Article

Comparative Reactive Oxygen Species (ROS) Content among Disposable Flavored Vape Bars

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Abstract:

Studies have shown that aerosols generated from flavored e-cigarettes contain Reactive Oxygen Species (ROS), promoting oxidative stress-induced damage within pulmonary cells. Our lab investigated the ROS content of e-cigarette vapor generated from disposable vape bars, a product exempt from the Federal Drug Enforcement Agency's (FDA) 2020 flavor ban. Specifically, we analyzed vape bars belonging to multiple flavor categories (Tobacco, Minty Fruit, Fruity, Minty/Menthol, Desserts, and Drinks), manufactured by various vendors and of various nicotine concentrations (0-6.8%). Aerosols from these flavored vape bars were generated by a single puff aerosol generator and individually bubbled through a fluorogenic solution to detect and semi-quantify ROS in H_2O_2 equivalents generated by the vape bars. We compared and contrasted the ROS levels generated by each flavor as an indirect determinant of oxidative stress potential by these disposable vape bars. Our results showed that ROS concentration (μM) of aerosols produced from the vape bars varied significantly between different flavors and a function of nicotine concentration. Likewise, our results suggest that flavoring chemicals and nicotine concentration play a role in alerting ROS production in e-cigarette aerosols. Our study provides insight into the differential health effects of flavored disposable vape bars and the need for their regulation.

Keywords: Vaping, disposable e-cigarettes, vape bars, flavoring, flavoring chemicals, Reactive Oxidative Species (ROS), disposables, oxidative stress

1. Introduction

Despite the significant decline in youth e-cigarette usage since the Federal Drug Enforcement Agency's (FDA) flavored e-cigarette ban which was enacted in February 2020, youth e-cigarette use within the United States remains significantly high [1]. Moreover, according to a cross-sectional study conducted by the Office on Smoking and Health at the Centers for Disease Control and Prevention (CDC), in 2020, 19.6% of high school students (3.02 million) and 4.7% of middle school students (550,000) reported current e-cigarette use [1]. The current prevalence of e-cigarette usage in this country, especially amongst its youth, is partly due to the switch many cartridge-based e-cigarette users made to using disposable e-cigarettes; the FDA's 2020 ban prompted this. Specifically, the FDA's flavoring ban only applies to nicotine-containing minty and fruity flavoring for cartridge or pre-filled pod devices [1]. However, products exempt from the previously mentioned ban include disposable e-cigarettes. A disposable electronic cigarette is a type of electronic nicotine delivery system (ENDS) which can be thrown away once it runs out of e-liquid or charge. According to the 2020 NYTS, the use of disposable e-cigarettes increased significantly from 2.4% in 2019 to 26.5% in 2020 among high-school students who were current e-cigarette users and from 3.3% in 2019 to 15.2% in 2020 among middle-school students who were current e-cigarette users [1]. One aspect of disposable devices which is attractive to youth e-cigarette users is the convenience at which they can be used; specifically, they do not require recharging or refilling with e-liquids like cartridge-based products. Additionally, disposable devices are much cheaper and practical to use than their cartridge-containing counterparts with e-liquids.

While e-cigarettes do appear to contain fewer toxic compounds than conventional cigarettes, with the increase in the variety of e-liquid flavors available during this past decade, it has been challenging to investigate e-cigarette induced pathophysiology more thoroughly[2]. Likewise, the

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long-term effects of e-cigarette vapor exposure on human health requires further investigation. However, studies so far have shown that e-cigarette aerosol production involves producing reactive oxygen species (ROS) [3]. ROS can be generated either intracellularly (via mitochondrial oxidative phosphorylation) or may arise from exogenous sources (cigarette smoke, e-cigarette aerosols, environmental pollution, certain foods, and drugs) [4]. Specific Reactive Oxygen Species include hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet-}$), and hydroxyl radical ($^{\bullet}OH$)[5]. ROS plays a crucial role in modulating the immune system and activating different signal transduction pathways and cell signaling processes [6]. For optimal cell signaling processes to occur, the balance between ROS and antioxidant substances within a cell is kept slightly in favor of ROS production [6].

However, the normal physiological balance between ROS and antioxidants can be disturbed through the inhalation of exogenous sources of ROS, thus leading to the damage of cellular structures. Specifically excess intracellular ROS levels cause oxidative damage to cellular membrane lipids, enzymes, and DNA. Moreover, excess ROS can also induce a vicious cycle of chronic inflammation in the lungs due to excessive ROS leading to the activation of specific immune cells, polymorphonuclear neutrophils (PMNs); activated PMNS can, in turn, generate more ROS in pulmonary cells [7]. This subsequent chronic inflammation leads to airways becoming more thickened and prone to mucus secretion, also known as airway modeling, this later resulting in lung dysfunction [8]. Regarding exogenous ROS sources, studies in the past have shown that tobacco-smoke-generated ROS can induce DNA damages within lung epithelial cells and premature pulmonary cell death, leading to the development of lung cancer and emphysema, respectively [9]. Additionally, one study had shown that through activating the heating element of an e-cigarette and then aerosolizing its e-liquid component, ROS is produced; additionally, those researchers found that ROS are drawn from the device into the lungs, directly[10].

Despite the well-known health effects of conventional cigarette smoking, one of the main factors driving both youth and adult appeal for e-cigarettes is the availability of many different flavors. To further explain, these flavors add to the allure many have for e-cigarettes by creating sensory perceptions of palatable tastes (sweet or cool) which conceal the bitter taste of nicotine[11]. Specifically, one survey found that the availability of fruit and candy e-liquid flavors significantly contributes to the prevalence of youth e-cigarette usage in the United States; adults seeming to prefer more traditional flavors, like tobacco [11] . Likewise, according to a Morbidity and Mortality Weekly Report by the CDC in September 2020, among current users of flavored disposable e-cigarettes, the most commonly used flavor types were fruit (82.7%; 650,000), mint (51.9%; 410,000); candy, desserts, or other sweets (41.7%; 330,000); and menthol (23.3%; 180,000) [1]. Accordingly, with the recent surge in flavored disposable e-cig use during this past year, more research should be conducted which investigates how ROS content within aerosols generated from disposable e-cigarettes depends on its flavor.

In addition to flavor, another factor contributing to the prevalence of disposable e-cigarette usage in this country is the range of nicotine concentrations which are available for these devices. Nicotine is a highly addictive alkaloid present within the aerosol generated by e-cigarettes as well as within the smoke generated from conventional cigarettes [12]. For disposable e-cigarettes sold within the United States, nicotine content ranges from 0mg/mL (0%, nicotine-free option) to 68 mg/mL (6.8%), depending on the vendor. Nicotine is highly addictive and can harm the neural development of those under the age of 25, which is most troubling given the prevalence of e-cigarette use among adolescents in this country [13]. Furthermore, exposure to nicotine through inhaling e-cigarette generated aerosols has contributed to prolonging e-cigarette usage among e-cigarette users, especially among those under the age of 25 [14]. Despite this, studies investigating how exogenous ROS generation varies as a function of nicotine concentration in ENDS products are lacking. Additionally, with the recent surge in flavored disposable e-cig use and the wide range of nicotine content available for these products, research should be conducted to determine how ROS generation among disposable e-cigarettes varies as a function of salt nicotine concentration. Consequently, in our study, we hypothesize that ROS levels within the aerosols generated from disposable e-cigarettes will vary as a function of flavoring chemicals as well as due to nicotine concentration. Furthermore, disposable e-cigarettes with a wide range of salt nicotine concentrations (0-6.8%) and within six main flavor categories (Tobacco, Minty Fruit, Fruity, Minty/Menthol, Desserts, and Drinks) from different commercial vendors were used.

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2. Materials and Methods

- 2.1 Disposable E-cigarettes (Vape Bars)

Disposable e-cigarettes (vape bars) were purchased from various locations and manufacturers within Rochester, NY. The disposable e-cigarettes used in this experiment contained a wide range of salt nicotine concentrations (0-6.8%) and were categorized into six main flavor categories (Tobacco, Minty Fruit, Fruity, Minty/ Menthol, Desserts, and Drinks). The commercial manufacturers of the disposable vape bars used were Puff Bar, Hyde, Tsunami Twin, NJOY, Blu, Fling, Hyppe Bar, SMOQ, Bolt, Zaero, Lit, Phantom, Eonsmoke, FreshBar, Fliq, Vice, SOL, and Jolly.

- 2.2 Generation of Vape Bar Aerosols

A fluorogenic dye was made using 0.01N NaOH, 2'7' dichlorofluorescein diacetate (H2DCF-DA) (EMD Biosciences, CA) (Cat # 287810), phosphate (PO_4) buffer, and horseradish peroxidase (Thermo Fisher, Ma) (Cat # 31491). The PO4 buffer was made using dibasic sodium phosphate (Sigma- Aldrich, MO) (Cat #2–0751) and sodium phosphate monobasic (JT Baker, NJ) (Cat #3828–01). Afterward, i.e. upon bubbling, the resulting fluorogenic dye was analyzed via fluorescence spectroscopy with a maximum excitation and emission spectra of 475nm and 535nm, respectively. The standards used in this experiment ranged from 0 to 50 µM, each made from 1.25 mM hydrogen peroxide stock solution. Afterward, the fluorogenic dye was added to each prepared standard. Next the fluorogenic dye and the prepared standards were allowed to react with one another for 15 minutes at 37°C. Standards were measured on a spectrofluorometer (Turner Quantech fluorometer, Mo. FM109535) in fluorescence intensity units (FIU).

Afterward, using a standard lab vacuum and a Buxco Individual Cigarette Puff Generator (Data Sciences International, CAT#: 601-2055-001), the aerosols generated from each disposable e-cigarette (vape bar) were individually bubbled through 10mL of 2′, 7′-Dichlorofluorescin Diacetate (DCFH) solution within a 50mL conical tube, at 1.5 L/min (Figure 1). More specifically, once a vape bar was inserted into the Buxco Puff Generator, aerosol was generated and bubbled into the fluorogenic dye under a specific puff profile regiment. Under this specific puff regiment, a total of 20 puffs was generated through the Puff Generator apparatus; the puff frequency was two puffs/min, and each puff had a volume of 55ml and lasted 3.0 seconds. Additionally, a flow measuring instrument (TSI Series 4100) attached to an inlet filter (MilliporeSigma™ SLFG05010) was used for determining the flow rate of the lab vacuum. Subsequently, after bubbling, each resulting fluorogenic dye sample was given 15 minutes to react within a 37°C degree water bath (VWR 1228 Digital Water Bath); the resulting solution was then immediately analyzed via fluorescence spectroscopy. For our negative control, air was bubbled through the fluorogenic dye; this was done through using the Buxco Puff Generator but without inserting a disposable vape bar into the machine. For our positive control, cigarette smoke generated through burning conventional research cigarettes (University of Kentucky 3R4F) was bubbled through the fluorogenic dye. All samples and controls were run in duplicates. Readings were based on the hydrogen peroxide standard curve and measured as hydrogen peroxide, H_2O_2 equivalents.

- 2.4 Statistical analysis

Statistical analysis of significance was calculated using one-way ANOVA as well as Tukey's post-hoc test for multiple comparisons by GraphPad Prism Software version 8.1.1. The results are shown as mean ± SEM with duplicates and triplicates analyses. Data were considered to be statistically significant for P values <0.05

The average ROS concentration and standard deviation for all vape bars within each flavor category were calculated using Microsoft Excel.

3. Results

3.1 Subsections

- **Total ROS concentration within aerosols generated from vape bars vary by flavor**

  Our data shows that aerosols generated from disposable flavored vape bars produced differential H_2O_2 equivalents. More specifically, the aerosols generated from different flavored vape bars contained significantly different total ROS concentrations (µM) (Figures 2-6). The disposable vape bars with the highest ROS content within each of the six previously mentioned flavor categories (Tobacco, Minty Fruit, Fruity, Minty/ Menthol, Desserts, and Drinks) were Hyde American Tobacco (5% Nicotine), Hyppe Bar: Cool Melon (5% Nicotine), Puff Bar: Blue Razz (5% Nicotine), NJOY: Cool Menthol (6% Nicotine), Strawberries and Cream (5% Nicotine), and SMOQ: Pink Lemonade (5% Nicotine), respectively (Figures 2-6) . Specifically, the aerosol produced by the 5% Nicotine Hyde American Tobacco flavored bar contained 10.43-10.72 µM H_2O_2 (Figure 2), the aerosol produced by the 5% Nicotine Hyppe Bar Cool Melon bar was 9.44-9.76 µM H_2O_2 (Figure 3) , and the aerosol generated from the 5% Puff Bar Blue Razz contained a ROS content of 8.15-9.11µM H_2O_2 (Figure 5). Moreover, the ROS content within the aerosols generated by SOL: Spearmint (5% Nicotine), Strawberries and Cream (5% Nicotine), and SMOQ: Pink Lemonade (5% Nicotine) was 8.78-9.25 µM, 8.11-8.39 µM, and 15.32-15.63 µM, respectively (Figures 4-7). Furthermore, among fruity-flavored vape bars, the ROS levels within the aerosols generated from the 0% Nicotine and 5% Nicotine-containing Blue Razz flavored bars were the highest among every 0% Nicotine Fruit-flavored Bars (5.68-5.82 µM) and every 5% Nicotine Fruit-flavored Bars (8.15-9.11 µM), respectively (Figure 5). Additionally, the highest ROS content among all vape bars analyzed in this experiment was found within the aerosol generated by the 5% Nicotine SMOQ: Pink Lemonade vape bar (15.32-15.63 µM) under the "Drinks" flavor category (Figure 6).

- **Total ROS concentration in aerosols generated by vape bars vary as a function of nicotine concentration**

  Comparatively, we observed significant variations in ROS levels as a function of nicotine content among disposable vape bars of the same specific flavor; this was observed for five specific flavors (Blue Razz, Mango Ice, Peach Ice, Lychee Ice, and Menthol) (Figures 7-8). When analyzing ROS content produced from aerosols generated by Blue Razz flavored vape bars, we found that the aerosol generated by the Nicotine containing bar (5% Nicotine) contained significantly higher ROS than the respective non-nicotine-containing bar (0% Nicotine) (Figure 7). Likewise, we found that the aerosol generated by the Nicotine containing (5% Nicotine) Peach Ice bar contained a significantly higher ROS content than that produced from a non-nicotine-containing Peach Ice bar (0% Nicotine) (Figure 8). Moreover, our results found that menthol bars with a 5-6% nicotine content contained significantly higher ROS levels than menthol flavors without nicotine (0% Nicotine). In contrast, for both the Mango Ice and Lychee Ice flavors, we found that the aerosol generated from the non-nicotine-containing bar generated a significantly higher level of ROS than its respective Nicotine containing counterpart (Figure 8).

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3.2. Figures, Tables and Schemes

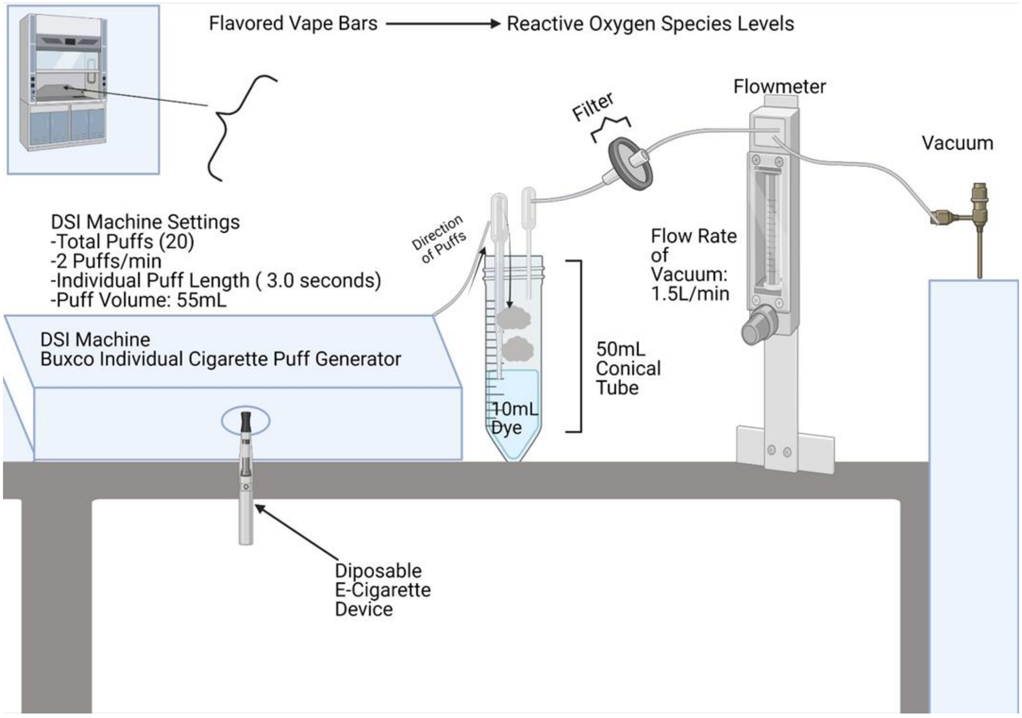

Figure 1. Disposable E-cigarette exposure generation system

This schematic shows the apparatus used in order to bubble the 10mL fluorogenic dye within each 50ml conical tube using the aerosol emitted from the e-cigarette inserted into the DSI Puff Generator machine. Using a standard lab vacuum, the fluorogenic dye was bubbled at 1.5 L/min and "puffs" were generated from each vape bar using the DSI Machine above. The DSI machine provided a total of 20 puffs, each puff lasting three seconds and having a volume of 55.0mL. Each conical tube was wrapped in aluminum foil to protect the fluorogenic dye from light. The entirety of the "bubbling" process using the DSI machine and vacuum apparatus was done inside a chemical fume hood.

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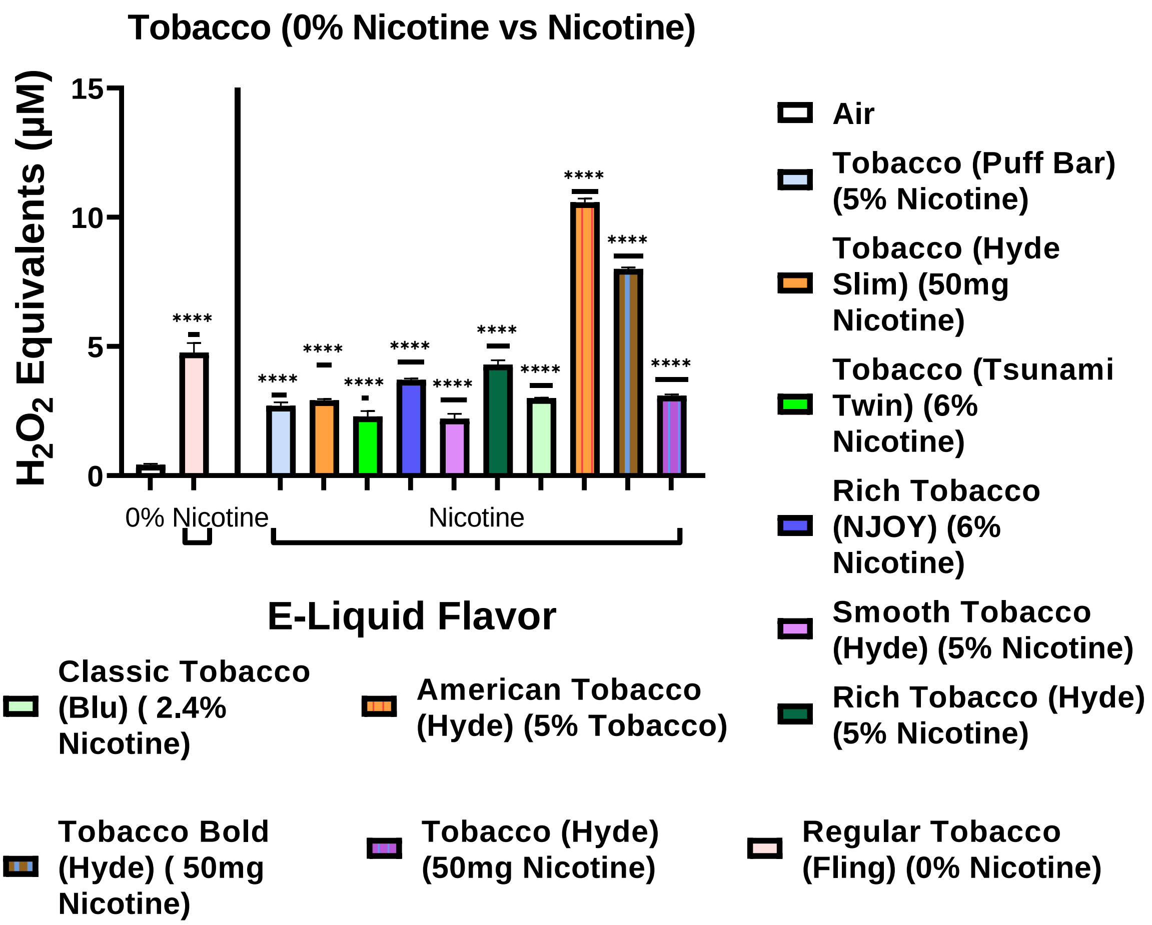

Figure 2. Generation of ROS by different tobacco-based flavors from various vendors.

Acellular ROS was measured from aerosols generated from various different tobacco flavored disposable e-cigarette devices using a hydrogen peroxide standard. Each tobacco-based vape bar's flavor, brand, and nicotine concentration are listed and color coded. All flavors were compared to the control value of air. Data are represented as mean ± SEM, and significance was determined by one- way ANOVA. * $p< 0.05$, ** $p< 0.01$, and *** $p< 0.001$ versus air controls.

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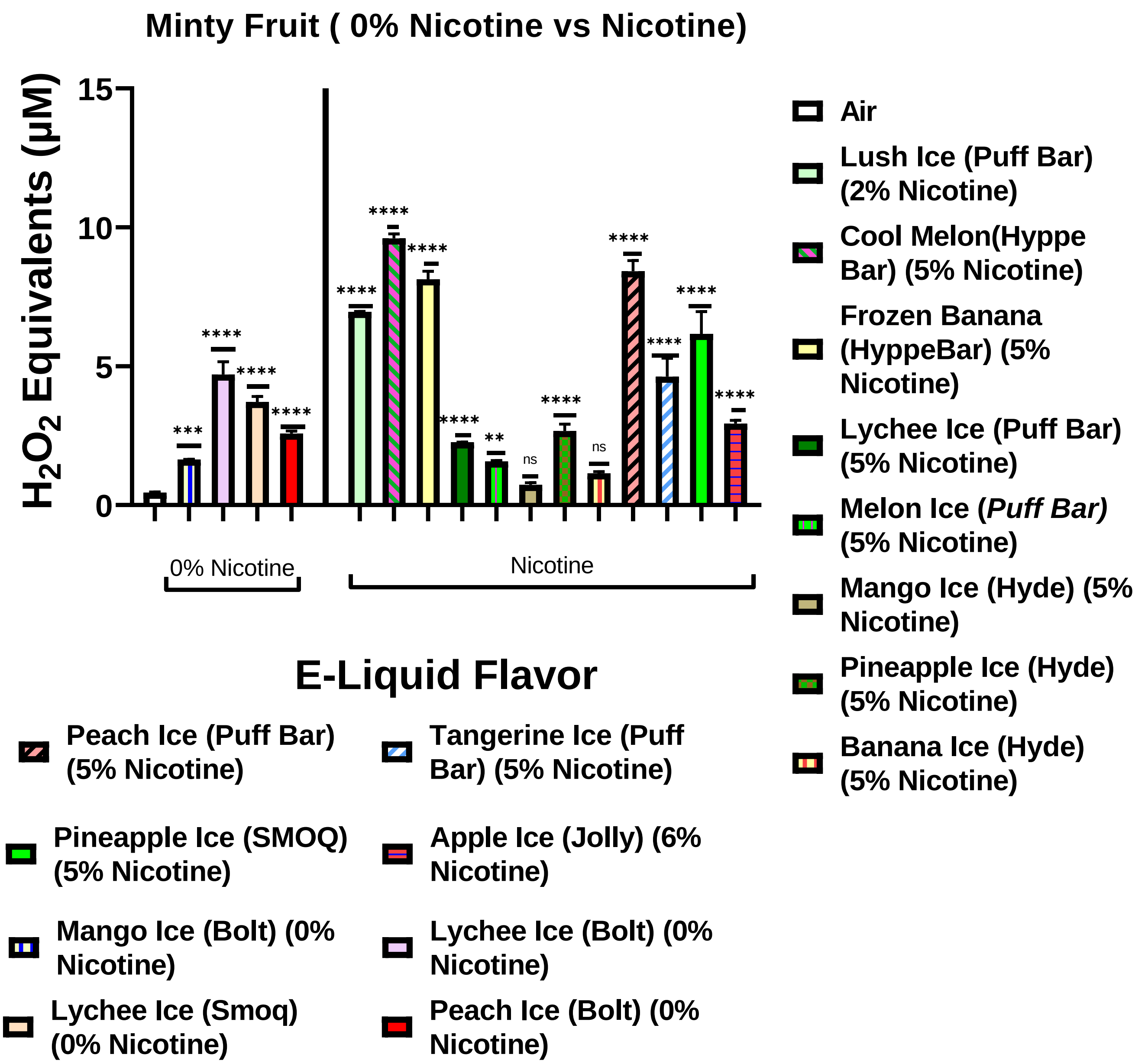

Fig 3. Generation of ROS by different minty fruit flavors from various vendors.

Acellular ROS was measured from aerosols generated from various different minty fruit flavored disposable e-cigarette devices using a hydrogen peroxide standard. The name of each minty fruit-flavored vape bar's specific flavor, brand, and nicotine concentration are listed and color coded. All flavors were compared to the control value of air. Data are represented as mean ± SEM, and significance was determined by one- way ANOVA. * p< 0.05, ** p< 0.01, and *** p< 0.001 versus air controls.

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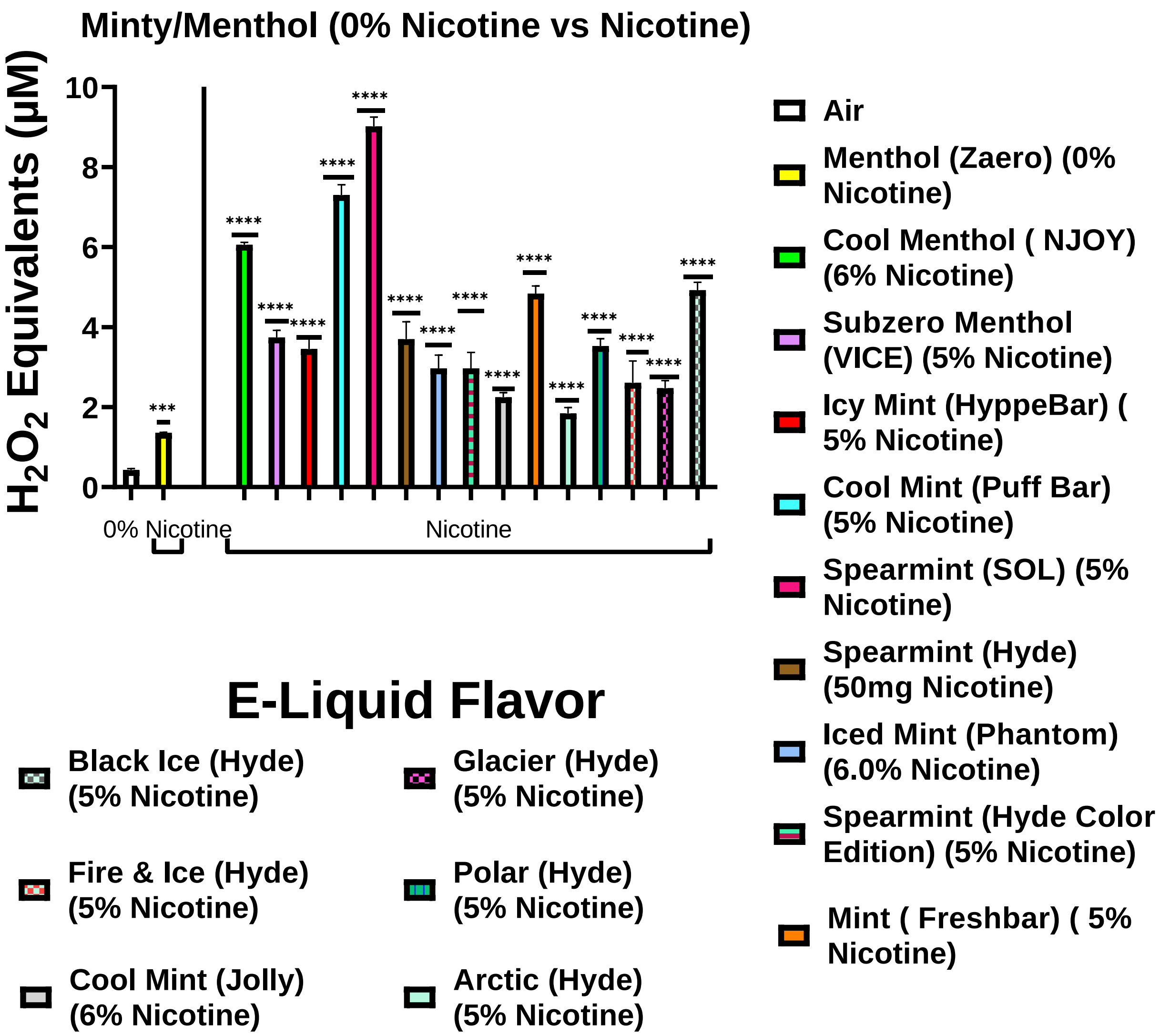

Fig 4. Generation of ROS by different Minty/ Menthol flavors from various vendors. Acellular ROS was measured from aerosols generated from various different minty/menthol flavored disposable e-cigarette devices using a hydrogen peroxide standard. The names of each minty/menthol flavored vape bar's specific flavor, brand, and nicotine concentration are listed below and color coded. All flavors were compared to the control value of air. Data are represented as mean ± SEM, and significance was determined by one- way ANOVA. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ versus air controls.

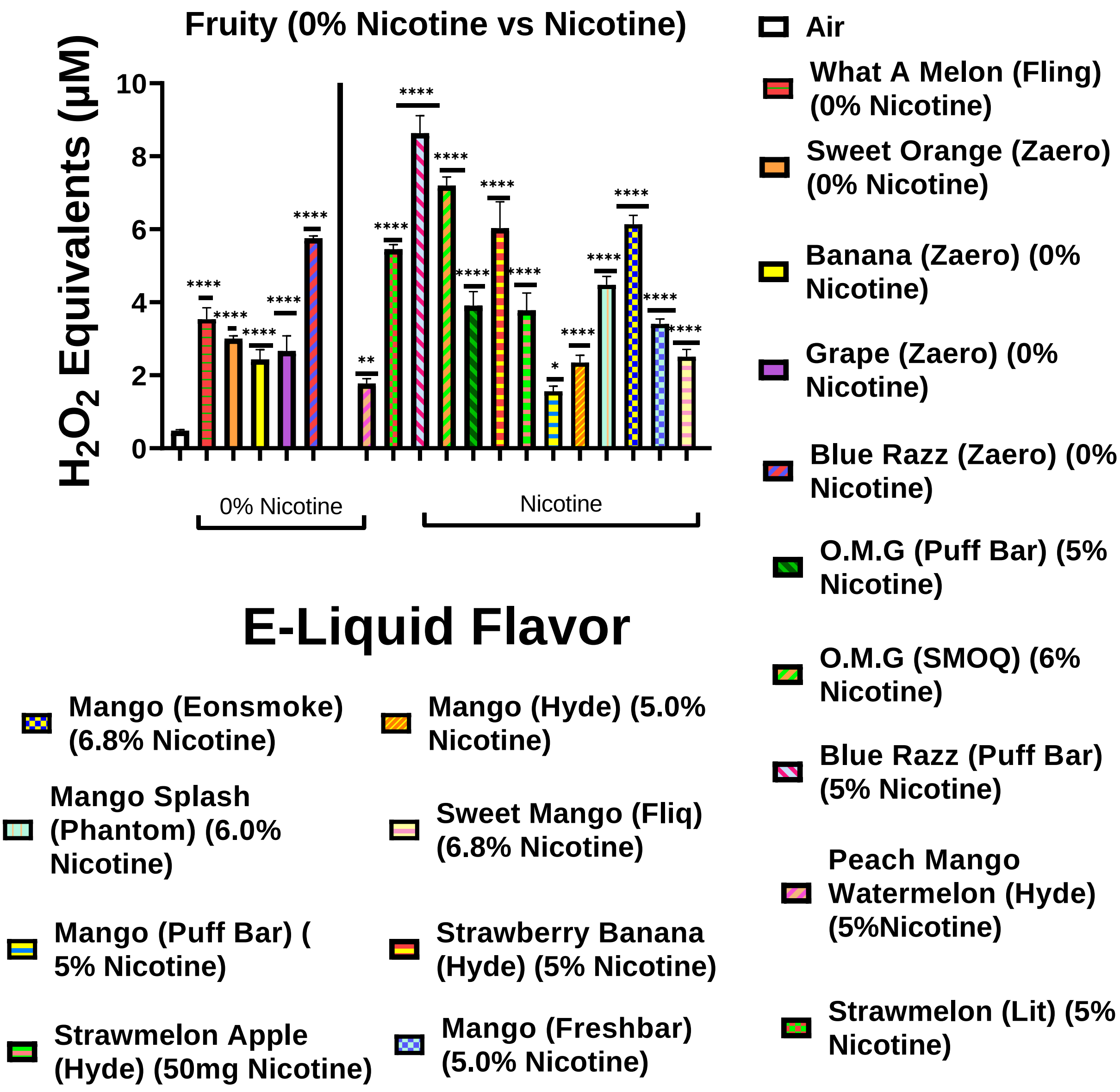

Fig 5. Generation of ROS by different fruity flavors from various vendors.

Acellular ROS was measured from aerosols generated from various different fruit flavored disposable e-cigarette devices using a hydrogen peroxide standard. Names of each vape bar's flavor, its brand, and its respective nicotine concentration are listed below and color coded. All flavors were compared to the control value of air. Data are represented as mean ± SEM, and significance was determined by one- way ANOVA. * $p< 0.05$, ** $p< 0.01$, and *** $p< 0.001$ versus air controls.

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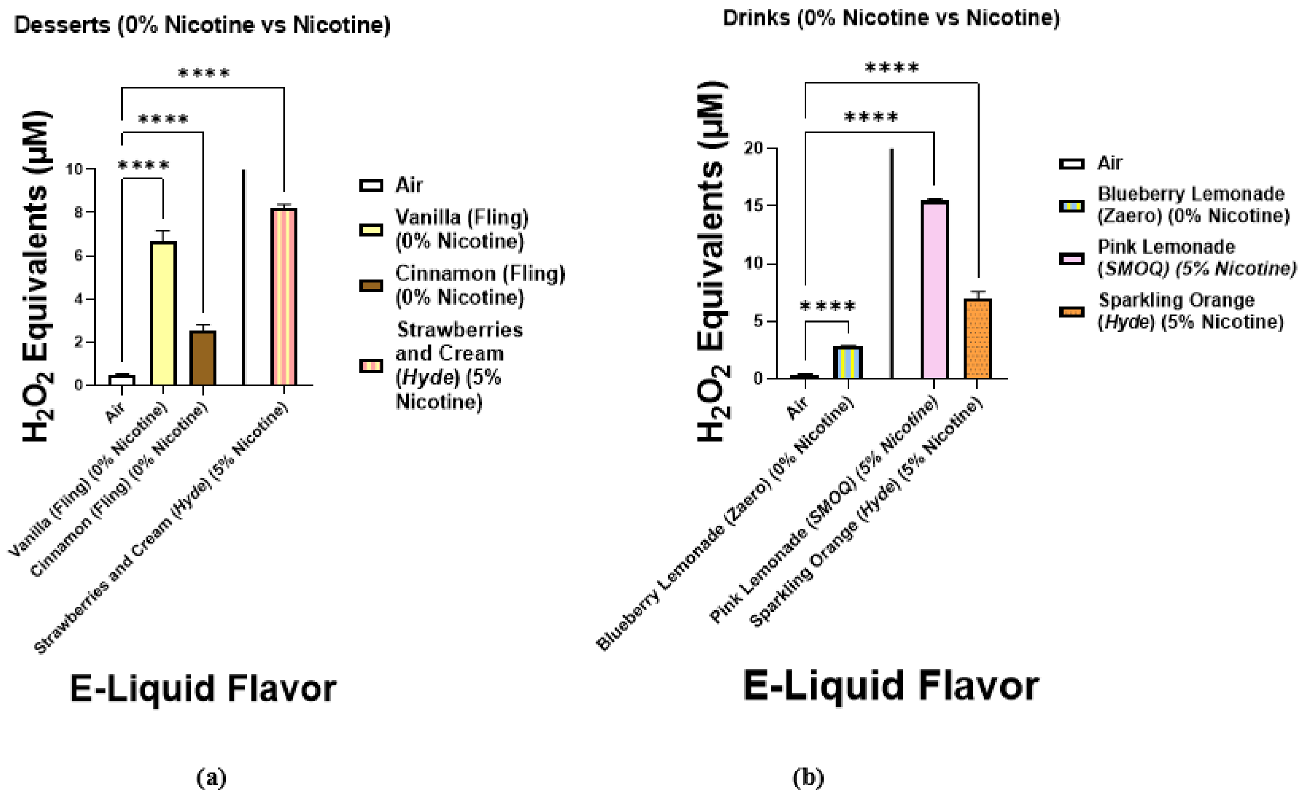

Fig 6. Generation of ROS by different dessert (a) and drink flavors (b) from various vendors.

Acellular ROS was measured from aerosols generated from different dessert flavored disposable e-cigarette devices (a) as well as from different drink flavored disposable devices (b) using a hydrogen peroxide standard. Names of each vape bar's specific flavor, its brand, and its nicotine concentration are listed below and color coded. All flavors were compared to the control value of air. Data are represented as mean ± SEM, and significance was determined by one- way ANOVA. * $p< 0.05$, ** $p< 0.01$, and *** $p< 0.001$ versus air controls.

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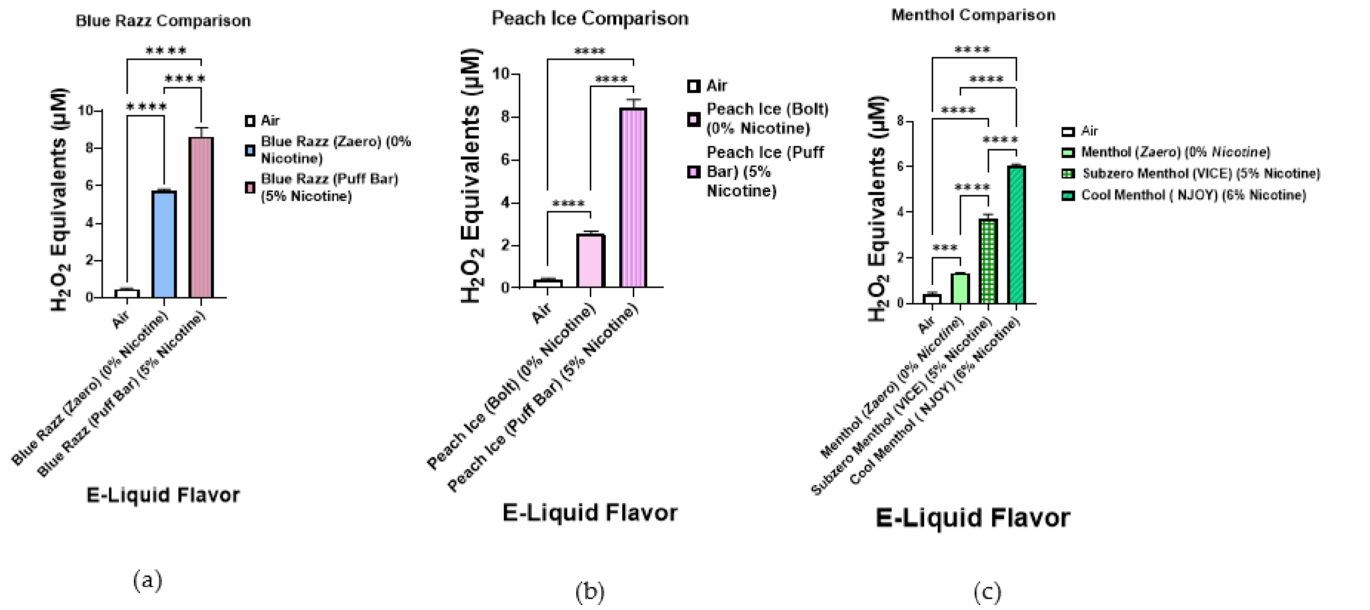

Fig 7. Direct relationship between ROS generation and Nicotine Concentration within aerosols generated from Blue Razz (a), Peach Ice (b), and Menthol (c) flavored disposable e-cigarettes

Acellular ROS was measured from aerosols generated from disposable e-cigarettes of the same flavor but different nicotine concentrations using a hydrogen peroxide standard. Regarding disposable vape bars which were of the same specific flavor ((Blue Razz (a), Peach Ice (b), and Menthol (c)), each one was manufactured from a different vendor. The names of each vape bar's flavor, its brand, and its respective nicotine concentration are listed to the side of each respective graph. All flavors were compared to the control value of air. Data are represented as mean ± SEM, and significance was determined by one- way ANOVA. * p< 0.05, ** p< 0.01, and *** p< 0.001 versus air controls.

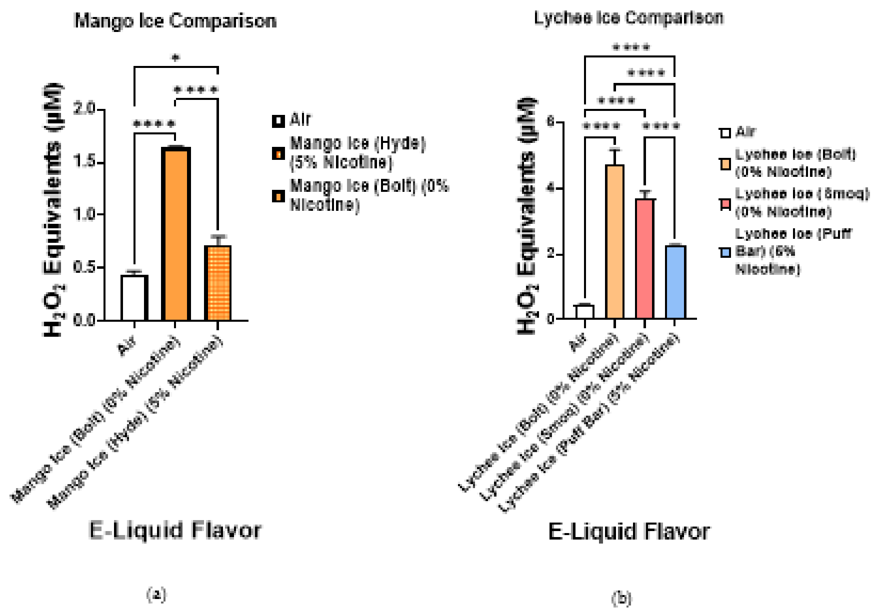

Fig 8. Inverse relationship between ROS generation and Nicotine Concentration in aerosols generated from Mango Ice (a) and Lychee Ice (b) flavored disposable e-cigarettes

Acellular ROS was measured from aerosols generated from disposable e-cigarettes of the same flavor but different nicotine concentrations using a hydrogen peroxide standard. Regarding disposable vape bars which were of the same specific flavor ((Mango Ice (a) and Lychee Ice (b)), each vape bar was manufactured from a different vendor. Additionally, the names of each vape bar's flavor, brand, and its respective nicotine concentration are listed to the side of each respective graph. All flavors were compared to the control value of air. Data are represented as mean ± SEM, and significance was determined by one- way ANOVA. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ versus air controls.

4. Discussion

When analyzing the ROS content emitted by vape bars within each flavor category (Tobacco, Fruity, Minty Fruit, Minty/Menthol, Drinks, and Dessert), we observed differential ROS production among the different flavored bars. More specifically, within each of the six flavor categories analyzed, different flavored disposable vape bars with the same nicotine content produced variable levels of ROS relative to the respective air control. The Tobacco, Fruity, Minty Fruit, Minty/Menthol, Drinks, and Dessert flavor categories were selected for our analyses due to the popularity of these flavor categories among e-cigarette users, especially among e-cigarette users in middle and high school, after the FDA's 2020 flavor ban [1]. Additionally, vape bars under the Minty (Iced) Fruit flavor category were analyzed due to its recent rise in popularity among youth e-cigarette users. More specifically, around the same time that disposable e-cigarette sales surged following the FDA's flavored e-cigarette ban in 2020, a significantly high number of iced e-cigarette flavors had entered marketplaces [15]. Likewise, the increased usage of iced fruit flavors among e-cigarette users in the country necessitated us to analyze these flavors because of their potential to make further regulatory action more complicated due to Iced Fruit flavors not fitting into existing flavors flavoring categorizations [15].

Research investigating how flavoring chemicals affect ROS generation in e-cigarette generated aerosols has been explored minimally; however, a few recent studies have delved into the dependence that ROS generation from e-cigarettes may have on flavoring chemicals. One study found that ROS levels generated from cigar/cigarillo smoke varied as a function of flavor [16]. Regarding studies conducted with e-cigarettes, one study found that ROS generation within the aerosols generated from cartridge-based e-cigarette devices was highly dependent on the vendor, puffing pattern, voltage, and the flavor of the cartridge-based e-cigarette device used [3]. Moreover, our lab's previous study found that the flavorings used in e-liquids can induce an inflammatory response in monocytes; the study further found that this response is mediated through ROS production [17].

Regarding our analysis of ROS generation's dependence on nicotine content, we can see that among specific flavors of vape bars (Blue Razz, Peach Ice, Lychee Ice, Mango Ice, and Menthol), ROS generation appears to vary as a function of nicotine content significantly. However, our data does not seem to suggest a consistently direct or inverse relationship between nicotine concentration and ROS generation among the flavored disposable e-cigarettes analyzed. Moreover, this previously mentioned lack of consistency when analyzing vape bars of the same flavor but different nicotine concentrations insinuate that specific flavoring chemicals found in flavored disposable e-cigarettes (Mango Ice, etc.) undergo chemical reactions with nicotine. Moreover, the type(s) and frequency of these chemical reactions vary as a function of nicotine concentration and the presence of specific flavoring chemicals; this contributes to differential ROS emission in generated aerosols.

While nicotine itself does not contribute to exogenous ROS production, the role that interactions between nicotine and the other usual constituents of e-liquids (flavoring agents, propylene glycol(PG), and vegetable glycerin (VG)) have in exogenous ROS production have been explored in the past [5, 18]. Similarly, one study had shown that the ROS emission from aerosolized e-liquids was significantly affected by the PG: VG ratio of the e-liquid [18]. Regarding the relationship between nicotine and ROS in aerosolized e-liquids, a few studies have found that adding nicotine to e-liquids produced significantly more particles within emitted aerosols, thus adding onto the Total Particulate Matter (TPM) within an aerosolized e-liquid [19]. Consequently, we analyzed the same flavored vape bars with different nicotine concentrations to see whether our results would support the theory that the increase in TPM seen through adding nicotine to e-liquids in the previously mentioned studies was due to increased ROS emissions. However, further experiments are required which use more flavors of vape bars within each flavor category to further elucidate the relationship between nicotine concentration in vape bars and ROS concentration within generated aerosols. For instance, one could analyze the ROS content within aerosols generated by disposable vape bars of the same flavor and the same vendor but of differing nicotine content to further control for the manufacturer of the e-cigarette in ROS production. Our lab did not have disposable devices of the same flavor and vendor but with different nicotine concentrations within the six previously mentioned flavor categories; however, we will obtain them for future experiments.

Regarding physicochemical interactions involving flavoring chemicals and their contribution to ROS emissions from e-cigarettes, research delving into how the interactions between different components of e-liquids contribute to ROS generation is lacking. However, one study (Son, Yeongkwon et al.) found that the flavoring chemicals within flavoring agents (those including maltol, benzyl acetate, anethole, etc.) may undergo redox cycling with transition metal ions found with e-liquids and produce •OH [5]. In conjunction with Son, Yeongkwon et al.'s results, the results of our study suggest that total ROS content within aerosols generated from disposable e-cigarettes varies due to the specific physicochemical interactions between specific flavoring chemicals and the other constituents of a vape bar's e-liquid component.

Specifically, our results support the theory that flavoring chemicals specific to one e-liquid flavor undergo different physicochemical interactions with PG:VG and nicotine than the flavoring chemicals found in a different e-liquid flavor. Likewise, these different physicochemical reactions specific to one specific e-liquid flavor contribute to the differential ROS levels observed among aerosols of the different flavored vape bars in our study.

However, further assays and experiments are needed to address the previously mentioned theory as acellular ROS assays alone are not sufficient to do this. Likewise, future studies can focus on using Gas Chromatography-Mass Spectrometry (GC-MS) to analyze the compounds within flavoring agents within flavored vape bars. In addition, Electron Paramagnetic Resonance (EPR) Spectroscopy can analyze the relative proportions of specific free radicals (H2O2, O2 •, and •OH) within the aerosol generated from vape-bars.

5. Conclusions

Overall, our results concur with our initial hypothesis that ROS generated from disposable e-cigarette bars varies among different flavors as well as a function of nicotine concentration. Our results seem to suggest that different physiochemical interactions occur between flavoring agents and the other constituents of e-liquids within disposable e-cigarettes (water, PG: VG, nicotine, etc.). Our results suggest that particular flavoring agents induce specific types of physicochemical interactions with the other constituents of e-liquids, contributing to ROS levels varying among aerosols generated from different flavored vape bars. Additionally, the differential ROS levels among aerosols generated from vape bars of the same flavor but with varying concentrations of nicotine suggest that nicotine plays a role in physicochemical interactions with flavoring chemicals and PG: VG. To better understand the relationship between nicotine and ROS generation and between flavoring chemicals and ROS generation within disposable e-cigarettes, future studies are required to analyze a significantly greater number of vape bars. More specifically, in addition to analyzing a greater number of vape bars, there should be more acellular ROS comparisons done between vape bars that control for vendor, thereby reducing the confounding influence a specific vendor may have on ROS generation.

Additionally, the chemical constituents of a vape bar's flavoring agents and the quantities of specific free radicals within its generated aerosols can be determined through GC-MS and EPR Spectroscopy, respectively. These assays can be used to understand how the physicochemical interactions inside an aerosolizing e-liquid contribute to differential ROS generation among different flavors. Likewise, in conjunction with the recommended future studies, the results of our study can generate evidence used in favor of public health policies that lead to the regulation of products, such as vape bars, which are exempt from the FDA's Flavor ban.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1. Original GraphPad Prism files where acellular ROS assay data was recorded and graphed are available upon request.

Author Contributions: Conceptualization, T.M, and I.R.; methodology, T.M .; Assay performance: S.Y. software, S.Y.; validation, S.Y., T.M., and I.R. .; formal analysis, S.Y.; investigation, S.Y.; resources, I.R.; data curation, S.Y.; writing—original draft preparation, S.Y.; writing—review and editing, S.Y, T.M., and I.R..; visualization, S.Y..; supervision, I.R..; project administration, I.R.; funding acquisition, I.R. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation.

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Institutional Review Board Statement:

All experiments performed in this study were approved and in accordance with the University of Rochester Institutional Biosafety Committee. Additionally all protocol, procedure and data analysis in this study were followed the NIH guidelines and standards of reproducibility and scientific rigor by an unbiased approach. (Biosafety Study approval #Rahman/102054/09-167/07-186; identification code: 07-186; date of approval: 01/05/2019). No animals or human subjects were used.

Data Availability Statement: We declare that we have provided all the data, but the primary data will be available upon request.

Conflicts of Interest: The authors declare no conflict of interest.

References:

1. Wang, T.W., et al., *E-cigarette Use Among Middle and High School Students - United States, 2020.* Mmwr-Morbidity and Mortality Weekly Report, 2020. **69**(37): p. 1310-1312.
2. Rehan, H.S., J. Maini, and A.P.S. Hungin, *Vaping versus Smoking: A Quest for Efficacy and Safety of E-cigarette.* Curr Drug Saf, 2018. **13**(2): p. 92-101.
3. Zhao, J., et al., *Assessment of reactive oxygen species generated by electronic cigarettes using acellular and cellular approaches.* J Hazard Mater, 2018. **344**: p. 549-557.
4. Bhattacharyya, A., et al., *Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases.* Physiol Rev, 2014. **94**(2): p. 329-54.
5. Son, Y., et al., *Hydroxyl Radicals in E-Cigarette Vapor and E-Vapor Oxidative Potentials under Different Vaping Patterns.* Chem Res Toxicol, 2019. **32**(6): p. 1087-1095.
6. Schieber, M. and N.S. Chandel, *ROS function in redox signaling and oxidative stress.* Curr Biol, 2014. **24**(10): p. R453-62.
7. Mittal, M., et al., *Reactive oxygen species in inflammation and tissue injury.* Antioxid Redox Signal, 2014. **20**(7): p. 1126-67.
8. Goldkorn, T., S. Filosto, and S. Chung, *Lung injury and lung cancer caused by cigarette smoke-induced oxidative stress: Molecular mechanisms and therapeutic opportunities involving the ceramide-generating machinery and epidermal growth factor receptor.* Antioxid Redox Signal, 2014. **21**(15): p. 2149-74.
9. Szafran, B.N., et al., *Electronic-Cigarette Vehicles and Flavoring Affect Lung Function and Immune Responses in a Murine Model.* Int J Mol Sci, 2020. **21**(17).
10. Lerner, C.A., et al., *Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung.* PLoS One, 2015. **10**(2): p. e0116732.
11. Soneji, S., et al., *Association Between Initial Use of e-Cigarettes and Subsequent Cigarette Smoking Among Adolescents and Young Adults: A Systematic Review and Meta-analysis.* JAMA Pediatr, 2017. **171**(8): p. 788-797.
12. Djordjevic, M.V. and K.A. Doran, *Nicotine content and delivery across tobacco products.* Handb Exp Pharmacol, 2009(192): p. 61-82.
13. Grant, J.E., et al., *E-cigarette use (vaping) is associated with illicit drug use, mental health problems, and impulsivity in university students.* Ann Clin Psychiatry, 2019. **31**(1): p. 27-35.
14. Jankowski, M., et al., *E-Cigarettes are More Addictive than Traditional Cigarettes-A Study in Highly Educated Young People.* Int J Environ Res Public Health, 2019. **16**(13).
15. Leventhal, A., et al., *'Ice' flavoured e-cigarette use among young adults.* Tob Control, 2021.
16. Lawyer, G.R., et al., *Classification of flavors in cigarillos and little cigars and their variable cellular and acellular oxidative and cytotoxic responses.* PLoS One, 2019. **14**(12): p. e0226066.
17. Muthumalage, T., et al., *Inflammatory and Oxidative Responses Induced by Exposure to Commonly Used e-Cigarette Flavoring Chemicals and Flavored e-Liquids without Nicotine.* Front Physiol, 2017. **8**: p. 1130.
18. Haddad, C., et al., *Reactive Oxygen Species Emissions from Supra- and Sub-Ohm Electronic Cigarettes.* J Anal Toxicol, 2019. **43**(1): p. 45-50.
19. Li, L., et al., *Effects of propylene glycol, vegetable glycerin, and nicotine on emissions and dynamics of electronic cigarette aerosols.* Aerosol Sci Technol, 2020. **54**(11): p. 1270-1281.