Rapid Oxidation of Skin Oil by Ozone

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Abstract

The reaction of gas-phase ozone with human skin oil has been studied at room temperature. Skin oil was exposed to ozone at mixing ratios similar to those in the ambient environment, and then analyzed for condensed-phase products using Direct Analysis in Real Time-Mass Spectrometry (DART-MS). Prior to ozone exposure, skin oil gives rise to prominent mass spectral signals indicative of highly unsaturated alkenes, sterols, triglycerides, long chain fatty acids, pyroglutamic acid, and probably waxy esters. Upon oxidation with 50 ppb ozone for 90 minutes, there is rapid loss of alkene, fatty acid, and triglyceride signals resulting from efficient multi-phase ozonolysis. Oxygenated products including a variety of carboxylic acids are identified via studies with pure compounds present in skin oil, i.e. squalene, cholesterol and triolein. The chemistry is rapid, occurring on timescales of tens of minutes, implying that these highly oxygenated reaction products are always present on human skin both indoors and outdoors.

Introduction

A major advance in our understanding of the chemistry of the indoor environment is recognition of the role of human occupants.[1] One class of impacts arises from multi-phase chemistry, especially skin oils present either on human subjects or flakes/oils deposited in indoor environments.[2-4] Gas-phase products arising from the ozonolysis of carbon-carbon double bonds within skin lipids have been identified.[3-6] As well, the rate of ozone deposition in indoor environments and ensuing chemistry depends on the level of human occupancy, where reactive loss of ozone can occur directly either on humans, their soiled clothes or skin materials that they deposit.[4,6-12]

The multi-phase reactions of ozone with condensed-phase unsaturated organics occur efficiently.[13,14] Typical functional groups formed from ozone reacting with an alkene include carbonyls, carboxyls, hydroxyl ketones and potentially hydroxyhydroperoxides. To better understand the nature of chemistry occurring indoors arising from skin oil chemistry, experiments have examined the oxidation of squalene with ozone.[15-19] The kinetics are fast, with reactive uptake coefficients of 10^{-5} to 10^{-3}. Both gas- and condensed-phase products have been identified, including carbonyls, acids, peroxides and ethers.[16,17,19] The condensed-phase products are more hydrophilic and redox-active than squalene.[18] As well, the oxidation products of fabrics soiled with squalene have been studied under ambient conditions.[20]

Many studies have been conducted on the chemical composition of skin surface lipids, with major components including long-chain alkenes (e.g. squalene, 10% by mass), saturated and unsaturated triglycerides (25%), fatty acid esters (22%), long-chain saturated and unsaturated fatty acids (25%), sterols (2%) and natural moisturizing agents (a few percent).[21-26] We do not attempt to reproduce these compositional studies. Rather, our goals in this work are to use DART-MS[27] to: i) analyze the change in the composition of skin oil upon exposure to ozone, identifying the species that react most efficiently and the oxidation products that remain in the condensed phase, and ii) assess the oxidative transformation timescales of key components at an environmentally relevant ozone mixing ratio. The motivations are to: i) provide kinetic information to interpret rates of ozone loss in inhabited indoor spaces, ii) identify components of skin oil that are unreactive with

ozone that could be used to track human input to surfaces, and iii) identify condensed-phase oxidation products to understand the factors impacting the composition of indoor surfaces. Condensed-phase products have not been previously identified upon exposure of skin oil to ozone, even though this chemistry will be occurring in both indoor and outdoor environments.

Experimental

Experiments were conducted using DART-MS analysis.[28] Samples are deposited onto the lower 5 mm of 1.6 mm-o.d. glass capillaries mounted in a Teflon holder. For skin oil, participants reproducibly touched the tip of the capillary with their fingers. Prior to applying the skin oil, the participants had not used personal care products for two days. They washed their hands with soap and water, and then rubbed hands over face and arms. Although we present the oxidation mass spectra of one specific male participant's skin oil, we also examined the spectra of three other male participants. The major spectral features were observed in all four skin oil samples although, as expected, there were variations in relative intensities of peaks from participant to participant, and from day-to-day; however, this variability is not relevant to the kinetics results. Rather it is the variability of the measurements amongst a set of coated tubes (commonly ±20% for 10 replicates) that impacts the precision of the kinetics. The pure substances used as references in the product analysis (100 ng) were applied as 1 μL dilute solutions in dichloromethane to the tip of the glass capillary, after which the solvent evaporated. The relative standard deviation of major peaks was also ±20%. We believe that this analytical method is largely sensitive to the free molecules present in the sebum (i.e. fatty secretions from the sebaceous glands) rather than lipid materials that are physically bound in the epidermis.

Ozone exposure was conducted at 295±3 K by placing the mounted capillaries in a glass flow tube through which ozone mixed in air flowed with a residence time of ~40 s.[28] We performed control experiments whereby both pure component and skin oil samples were analyzed for time periods up to 90 minutes with no ozone, and we observed no loss of signal due to volatilization beyond the variability stated above. After ozone/air exposure the samples were stored in a desiccator where a small flow of nitrogen was added to avoid contamination from room air.

DART-MS analysis was performed by passing the coated capillaries in front of the ion source (IonSense Inc.) on a motorized rail at a speed of 0.3 mm/second. The DART source was held at 500ºC, resulting in (280±20)ºC at the capillary sample, and used a helium flow of 3.0 liter/min. Ambient air from the lab was present in the source region. Mass spectra were measured with a JMS-T100LC time-of-flight mass spectrometer (JEOL USA Inc.) having mass resolution of approximately 6000 at a mass-to-charge ratio (*m/z*) 600.

Results and Discussion

1. Skin oil characterization

A typical positive ion mass spectrum of skin oil is shown as the red trace in Figure 1a which has four noteworthy features. First, two of the strongest peaks are due to squalene (Molecular Weight, MW = 410.4 amu for the C12 isomer) with the first peak (*m/z* 411.4) arising from protonation in the ion source and the second (*m/z* 428.4) due to addition of ammonium arising from trace ammonia in the lab air. Control experiments with pure squalene confirm this assignment (Figure S1). Second, we attribute the strong peak at *m/z* 369.3 to skin lipid sterols, such as cholesterol and lathosterol.[25] Control experiments (Figure S2) demonstrate that cholesterol is detected at *m/z* 369.3 following loss of water from protonated cholesterol (*m/z* 387.3), a common fragmentation for protonated alcohols. This peak in the skin oil may also arise from sterol esters.[29] Third, there is a cluster of peaks from just below *m/z* 800 to 1000 (see Table S1). These peaks likely arise from triglycerides, with roughly equal abundance in skin lipids of the unsaturated and saturated forms.[25] The unsaturated forms have hydrocarbon chains 14-24 carbon atoms long (i.e. MW=700-1100 amu). Results from a control experiment with a pure C18 mono-unsaturated triglyceride, triolein (MW = 884.8 amu for C12 isomer), are shown in Figure S3. The DART-MS spectrum shows intensity for ammoniated triolein (*m/z* 902.7) and a fragment ion of protonated triolein (*m/z* 603.5) following the loss of a C18 mono-unsaturated fatty acid (MW = 282.4 amu). The fourth cluster of peaks in the positive ion mass spectrum, i.e. those between *m/z* 450 and 600 (Table S1), may arise from the triglycerides or waxy esters (typically mono- and di-esters with 10 to 20 carbon chains, MW = 450 to 600).[25] These peak assignments are in agreement with the DART-MS literature.[29]

A fifth important class of compounds, the long-chain fatty acids, is observed with three large peaks in the negative ion spectrum at *m/z* 253.2, 255.2 and 281.2 (Figure 1b and Table S1). These likely

correspond to the most abundant fatty acids in skin lipids, cis-hexadec-6-enoic acid (MW=254.2 amu for C12 isomer), palmitic acid (MW=256.2 amu for C12 isomer), and cis-octadec-8-enoic acid (MW=282.3 amu for C12 isomer).[25] Organic acids are detected by proton abstraction in negative ion mode DART-MS, i.e. at $[M\text{-}H^+]^-$.

The most prominent peak in the negative ion spectrum is observed at *m/z* 128.0. Control experiments show that glutamic acid and pyroglutamic acid contribute to this mass (Figure S4). Using HPLC-MS analysis (see SI-Text, Figures S5, S6), we established that this peak is mainly due to pyroglutamic acid, a natural moisturizing substance present at the 1% level in the human epidermis.[21,23,26]

2. Oxidation kinetics of skin oil and common skin oil materials

The blue traces in Figure 1a and 1b correspond to an ozone exposure of 50 ppb for 90 minutes. There is loss of intensity in a large number of peaks and formation of intensity at others. The squalene peaks lose their prominence entirely, the triglyceride peaks are significantly depleted, as are peaks from *m/z* 450 to 600 (see Table S1). The intensity of the sterol peak at *m/z* 369.3 is reduced slightly but decreases by 50% when exposed to 300 ppb ozone for 45 minutes (data not shown). In the negative ion mode, the peaks due to the fatty acids all decrease substantially with the peak at *m/z* 255.2 (likely palmitic acid) decreasing to ~60% of its starting value and those at *m/z* 253.2 and 281.2 decreasing more than 70%.

Squalene is an unsaturated compound that reacts rapidly with ozone.[16-18] A decay plot demonstrates the rapid kinetics of pure squalene heterogeneous ozonolysis, with an e-folding lifetime of 10 minutes with 50 ppb ozone (Figure 2c). Triolein, the oleic acid tri-ester of glycerol, shows similar reactivity (Figure 2d). These liquids react more rapidly than pure cholesterol which is a solid at ambient temperature (Figure 2b). Although cholesterol is known to react with ozone,[30,31] its phase may be the reason for the lower reactivity.

The sterol peak (*m/z* 369.3) shows similar loss kinetics to that of pure cholesterol (Figure 2b), with a small decay at early times. By comparison, the peaks due to squalene (*m/z* 411.4) and cis-hexadec-6-enoic acid (*m/z* 253.2) show more reactivity, with some saturation of the reactivity at

long times (Figure 2d). This is likely due to burial of some of the molecules in the skin oil matrix, limiting exposure to ozone at the surface. For probably the same reason, the squalene signal does not decay as rapidly as when present as a pure compound, with an e-fold lifetime of about 30 minutes (Figure 2c). The peak assigned to palmitic acid (*m/z* 255.2) also exhibits initial decay and the same plateau in the signal at long times (Figure 2d). Since palmitic acid does not react directly with ozone, this may be due to reaction with reactive intermediates such as Criegee biradicals. Whether other species also react with intermediates of this type is not known.

The peak due to pyroglutamic acid does not decay with ozone (Figure 2a) consistent with it not containing a carbon-carbon double bond. This chemical may be a convenient tracer of human skin oil contamination in indoor environments, given its relatively high abundance and low reactivity with ozone.

3. Products formed from oxidation of skin oil and common skin oil materials

The pure substance experiments help to interpret the oxidation products. Prominent products from squalene are observed only in the negative ion mode. The dominant peaks at *m/z* 115.0 and 117.0 are consistent with levulinate and succinate anions, respectively (Figure S1). The corresponding acids are reaction end products, formed via successive ozonolysis reactions at the multiple carbon-carbon double bonds in squalene, yielding at each step carbonyl and carboxylic acid functional groups upon bond breakage. These products are present in the skin oil oxidation experiment, as seen in the inset in Figure 1b. Other dicarboxylic acids, including adipic acid and suberic acid, arising from oxidation of the most common unsaturated acids, cis-hexadec-6-enoic acid and cis-octadec-8-enoic acid, respectively, are also observed at *m/z* 145.0 and 173.1. We note that a study of squalene oxidation indicated substantial product formation at high molecular weights.[19] The positive ion DART-MS spectra of squalene similarly show the formation of high MW products upon ozone oxidation (>450 amu) (Figure S1).

Although cholesterol reacts slowly, the products formed (*m/z* 385.3, 401.3, 417.3 in the positive ion spectrum and *m/z* 433.3 in the negative ion spectrum, Figure S2) arise from addition of 1, 2 or 3 oxygen atoms, respectively, to its carbon-carbon double bond. For example, the three-oxygen product, i.e. *m/z* 417.3 in the positive ion spectrum, could arise from ozonolysis of the carbon-

carbon double bond into an aldehyde and carboxylic acid. Given the small decay of cholesterol, it is not possible to identify these product peaks in the skin oil spectrum.

The positive ion DART-MS spectra of triolein before and after exposure are shown in Figure S3. It is not surprising that, with three double bonds, triolein yields a large number of ozonolysis products that are observed from *m/z* 450-800. A common feature is that they all have molecular weights lower than the parent triolein molecule, indicating that ozonolysis leads to cleavage of an alkyl chain and formation of a distribution of smaller products. These observations are consistent with those made from the skin oil experiments in which high MW skin oil components (*m/z* 750- to 1000) disappeared after ozone reaction whereas lower MW species from *m/z* 450-800 formed. The most intense peak in the negative ion mass spectrum (*m/z* 157.1) is consistent with the formation of nonanoic acid, a low volatility product arising from ozonolysis of the oleate chain in triolein.

To illustrate the kinetics of product formation, the time-dependent signals of oxidation products from squalene and cis-hexadec-6-enoic acid are shown in Figure 2c,d, along with a likely oxidation product at *m/z*(-) 585.3 from unsaturated triglycerides. This oxidation product was observed from triolein (Figure S3).

4. Environmental Significance

This investigation is the first mass spectrometry study of the chemical changes occurring in the condensed phase when skin oil is exposed to ozone. By focussing on the condensed phase products, this work complements earlier studies that addressed the gas-phase products formed when ozone interacts with human lipid materials containing species such as squalene.[4]

Driven by the unsaturated skin oil components present, the oxidative transformation is rapid as seen by the significant change observed in the DART-MS spectrum after 90 minutes exposure to 50 ppb ozone. Specifically, squalene and the unsaturated fatty acids in skin oil have an oxidation timescale on the order of tens of minutes at this ozone mixing ratio, a common outdoor value during smog episodes; this lifetime will be longer indoors which typically have ozone levels 0.2 to 0.8 of those outdoors.[32] We note that given the amount of time spent indoors, human ozone

exposure is frequently dominated by indoor exposure.[32] The values used here, i.e. 75 ppb·hours, are environmentally relevant being equivalent to one hour outdoors at 75 ppb ozone or 7.5 hours indoors at 10 ppb. There is evidence for the formation of a suite of highly oxygenated species, composed of carboxylic acids such as succinic acid, levulinic acid, higher dicarboxylic acids, and highly functionalized species. One species, pyroglutamic acid, is not reactive with ozone which may make it useful as a tracer of human contamination indoors.

These results imply that the oils covering human skin and those deposited to indoor surfaces are being continually oxidized under ambient conditions, with a mixture of both endogenous components alongside their heterogeneous oxidation products. The oxidation products are less reactive than the precursors, thus making the surfaces less reactive than a fresh skin oil surface. As well, the impacts of the observed loss kinetics of the many skin oil species could be related to ozone loss indoors, using a coupled indoor air – multiphase chemistry model. It will be important to determine the toxicological properties of the oxidation products given earlier work that has indicated that squalene oxidation gives rise to redox-active condensed-phase materials and gas-phase products that are irritants.[18,33]

Supporting Information:

Mass spectra (with and without ozone) of squalene, cholesterol, triolein and their ozonolysis products obtained by DART-MS (Figure S1-S3); mass spectra of glutamic and pyroglutamic acids obtained by DART-MS (Figure S4) and ESI-MS (Figure S5); extracted ion chromatograms of HPLC-MS analysis of glutamic and pyroglutamic acids (Figure S6); lists of major peaks of skin oil, squalene, cholesterol and triolein and their ozonolysis products (Table S1-S4); description of the HPLC-MS analysis (SI-Text).

Acknowledgements

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Supporting Information

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Summary (9 pages; 6 figures; 4 tables)

This supporting information contains: Mass spectra (with and without ozone) of squalene, cholesterol, triolein and their ozonolysis products obtained by DART-MS (Figure S1-S3); mass spectra of glutamic and pyroglutamic acids obtained by DART-MS (Figure S4) and ESI-MS (Figure S5); extracted ion chromatograms of HPLC-MS analysis of glutamic and pyroglutamic acids (Figure S6); lists of major peaks of skin oil, squalene, cholesterol and triolein and their ozonolysis products (Table S1-S4); description of the HPLC-MS analysis (SI-Text).

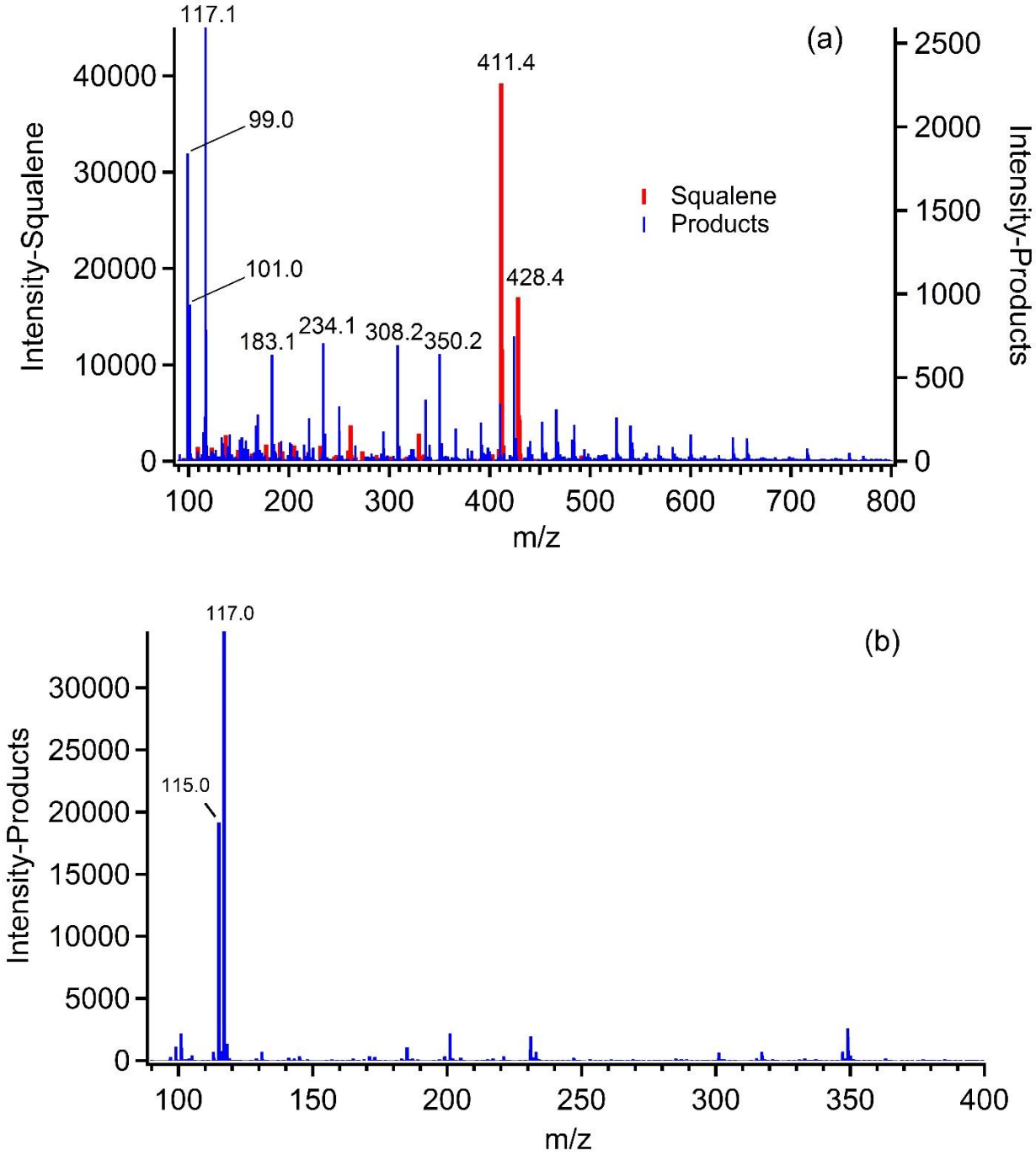

Figure S1. Mass spectrum (red) of squalene obtained under positive (a) and negative (b) modes. The blue trace is the product difference mass spectrum, i.e. it is the mass spectrum formed by subtracting that observed of pure squalene from that observed after squalene is exposed to ozone at 50 ppb for 90 minutes. Note the different scales on the y-axes in (a).

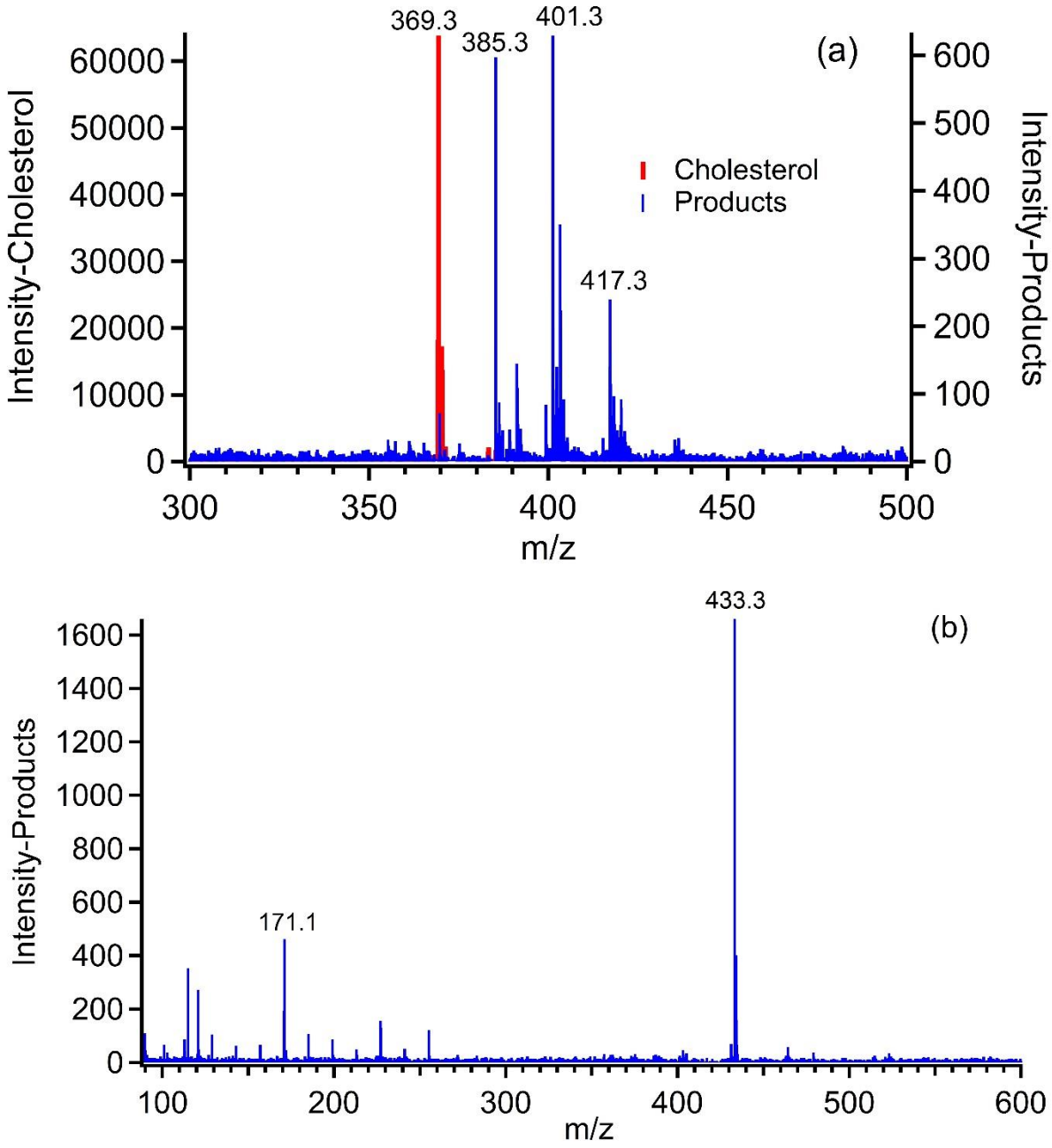

Figure S2. Mass spectrum (red) of cholesterol obtained under positive (a) and negative (b) modes. The blue trace is the product difference mass spectrum, i.e. it is the mass spectrum formed by subtracting that observed of pure cholesterol from that observed after squalene is exposed to ozone at 50 ppb for 90 minutes. Note the different scales on the y-axes in (a).

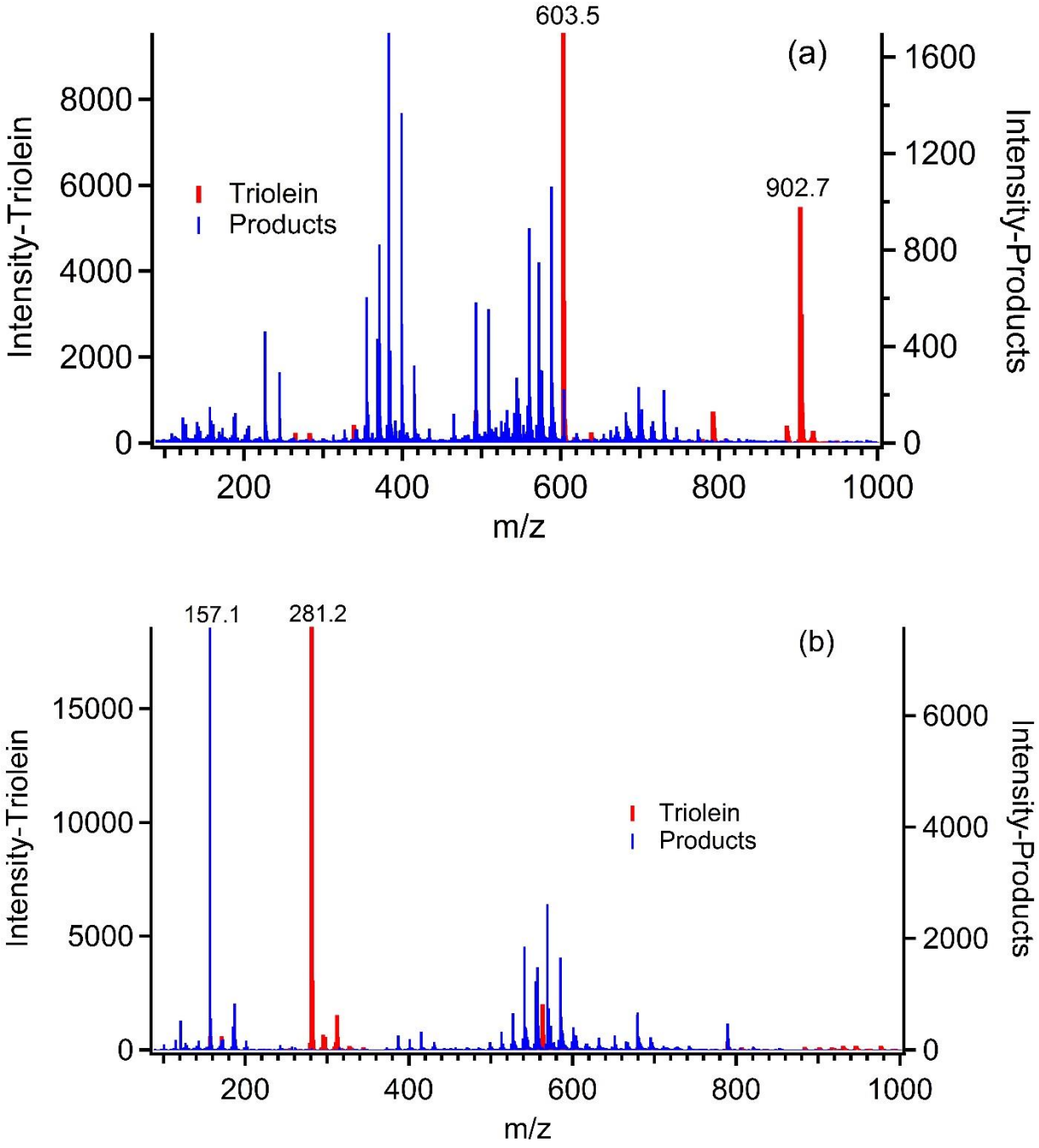

Figure S3. Mass spectrum (red) of triolein obtained under positive (a) and negative (b) modes. The blue trace is the product difference mass spectrum, i.e. it is the mass spectrum formed by subtracting that observed of pure triolein from that observed after squalene is exposed to ozone at 50 ppb for 90 minutes. Note the different scales on the y-axes in (a).

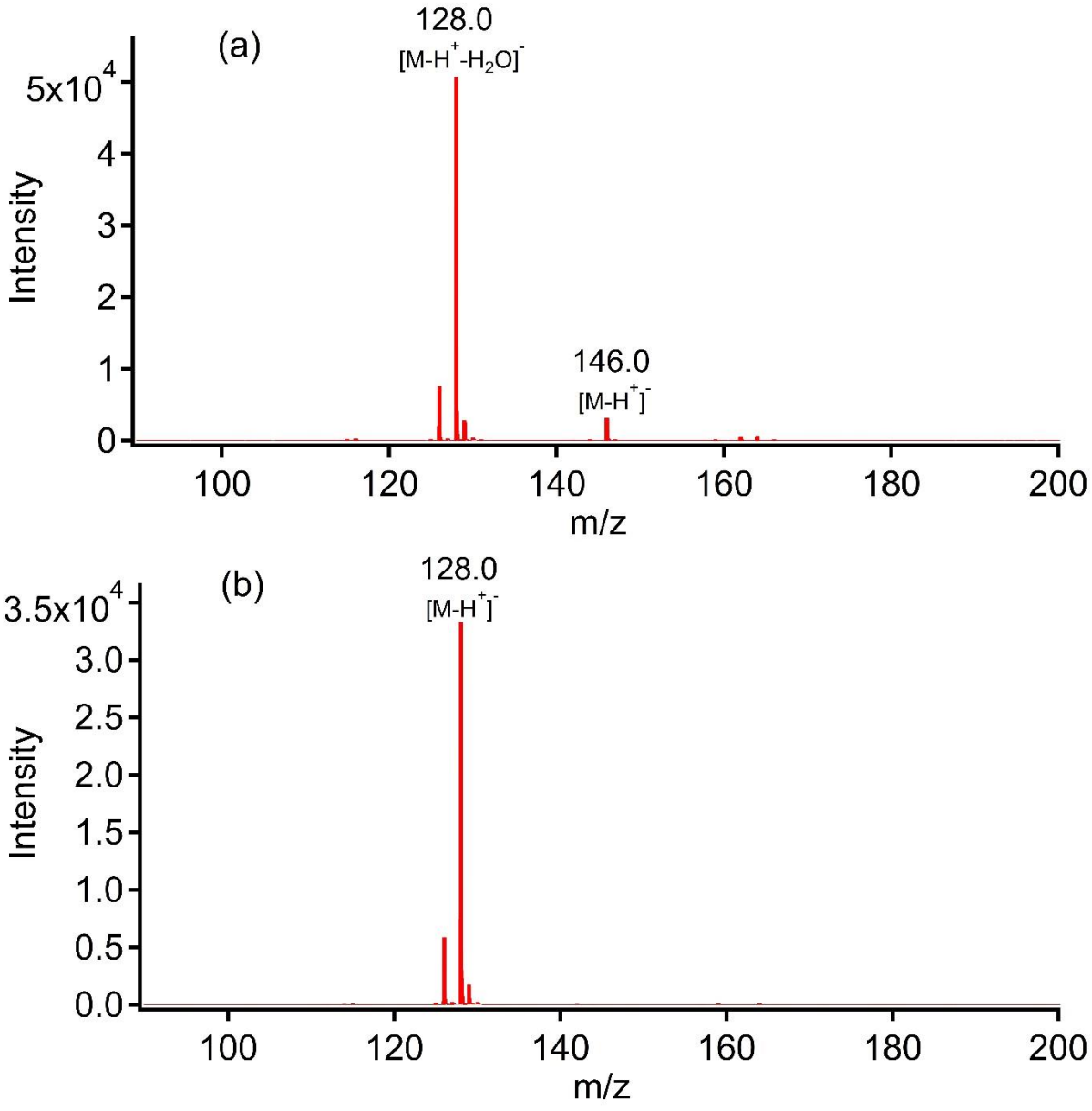

Figure S4. Mass spectrum of glutamic (a) and pyroglutamic acid (b) measured by DART-MS under negative mode. For glutamic acid *m/z* 146 and 128 (a) are due to $[M-H^+]^-$ and $[M-H^+-H_2O]^-$, respectively; for pyroglutamic acid (b) *m/z* 128 is due to $[M-H^+]^-$.

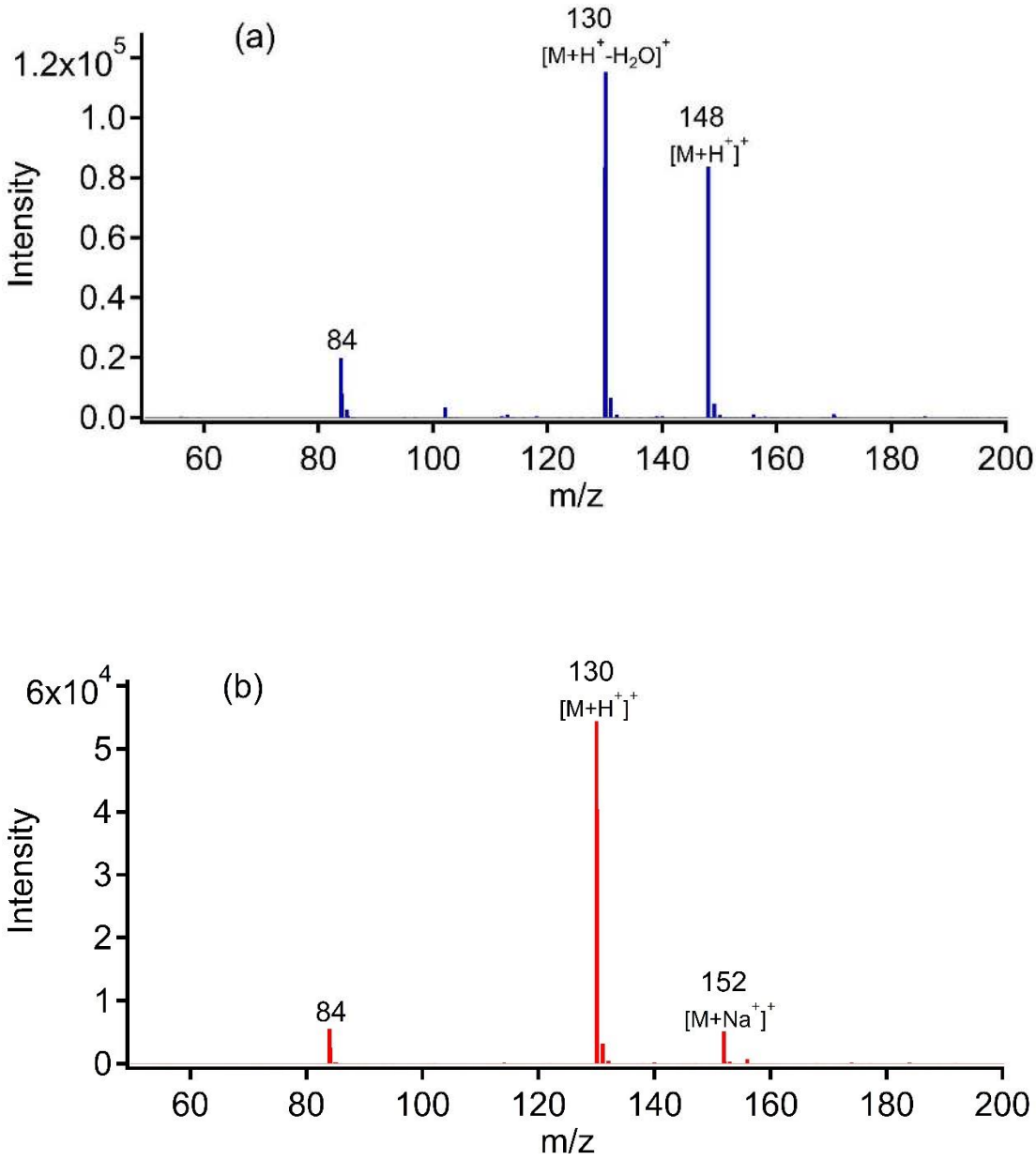

Figure S5. Mass spectra of glutamic acid (a) and pyroglutamic acid (b) obtained by the ESI-MS under positive mode.

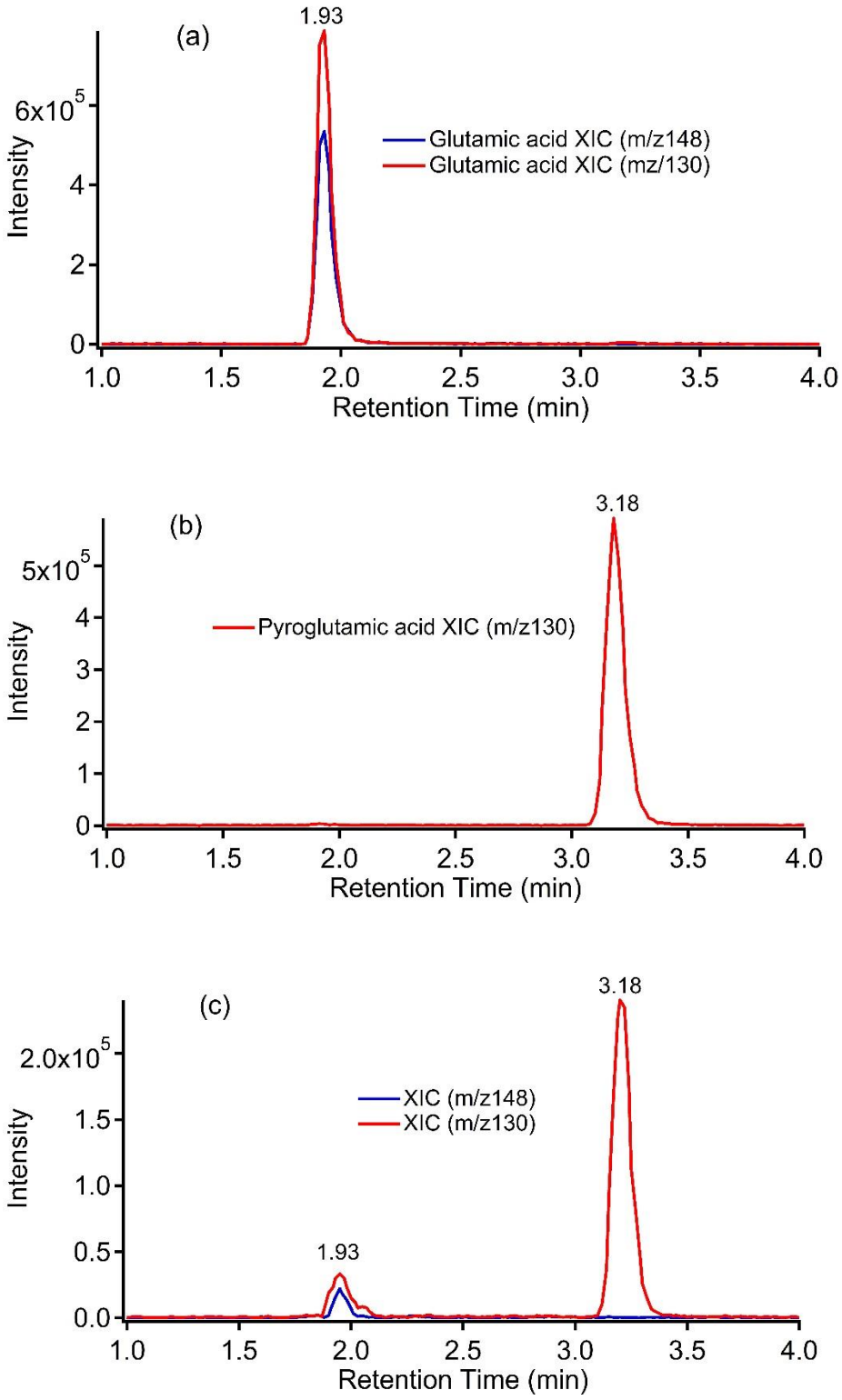

Figure S6. Extracted ion chromatogram (i.e. mass spectral intensity at the indicated mass-to-charge ratio) as a function of retention time) for the HPLC-MS analysis of (a) glutamic acid, (b) pyroglutamic acid and (c) aqueous extract of skin oils (see SI-Text for explanation).

Table S1. Major peaks of the un-oxidized and oxidized skin oil DART-MS spectra

	Un-oxidized	Oxidized
Positive mode	130.0, 311.1, 369.3, 411.4, 428.4, 451.4, 465.4, 479.4, 493.5, 507.5, 521.4, 523.4, 533.5, 535.5, 537.4, 547.4, 549.5, 550.5, 551.5, 563.5, 565.3, 575.4, 577.5, 579.5, 806.6, 808.7, 820.7, 822.7, 834.7, 836.8, 846.7, 848.7	101.0, 117.0, 130.0, 130.0, 311.1, 369.3, 383.3, 425.3, 441.3, 453.3, 455.3, 467.4, 469.3, 474.3, 523.4, 530.4, 537.4, 544.4, 551.5, 555.8, 565.5, 572.4, 586.4, 588.4, 616.4, 684.5, 686.5, 728.5
Negative mode	90.0, 137.0, 154.1, 227.2, 253.2, 255.2, 281.2, 405.0, 537.5, 798.7	90.0, 115.0, 117.0, 137.0, 145.0, 187.1, 255.2, 405.0, 457.3, 571.4, 585.4, 599.4, 613.4, 731.5, 798.7

Table S2. Major peaks of squalene and its ozonolysis products obtained by DART-MS

	Reactants	Products
Positive mode	411.4, 428.4	99.0, 101.0, 117.1, 183.1, 234.1, 308.2, 350.2, 424.2, 452.2, 466.3, 484.3, 526.2, 540.3
Negative mode	None	101.0, 115.0, 117.0, 201.1, 231.1, 349.1

Table S3. Major peaks of cholesterol and its ozonolysis products obtained by DART-MS

	Reactants	Products
Positive mode	369.3	385.3, 391.3, 401.3, 403.3, 417.3
Negative mode	None	115.0, 121.0, 171.1, 433.3

Table S4. Major peaks of triolein and its ozonolysis products obtained by DART-MS

	Reactants	Products
Positive mode	603.5, 902.7	227.1, 245.1, 355.2, 371.2, 383.2, 399.2, 415.2, 493.3, 509.3, 544.3, 560.3, 572.3, 588.3, 682.5, 698.5, 730.4
Negative mode	281.2, 563.5	157.1, 187.1, 527.3, 541.3, 557.3, 569.3, 585.3, 679.4, 789.6

SI – Text

HPLC-ESI-MS Analysis of Glutamic and Pyroglutamic Acids in Skin Oil:

Kimwipes were used to collect skin oils from the participant. The Kimwipes were cleaned by sonication with 20mL dichloromethane for 15 minutes followed by 15 minutes sonication with 20mL methanol and then drying under nitrogen. The skin oils were collected by wiping the participant's face and arms with the Kimwipes that were then extracted by sonication with 20 mL Milli-Q water. The aqueous extract was directly injected into an HPLC-ESI-MS (Agilent 1260 Infinity binary HPLC coupled to Agilent 6538 UHD quadrupole time-of-flight mass spectrometer) for analysis. The standards were prepared by dissolving the glutamic and pyroglutamic acids in Milli-Q water. Blank samples were extracted and analyzed in an analogous manner as the skin oil samples.

Figure S5 shows the mass spectra of glutamic (a) and pyroglutamic acid (b) obtained by ESI-MS in the positive ion mode. Figure S6 shows the extracted ion chromatograms of the HPLC-MS analysis of glutamic acid standard (~0.1mM) (a), pyroglutamic acid standard (~0.1mM) (b) and the aqueous extract of the skin oil (c). The HPLC separation was made using a 100 mm × 4.6 mm Gemeni NX 5µm C18 column (Phenomenex, Torrance, CA) and mobile phase consisting of 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.5 mL/min. The mobile phase gradients were as follows: 0 min 5% B-1 min 5% B-11 min 95% B-11.01 min 95% B-14 min 100% B-14.01 min 5% B-15 min 5% B.