## 1 Supporting Information

- 2 Kinetics and Condensed-phase Products in Multiphase Ozonolysis of an Unsaturated
- 3 Triglyceride
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# 26 1) Overview of Experiments

Table S1: Summary of coating composition, average triolein thickness, extraction method andanalytical technique used in the study.

Experiment	Coating composition	Average triolein thickness (nm)	Extraction method	Analytical technique
Kinetics (Triolein decay)	0.9 μg triolein and 0.45 μg BES	4	5 mL 2-propanol	LC-MS
	1.8 μg triolein and 0.9 μg BES	8	10 mL 2-propanol	LC-MS
Kinetics and product evolution	9.0 μg triolein and 4.5 μg BES	40	2 mL 2-propanol	LC-MS
Product identification	180 μg triolein	800	2 mL mixed solvent (1.5 μM NH <sub>4</sub> COOH in 70% methanol, 15% 2-propanol, 15% acetonitrile and 0.1% v/v formic acid) <sup>1,2</sup>	Direct infusion MS
Photolysis of SOZ	3.6 μg BES and ~18 μg fully oxidized triolein	/	2 mL 2-propanol	LC-MS (kinetics)
			3 replicates in 2 mL mixed solvent (1.5 μM NH₄COOH in 70% methanol, 15% 2- propanol, 15% acetonitrile and 0.1% v/v formic acid) <sup>1,2</sup>	Direct infusion MS (product identification)

### 38 2) Geometry of Triolein Coatings



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42 **Figure S1:** Optical microscopy images of **a**) 0.9 μg, **b**) 1.8 μg and **c**) 9.0 μg triolein coatings, mixed

43 with 0.45, 0.9 and 4.5 μg BES respectively.

#### 44 3) Photolysis Lighting Conditions



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Figure S2: Spectral irradiance of artificial sunlight inside the solar simulator, daytime indoor direct
sunlight by a closed window (measured at noon in mid-July 2018) and daytime indoor indirect
light measured on an office desk, 3 m away from a window.

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The Suntest CPS solar simulator (Atlas Electric Devices) in condition (1) consists of a Xenon lamp 50 51 that simulates the direct irradiance of sunlight. A benchtop chiller (VWR) is connected to the 52 bottom of the simulator. It offsets the lamp heat so that the temperature inside the simulator remains at ~295K. Condition (2) was carried out by the windows (facing south) at the Department 53 of Chemistry, University of Toronto between 11:00 and 15:00 in mid-July 2018 for the highest 54 daytime light irradiance. Temperature on the surfaces near the windows was measured to be 55 56 313 - 318K. Condition (3) was done on an office desk 3 m away from a north-facing window in 57 the same building. The fluorescence lights on the ceiling were about 2 m above the desk. At this 58 distance, the irradiance from artificial lighting reaching the object was less than the diffuse 59 sunlight coming through the window. This office lighting condition is representative of a typical indoor environment without direct sunlight. The light spectra of all 3 conditions above were 60 measured by a spectral radiometer (StellarNet Inc., Figure S2). It is seen that the amount of light 61

- 62 in condition (3) is roughly 2 orders of magnitude lower than direct sunlight in the solar simulator,
- whereas condition (2) is intermediate between the two. In condition (4), the exterior of the petri 63
- dish was fully covered by aluminum foil. After photolysis, each sample was extracted by 2 mL 2-64
- propanol for LC-MS analysis. For direct infusion MS analysis, 3 three identically-prepared samples 65
- were extracted by 2 mL mixed solvent (1.5 µM NH₄COOH in 70% methanol, 15% 2-propanol, 15% 66
- acetonitrile and 0.1% v/v formic acid).<sup>1,2</sup> 67

(v/v) formic acid (B) Distilled water (A); 5

mM NH₄COOH in 90%

methanol, and 0.1%

(v/v) formic acid (B)

2-propanol, 10%

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#### 69 4) HPLC-ESI-MS Conditions

Product

evolution

and

photolysis

of SOZ

/1	retention times ( $t_R$ ) and retention time windows in selected ion monitoring (SIM) Q1 scan.					
	Experiment	LC mobile phase	Analyte	m/z [M + NH <sub>4</sub> ]+	t <sub>R</sub> (min)	t <sub>R</sub> window (min)
	Kinetics of	Distilled water (A); 5 etics of mM NH <sub>4</sub> COOH in 60%	BES	444.5	2.3	1
	decay methanol, and 0.1%	Triolein	903.0	6.8	2	

BES

Triolein

Mono-ozonide

Di-ozonide

Tri-ozonide

444.5

903.0

951.0

999.0

1047.0

3.5

4

70 **Table S2:** Solvent systems in LC mobile phase, analytes, center masses (m/z with  $NH_{a^{+}}$  adduct),

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5  $\mu$ L extract was injected onto the LC column. With a flow rate of 300  $\mu$ L  $\cdot$  min<sup>-1</sup>, the mobile phase 73 74 contained two solvent systems A and B. Under positive ESI ionization, the addition of NH<sub>4</sub><sup>+</sup> in the 75 mobile phase facilitates the formation of ammonium adducts in the analyte ions. In the kinetics experiments monitoring the triolein decay, the flow gradient was: 0 – 0.1 min and 8.5 – 9 min 76 10% **A** and 90% **B**; 0.1 – 8.5 min 100% **B** with a gradient curve 5 in 0.4 min. The divert valve 77 (Rheodyne) was set to MS between 1 and 8.1 min, while the remainder was diverted to the waste. 78 In the SOZ evolution and photolysis experiments, the flow gradient was: 0 - 0.1 min and 6.5 - 879 min 10% A and 90% B; 0.1 – 6.5 min 100% B with a gradient curve 5 in 0.4 and 0.3 min. The flow 80 for the period between 1 and 6.2 min was diverted to MS. Detailed descriptions of mobile phase 81

solvent systems, analytes and settings in selected ion monitoring (SIM) mode are shown in Table
S2. Sample chromatograms of fresh and partially oxidized triolein (9.0 μg) with BES internal
standard are shown in Figure 3 (a-b).

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#### 86 5) Quantitation Methods

87 Peak areas in SIM chromatograms were analyzed by Thermo Xcalibur Qual Browser 4.1.50 with 88 Boxcar-type peak smoothing at 7 pts. To remove peak overlap (e.g. Figure 3b), a scan filter was 89 applied to redraw the individual peak with its corresponding m/z. The relative intensities of the 90 unreacted triolein and products (SOZs) were determined by normalizing the peak areas of these 91 analytes to BES (unreactive internal standard). The uncertainty for each data point was calculated 92 based on one standard deviation (1 $\sigma$ ) of all individually prepared replicates. 6 or 9 replicates were 93 prepared in kinetics experiments (0.9 µg and 1.8 µg triolein coatings under dry conditions), while 94 all other LC-MS analyses contained at least 3 replicates. In the kinetic plots, the pseudo-first-95 order rate constants  $(k_1)$  are attained through the curve fitting function in eq. S1 where the offset b is negligible. 96

97 
$$\frac{[\text{Triolein}]_{t}}{[\text{Triolein}]_{0}} = e^{-k_{1}t} + b \text{ (eq. S1)}$$

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#### **6) Direct Infusion MS and MS/MS Conditions**

100 Although the LC-MS method was sufficient to measure triolein and the major SOZ products, it 101 was difficult to observe clear chromatographic peaks for minor products. Therefore, the detailed 102 product identification was done through direct infusion mass spectrometry, which permits signal 103 averaging to detect compounds with a low yield. For mass spectra recorded in the full scan mode 104 (Q1), the extract before/after oxidation was directly infused into the ESI ion source through a syringe pump (Chemyx) and PEEK tubing over a m/z range of 450-1100. SOZ ions with an 105 106 ammonium adduct  $[M + NH_4]^+$  were analyzed in the positive ionization mode. Most other 107 oxygenated products such as acids and aldehydes were detected in negative ion mode, and ions

were observed in the deprotonated form [M - H]<sup>-</sup> and/or with a formate adduct [M + HCOO]<sup>-</sup>.
The ion source settings were as follows: spray voltage at +3000 V (or -2200V in the negative ion mode); ion transfer tubing temperature at 250 °C; vaporizer temperature at 150 °C; RF lens amplitude at 252.0 V; sheath gas, auxiliary gas and sweep gas flows at 25, 2 and 0 (or 25, 10 and 2 in the negative ion mode) arbitrary units respectively.

In MS/MS analysis, product ion scan in the positive ionization mode was used with precursor ions
 selected at m/z = 903 (triolein), 951 (mono-ozonide), 999 (di-ozonide) and 1047 (tri-ozonide). 1
 mTorr CID gas (Ar) and 20 V CID voltage were applied. Other settings were identical to those
 described above. All mass spectra were analyzed through Thermo Scientific Freestyle 1.3. SP2.





Figure S4: (+)-ESI-MS/MS spectra of a) triolein (m/z = 903.0), b) mono-ozonide (m/z = 951.0), c)
di-ozonide (m/z = 999.0) and d) tri-ozonide (m/z = 1047.0). Product ion identification is described
in Table S3.

Table S3: Identification of product ions from MS/MS fragmentations of triolein and SOZs as
 precursor ions in Figure S4. The fragmentation assignments are consistent with those from Ref
 3-5.

Precursor ion	Product ion m/z	Neutral losses
Triolein	602 7	
$[C_{57}H_{104}O_6 + NH_4]^+$	005.7	$C_{18} G_{34} O_2 + N G_3$
	791.8	$C_9H_{18}O + NH_3$
Mono-ozonide	603.7	$C_{18}H_{34}O_5 + NH_3$
[C <sub>57</sub> H <sub>104</sub> O <sub>9</sub> + NH <sub>4</sub> ] <sup>+</sup>	509.5	$C_{18}H_{34}O_2 + C_9H_{18}O + NH_3$
	493.5	$C_{18}H_{34}O_2 + C_9H_{18}O_2 + NH_3$
Di-ozonide	509.5	$C_{18}H_{34}O_5 + C_9H_{18}O + NH_3$
$[C_{57}H_{104}O_{12} + NH_4]^+$	493.5	$C_{18}H_{34}O_5 + C_9H_{18}O_2 + NH_3$
Tri-ozonide	399.4	$C_{18}H_{34}O_5 + C_9H_{18}O_2 + C_9H_{18}O + NH_3$
$[C_{57}H_{104}O_{15} + NH_4]^+$	383.4	$C_{18}H_{34}O_5 + C_9H_{18}O_2 + C_9H_{18}O_2 + NH_3$





### 171 9) Product Identification from ESI-MS Spectra

- 172 **Table S4:** Product identification of major peaks in Figure 2, 4 and S5. **A-E** represent the possible
- 173 structures on the acyl chains after oxidation/photolysis. Products highlighted in bold represent
- the final products after full ozone oxidation of triolein (dry or humid air). All molecular weights
- 175 (MW) and experimental m/z ratios correspond to the mass with the most abundant isotopes.



MW	m/z	lon identity	Proposed combinations on acyl chains (R <sub>1</sub> , R <sub>2</sub> , R <sub>3</sub> )
884.8	(+) 903.0 (-) 930.0 (-) 946.9	[M + NH <sub>4</sub> ] <sup>+</sup> [M + HCOO] <sup>-</sup> [M + NH <sub>3</sub> + HCOO] <sup>-</sup>	A-A-A (Triolein)
1028.7	(+) 1046.9 (-) 1090.9	$[M + NH_4]^+$ $[M + NH_3 + HCOO]^-$	B-B-B
980.8	(+) 998.9 (-) 1042.9	[M + NH <sub>4</sub> ] <sup>+</sup> [M + NH <sub>3</sub> + HCOO] <sup>-</sup>	A-B-B
932.8	(+) 950.9 (-) 994.9	$[M + NH_4]^+$ $[M + NH_3 + HCOO]^-$	A-A-B
902.6	(-) 947.8	[M + HCOO] <sup>-</sup>	B-B-E
886.6	(-) 931.8	[M + HCOO] <sup>-</sup>	B-B-D
854.6	(-) 899.8	[M + HCOO] <sup>-</sup>	A-B-E
838.6	(-) 883.8	[M + HCOO] <sup>-</sup>	A-B-D
806.6	(-) 851.8	[M + HCOO] <sup>-</sup>	A-A-E
790.6	(-) 835.8 (-) 789.8	[M + HCOO] <sup>-</sup> [M - H] <sup>-</sup>	A-A-D
774.6	(-) 819.8	[M + HCOO] <sup>-</sup>	A-A-C
744.5	(-) 743.7	[M - H] <sup>-</sup>	B-D-D
696.5	(-) 695.7	[M - H] <sup>-</sup>	A-D-D
680.5	(-) 679.7	[M - H] <sup>-</sup>	A-C-D
602.3	(-) 601.5	[M - H] <sup>-</sup>	D-D-D (after photolysis)

177 It should be noted that no peaks of hydroperoxides, resulting from reactions between the Criegee 178 intermediates (CI) and 2-propanol, were observed from mass spectra in both positive and 179 negative ion modes. This indicates that no CI scavenging was caused by the incomplete 180 evaporation of solvent.<sup>[6]</sup>

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### 182 10) Direct Infusion (-)-ESI-MS Spectra of Tri-ozonide Under Photolysis



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**Figure S5:** Full scan direct infusion (-)-ESI mass spectra of 18  $\mu$ g fully oxidized triolein (mainly triozonide) under artificial solar light inside the solar simulator (450-550 W/m<sup>2</sup>) for **a**) 0 hour, **b**) 1 hour, **c**) 5 hours and **d**) 16 hours.  $\Delta m/z$  of 142 (with accompanying loss of m/z = 17 and or 46 adducts) represents the conversion from the SOZ to a carboxylic acid on one of the acyl chains. As indicated in Table S4, the proposed structures of m/z = 932, 744 and 602 correspond to mono-,

189 di- and tri-acids, respectively.

### 190 References

- 191 [1] Li, M.; Butka, E.; Wang, X. Comprehensive quantification of triacylglycerols in soybean seeds
- by electrospray ionization mass spectrometry with multiple neutral loss scans. *Sci. Rep.* 2014, *4*,6581.
- 194 [2] Wabaidur, S. M.; AlAmmari, A.; Aqel, A.; AL-Tamrah, S. A.; Alothman, Z. A.; Ahmed, A. B. H.
- 195 Determination of free fatty acids in olive oils by UPHLC–MS. *J. Chromatogr. B* **2016**, *1031*, 109-115.
- [3] Sun, C.; Zhao, Y.; Curtis, J. M. A study of the ozonolysis of model lipids by electrospray
  ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 2012, *26*, 921-930.
- 198 [4] Pleik, S.; Spengler, B.; Bhandari, D. R.; Luhn, S.; Schäfer, T.; Urbach, D.; Kirsch, D. Ambient-air
- 199 ozonolysis of triglycerides in aged fingerprint residues. *Analyst* **2018**, *143*, 1197-1209.
- 200 [5] Harrison, K. A.; Murphy, R. C. Direct mass spectrometric analysis of ozonides: Application to
- unsaturated glycerophosphocholine lipids. *Anal. Chem.* **1996**, *68*, 3224-3230.
- [6] Criegee, R. Mechanism of Ozonolysis Angew. Chem. Int. Ed. Engl. 1975, 14, 745-752.