

Assessing the Effect of Reactive Oxygen Species and Volatile Organic Compound Profiles Coming from Certain Types of Chinese Cooking on the Toxicity of Human Bronchial Epithelial Cells

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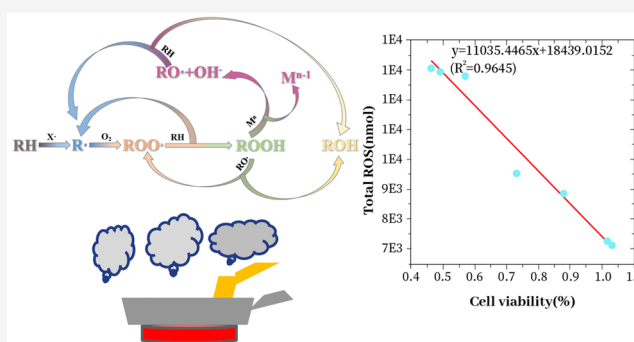


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Supporting Information

ABSTRACT: The International Agency of Research on Cancer identifies high-temperature frying, which features prominently in Chinese cooking, as producing group 2A carcinogens. This study simultaneously characterized particulate and gaseous-phase cooking emissions, monitored their reactive oxygen species (ROS) concentrations, and evaluated their impact on genetic damage and expression in exposed human bronchial epithelial cells. Five types of edible oil, three kinds of seasonings, and two dishes were assessed. Among tested edible oils, heating of soybean oil released the largest particle number concentration (2.09×10^{13} particles/(g cooking material and oil)·h) and volatile organic compounds (VOCs) emissions ($12103.42 \mu\text{g}/(\text{g cooking material and oil})\cdot\text{h}$). Heating of lard produced the greatest particle mass concentration ($0.75 \text{ mg}/(\text{g cooking material and oil})\cdot\text{h}$). The main finding was that sunflower and rapeseed oils produced the highest ROS concentrations (80.48 and $71.75 \text{ nmol}/(\text{g cooking material and oil})\cdot\text{h}$, respectively). ROS formation most likely occurred during the autoxidation of both polyunsaturated and monounsaturated fatty acids. Among all the tested parameters, only ROS concentrations exhibited consistency with cell viability and showed significant correlations with the expression levels of CYP1A1, HIF-1a, and especially with IL-8 (the marker for oxidative stress within the cell). These findings indicate that ROS concentration is potentially a suitable metric for direct assessment of exposure levels and potential toxicity.



1. INTRODUCTION

Lung cancer was responsible for 24.1% of all cancer deaths in China for both sexes and presents the most common cause of cancer-related deaths in China per 2018 Global Cancer Statistics.¹ It was also responsible for 1.6 million deaths globally in 2012.² About 15% of lung cancer cases in men and 53% in women are not associated with cigarette smoking.³ Epidemiologic evidence suggests that cooking emissions may increase the risk of lung cancer,^{4,5} especially in areas with a lower prevalence of smoking.⁶ In addition, only 4% of Chinese females are smokers, but the incidence of lung cancer is higher than in countries with higher smoking rates.⁷

We have reviewed 75 publications related to Chinese cooking emissions and found that most compared emissions from various ingredients and cooking techniques.⁸ Cooking produces harmful substances including particulate matter, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAH), all of them being associated with negative health effects and increased morbidity. Depending on different environmental factors, including temperature and humidity, these pollutants can be present in both the gas and particle phase. Toxicity of cooking particles typically increases

with the rise in the concentration and reduction in the particle size.^{10,11} Ultrafine particles (UFPs) emitted from cooking are reported to induce oxidative stress in lung cells¹² and enhance lung inflammation and allergic reactions.¹³ There is a consistent positive correlation between the particulate pollutants released from cooking and the risk of lung cancer. This explains why nonsmoking women have a high chance of developing lung cancer in China and the western and southeastern countries.^{14–16} Ko et al. reported that exposure to cooking oil fumes (COFs) is associated with respiratory diseases and increased lung cancer mortality among hotel and restaurant staff.¹⁷ The poor lung function of chefs was associated with the prolonged exposure to the mixture of indoor air contaminants, many of which were produced during

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cooking processes.^{16,18} The International Agency of Research on Cancer (IARC) still categorizes emissions from high-temperature frying as group 2A carcinogens (probably carcinogenic), indicating the limited evidence of carcinogenicity in humans.¹⁹ Although there is considerable epidemiological and toxicological evidence of the potential detrimental effects of cooking emissions on human health, there is still insufficient evidence to conclude that there is an exposure/response relationship. Therefore, there is a need to fully characterize the toxicity–exposure relationship of cooking emissions, including short-term prospective cohort effect research, long-term observational studies, and study of toxicological mechanisms *in vitro* and *in vivo* at the molecular protein level.

The current understanding states that exposure to air toxins leads to *in situ* formation of reactive oxygen species (ROS), which could cause oxidative stress,²⁰ induce inflammation, and possibly lead to other diseases, mainly heart disease, stroke, chronic obstructive pulmonary disease, lung cancer, and acute respiratory infections in children. ROS can be formed directly on particle surfaces or be carried by a gaseous phase.²¹ Hopke et al. considered particle-bound ROS as a critical exposure marker of ambient pollution.²² However, this group of species has a short half-life, low concentration, and high reactivity. Sampling with Teflon filters cannot fully capture these redox active semivolatile compounds, and they might not survive the period between filter collection and ROS measurement unaltered.

To avoid this disadvantage, a cell-free ROS monitoring system adopting a profluorescent nitroxide probe (BPEAnit) has been previously applied to detect ROS related to combustion-generated aerosols,²³ diesel exhaust,²⁴ biodiesel exhaust,²⁵ and secondary organic aerosol (SOA),²⁶ but not to cooking emissions. These studies concluded that the organic compounds of the particle pollutant were significantly related to ROS. We know that cooking emissions produce a large amount of organic matter. Therefore, it is meaningful to conduct the first detailed *in situ* study simultaneously characterizing particulate and gaseous-phase cooking emissions, monitoring their ROS, and evaluating their impact on genetic damage and expression, by assessing five types of edible oil, three kinds of seasonings, and two dishes.

2. MATERIALS AND METHODS

2.1. Experimental Setup and Design. Figure S1 shows the stainless-steel hood ($D = 0.8$ m, $H = 0.75$ m, bottomless) adopted in this study for characterizing cooking emissions. A cooker was placed centrally below the hood.

The hood was designed to avoid overflow of emissions. The fan at the top of the chamber generated uniformly mixed internal fumes as well as providing sufficient airflow to suck the produced emissions into the horizontal ventilation duct. The make-up air was set at the base of the hood, and flow was maintained during the measurements with an air velocity of 0.2 m/s. The collected fumes entered the ventilation duct first, and a portion of the fumes went into a Dekati ejector diluter with a constant dilution ratio of 11. The diluter was connected to a manifold that was designed to ensure thorough mixing before splitters directed samples into each instrument. The splitters were made of stainless steel and were as short as possible to reduce losses.

Prior to measurement, the chamber was opened, and the fan was switched on until the monitored particle number

concentration inside the chamber became stable, after which all instruments began to record data. After each cooking experiment, the chamber was opened completely to increase the air exchange rate. Acetone was used to clean the solid surface of the chamber and the surface of the instruments for each round of measurement. Particle number and mass concentration of the background were continuously monitored during the experiment. Also, VOC measurements of the background and background cell exposure samples were taken before each experiment. Each measurement was repeated for three times. Each measurement was conducted three times.

Three categories of cooking were investigated: heating oil, heating oil with added seasonings, and cooking two dishes (Figure S1). During the cooking process, the cooking pan was preheated to 260 °C before adding the oil. For the first category, five types of edible oils were investigated: soybean, peanut, rapeseed, and sunflower oils and lard. The entire heating period for 300 mL (279 g) of oil was 30 min. For the second category, seasonings of paprika, pepper and garlic were added to the two most commonly used edible oils, soybean and peanut oils. The oil was heated for 20 min, then 10 g of one of the seasonings was added for the final 10 min. For the third category, the first dish was stir-fried eggs with tomato, which was cooked as follows: 20 mL (18.6 g) of oil was heated, 15 g of seasonings and 10 mL of soy sauce were added, and 250 g of tomato and 150 g of eggs were added 1.5 min after adding the oil. The second dish was pork cooked with green chili, which was cooked as follows: 20 mL (18.6 g) of oil was heated; 15 g of seasoning, 10 mL of soy sauce, and 150 g of pork were added after 1 min; and 200 g of green chili was added after another 2 min. Both dishes were seasoned with 3 g of salt 8 min after the oil was heated.

2.2. Particle Number Concentration and Size-Fractionated Mass Concentration. Size-fractionated particle number concentrations were measured using the Dekati Electrical Low-Pressure Impactor (ELPI) (Dekati Ltd., Kangasala, Finland) with a time resolution of 1 s. The ELPI was flushed with zero air and calibrated in accordance with the manufacturer's instructions.²⁷ The sampling size range is 30 nm to 10 μm , which is divided into 12 size bins: 0.0290–0.0570 μm , 0.0570–0.0930 μm , 0.0930–0.1540 μm , 0.1540–0.2600 μm , 0.2600–0.3800 μm , 0.3800–0.6090 μm , 0.6090–0.9430 μm , 0.9430–1.5900 μm , 1.5900–2.3800 μm , 2.3800–3.9700 μm , 3.9700–6.6500 μm , and 6.6500–9.8600 μm . The airflow was 10 L/min. A condensation particle counter (CPC, TSI 3007) was used to determine the total particle number concentrations, ranging from 10 to 1000 nm, and the time resolution was 1 s. The airflow was 0.7 L/min.

2.3. VOC Species. A proton transfer reaction mass spectrometer (PTR-MS, Ionicon 500, Innsbruck, Austria) operating in H_3O^+ mode was adopted for online VOC species monitoring, and was operated with a drift voltage of 535 V. The chamber temperature was 60 °C, and the drift pressure was 2.2 mbar, which caused a reduced electric field (E/N) of about 120 td. The mass resolution, as well as the mass accuracy and the relative transmission efficiency, were routinely verified using a 76-compound gas standard including aldehydes, ketones, aromatics, and others from m/z 33 to m/z 181 (1 ppmV each compound). Data were analyzed using the PTR-MS Viewer 3 postprocessing software (distributed by Ionicon Analytik GmbH, Innsbruck, Austria). More details on the calibration and quality control can be found in the Supporting Information section 2.3S.

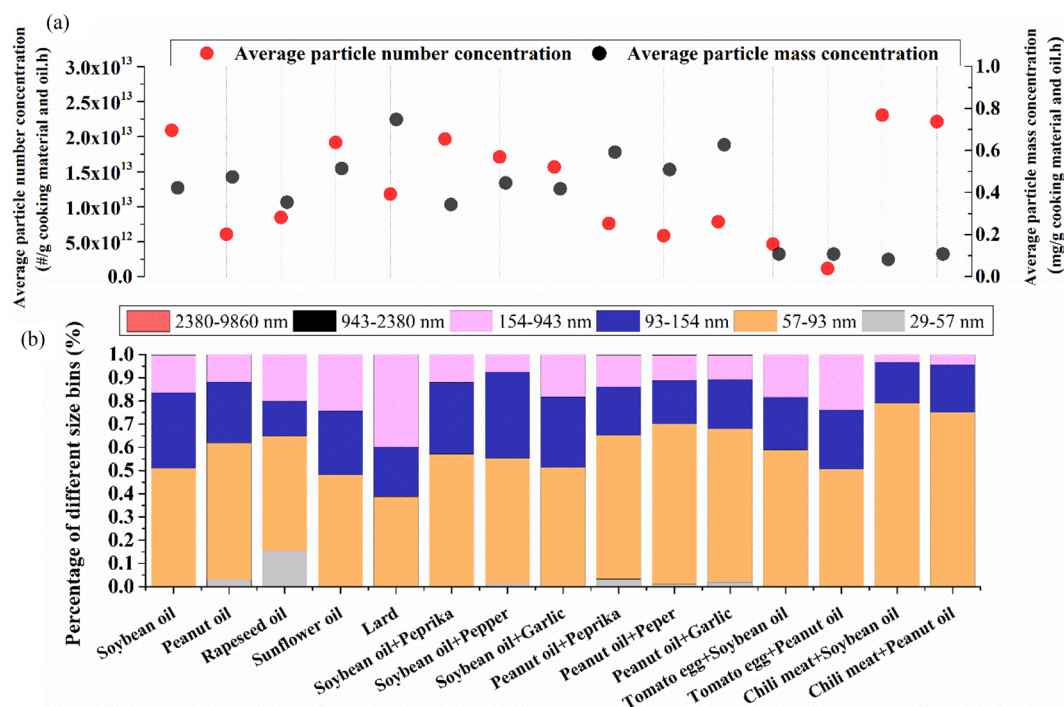


Figure 1. (a) Particle number and mass concentration for each cooking process; (b) particle size fraction for each cooking process

2.4. ROS Concentration Monitoring. A cell-free ROS monitoring system adopting a profluorescent nitroxide probe (BPEAnit) was applied in situ to assess the PM-bound ROS of various cooking emissions. ROS samples were collected by bubbling aerosol through an impinger containing 20 mL of 4 μ M BPEAnit solution [using analytical reagent (AR)- grade dimethyl sulfoxide (DMSO) as a solvent], followed by fluorescence measurements with a spectrofluorometer (Ocean Optics).²⁸ Because the efficiency of particle collection in the impingers is size-dependent, all of the reported data were corrected for the losses in impingers, according to the loss function provided by Miljevic et al.²⁹ The amount of BPEAnit reacting with the cooking emissions was calculated from a standard curve obtained by plotting known concentrations of the methanesulfonamide adduct of BPEAnit (fluorescent) against the fluorescence intensity at 485 nm. The collection efficiency of particles in the impingers is size-dependent, and the data was corrected for the losses. The airflow was 1 L/min. ROS results were reported as total ROS, which presents ROS content of both the gas and particle phase, meaning that total ROS in this case is ROS content found in tested cooking fumes.

2.5. Cell Culture and Online Exposure. Human bronchial epithelial cells (BEAS-2B) were adopted for real-time cell exposure tests. The medium was 90% RPMI-1640 + 10% fetal bovine serum. The culture conditions were 37 °C and 5% CO₂. Transwell inserts containing BEAS-2B cells were placed into the CULTEX Radial Flow System (CULTEX, Germany), which is capable of conducting simultaneous cell exposures in triplicate after the incoming pollutants have passed through the main channel inlet. The flow rate was 0.025 L/min, and the exposure period was 10 min. The buffer fluid in the basal well of the exposure chamber was maintained at 37 °C by an external water bath. Baseline readings of cell viability were measured from unexposed BEAS-2B. The airflow into the CULTEX system, including the bypass flow, was 1.5 L/min.

Details on the cell viability and CYP1A1, IL-8, and HIF-1 α gene expression protocol can be found in the [Supporting Information section 2.5S](#).

2.6. Data Processing and Analysis. Details on the data processing and analysis can be found in the [Supporting Information section 2.6S](#).

3. RESULTS AND DISCUSSION

3.1. PM and PNC. Figure 1a shows the average particle number concentrations and average particle mass concentrations of each cooking process. Among the five types of oil, heating of soybean oil released the greatest particle number concentrations (2.09×10^{13} particles/(g cooking material and oil)·h), and peanut oil the least (6.08×10^{12} particles/(g cooking material and oil)·h). In this study, the particle number emission factors from heating oils were in the range 2.8×10^{13} – 9.8×10^{13} particles/min; heating oil with seasonings 2.7×10^{13} – 9.2×10^{13} particles/min and cooking two dishes released 1.5×10^{13} – 1.15×10^{14} particles/min. In 2011, Buonanno et al. reported emission factors in the range $(3.6$ – $9.6) \times 10^{12}$ particles/min for frying and grilling chips, onions, eggplants, cheese, bacon and pork.³⁰ Furthermore, in 2009, Buonanno et al. reported that 2.3×10^{12} , 1.8×10^{12} , 1.1×10^{12} particles/min were emitted during heating of peanut, olive, and sunflower oils, respectively.³¹ Similarly, in the study of Torkmahalleh et al., heating olive, coconut, corn, soybean, canola, peanut, and safflower oils^{30–32} released 1.6×10^{12} , 1.4×10^{12} , 2.2×10^{12} , 3.3×10^{12} , 9.5×10^{11} , 3.5×10^{12} , and 8.1×10^{11} particles/min, respectively. Emission factors in this study are higher than the results found in the literature due to the experimental design: In this study, 100% of emissions were collected in the hood, while in the other studies, sample collection was conducted over the wok or at a distance to the wok.

Heating lard released the greatest particle mass concentration (0.75 mg/(g cooking material and oil)·h), and rapeseed

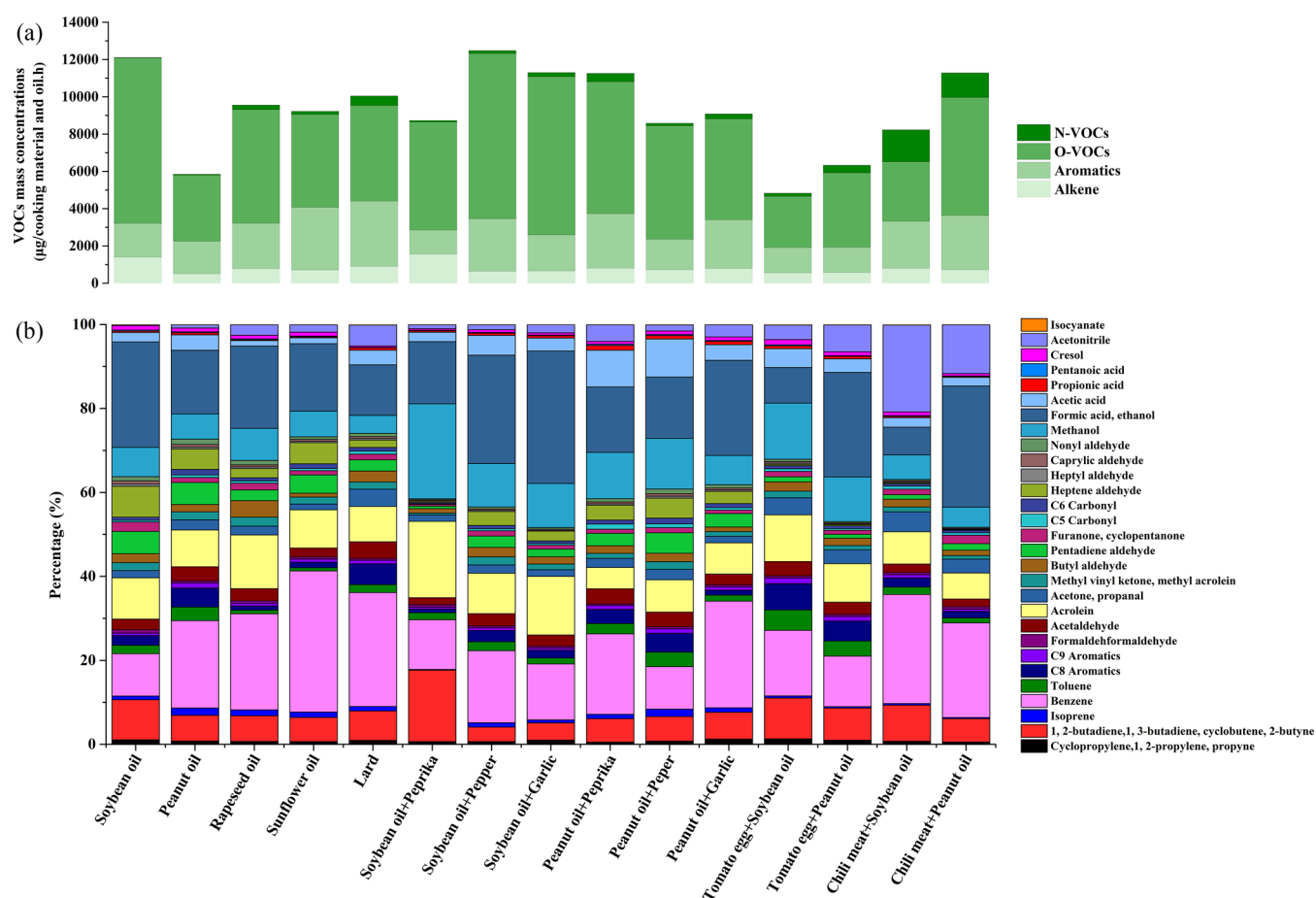


Figure 2. (a) VOCs concentration of different categories for each cooking process. (b) Fractional contribution of different VOCs species for each cooking process.

oil released the least ($0.35 \text{ mg}/(\text{g cooking material and oil}\cdot\text{h})$). This could be because the particles from heating lard are the largest, while those from rapeseed oil are the smallest. The particle mass emission factors released during heating oils were in the range $1.63\text{--}3.5 \text{ mg}/\text{min}$: Heating oil with seasonings released $1.64\text{--}3.14 \text{ mg}/\text{min}$, while cooking of the two selected dishes released $0.5\text{--}0.6 \text{ mg}/\text{min}$. Torkmahalleh et al.³² reported emission rates of 54, 27, 26, 5.7, 5.1, 3.7, and $2.8 \text{ mg}/\text{min}$ during heating of olive, coconut, corn, soybean, canola, peanut, and safflower oils. The particle mass emission factors of cooking several dishes in O'Leary's study ranged from 0.54 to $3.7 \text{ mg}/\text{min}$.³³ For heating oil with added seasonings, the particle number concentrations were slightly lower when pepper and garlic were added to both soybean and peanut oils, and the particle mass concentrations were lower only for peanut oil when pepper was added. Torkmahalleh et al. observed that adding pepper and sea salt reduced $\text{PM}_{2.5}$, but adding garlic powder and ginger powder did not.³⁴ For both dishes, the use of soybean oil resulted in greater particle number concentrations.

Figure 1b shows the size-fractionated particle mass concentration. Particles smaller than $1 \mu\text{m}$ accounted for nearly 100% of the total particle mass. Ultrafine particles within the size range of $29\text{--}93 \text{ nm}$ took up $38.8\text{--}64.9\%$ of the particle mass concentration. Particles within the size range of $57\text{--}93 \text{ nm}$ dominated, accounting for $38.8\text{--}58.7\%$. Heating rapeseed oil released 15.4% of particles within size bins of $29\text{--}57 \text{ nm}$, and heating lard generated 39.8% of particles within size bins of

$154\text{--}943 \text{ nm}$. For oil heated with added seasonings, the fraction of small size particles increased. Peanut oil produced a greater percentage of ultrafine particles in comparison with soybean oil. With regard to the two dishes, chili pork released a greater number of particles in the range of $57\text{--}93 \text{ nm}$. Other studies also concluded that frying bacon, pork, and beef produced many more ultrafine particles than frying eggs and vegetables.^{35,36}

3.2. VOC Species. The total VOC mass concentration for the 70 species of $m/z = 31\text{--}169$ produced from heating the oils was in the range of $5.85\text{--}12.1 \text{ mg}/(\text{g cooking material and oil}\cdot\text{h})$. The ranking was as follows: soybean oil > rapeseed oil > lard > sunflower oil > peanut oil. Among the 70 species, $m/z = 33, 47, 55, 57,$ and 79 dominated, the sum of which accounted for $43.87\text{--}59.27\%$ of the total VOC mass concentration (Figure S2). Additionally, $m/z = 113$ was also predominant for heating soybean and sunflower oils (Figure S3), and $m/z = 42$ and 107 were observed in significant amounts for heating lard. We identified 29 VOC species (Table S1), including 3 alkenes, 4 aromatics, 20 oxygenated VOCs (O-VOCs), and 2 nitrogenous organic compounds (N-VOCs). These 29 VOC species accounted for $71.6\text{--}85.5\%$ of the total mass concentration. In the following text, only these 29 VOC species are discussed.

Figure 2a shows the average VOC mass concentration produced per unit of cooking material and oil measured using PTR-MS. Among the five types of oil, heating soybean oil released the largest amount of VOC emissions, reaching up to

12103.42 $\mu\text{g}/(\text{g cooking material and oil})\cdot\text{h}$, while heating peanut oil recorded the smallest amount, which was 5845.77 $\mu\text{g}/(\text{g cooking material and oil})\cdot\text{h}$. The other three types of oil released comparatively equal amounts of total VOCs. O-VOCs dominated, followed by aromatics, alkenes and N-VOCs. Soybean oil produced the largest amount of O-VOCs: about 8865.44 $\mu\text{g}/(\text{g cooking material and oil})\cdot\text{h}$ (up to 73.25% of the total 29 VOCs). It was found that 50% of the O-VOCs were carbonyl compounds. Compared to heating of soybean oil, the total concentration of $m/z = 33, 47, 55, 57,$ and 79 reduced by 49.9, 25.0, 25.4, and 37.5 for peanut, rapeseed, and sunflower oils and lard, respectively

The percentages of specific VOC species are listed in Figure 2b. Benzene, (formic acid and ethanol), and acrolein were the major species, accounting for 44.8–58.8%, followed by (1,2-butadiene, 1,3-butadiene, cyclobutene, and 2-butylene), and methanol, accounting for 5.8–9.6% and 4.3–7.0%, respectively. Soybean oil produced the largest fraction of formic acid and ethanol, accounting for 25.1%, followed by benzene (10.1%) and acrolein (9.8%). Peanut oil produced the largest fraction of benzene (20.8%), followed by (formic acid and ethanol) (15.2%) and acrolein (8.8%). Heating rapeseed and sunflower oils and lard followed the same sequence as peanut oil, with rapeseed oil producing the largest fraction of benzene (up to 33.6%). Heptenal and pentadienal were observed during the processes of heating soybean, peanut, and sunflower oils, accounting for 4.9 and 7.4%, respectively. C8 aromatics accounted for 4.6 and 5.0% for heating peanut oil and lard, respectively. Acetonitrile accounted for 5.0% of the 29 VOC emissions from heating lard, which could be a factor causing its higher level of N-VOC emissions. Despite this, the average N-VOC concentration was only 515.02 $\mu\text{g}/(\text{g cooking material and oil})\cdot\text{h}$. Aldehyde emissions are affected by oil types. While heating oil, different types of aldehydes are produced; this process is influenced by the location of a double bond in the triglycerides.⁴⁰ Acrolein is related to the content of linolenic acid in edible oil. Methanol is probably the byproduct of cell wall synthesis, released by seasonings.⁴¹ Edible oils usually have the content of benzo(a)pyrene, which could release 1,2-butadiene, 1,3-butadiene, cyclobutene, and 2-butylene after being heated.⁴² Aromatics and O-VOCs are the dominant VOC species for cooking emission. Benzene is the most commonly detected aromatic compound, and formic acid and ethanol are common O-VOCs. They are also mainly affected by oil types.

For heating oils with added seasonings, the total VOC mass concentration for the 70 species of $m/z = 31-169$ was in the range of 8580.65–12 475.72 $\mu\text{g}/(\text{g cooking material and oil})\cdot\text{h}$. Soybean oil with seasonings generally released greater VOC mass concentrations in comparison to peanut oil. For each specific oil, the added seasonings produced greater VOC mass concentrations than heating the oil alone. The total amount of $m/z = 33, 47, 55, 57,$ and 79 increased 41.93, 13.89, and 37.73% for soybean oil with added paprika, pepper, and garlic, and 65.95, 9.86, and 46.85% for peanut oil with added paprika, pepper, and garlic, respectively. Soybean oil with added paprika also showed peaks at $m/z = 43$ and 74 , but $m/z = 61$ peaked in the case of other oils with added seasonings. For soybean oil, adding garlic produced the largest amount of VOCs, and adding paprika produced the least, owing to an $m/z = 47$ that was much lower compared with adding pepper and garlic. However, when peanut oil was used the opposite results were observed. Soybean oil with added paprika produced the

greatest amount of methanol, accounting for up to 22.6%, while soybean oil with added pepper and garlic released the largest amount of formic acid and ethanol, accounting for 25.8 and 31.5%, respectively. For peanut oil, adding paprika and pepper produced similar concentrations of formic acid and ethanol and methanol, but the level of formic acid and ethanol was significantly higher than for oil alone, while benzene accounted for 25.4%. Among the 29 VOC species, methanol, (formic acid and ethanol), benzene, and acrolein, and (1,2-butadiene, 1,3-butadiene, cyclobutene, and 2-butylene) dominated, accounting for 50.4–84.5%.

Monitoring of the time-dependent VOC species showed that levels of methanol and acetic acid increased sharply with the addition of paprika, pepper and garlic. Methanol increased by factors in the range of 1.64–12.39 (garlic < pepper < paprika) through the byproducts of cell wall synthesis emitted from plant seasonings. Acrolein was produced in similar concentrations with the oils only and with the added seasonings, indicating that acrolein is mainly produced by heating oils. For both soybean and peanut oils, the production of acetaldehyde, propanal, butyraldehyde, and valeraldehyde increased after the addition of paprika. Acetaldehyde levels increased after the addition of pepper, and propionic acid increased after the addition of garlic.

For the two dishes, VOC emissions produced from chili pork were much greater than those produced from the egg and tomato dish. The total concentration of $m/z = 33, 47, 55, 57,$ and 79 released from chili pork increased 64.5% with soybean oil and 91.2% with peanut oil when compared with the egg and tomato dish. The major O-VOCs species were methanol, acrolein, and (formic acid and ethanol). The major aromatics were benzene and C8 aromatics. Benzene, formaldehyde, acrolein, isoprene, and acetonitrile were released from chili pork at a much higher level compared with the egg and tomato dish.

3.3. ROS Concentration and a Potential Formation Pathway. Figure 3 shows that among the five edible oils, the greatest amounts of ROS were produced by sunflower oil

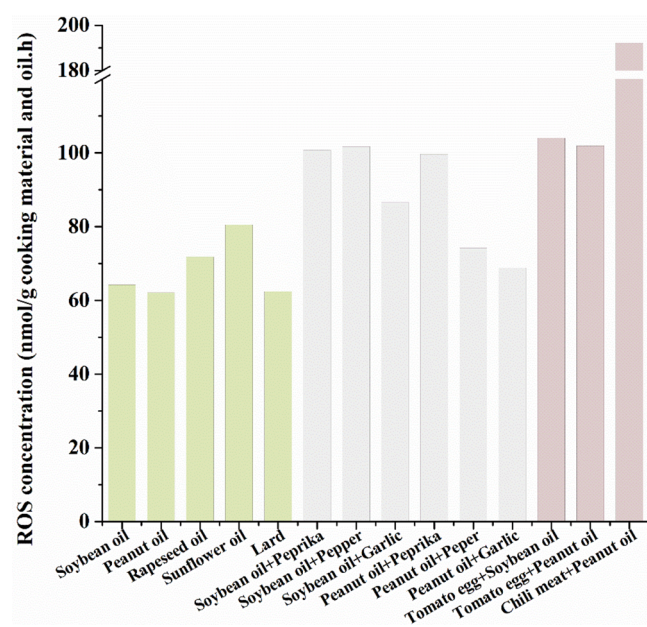


Figure 3. ROS concentration for each cooking process.

(80.48 nmol/(g cooking material and oil)·h) and rapeseed oil (71.75 nmol/(g cooking material and oil)·h).

It has been reported that burning fatty acid methyl esters (FAMES) with a high oxygen content is more likely to produce higher concentrations of ROS.^{24,37–39} However, the oxygen content within the five types of edible oils are approximately equal.^{40,41} Greater amounts of ROS were emitted from sunflower and rapeseed oils, possibly due to their higher content of unsaturated fatty acids,^{42,43} as shown in Table S2, 88.6% for sunflower oil and 94.48% for rapeseed oil, followed by soybean oil (79.42%), peanut oil (77.51%), and lard (51.50%). The double bond of the unsaturated fatty acid is easily broken and oxidized during the heating process.

Table S2 indicates that the monounsaturated fatty acid content of rapeseed oil was the greatest (up to 73.04%), and the polyunsaturated fatty acid content was up to 17.99%. In contrast, the polyunsaturated fatty acid content of sunflower oil was dominant (up to 60.39%), but monounsaturated fatty acid accounted for only 26.17%. Both polyunsaturated and monounsaturated fatty acids are highly prone to autoxidation due to the high degree of unsaturation in their molecular structure,⁴⁴ resulting in ROS formation via different reaction pathways (Figure 5).

The autoxidation of polyunsaturated and monounsaturated fatty acids includes three steps: initiation, propagation, and termination. As shown in Figure 4, the process is initiated after

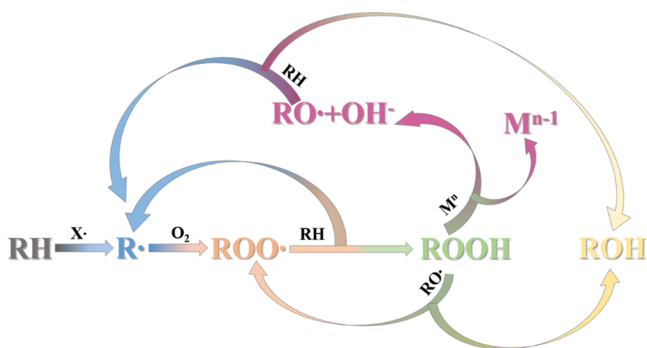


Figure 4. ROS reaction pathway for both polyunsaturated fatty acid and monounsaturated fatty during autoxidation.

hydrogen abstraction of a fatty acid RH, resulting in the generation of the pentadienyl radical R. Then, R• combines with O₂ to produce a peroxy radical ROO•. ROO• can react with RH to form R• and a hydroperoxide ROOH. In the presence of transition metal ions Mⁿ, ROOH transforms into alkoxy radicals RO• and OH•. RO• further reacts with RH to form R• and ROH. Additionally, ROOH could combine with RO• to generate ROO• and ROH. In the whole autoxidation process of unsaturated fatty acids, ROS species including R, RO, ROO, and OH• could be generated. Oxidation of polyunsaturated fatty acids is linearly dependent on the amount of bis-allylic methylene present in the fatty acid, so the oxidation is controlled by the hydrogen abstraction from the relatively weak C–H bond of the bis-allylic methylene with a bond dissociation energy (BDE) of about 75 kcal/mol. For monounsaturated fatty acids, the oxidizability is much less as the monoallylic methylene hydrogens are more resistant to abstraction, as the BDE is 88 kcal/mol. We found that heating sunflower oil released the greatest ROS concentrations, which is consistent with the above explanation.

Termination of autoxidation results in the cleavage of ROOH and enhancement in small molecules of carbonyl compounds. Results of this study show that the aldehyde concentrations produced were 284.2, 197.3, 266.4, 299.8, and 153.2 μg/(g cooking material and oil)·h for soybean, peanut, rapeseed, and sunflower oils and lard, respectively. The corresponding values of ketone concentrations were 117.68, 67.06, 169.67, 84.53, and 97.68 μg/(g cooking material and oil)·h, and those for carbonyl compounds were 401.9, 264.4, 436.1, 384.3, and 250.9 μg/(g cooking material and oil)·h. These findings further confirm the previous assumption that the presence of unsaturated fatty acids could produce more ROS and carbonyl compounds than in the case of their saturated counterparts. Therefore, although unsaturated oils are important for human health as the structural components of their cell membrane serve as precursors to bioactive lipid mediators and provide a source of energy,⁴⁵ their potential impact on cell inflammation through higher ROS emissions should be taken into account.

When seasonings were added to soybean oil, ROS concentrations increased by 56.9% (paprika), 58.4% (pepper), and 34.8% (garlic). For peanut oil, ROS concentrations increased by 60.5% (paprika), 19.5% (pepper), and 10.9% (garlic). These increases in ROS concentrations compared to heating oil alone could be attributed to an increase in VOC emissions, which strengthens interactions between particles and accelerates ROS formation. The ROS concentration when pepper was added to soybean oil reached 101.71 nmol/(g cooking material and oil)·h. The addition of garlic to edible oils resulted in the smallest increases in the ROS concentrations. Of the two dishes, chili pork released much higher levels of ROS (about 192.12 nmol/(g cooking material and oil)·h) than did the egg and tomato dish, which was consistent with particulate and gaseous emissions.

3.4. Cell Viability and Their Genetic Expression. Figure 5a shows the viability of the human bronchial epithelial cell BEAS-2B after exposure to cooking emissions and the total ROS produced during each cooking process. There was no significant difference for the unlabeled test group. BEAS-2B cells exposed to cooking emissions exhibited significantly reduced cell viability in comparison with the blank group. Cell viability was significantly reduced by exposure to emissions from soybean oil with added seasonings and peanut oil with added paprika or pepper ($P < 0.01$). Peanut oil with added garlic also reduced the cell viability ($P < 0.05$). Generally, exposure to emissions from any of the edible oils heated with added seasonings resulted in a reduction in cell viability of about 10–60%. Emissions from the egg and tomato dish caused no significant reduction in cell viability, possibly due to cells being exposed for 30 min during heating of the oil and 10 min during cooking of the dish. Therefore, the impact of long-term exposure cannot be ignored.

The correlations between total particle number, total particle mass, ROS, total VOCs, and cell viability were further investigated, as shown in Figure 5c. Only total ROS concentrations showed a consistent relationship with cell viability: The greater the total ROS concentrations, the lower the cell viability. The chemical composition and species of particles and VOCs vary widely, so it is difficult to estimate their respective impact on toxicity due to their complexity. However, ROS is a direct and meaningful factor for assessing cell viability.

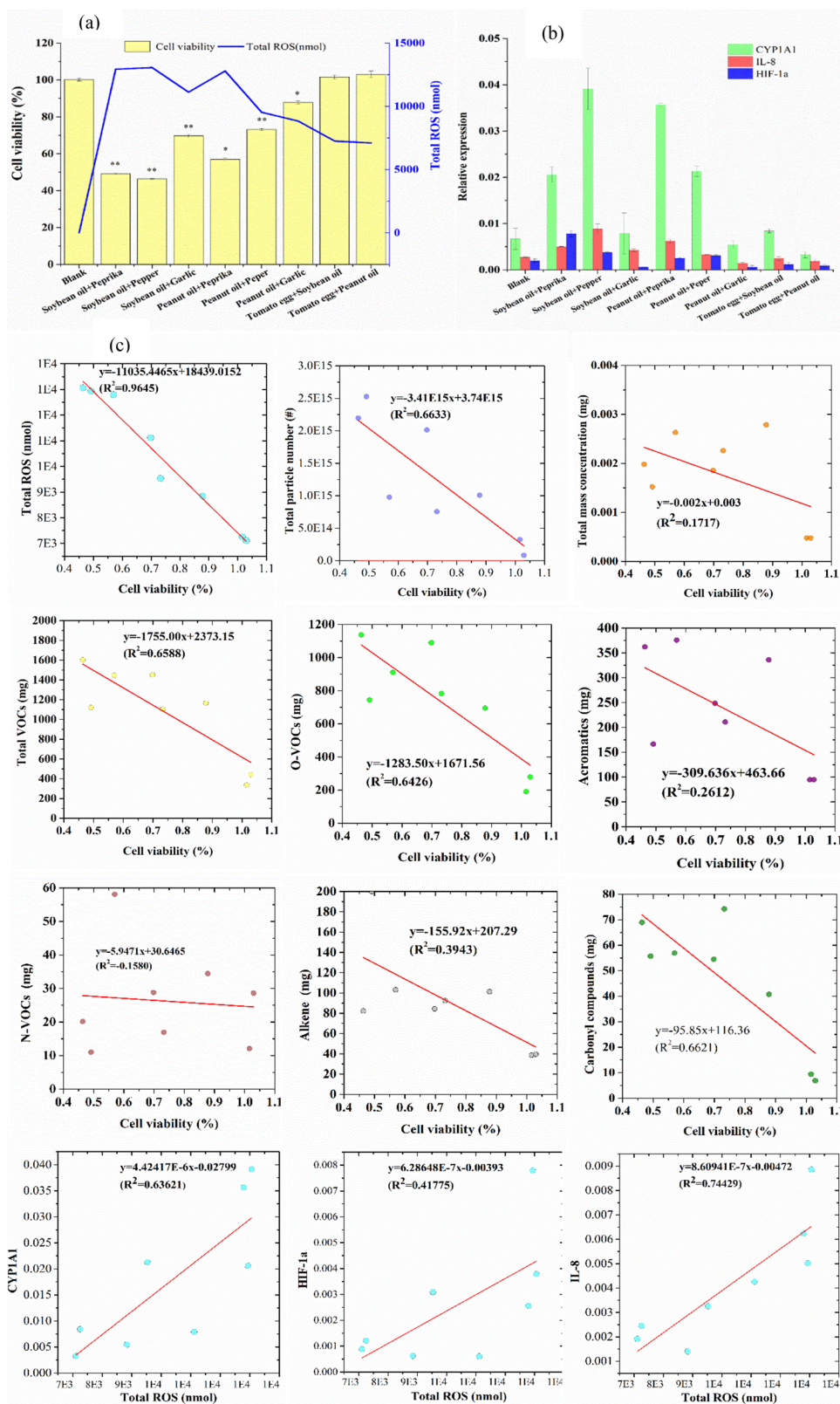


Figure 5. (a) Cell viability and total ROS for each cooking process. (b) Relative expression of CYP1A1, IL-8, and HIF-1a for the exposed BEAS-2B cells during each cooking process. (c) Correlations between cell viability and different tested parameters.

The relative expression levels of CYP1A1, IL-8, and HIF-1a of the exposed BEAS-2B cells were examined, as shown in Figure 5b. All the oils with added seasonings, except for peanut oil with garlic, showed significant increases in the relative

expression of IL-8 ($P < 0.05$). Additionally, both soybean oil with added paprika or pepper and peanut oil with added paprika or pepper showed significant increases in the relative expression of CYP1A1 and HIF-1a. The question imposed is

what changes in the cooking fumes, due to the addition of seasoning, that had this effect on human cells. An explanation could be found in Figure 5c where the correlation between total ROS and the expression levels of CYP1A1, IL-8, and HIF-1 α is shown. A reasonable correlation can be seen between all three pointing out to the importance of total ROS exposure. What is most notable is that the highest correlation was observed between total ROS exposure and IL-8. As IL-8 is a marker of oxidative stress within the cell, this clearly indicates the importance of ROS in inducing inflammation within the cells that ultimately leads to a reduction in cell viability. This leads to a conclusion that exposure to ROS via cooking emissions or in general from any other combustion source such as diesel could induce mRNA expression of IL-8, CYP1A1, and HIF-1 α as these three factors are related to inflammation and cancer. Hence, total ROS exposures should be used as a marker of cell toxicity and an important measure of the impact of these emissions on human health. There is no reason why these observations cannot be extended beyond cooking emissions to other combustion (or pollutant) sources such as vehicle emissions.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.9b07553>.

Set-up of the measurement; time design for different cooking processes; fractional contribution of different VOCs classes for each cooking process; concentrations of various VOCs species for different cooking processes; Time-variations of particle number concentrations for different sizes during the heating oil process, during the heating oil with seasonings process, and during dish cooking process; calibration and quality control of VOC sampling; cell viability and CYP1A1, IL-8, and HIF-1 α gene expression testing protocol; data processing and analysis; assignment of various m/z compounds to corresponding VOCs species; composition of different types of oil (PDF)

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Notes

The authors declare no competing financial interest.

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