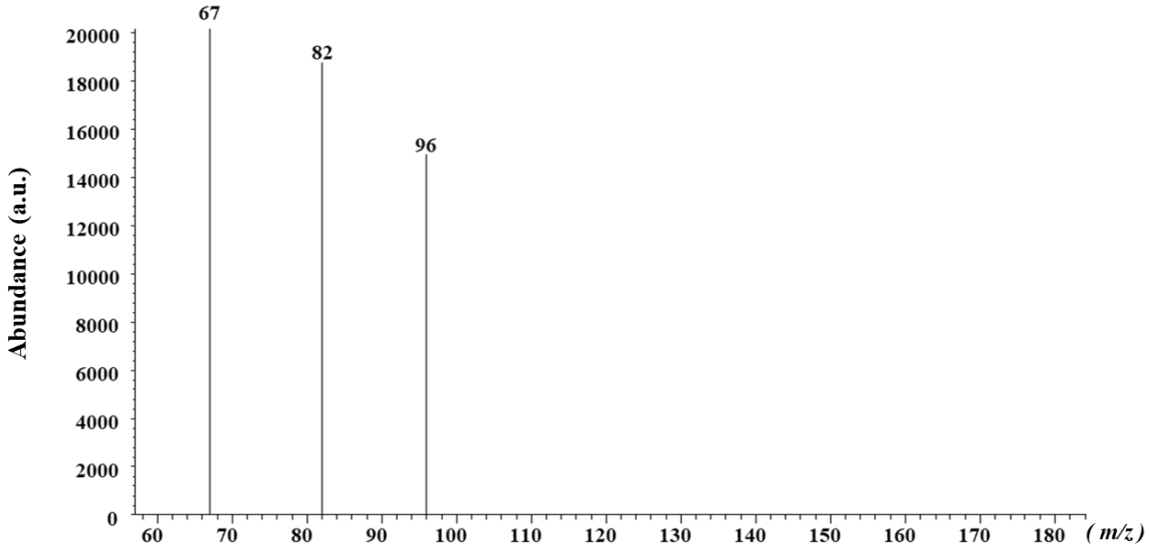

120000
100000
80000
60000
40000
20000
0
TL signal intensity (a.u.)
50
93.4
135.4
177.4
219.4
261.4
303.4
345.4
387.4
Temperature (°C)
5 kGy
3 kGy
1 kGy
0.75 kGy
0.5 kGy
0.25 kGy
0.1 kGy
0 kGy

Table S6.

Fatty acid	Content (g/100 g)
Palmitic acid	1.22 ± 0.02
Stearic acid	0.38 ± 0.01
Oleic acid	3.01 ± 0.06
Linoleic acid	5.72 ± 0.11
Linolenic acid	1.01 ± 0.02
Crude fat (%, w/w)	12.60 ± 0.53

Figure S2.


67
82
96
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Abundance (a.u.)
60
70
80
90
100
110
120
130
140
150
160
170
180
(m/z)