Supporting Information

Detecting Lipid-Derived Electrophiles with a Turn-On Fluorescent Probe. Alkylation of 7-Mercapto-4-MethylCoumarin by α, β-Unsaturated Carbonyls Yields Fluorescence Increase to Quantity 'Electrophilic Content' in Extracted Edible Oils.

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Materials and Reagents

7-Mercatpto-4-methylcoumarin (97%), trans-2-nonénal, L-proline, 1-nonanal, Hexane ACS and Chloroform HPLC, were obtained from Sigma-Aldrich. Dichloromethane ACS was supplied by Caledon. Ethanol 100% and Methanol ACS were provided respectively by Commercial alcohols and Anachemia. Acrolein, Malondialdehyde, and Acrylamide were also obtained from Sigma-Aldrich. The semi-transparent polypropylene microplate was purchased from Eppendorf.

Frozen French Fries Data

McCain®

Cavendish Farms®

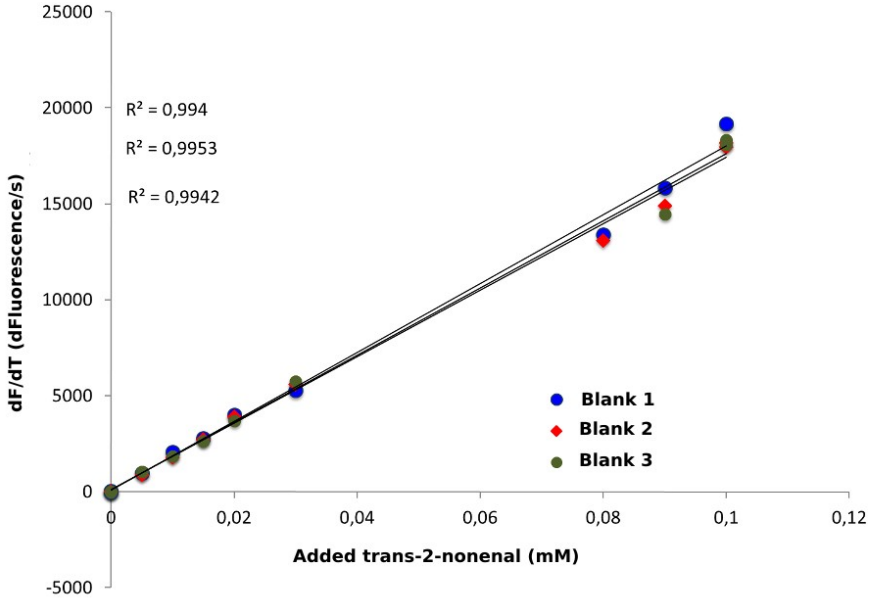
Instruments

UV–Vis absorption spectra were measured on an Agilent Cary 60 UV-Vis spectrophotometer and the microplate reader used in most experiments was a Tecan infinite M1000 Pro.

Purification of 7-mercapto-4-methylcoumarin

To remove some impurities from the commercial product, 7-mercapto-4-methylcoumarin (C-SH) (97% pure) is first recrystallised from a mixture of hexane and dichloromethane. C-SH is not very soluble in low polar organic solvents, so hexane is used as the solvent for its purification. Typically, 1.5 g of C-SH is placed in an Erlenmeyer flask equipped with magnetic bar. After the addition of hexane (~ 50 mL), the mixture is stirred and heated. A minimum of dichloromethane is then added via squirt bottle to achieve complete dissolution of the C-SH. The evaporating dichloromethane also cleans the walls of the Erlenmeyer flask from undissolved C-SH powder. The solution is then removed from the hot plate to cool and to allow the dichloromethane to evaporate so that the C-SH, which is poorly soluble in hexane, recrystallizes and the crystals are recovered. Typical recovery is 50-70%.

Calculation of Limit of Quantification (LOQ)



To determine the reproducibility of the method, a calibration curve of the rate of fluorescence increase as a function of added *trans*-2-nonenal was measured in triplicate. The figure shown above demonstrates a relatively reproducible method, as there is minimal deviation between the three calibration curves.

In these experiments, the standard deviation for the response with no added electrophile (n = 6) was equal to 19.7 (fluorescence increase over time, a.u./sec). To calculate the LOQ, we use the calibration curve eqation (y = 188 777 x + 68.3) and the standard equation :

LOQ signal = 10 × Standard Deviation of blank

$$LOQ\ concention = \frac{LOQ\ signal - 68.3}{188777} = \frac{(10\ \times 19.7) - 68.3}{188777} = 0.0008\ mM$$

The limit of quantification is approximately 0.1 µM in *trans*-2-nonenal equivalents.

Relative response for the C-SH test vs the p-AV test for various electrophiles

To calibrate our pre-fluorescent probe, we tested some electrophiles under the same optimal conditions to evaluate their responses according to their reactivities. The response of electrophiles with the C-SH method were compared with those of the standardized p-anisidine index method. For this purpose, stock solutions of acrolein (0.3 mM), acrylamide (0.3 mM), 1-nonanal (0.029 M) and malonaldehyde (0.1 mM as tetrabutylammonium salt) were prepared. From these solutions, six standard solutions of each (from 0.03 mM to 0.024 mM) were prepared in 10 mL volumetric flasks. The solutions were analyzed according to the procedure of the two existing methods. The slope of the calibration curve for each electrophile was used to estimate the relative response of each electrophile using the two methods.

Table S1. Relative response for the C-SH test vs the p-AV test for various electrophile

	C-SH		p-AV	
	C-SH signal[a]	Normalized to *trans*-2-nonenal	p-AV signal[b]	Normalized to *trans*-2-nonenal
trans-2-nonenal	17.0	1	3.72	1
nonanal	no increase	-	-	0.13
acrolein	155	9.11	183.39	49.3
malondialdehyde	no increase	-	no increase	-
acrylamide	3.1	0.18	9.73	2.62

[a]Slope of the rate of fluorescence increase vs concentration of electrophile. [b]Slope of the absorption at 350 nm after the standard 10-minute reaction vs concentration of electrophile.

Choice of solvent

H-bond accepting solvents such as methanol or ethanol are necessary in our method due to the mechanism of fluorescence quenching in C-SH. In pure chloroform, the fluorescence quantum yield of C-SH is 0.096. This significant fluorescent quantum yield gives too strong a signal which obscures the fluorescence increase following alkylation with dilute lipid derived electrophiles. In methanol, however, C-SH is practically non-fluorescent (Φ_F < 0.005) since its excited state can lose a proton to become a non-emissive anion (Figure S2). Extracted lipides are poorly soluble in polar solvents such as ethanol and methanol (left and center test tubes as seen below). A compromise between lipid solubilizing solvent and polar solvent necessary to attenuate C-SH fluorescence was found in 1:1 ethanol:chloroform (right test tube).

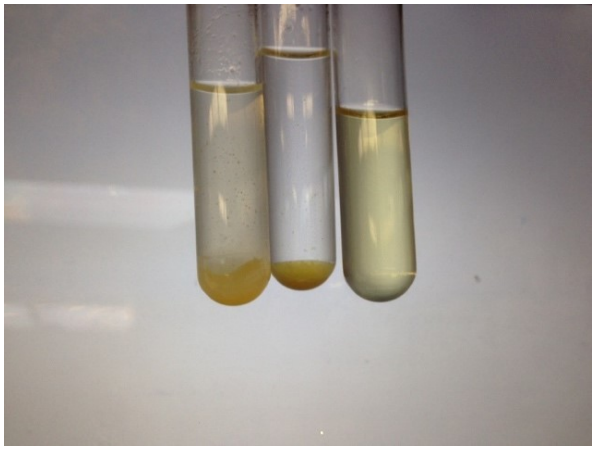


Figure S1. On the left the mixture of oil in ethanol, in the centre in methanol and on the right in the 1:1 (v/v) EtOH:$CHCl_3$.

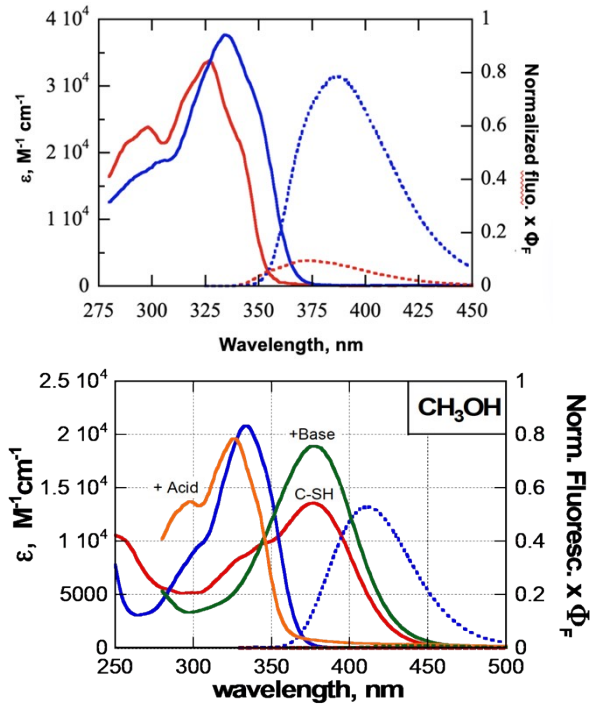


Figure S2. Absorption and emission spectrum of **C-SH** and its methylated product **C-SMe** in chloroform (top) and methanol (bottom) (solid line: absorption, dashed line: fluorescence). Data from: Frenette, M. Advances in free radical oxidation: Mechanistic studies, fluorescent probe design and radically different antioxidants. PhD Thesis, Ottawa, **2009**. https://ruor.uottawa.ca/bitstream/10393/29778/1/NR51812.PDF

Addition of Catalyst: L-proline

Proline catalyzes nucleophilic addition to α,β-unsaturated ketones by the formation of an iminium that activates the β-position.

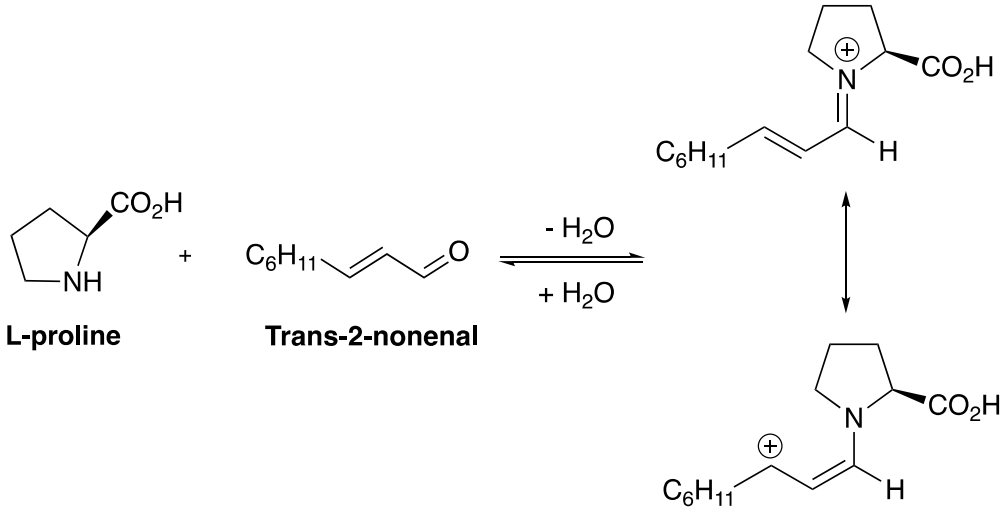


Figure S3. Reaction of L-proline with *trans*-2-nonenal to form an iminium cation.

Selectivity test

Observation of the increase in fluorescence as a function of time in relation to similar electrophiles: nonanal (red), trans-2-nonenal (blue).

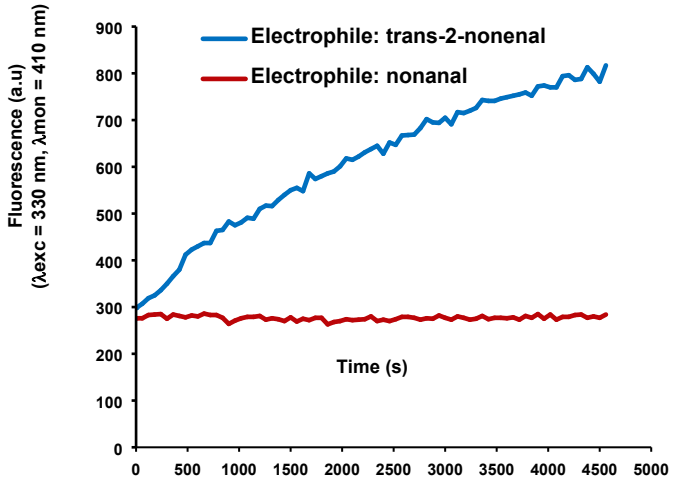


Figure S4. Fluorescence response of C-SH in the presence of *trans*-2-nonenal and nonanal; no fluorescence increase is observed for the reaction with simple aldehyde.

Additional results

Results from nut butters and chips. The oils from these samples were extracted using the excess dichloromethane extraction method. However, the oils extracted from the peanut butters were centrifuged at 3000 RPM for 10 minutes.

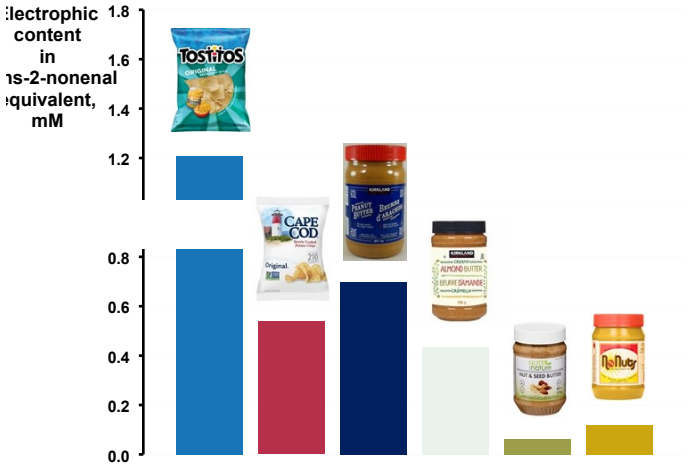


Figure S5. Comparison of electrophilic content, expressed in *trans*-2-nonenal equivalents, for extracted oils from peanut butters, Tostitos, and Cape Cod brand chip.

Relative response of the three methods (peroxide value (POV), p-anisidine value (p-AV), C-SH method) of a heated Canola oil for 6h. The p-AV and POV responses are normalized to the C-SH response.

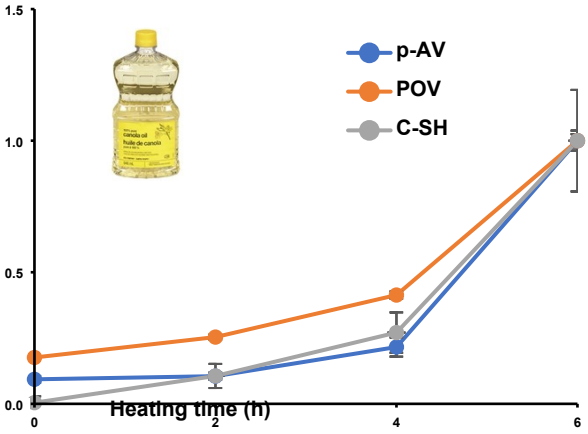


Figure S6. Normalized response of C-SH, p-AV and POV tests for canola oil heated at 180°C.

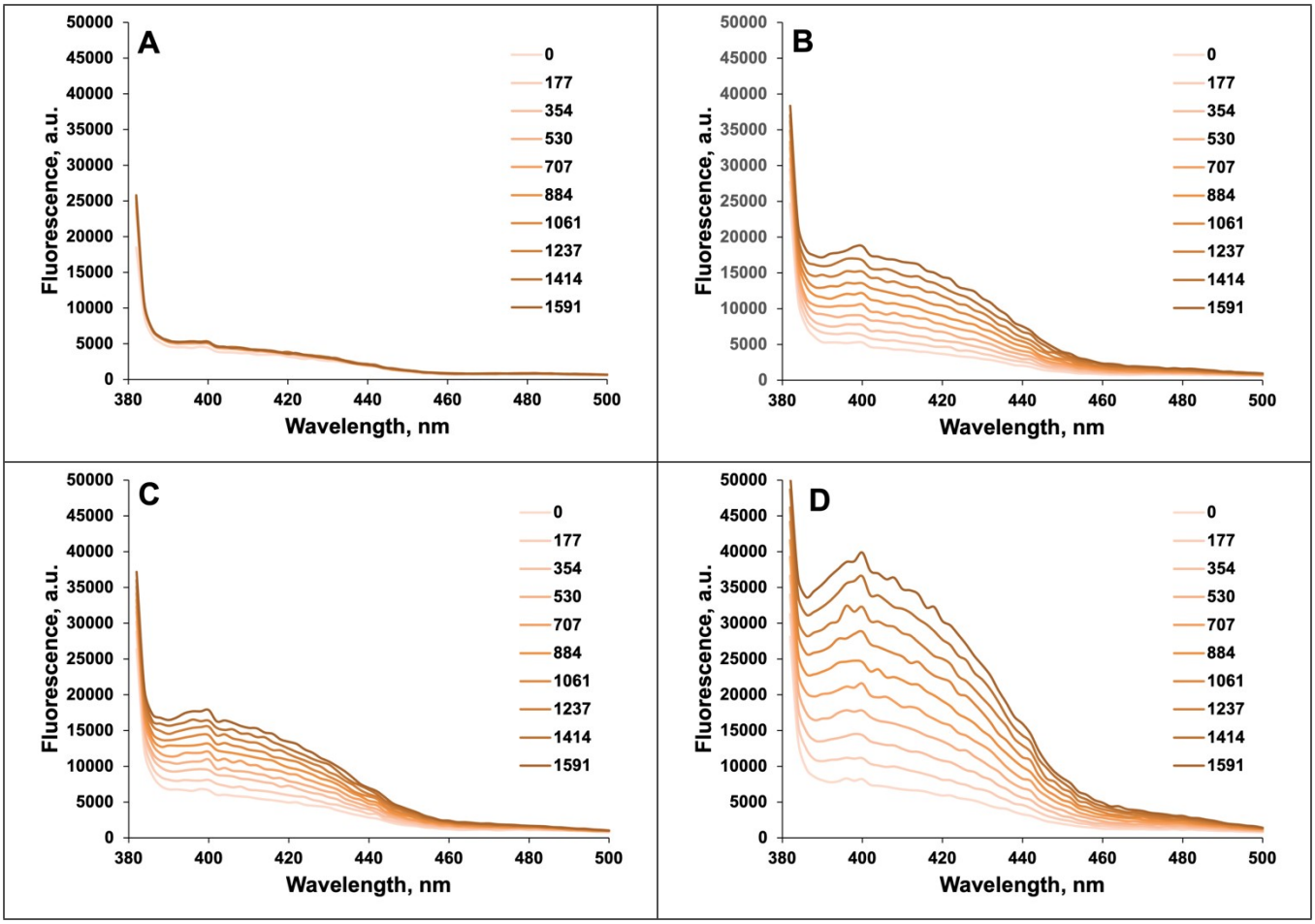


Figure S7. Emission spectra (λ_{exc} = 365 nm) taken at ~2 min increments (legend indicates reaction time in seconds) after the addition of 1 mM **C-SH** and 4 mM L-proline in (A) ethanol:$CHCl_3$ solvent, (B) 100 µM **2-NE**, (C) 10 µL Pringle®-extracted oil in 300 µL total well volume, (D) 100 µM **2-NE** + 10 µL Pringle®-extracted oil in 300 µL total well volume.

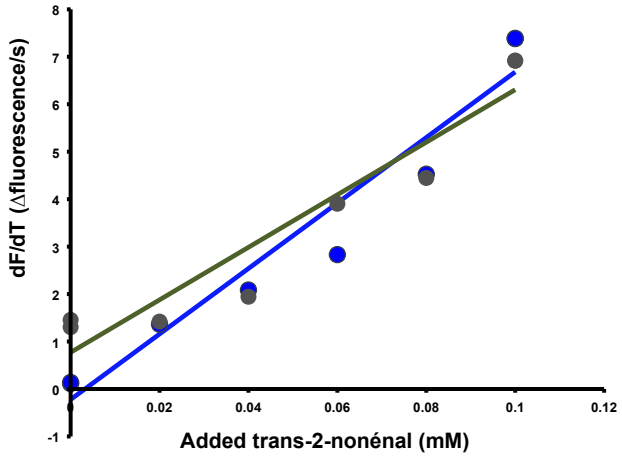


Figure S8. Example of data where inaccurate pipetting was the likely cause of poor linearity in the response. These types of results were unfortunately common for several students who worked in the development of this method. The combination of rapid pipetting needed to initiate kinetics as soon as possible and the volatility of the solvent used were a major source of error.