metabolites

MDPI

Supporting Information

Food Monitoring: Limitations of Accelerated Storage to Predict Molecular Changes in Hazelnuts (*Corylus avellana* L.) under Realistic Conditions Using UPLC-ESI-IM-QTOF-MS

Henri Loesel [1], Navid Shakiba [1,2], Soeren Wenck [1], Phat Le Tan [1], Tim-Oliver Karstens [1], Marina Creydt [1], Stephan Seifert [1], Thomas Hackl [1,2] and Markus Fischer [1,*]

[1] Hamburg School of Food Science, Institute of Food Chemistry, University of Hamburg, Grindelallee 117, 20146 Hamburg, Germany; henri.loesel@uni-hamburg.de (H.L.); navid.shakiba@uni-hamburg.de (N.S.); soeren.wenck@uni-hamburg.de (S.W.); phat.le.tan@studium.uni-hamburg.de (P.L.T.); tim-oliver.karstens@studium.uni-hamburg.de (T.-O.K.); marina.creydt@uni-hamburg.de (M.C.); stephan.seifert@uni-hamburg.de (S.S.); thomas.hackl@uni-hamburg.de (T.H.)

[2] Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany

* Correspondence: markus.fischer@uni-hamburg.de; Tel.: +49-40-42838-4359

Supplementary Figures

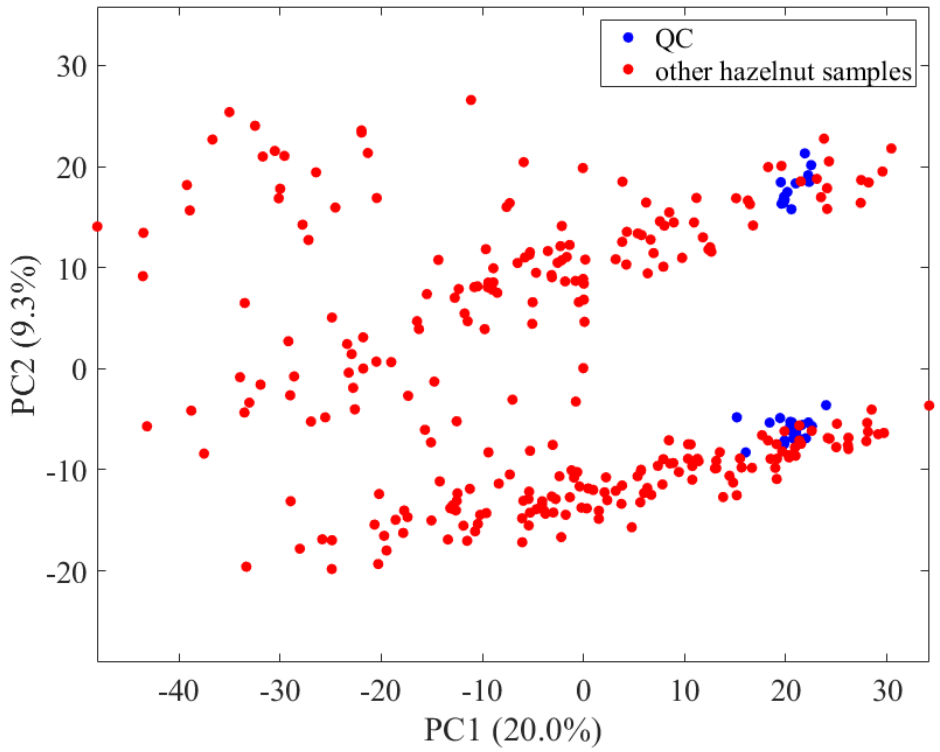

Figure S1. Scores of the principal component analysis depicted according to distinguish the quality control (QC) samples from the others. The data was previously vector normalized and autoscaled together regarding the two batches.

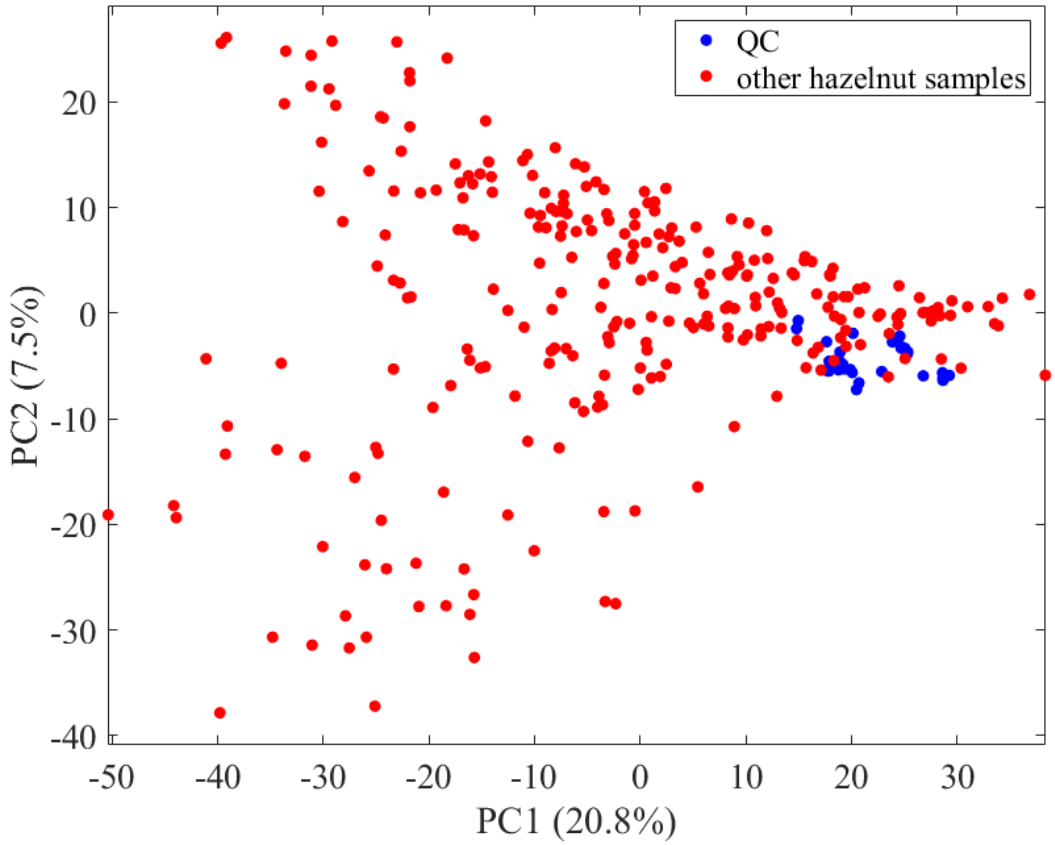

Figure S2. Scores of the principal component analysis depicted according to distinguish the quality control (QC) samples from the others. The data was previously vector normalized and autoscaled separately regarding the two batches..

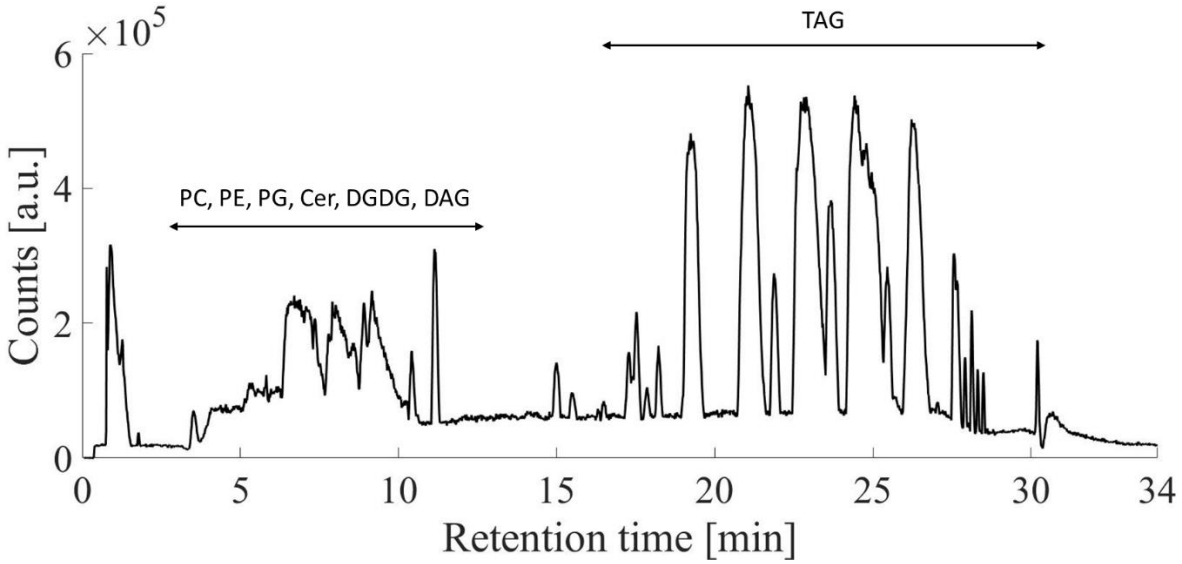

Figure S3. Exemplary TIC of a hazelnut sample with the following assignments of the main substance classes: PC: phosphocholine, PE: phosphoethanolamine, PG: phosphoglycerol, Cer: ceramide, DGDG: digalactosyldiacylglyceride, DAG: diacylglyceride, TAG: triacylglyceride.

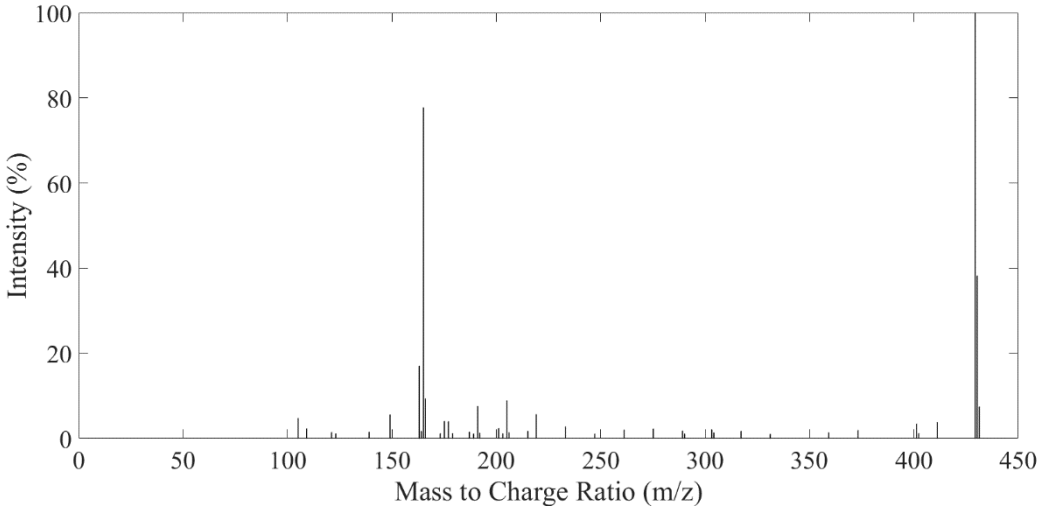

Figure S4. MS/MS spectrum of the metabolite, which was identified as α-tocomonenol. The collision energy was 10 eV.

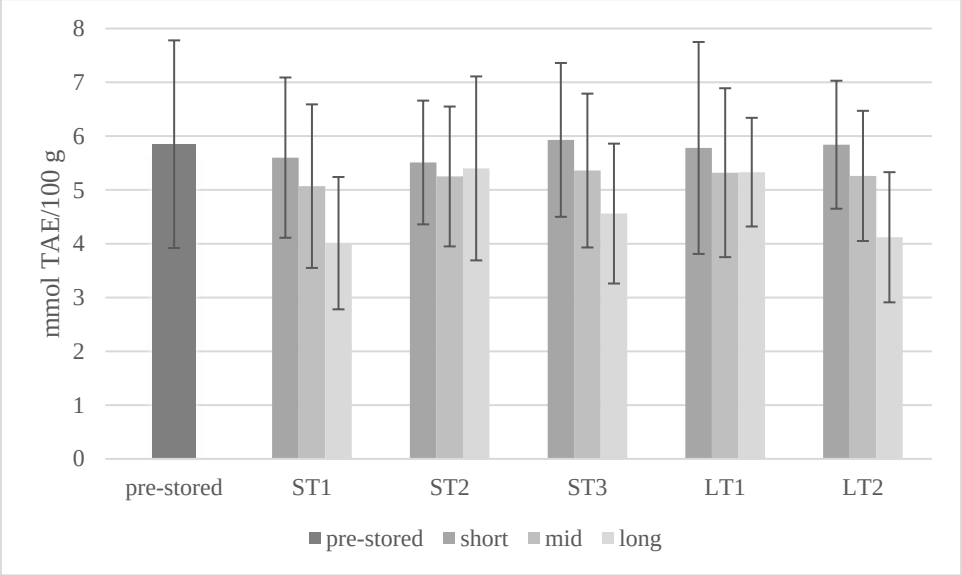

Figure S5. Bar graph of the results of the TEAC assay. The storage durations of accelerated short-term storage were summarized as follows: short (day 2, day 4, week 2), mid (week 6, week 10, week 12), and long (week 16, week 20, week 24). The storage periods of realistic long-term storage were summarized as follows: short (month 3, month 6), mid (month 9, month 12), and long (month 15, month 18).

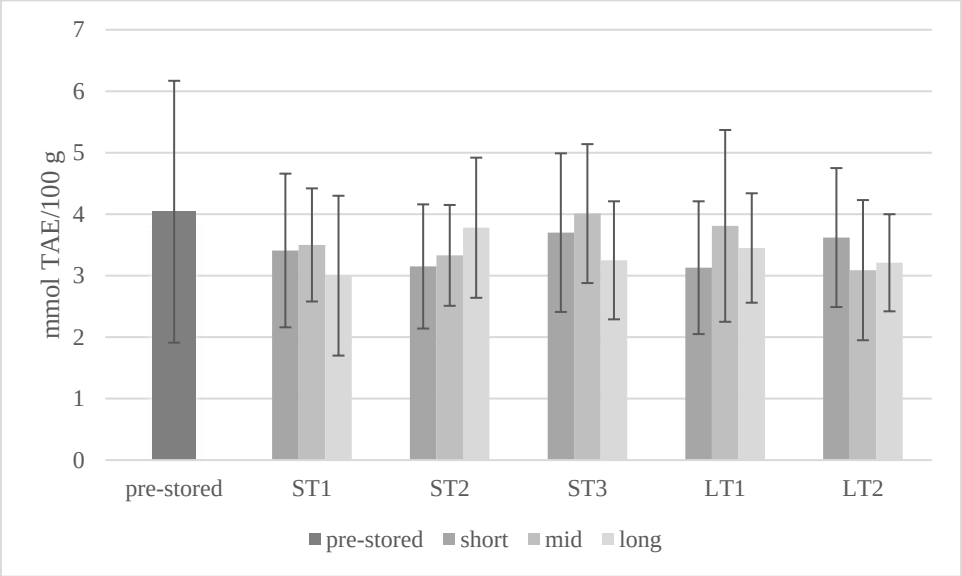

Figure S6. Bar graph of the results of the ORAC assay. The storage durations of accelerated short-term storage were summarized as follows: short (day 2, day 4, week 2), mid (week 6, week 10, week 12), and long (week 16, week 20, week 24). The storage periods of realistic long-term storage were summarized as follows: short (month 3, month 6), mid (month 9, month 12), and long (month 15, month 18).

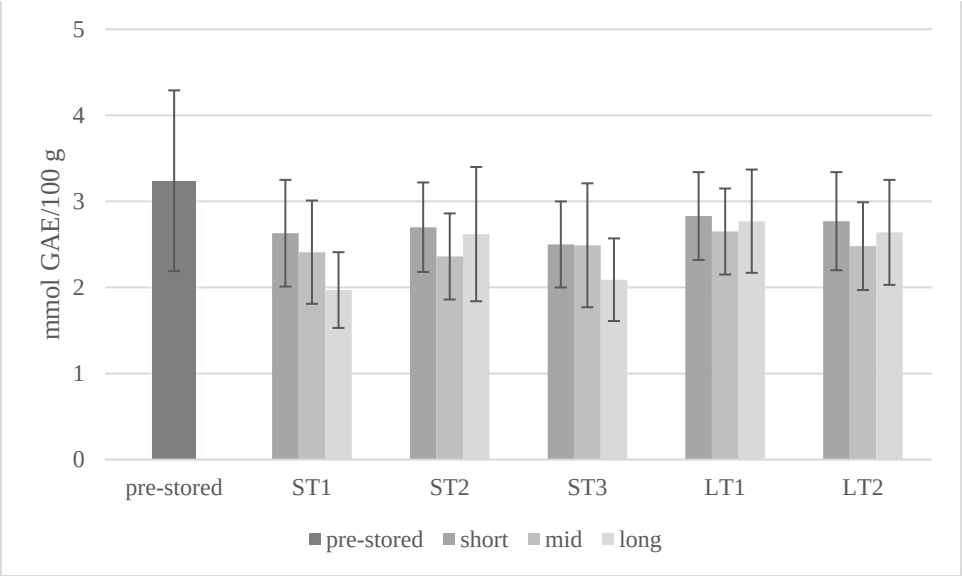

Figure S7. Bar graph of the results of the FOLIN-CIOCALTEU assay. The storage durations of accelerated short-term storage were summarized as follows: short (day 2, day 4, week 2), mid (week 6, week 10, week 12), and long (week 16, week 20, week 24). The storage periods of realistic long-term storage were summarized as follows: short (month 3, month 6), mid (month 9, month 12), and long (month 15, month 18).

Supplementary Tables

Table S1. Overview about the metadata of the stored hazelnut samples.

Sample-ID	Variety	Harvest year	Origin	Diameter [mm]
1	Mixture	2018	Turkey	11 – 13
2	Mixture	2019	Turkey	11 – 13
3	Tombul	2018	Turkey	13 – 15
4	Mixture	2019	Turkey	13 – 15

5	Mixture	2018	Turkey	>14
6	Mixture	2019	Turkey	>14

Table S2. Overview about the chosen storage durations.

ST1 – ST3 (weeks)	LT1 and LT2 (months)
2 days	3
4 days	6
2	9
4	12
6	15
8	18
10	
12	
16	
20	
24	

Table S3. Results of the classification of pre- and post-storage samples (post storage is defined by accelerated short-term storage ≥12 weeks and realistic long-term storage: ≥12 months; T_0: pre-storage, M_{12}: 12 months). The Sample-ID column shows which sample was not included in the training set and was subsequently classified using the trained model.

	Sample-ID	1	2	3	4	5	6
ST1	Training accuracy [%]	100	100	96	96	96	100
	Validation [Amount of correct classified samples]	5/5	5/5	5/5	5/5	5/5	5/5
	Validation [Misclassified samples]	/	/	/	/	/	/
ST2	Training accuracy [%]	72	80	80	80	80	84
	Validation [Amount of correct classified samples]	4/5	4/5	4/5	4/5	4/5	5/5
	Validation [Misclassified samples]	T_0	T_0	T_0	T_0	T_0	/
ST3	Training accuracy [%]	96	96	96	92	92	96
	Validation [Amount of correct classified samples]	4/5	5/5	5/5	5/5	5/5	5/5
	Validation [Misclassified samples]	T_0	/	/	/	/	/
LT1	Training accuracy [%]	75	75	80	80	75	80
	Validation [Amount of correct classified samples]	3/4	3/4	3/4	3/4	3/4	3/4
	Validation [Misclassified samples]	T_0	M_{12}	T_0	T_0	T_0	T_0
LT2	Training accuracy [%]	85	90	85	85	90	90
	Validation [Amount of correct classified samples]	3/4	4/4	3/4	4/4	3/4	4/4
	Validation [Misclassified samples]	T_0	/	T_0	/	T_0	/

Identification parameters

Phosphocholines were identified via the fragment at *m/z* 184 formed by cleavage of the polar head group. The corresponding $[M+H-183]^+$-fragment, on the other hand, showed only low intensity. The fatty acids were determined by difference calculation.

The characteristic fragment of phosphoethanolamines was the ion $[M+H–141]^+$, which was formed by the cleavage of the polar head group. The fragment formed was the diacylglyceride and subsequently cleaved off a fatty acid, which made it possible to obtain information about the fatty acids it contained.

Phosphoglycerols were identified by the fragment of the polar head group at *m/z* 195 , and fatty acids were determined by difference calculation.

Identification of ceramides based on fragment spectra was performed analogously to another study. The most intense signal in the fragmentation spectra showed a signal corresponding to the phytosphingosine moiety (*m/z* 326 or 324) formed after cleavage of the amide bond.

Fragment spectra of digalactosyldiacylglycerides revealed $[M+Na–162]^+$ fragments formed by cleavage of a galactosyl unit. Other fragments are formed when the monogalactosyl fragment cleaves off a fatty acid. In addition, fatty acids are cleaved directly from the molecular ion.

Oxidized lipids were identified by MS/MS spectra according to previous studies, where the mechanisms of formation and fragmentation were also described in detail. The epoxides were identified by the fragments of the diacylglycerides, which have an *m/z* +14 compared to the corresponding unoxidized diacylglyceride. This difference results from the addition of one oxygen atom and the abstraction of two hydrogen atoms.

Triacylglycerides were identified based on the fragments that result from the cleavage of a fatty acid and the neutral loss of this fatty acid. In addition, $[RCOO + 58]^+$ fragments were used for structure elucidation.

Table S4. Summary of identification parameters of marker compounds that are affected by storage.

Tentative metabolite	Storage condition	Sum formula	Ion	*m/z* (analytical)	*m/z* (calculated)	*m/z* difference [ppm]	RT [min]	DT [ms]	CCS-value [analytical, Å²]	CCS-value [LipidCCS database, Å²]	CCS-value [difference, %]	Main fragments [*m/z*]
PC (0:0/0:0)	ST1	$C_8H_{20}NO_6P$	+H	258.1104	258.1101	1.17	0.84	19.166	156.04	no entry	/	184.0733 104.1071
α-tocomonoenol	ST1	$C_{29}H_{48}O_2$	+H	429.3733	429.3727	1.38	8.47	26.790	214.62	no entry	/	205.1219 165.091
PC (16:0/0:0)	ST1	$C_{24}H_{50}NO_7P$	+H	496.3408	496.3398	2.09	3.41	28.721	229.28	230.5	0.53	184.0733 104.1072
PC (18:2/0:0)	ST1	$C_{26}H_{50}NO_7P$	+H	520.3408	520.398	1.99	2.65	28.468	226.96	229.7	1.19	184.0732 104.1073
PC (18:1/0:0)	ST1	$C_{26}H_{52}NO_7P$	+H	522.3566	522.3554	2.27	3.55	29.125	232.23	232.7	0.20	184.0735 104.1071
Cer (d36:3)	ST1	$C_{36}H_{67}NO_3$	+H	562.5194	562.5194	0.05	8.21	32.086	255.57	254.6 [Cer (d18:2/18:1)]	/	324.2905 306.2794 300.2902 282.2794

Tentative metabolite	Storage condition	Sum formula	Ion	*m/z* (analytical)	*m/z* (calculated)	*m/z* difference [ppm]	RT [min]	DT [ms]	CCS-value [analytical, Å²]	CCS-value [LipidCCS database, Å²]	CCS-value [difference, %]	Main fragments [*m/z*]
Cer (d36:2)	ST1	$C_{36}H_{69}NO_3$	+H	564.5361	564.535	1.91	9.45	32.266	257	258.6 [Cer (d18:1/18:1)]	/	326.3058 308.2956 300.2898 282.2798
Cer (d38:4)	ST1	$C_{38}H_{69}NO_3$	+H	588.5363	588.535	2.18	8.28	32.430	258.07	no entry	/	324.2903 306.2792
Cer (d38:3)	ST1	$C_{38}H_{71}NO_3$	+H	590.5519	590.5507	2.08	9.54	32.738	260.52	261.2 [Cer (d18:2/20:1)]	/	326.306 308.2954
DAG (18:3/18:1/0:0)	ST1	$C_{39}H_{68}O_5$	+H	617.5153	617.514	2.19	5.62	32.661	259.64	259.9	0.10	599.5038 339.2902 335.2592
DAG (18:0/18:3/0:0)	ST1, ST3	$C_{39}H_{70}O_5$	+H	619.5313	619.5296	2.75	6.94	33.523	266.53	264.6	-0.73	341.3056 335.2589
TAG (18:1/18:1/2:0)	ST3	$C_{41}H_{74}O_6$	+NH_4	680.5859	680.5824	5.33	11.1	34.361	272.7	no entry	/	663.5579 603.5361 381.3011 339.2898
PE (18:2/18:2)	ST1	$C_{41}H_{74}NO_8P$	+H	740.5242	740.5225	2.32	6.82	34.529	273.61	276.0	0.87	599.5044 337.2742
PE (18:2/18:1)	ST1	$C_{41}H_{76}NO_8P$	+H	742.5406	742.5381	3.33	7.72	34.875	276.36	278.1	0.63	601.5207 339.2893 337.2736
PE (18:1/18:1)	ST1	$C_{41}H_{78}NO_8P$	+H	744.5562	744.5538	3.25	8.84	35.185	278.82	281.5	0.95	603.5364 339.2911
PG (36:5)	ST1	$C_{42}H_{73}O_{10}P$	+H	769.5017	769.5014	0.38	4.03	35.320	279.74	281.6 [PG (18:2/18:3)]	/	597.4869 195.0026
PC (36:4)	ST1	$C_{44}H_{80}NO_8P$	+H	782.5729	782.5694	4.44	7.34	36.074	285.66	285.4 [PC (18:2/18:2)]	/	184.0731
TAG (18:2/18:1/12:0)	ST1	$C_{51}H_{92}O_6$	+NH_4	818.726	818.7259	3.48	18.82	38.493	304.72	305.8	0.35	601.5203 521.4572 519.4416
TAG (18:2/18:2/16:1)	ST1	$C_{55}H_{96}O_6$	+NH_4	870.757	870.7545	2.91	18.88	39.657	313.68	314.5	0.26	599.5049 573.4898
TAG (18:2/16:1/20:5)	ST1, ST3	$C_{57}H_{94}O_6$	+H	875.7133	875.7123	1.12	18.85	39.562	312.9	316.3	1.07	621.4848 595.4694 573.4892
TAG (18:1/18:1/16:0)	ST1	$C_{55}H_{102}O_6$	+NH_4	876.8046	876.8015	3.65	24.72	40.550	320.76	319.3	-0.46	603.5367 577.5215

Tentative metabolite	Storage condition	Sum formula	Ion	*m*/*z* (analytical)	*m*/*z* (calculated)	*m*/*z* difference [ppm]	RT [min]	DT [ms]	CCS-value [analytical, Å²]	CCS-value [LipidCCS database, Å²]	CCS-value [difference, %]	Main fragments [*m*/*z*]
TAG (52:2) epoxidized	ST3, LT2	$C_{55}H_{100}O_7$	$+NH_4$	890.7836	890.7807	3.29	17.26	40.527	320.49	no entry	/	873.7573 617.5151 603.537 591.5009 575.5053 339.29 313.274 491.3749 477.3944
TAG (52:2) epoxidized	ST1, ST3, LT2	$C_{55}H_{100}O_7$	$+NH_4$	890.7838	890.7807	3.52	16.26	40.546	320.64	no entry	/	855.7471 603.5366 591.5001 573.4895 499.4512
TAG (52:1) epoxidized	ST1, ST3	$C_{55}H_{102}O_7$	$+NH_4$	892.7996	892.7964	3.68	18.96	40.680	321.7	no entry	/	619.5325 601.5203 593.5161 577.5212 599.505 493.3899 477.3951 451.3794 339.2901 337.274 313.2742 263.2372
TAG (18:3/18:2/18:2)	ST1, ST3	$C_{57}H_{96}O_6$	$+NH_4$	894.7579	894.7545	3.86	17.44	40.064	316.79	318.5	0.54	877.7313 599.5028 597.4896 337.2726 335.2578
TAG (54:5) epoxidized	ST1, ST3	$C_{57}H_{98}O_7$	$+H$	895.7391	895.7385	0.63	16.24	40.276	318.47	no entry	/	615.5964 613.4791 293.2085
TAG (18:2/18:2/18:2)	ST1, ST3	$C_{57}H_{98}O_6$	$+NH_4$	896.7738	896.7702	4.14	19.19	40.316	318.79	319.3	0.16	879.7462 599.5052 337.274
TAG (54:4) epoxidized	ST1, ST3, LT2	$C_{57}H_{100}O_7$	$+H$	897.7564	897.7542	2.47	10.54	40.614	321.15	no entry	/	879.745 617.5155 615.4998 601.5194 339.2898

Tentative metabolite	Storage condition	Sum formula	Ion	*m/z* (analytical)	*m/z* (calculated)	*m/z* difference [ppm]	RT [min]	DT [ms]	CCS-value [analytical, Å²]	CCS-value [LipidCCS database, Å²]	CCS-value [difference, %]	Main fragments [*m/z*]
TAG (18:1/18:2/18:2)	ST1	$C_{57}H_{100}O_6$	$+NH_4$	898.7911	898.7858	6	20.99	40.674	321.62	320.7	-0.29	881.7639 601.5209 599.5057 339.2896 337.2741
TAG (18:2/18:2/20:5)	ST1	$C_{59}H_{96}O_6$	$+H$	901.7301	901.7338	2.37	19.27	40.187	317.73	321.3	1.11	621.4858 599.5038
TAG (53:2) epoxidized	ST1, ST3	$C_{56}H_{104}O_7$	$+NH_4$	906.8157	906.812	4.13	20.03	41.235	326.04	no entry	/	889.788 871.7766 619.5312 607.5302 601.5202 591.5351 493.3889 477.3949 339.2897 337.2738 327.2894
TAG (52:0) epoxidized twice	ST1, ST3, LT2	$C_{55}H_{102}O_8$	$+NH_4$	908.7942	908.7913	3.26	13.68	40.921	323.53	no entry	/	891.7645 873.7533 635.5248 617.5142 593.5144 575.5033 509.3834 493.3884 467.3729 451.3779 355.2843 337.2743 313.274
TAG (54:6) epoxidized	ST1	$C_{57}H_{96}O_7$	$+NH_4$	910.753	910.7494	4	12.54	40.195	317.74	no entry	/	613.484 599.5043 595.4729 491.3727 337.2733

Tentative metabolite	Storage condition	Sum formula	Ion	*m/z* (analytical)	*m/z* (calculated)	*m/z* difference [ppm]	RT [min]	DT [ms]	CCS-value [analytical, Å²]	CCS-value [LipidCCS database, Å²]	CCS-value [difference, %]	Main fragments [*m/z*]
TAG (54:4) epoxidized	ST1	$C_{57}H_{100}O_7$	$+NH_4$	914.7839	914.7807	3.53	15.95	40.756	322.19	no entry	/	897.7588 881.7638 617.5155 615.5007 601.5225 599.5039 597.4887 491.3743 339.2901 261.222
TAG (54:3) epoxidized	LT1, LT2	$C_{57}H_{102}O_7$	$+NH_4$	916.7984	916.7964	2.25	15.43	41.107	324.97	no entry	/	881.7641 617.5148 603.5364 599.506 339.2905 261.222
TAG (54:3) epoxidized	ST1, ST3	$C_{57}H_{102}O_7$	$+NH_4$	916.7992	916.7964	3.14	14.62	40.707	321.79	no entry	/	899.7704 881.7619 617.5129 601.5199 599.5034 339.2903 337.2727 261.2218
TAG (54:3) epoxidized	ST1, ST3	$C_{57}H_{102}O_7$	$+NH_4$	916.7997	916.7964	3.69	17.33	41.113	325.02	no entry	/	899.773 619.5305 617.5159 601.5212 599.5047 493.3891 491.374 477.3944 475.379
TAG (54:2) epoxidized	ST1, ST3	$C_{57}H_{104}O_7$	$+NH_4$	918.8159	918.812	4.29	19.11	41.498	328.07	no entry	/	901.7896 883.779 619.5324 603.5371 601.5197 493.3905 477.3956

Tentative metabolite	Storage condition	Sum formula	Ion	*m/z* (analytical)	*m/z* (calculated)	*m/z* difference [ppm]	RT [min]	DT [ms]	CCS-value [analytical, Å²]	CCS-value [LipidCCS database, Å²]	CCS-value [difference, %]	Main fragments [*m/z*]
TAG (54:1) epoxidized	ST1, ST3	$C_{57}H_{106}O_7$	$+NH_4$	920.8307	920.8277	3.34	21.07	41.840	330.78	no entry	/	903.8044 885.7931 621.546 619.5316 605.5529 601.5199 493.3895 477.3946
TAG (54:5) epoxidized twice	ST1	$C_{57}H_{96}O_8$	$+NH_4$	926.7466	926.7433	2.48	8.89	40.345	318.85	no entry	/	629.4783 615.4966 613.4813 597.4893 595.4705
TAG (54:4) epoxidized twice	ST1	$C_{57}H_{98}O_8$	$+NH_4$	928.7623	928.76	2.53	9.98	40.593	320.82	no entry	/	893.7264 631.4945 615.5003 613.4841 599.5054 491.3742 337.2746
TAG (54:2) epoxidized twice	ST1	$C_{57}H_{102}O_8$	$+NH_4$	932.7945	932.7913	3.5	12.49	41.238	325.92	no entry	/	915.7696 897.7572 633.5098 615.5 603.5363 491.3737 339.2884 277.2164
TAG (54:1) epoxidized twice	ST3	$C_{57}H_{104}O_8$	$+NH_4$	934.8096	934.8069	2.9	15.12	41.438	327.51	no entry	/	635.5244 619.5267 617.5128 603.5337 509.3846 493.3878 479.3731 339.2889
TAG (54:2) epoxidized thrice	ST1	$C_{57}H_{100}O_9$	$+NH_4$	946.7732	946.7706	2.84	7.74	40.878	323	no entry	/	929.7463 911.7361 633.5109 631.4954 617.5127 615.4996 599.5055 491.3748

Tentative metabolite	Storage condition	Sum formula	Ion	*m/z* (analytical)	*m/z* (calculated)	*m/z* difference [ppm]	RT [min]	DT [ms]	CCS-value [analytical, Å²]	CCS-value [LipidCCS database, Å²]	CCS-value [difference, %]	Main fragments [*m/z*]
TAG (56:2) epoxidized	ST1, ST3	$C_{59}H_{108}O_7$	$+NH_4$	946.8462	946.8433	3.09	20.57	42.088	332.61	no entry	/	647.5634 629.5537 603.5367 505.4268
TAG (54:1) epoxidized thrice	ST1, LT2	$C_{57}H_{102}O_9$	$+NH_4$	948.789	948.7862	3	8.51	41.130	324.99	no entry	/	633.511 617.5126 615.5011 599.504
TAG (54:0) epoxidized thrice	ST1, ST3, LT2	$C_{57}H_{104}O_9$	$+NH_4$	950.8034	950.8019	1.65	9.39	41.210	325.61	no entry	/	635.5253 933.776 617.5144 599.5033 509.3838 493.3894
DGDG (18:2/18:2)	ST1	$C_{51}H_{88}O_{15}$	+Na	963.6044	963.6015	3.04	6.03	39.374	310.96	no entry	/	801.551 683.3613 521.3087
DGDG (18:1/18:2)	ST1	$C_{51}H_{90}O_{15}$	+Na	965.6202	965.6172	3.19	6.74	39.614	312.86	no entry	/	803.5631 685.3791 683.3616 523.3236 521.3058
DGDG (18:1/18:1)	ST1	$C_{51}H_{92}O_{15}$	+Na	967.6361	967.6328	3.45	7.64	39.857	314.78	no entry	/	805.5795 685.3767 523.3228

Table S5: Results from the determination of antioxidant capacity (AOA) with the TEAC, ORAC, and FOLIN-CIOCALTEU (FC) assays. The storage durations of accelerated short-term storage were summarized as follows: short (day 2, day 4, week 2), mid (week 6, week 10, week 12), and long (week 16, week 20, week 24). The storage periods of realistic long-term storage were summarized as follows: short (month 3, month 6), mid (month 9, month 12), and long (month 15, month 18).

		TEAC (mmol TAE/100 g)			ORAC (mmol TAE/100 g)			FC (mmol GAE/100 g)		
Pre-stored	AOA	5.85 ± 1.93			4.04 ± 2.13			3.24 ± 1.05		
		short	mid	long	short	Mid	long	short	mid	long
ST1	AOA	5.60 ± 1.49	5.07 ± 1.52	4.01 ± 1.23	3.41 ± 1.25	3.50 ± 0.92	3.00 ± 1.30	2.63 ± 0.62	2.41 ± 0.60	1.97 ± 0.44
	p-value	7.43×10^{-01}	3.21×10^{-01}	1.19×10^{-02}	4.35×10^{-01}	4.54×10^{-01}	1.59×10^{-01}	9.43×10^{-02}	2.26×10^{-02}	3.24×10^{-04}
ST2	AOA	5.51 ± 1.15	5.25 ± 1.30	5.40 ± 1.71	3.15 ± 1.01	3.33 ± 0.82	3.78 ± 1.14	2.70 ± 0.52	2.36 ± 0.50	2.62 ± 0.78
	p-value	6.06×10^{-01}	3.98×10^{-01}	5.93×10^{-01}	2.34×10^{-01}	3.12×10^{-01}	7.01×10^{-01}	1.02×10^{-01}	1.01×10^{-02}	1.32×10^{-01}
ST3	AOA	5.93 ± 1.43	5.36 ± 1.43	4.56 ± 1.30	3.70 ± 1.29	4.01 ± 1.13	3.25 ± 0.96	2.50 ± 0.50	2.49 ± 0.72	2.09 ± 0.48
	p-value	9.10×10^{-01}	5.17×10^{-01}	7.77×10^{-02}	6.69×10^{-01}	9.64×10^{-01}	2.13×10^{-01}	2.73×10^{-02}	6.24×10^{-02}	1.11×10^{-03}
LT1	AOA	5.78 ± 1.97	5.32 ± 1.57	5.33 ± 1.01	3.13 ± 1.08	3.81 ± 1.56	3.45 ± 0.89	2.83 ± 0.51	2.65 ± 0.50	2.77 ± 0.60
	p-value	9.50×10^{-01}	5.42×10^{-01}	4.62×10^{-01}	2.37×10^{-01}	4.11×10^{-01}	7.94×10^{-01}	2.67×10^{-01}	1.18×10^{-01}	2.39×10^{-01}
LT2	AOA	5.84 ± 1.19	5.26 ± 1.21	4.12 ± 1.21	3.62 ± 1.13	3.09 ± 1.14	3.21 ± 0.79	2.77 ± 0.57	2.48 ± 0.51	2.64 ± 0.61
	p-value	9.89×10^{-01}	4.39×10^{-01}	3.26×10^{-02}	5.80×10^{-01}	2.28×10^{-01}	2.35×10^{-01}	2.24×10^{-01}	5.09×10^{-02}	1.40×10^{-01}