A rapid semi-quantitative screening method to assess chemicals present in heated e-liquids and e-cigarette aerosols

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Abstract. Introduction: Electronic cigarettes (e-cigarettes) lack regulatory status as therapeutic products in all jurisdictions worldwide. They are potentially unsafe consumer products, with significant evidence ~~showing~~ they pose a risk to human health. Therefore, developing rapid, economical test methods to assess the chemical composition of e-liquids in heated and unheated forms and the aerosols produced by e-cigarettes is crucial. **Methods**: Four different e-liquids were heated using two different methods: 1) "typical" vaping using an e-cigarette device, by cycling "on" for three seconds every minute for two hours (e-liquid obtained from remainder in the tank and aerosol collected in an impinger) and, 2) "accelerated" heating, using an e-cigarette coil, submerged in e-liquid, and heating in short 20 second bursts "on" then 20 seconds "off" for two minutes only (liquid traps aerosol produced). All e-liquids were then analyzed to test for the presence and quantity of 13 chemicals by gas-chromatography mass-spectrometry and compared to an unheated sample. **Results:** E-liquids heated with the "accelerated" method showed a comparable trend to the "typical" heating method, ~~of~~ i.e. increase or decrease in chemical compound quantity, for ~~greater~~ more than two-thirds of the detected compounds analyzed over all e-liquids. Six chemicals were detected as aerosol from the impinger fluid with the "typical" heating method, ~~most~~ at negligible levels. **Conclusion:** We propose that this accelerated version of the typical vaping~~rapid~~ method could form the basis of a standardized screening tool to test heated e-liquids (and e-cigarette aerosols) for harmful or banned substances. This will ensure that only approved products reach the consumer and reduce potential e-cigarette harms. ~~to ensure only approved products reach the consumer, and the potential harms of e-cigarette use are reduced.~~

1 Introduction

~~The potential for e-cigarettes to negatively impact health is of concern due to the known presence of unsafe chemical constituents, and the possibly carcinogenic, mutagenic or reprotoxic nature of the aerosols that are produced by heating,~~

~~aerosolizing and inhaling e-liquids~~Chemicals are present in the aerosol produced by ~~the~~ electronic cigarettes (e-cigarette) when the e-liquid ~~it contains~~ is heated and aerosolised (Goniewicz et al., 2014, European Parliament, 2014). The e-cigarette is referring to the aerosol generating device, which uses the "e-liquid" to create aerosol by evaporation condensation method. If in sufficient quantit~~y~~ies, and dependent on the hazard type, chemicals present in an e-cigarette-produced aerosol have the potential to negatively impact health when inhaled (European Association for the Co-ordination of Consumer Representation in Standardisation, 2021). For example, ~~International Agency for Research on Cancer~~ group one carcinogens~~, such as~~ acetaldehyde and formaldehyde have been found in e-cigarette aerosols and are degradation products of the e-liquid base components, propylene glycol and glycerol ~~and are known degradation species of the main e-liquid components, propylene glycol and glycerin~~ (Goniewicz et al., 2014). Other chemical ~~Many other i~~ingredients found in unheated e-liquids that pose a risk to human health (e.g. respiratory irritants, sensitizers etc.)~~, or for which the inhalation health effects are unknown, are regularly found in unheated e-liquids, occasionally at unsafe levels — most are ingredients added to the~~may include ~~e-liquids as~~ flavors or solvents added to the e-liquids ~~(European Association for the Co-ordination of Consumer Representation in Standardisation, 2021). These potentially unsafe ingredients~~These chemicals ~~may~~ can be present in unheated e-liquid, heated e-liquid, aerosol~~-ised e-liquid~~generated by the heating process, or ~~in~~ any combination of the three forms. ~~However~~Yet, as recently as 2015, no country in the world regulated e-liquid ingredients beyond nicotine levels.

As more evidence emerges suggesting that e-cigarette aerosols~~s~~ negatively impact health, regulation is rapidly evolving in this area (European Parliament, 2014, Budzyńska, Sielemann, Puton, & Surminski, 2020, Therapeutic Goods Administration, 2021). For example, the European Union (EU) Tobacco Product Directive (TPD) states that only ingredients in nicotine-containing e-liquids that do not pose a risk to human health in heated or unheated form~~s~~ can be used (European Parliament, 2014) and .~~many~~As a result of the EU TPD, countries (including United Kingdom, Germany, and France) banned some ingredients as a result ~~that have banned ingredients include the United Kingdom, Germany and France~~ (European Parliament, 2014, European Association for the Co-ordination of Consumer Representation in Standardisation, 2021, Budzyńska, Sielemann, Puton, & Surminski, 2020). A modest ingredient ban ~~on certain ingredients,~~for ~~in~~ nicotine-containing e-liquids ~~only, also~~ came into effect in October 2021 in Australia, prior to~~and~~ a complete ban on non-prescription e-cigarette (and e-liquid) importation and sale in Australia ~~was~~ being announced in May 2023 (Nogrady, 2023, Therapeutic Goods Admnistration, 2021). Known health effects of banned ingredients include ~~E~~e-cigarette or Vaping Associated Pulmonary (or Lung) Injury (aka VAPI, or VALI/EVALI).~~and T~~there is emerging evidence of self-reported lung conditions associated with e-cigarette use (Greenhalgh, 2019, Osei et al., 2020, Bircan, Bezirhan, Porter, Fagan, & Orloff, 2021). ~~The~~ Health concerns ~~for e-liquids (heated and unheated) and their aerosols, to negatively impact health~~ are amplified by ~~unregulated e-liquid use,~~ a multibillion-dollar market driving product sales and increasing use in young people. Consequently, pre-market approvals (including unheated ingredient listing) are preferred by regulators which allow independent determination of product safety (Australian Government, 2023, United States Food and Drug Administration, 2023).~~and growth , emerging evidence of self-reported lung conditions associated with e-cigarette use, and E-cigarette or Vaping Associated Pulmonary (or Lung) Injury (aka VAPI, or VALI/EVALI) (Greenhalgh, 2019, Osei et al., 2020, Bircan, Bezirhan, Porter, Fagan, & Orloff, 2021).~~

Whilst chemical testing of unheated e-liquids is relatively common, the safety of e-liquids remains largely unassessed due to the sheer scale of the market~~, and~~. Additionally, ~~with the exception of~~except for the EU TPD guidance~~,~~ (to the best of our knowledge), testing heated e-liquids for inclusion in pre-market product approval is not required in Australia or elsewhere (European Parliament, 2014, Greenhalgh, 2019, Scientific Committee on Health Environmental and Emerging Risks 2021,

70 Larcombe et al., 2021). Methods to assess chemical content generally involve testing of unheated e-liquids or the e-cigarette aerosol generated, but not the heated e-liquid (Scientific Committee on Health Environmental and Emerging Risks, 2021). ~~This is important however, as~~However, the heated e-liquid~~s is~~ are more representative of what the user inhales~~,~~ compared to the unheated e-liquid~~s, but and~~also easier to assess when compared to e-cigarette aeroso~~ll~~ls (Larcombe et al., 2021, Erythropel et al., 2019). ~~Further,~~Importantly, chemicals present in e-liquids are known to degrade due to heating, either by boiling or

75 evaporative-convection (depending on wetted-wick temperature) and this can be exacerbated by presence of catalytic surfaces such as Kanthal. ~~and~~ The secondary products form~~ed secondary products~~, at high (>200ºC) or low temperatures (<200 ºC) ~~that,~~ may have increased (or decreased) toxicity compared to the parent compound (~~Goniewicz et al., 2014,~~ Erythropel et al., 2019~~,~~). ~~For example, the degradation processes for e-liquid base components propylene glycol and glycerin, have been reported for both low (< 200ºC) and high temperatures (>200ºC) and the physical mechanisms underlying aerosol production (and~~

80 ~~therefore chemical formation) have been described to be either by boiling or evaporative-convection depending on wetted-wick temperature (Jaegers, Hu, Weber, & Hu, 2021,~~ Floyd, Queimado, Wang, Regens, & Johnson, 2019, Goniewicz et al., 2014, Jaegers, Hu, Weber, & Hu, 2021, Li et al., 2021, Zhao, Shu, Guo, & Zhu, 2016, Saliba et al., 2018). ~~For a given e-liquid, the abundance of degradation products depends on numerous factors including the temperature the coil is heated to, the availability of oxygen, and the exposure to a potentially catalytic surface such as Kanthal (iron-chromium-aluminum alloy)~~

85 ~~(Saliba et al., 2018). Kanthal is commonly used in e-cigarette heating coils, and it has been shown to reduce the temperature needed to thermally decompose e-liquid components (Jaegers, Hu, Weber, & Hu, 2021).~~

Many approaches have been trialled to address the difficulties in directly assessing the chemical composition of e-cigarette produced aerosol~~to test the chemical composition of e-liquids since the introduction of the modern e-cigarette in 2003~~. Such challenges include collecting enough aerosol to perform an assay (and therefore detect potentially toxic compounds), and

90 overload of the main excipients (propylene glycol and vegetable glycerin). These difficulties induce increasingly complicated test methods, yet c~~C~~urrent methods have been ~~summarized recently and~~ shown to be outdated or limited (Floyd, Queimado, Wang, Regens, & Johnson, 2019, Herrington & Myers, 2015, Scientific Committee on Health Environmental and Emerging Risks, 2021). ~~. There are many difficulties in directly assessing an e-cigarette produced aerosol. It is challenging to collect enough aerosol to perform an assay and detect potentially toxic compounds, especially because of the overload of the main~~

95 ~~excipients (propylene glycol and vegetable glycerin). This induces the use of increasingly complicated test methods (Floyd, Queimado, Wang, Regens, & Johnson, 2019, Herrington & Myers, 2015).~~ D~~D~~espite the expansive range of tests, a simple test capable of assessing heated and unheated e-liquids, and the aerosol produced all at once~~.~~ is ~~is~~ yet to be established, but vital~~,~~ ~~but vital. Therefore, it is critical to standardize the procedures allowing to test the chemical composition of e-liquids. There are many difficulties in directly assessing an e-cigarette produced aerosol. It is challenging to collect enough aerosol to perform~~

100 ~~an assay and detect potentially toxic compounds, especially because of the overload of the main excipients (propylene glycol and vegetable glycerin). This induces the use of increasingly complicated test methods (Floyd, Queimado, Wang, Regens, & Johnson, 2019, Herrington & Myers, 2015). Despite the expansive range of tests, a simple test capable of assessing heated and unheated e-liquids, and the aerosol produced all at once is yet to be established, but vital.~~

In this study we aimed to: 1) establish and validate a simple~~n~~ "accelerated", method of heating and aerosolising e-liquids (i.e.

105 "vaping") that would be comparable to, but quicker than, the "typical" method of heating and aerosolising e-liquid ~~vaping of a user~~; 2)~~) that it~~ could assess the chemical composition of the heated e-liquid and the aerosol produced all at once. We hypothesized that the "accelerated" method and the commonly used "typical" vaping method would result in similar heating-induced chemical changes in the e-liquids. ~~Comparability~~ The ~~of~~ two heating method ~~was assessed~~were compared by measuring the presence and concentration of ~~the same~~a wide range of chemicals ~~chemicals with both methods~~. We hope that

110 this "accelerated" test methodology can form the critical first step in establishing a rapid test for screening of e-liquids for banned substances.

2 Materials and Methods

2.1 "Typical" vaping process

A set up was designed to replicate the heating/cooling process an e-liquid would undergo when an e-cigarette is used in a

115 "typical" way (Figure 1 (a)) (Etter & Bullen, 2014, St Helen et al., 2016, Cooperation Center for Scientific Research Relative to Tobacco (CORESTA), (2015)). The method allowed sample collection at two points for analysis of heated e-liquid and aerosol respectively: 1) from the remainder in the e-liquid tank (atomiser), and 2) from the impinger (Figure 1 (a)). To begin, the e-cigarette (MVP4, Innokin, Shenzhen, China, operating wattage range 6–100 W, temperature range 150–315 ºC, maximum current 35.5A) atomizer (the e-liquid tank, containing e-cigarette coil) was filled with ~3.5 mL of e-liquid and the

120 impinger was filled with 5 mL of e-liquid excipient (50:50 glycerin–propylene glycol (v/v)), Sigma Aldrich, Milwaukee WI, USA). A flow of ~3 L/min ambient filtered air was drawn through the system via laboratory bench vacuum and kept stable through monitoring with a flow meter (Max 5 Lpm, TSI, 800669, Shoreview, MN). New coils (Kanthal BVC, 100–200 W, 0.28 Ω, Innokin Scion) were used each time to avoid cross contamination of chemical species and to control for coil ageing effects. The e-cigarette device was set to 80 W (reading 0.28–0.35 Ω) each time the device was connected to the atomiser. To

125 vape the device in line with recommendations by the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA), the ignition button was held for ~3 seconds, and the aerosol drawn from the device into a 60 mL syringe and then expelled through two ~ 4 mm ID, 15 cm tubing lengths into the custom made (27 L – 30x30x30 cm) chamber, using a three-way tap (Dispoflex™, Disposafe health and life care Ltd, Haryana, India) (Cooperation Center for Scientific Research Relative to Tobacco (CORESTA) (2015)). This process was repeated every minute for two hours (with the atomizer tank

130 refilled after ~60 minutes), for 120 puffs total. ~~so that a volume of 7.2 L of e-cigarette aerosol containing air was introduced to the system.~~ While we acknowledge that vaping topography is extremely variable, 120 puffs over a 2 hour period (120 x 60

mL, puffs, therefore 7.2 L of inhaled aerosol containing air) was chosen to be representative of what a typical vaper might use (Etter, 2014). After heating, the liquid was transferred to glass sample vials, and kept at 4°C to minimise the loss of volatiles.

2.2 "Accelerated" vaping process

An accelerated ageing/vaping process was developed, based on standard tests for ageing/oxidation of oils (Figure 1(b)) (American Society for Testing Materials, 2009). Our premise for collection of e-cigarette aerosols in the liquid was as follows:

1. An e-cigarette is an evaporation condensation aerosol generator – intended to modify the e-liquid as little as possible during aerosolization, however, it does thermo-oxidise, hence the need for this research;
2. Our "accelerated" method of heating the e-liquid via a submerged coil creates a "bubbling aerosol generator" (Vidamantas, 1997). Like an evaporation aerosol condensation generator, a bubbling generator will modify the e-liquid minimally, however, may allow more volatile compounds to preferentially aerosolise;
3. The creation of an aerosol via bubbling can allow aerosol capture either whilst bubbling through the bulk liquid (when cooling) or at the gas-liquid surface (Ghiaassiaan, 1997 and Koch, 2012);
4. Surface bubbles can generate aerosol either by jet or film droplets when they burst, and based on combinations of surface tension and bubble size, aerosol will recombine with the liquid the bubble arises from when it bursts (Koch, 2012, Mead-Hunter, 2018).
5. Thereby, through a combination of these processes it is reasonable to assume we retain a representative sample of the same material that is aerosolised, as well as possibly more of the thermo-oxidised (aged) material we are interested in.

To create the bubbler, an identical Kanthal BVC coils, as used in the "typical" vaping process, were was connected to a power supply (MP3090, PowerTech, China), by means of solid coper wires connected to each end of the coil (end cap removed). The power supply was set at 7.4 V and 27 A (0.274 Ω) to stay within the maximum power (200 W) of the coil used for the "typical" process and to ensure that the resistance matched that of the "typical" vaping process. The coil was placed in 100 mL beaker, which was open to air, held on a 45° angle with a clamp-stand and ~30 mL of e-liquid was poured into the beaker, enough to completely submerge the coil and ensure the full volume of liquid would not heat to boiling temperature within the one minute total heating period (20 seconds on 20 seconds off x 3), and limitedno planar surface evaporation would occur (Figure 1 (b)2). The 45° angle was used both to minimize the liquid volume needed to immerse the coil and to ensure any aerosol (or vapor) produced would recondense on the wall of the beaker allowing it to be collected for sampling. The power supply was then turned on to operate the coil for 5 x 20 second "burst" intervals with 20 second pauses interspersed for a total "on" time of 1 minute, mimicking a "short cluster" vaping pattern for a user (St Helen et al., 2016). After heating, the liquid was transferred to glass sample vials, and kept at 4°C to minimise the loss of volatiles.

2.3 Sample and chemical selection

Four flavoured e-liquids, labelled "nicotine-free", were assessed – "Butterscotch Tobacco", "Menthol", "Choc Caramel", and "Tiramisu" which were purchased from online suppliers and analyzed as 50:50 propylene glycol–glycerin (v/v) ratios. The propylene glycol: glycerol mixture was selected as 50:50 since it is a commonly sold ratio. Each e-liquid chemical composition was assessed using both methods to quantify 13 chemicals: 4-(4-methoxyphenyl)-2-butanone, ethyl vanillin, eugenol,

nicotyrine, nicotine, menthol, thymol, ethyl maltol, trans-cinnamaldehyde, 2-chloro-phenol, benzyl alcohol, benzaldehyde, and furfural, with a molecular weight range from 178.23 to 96.09 g/mol. The 13 chemicals were chosen based on (i) being previously identified, known ingredients in e-liquids (ii) the availability of a standard for the chemical (Larcombe et al., 2021).

170 **2.4 Chemical analysis method of "Accelerated" and "Typical" vaping process.**

Thirteen chemicals were tested for, in four different e-liquids, using gas-chromatography mass-spectrometry. For each of the four e-liquids, we tested for chemicals in three forms – (i) "unheated" e-liquid (i.e. straight out of the bottle), (ii) remainder of e-liquid in the atomizer and collected from the impinger after "typical" vaping and (iii) e-liquid remaining in the beaker after "accelerated" vaping. The latter sample (iii) was taken in order to detect aerosols, and assuming that aerosols (not vapor) would
175 be captured in the e-liquid with/during the accelerated method. The aerosol generated from the "typical" method was captured in an impinger containing 50:50 (v/v) glycerin–propylene glycol. Our intention was for this collected aerosol in the impinger to be added to the atomiser tank sample, for equivalent comparison to accelerated sample, however negligible values for the impinger result meant that they were excluded from the final analysis. ~~and are shown in supplementary only (Supplementary A).~~
180 Samples obtained from both methods used to heat e-liquids were compared to unheated e-liquids, both within e-liquid type, and within chemical compound, with the purpose of the comparison being to identify trends of increase or decrease from unheated e-liquid.

2.5 Chemical detection and analysis

Chemical analysis of "accelerated" and "typical" vaping process e-liquids, including sample and chemical detection, has been
185 previously described in detail elsewhere (Larcombe et al., 2021). The samples (0.25 g) were accurately weighed and placed into amber vials with 4.75 mL ultrapure water. Thereafter, 10 µL of a 1 g/L 4-bromophenol-d4 stock solution was added as an internal standard. Prior to the analysis, 1.6 g of analytical grade sodium chloride was added to increase volatilisation and the vials tightly capped. To facilitate adsorption, the samples were incubated at 90°C for 15 min prior to solid-phase micro-extraction using a divinylbenzene/carboxen/polydimethylsiloxane fiber from Supelco® allowing for 13.6 min adsorption of
190 the analytes on the fiber. The fiber was then desorbed at 250 °C in the injector in spitless mode for 5 min followed by 15 min in split mode. A Gerstel MPS2 multifunction autosampler was used to perform automated solid-phase micro-extraction injections. Analysis were carried out with an Agilent 6890N gas-chromatograph interfaced with an Agilent 5973 Network Mass Selective Detector, fitted with a HP-INNOWax polyethylene glycol stationary phase capillary column (30 m; 0.25 mm; 0.25 µm, Agilent J&W, Australia), to separate polar compounds. A constant flow (1.2 mL.min^{-1}) of helium (99.999% pure,
195 BGC, Australia) was used as a carrier gas. Optimal gas-chromatography mass-spectrometry conditions were determined, as measured by maximum sensitivity, baseline separation of analytes and gaussian peak shapes. In order to ensure a good separation of the different analytes, the oven was held isothermal at 37 °C (2 min), then heated to 260 °C at 5 °C.min^{-1}, and held at the final temperature for 10 min. Detection of analytes was carried out using a mass spectrometer in electron impact

ionization mode at 70 eV. The mass spectrometer quadrupole temperature was set at 150°C and the mass spectrometer source
200 at 230°C. The compounds were identified using a combination of their retention times, comparison of the mass spectra data of pure compounds and the specific diagnostic ion fragments of each component, with the National Institute of Standards and Technology Mass Spectral search program from the NIST/EPA/NIH EI and NIST Tandem Spectral Library which came integrated with the analysis software.

3 Results

205 Over all e-liquids, three of the thirteen compounds tested for were not detected in any e-liquid type form (4-(4-methoxyphenyl)-2-butanone, thymol, 2-chlorophenol) (Table 1).

3.1 Inclusion and exclusion criteria for analysis

There were 16 instances where a chemical was detected in unheated and both heated forms (Table 1) and all were included in analysis. Analysis involved: 1) simple comparison in table format of the heated (two methods) and unheated form of an e-
210 liquid sample and, 2) comparison via fold change compared to unheated ((Y-X)/X, where X is the unheated sample (mg/L concentration) and Y is the heated sample (mg/L concentration)) for both "typical" and "accelerated" heating methods.
Fold-change aAnalyses were not possible on the following: one chemical was undetected in unheated form but detected in both heated forms (menthol in butterscotch tobacco); one chemical was undetected in unheated form and detected in only one heated form (trans-cinnamaldehyde in choc caramel). A further three chemicals were detected in unheated form and only one
215 heated form (benzyl alcohol (Tiramisu), eugenol (Tiramisu) and furfural (Tiramisu)) (Table 1, represented by italicised values).

3.2 Behaviour of the different chemicals detected in e-liquids (Figure 3)

To compare the effect of heating, results are displayed as fold change compared to unheated ((Y-X)/X, where X is the unheated sample (mg/L concentration) and Y is the heated sample (mg/L concentration)) for both "typical" and "accelerated" heating methods (Figure 23). Specific chemicals (benzaldehyde, benzyl alcohol, ethyl vanillin, ethyl maltol, furfural, menthol, nicotine,
220 and nicotyrine) were present in unheated form, and both heated forms in 16 instances.
Over all e-liquids, types in these 16 instances, 70% (11/16) demonstrated a consistent trend within chemical type i.e. both methods of heating either increased or decreased in concentration compared to unheated sample. Ethyl vanillin (choc caramel and tiramisu), furfural (butterscotch tobacco), ethyl maltol (tiramisu), and benzaldehyde (choc caramel) are the five exceptions.

3.3 Chemical characterization by e-liquid type

225 In the "Menthol" e-liquid, of the 13 chemicals tested, nine were not detected in the heated or unheated sample (Table 1). Of the four that were detected (nicotine, nicotyrine, menthol and benzaldehyde), all (4/4, 100%) exhibited the same trend

(increasing concentration) after heating when compared to the unheated sample for both "typical" and "accelerated" heating methods (Table 1, Figure 23).

In the "Butterscotch Tobacco" e-liquid, of 13 chemicals tested, eight were not detected in heated or unheated form, and an additional one (menthol) was undetected in unheated form (Table 1). Of the four chemicals detected in each sample, three (3/4, 75%) (ethyl vanillin, benzyl alcohol and benzaldehyde) exhibited the same trend for both "typical" and "accelerated" heating methods when compared with the unheated e-liquid (Table 1, Figure 23). However, furfural increased after "typical" heating but decreased with the "accelerated" method when compared to the unheated sample.

In the "Tiramisu" e-liquid, of 13 chemicals tested, seven were not detected in heated or unheated form, and an additional three were undetected in one form of heating (Table 1). Of the three detected in each sample, one (1/3, 33%) (benzaldehyde) exhibited the same trend after both "typical" and "accelerated" heating (Table 1, Figure 23). The remaining two chemicals detected (ethyl vanillin and ethyl maltol) both decreased after "typical" heating but increased after "accelerated" heating when compared to the unheated sample.

In the "Choc Caramel" e-liquid, of 13 chemicals tested, seven were not detected in heated or unheated form and an additional one (trans-cinnamaldehyde) was undetected in both unheated and heated form (Table 1). Of the five chemicals detected in each sample, three (3/5, 60%) (benzyl alcohol, ethyl maltol, and furfural) exhibited the same trend after both "typical" and "accelerated" heating methods (Table 1, Figure 23). Benzaldehyde increased after "typical" heating but decreased after "accelerated" heating compared to the unheated sample. Conversely, ethyl vanillin increased after "accelerated" heating, but decreased after "typical" heating when compared to the unheated sample.

3.4 Impinger results from "typical vaping" heating method

Only six of the 13 chemicals tested were detected at negligible quantities in the impinger fluid; furfural, benzaldehyde, menthol, benzyl alcohol, ethyl maltol and ethyl vanillin. However, and as the impinger results were negligible, compared to heated e-liquid, for most compounds, results from the impinger fluid as such they have been includedwere unable to be included in final analysis only in supplementary material (Supplement A).

4 Discussion

The "accelerated" method used here is simple, cost effective, and has the potential to produce heated e-liquid and aerosol in a similar manner to an e-cigarette but on an accelerated timeframe, allowingfor chemical assessment in a single experiment. Due to the prohibitive costs of commercially available vaping machines, mMany "in house" other simplified "in house" methods and set-ups (e.g. e-cigarette puffing machines) exist for vapinghave been developed. However, , due to the prohibitive costs of commercially available vaping machines, but to the best of our knowledge, they all focus on generation of the e-cigarette aerosol and not on assessment of the heated e-liquid (Palazzolo, Caudill, Baron, & Cooper, 2021).

Comparison of the accelerated and typical heated samples with their unheated counterpart showed~~, overall,~~ that in ~~over two-thirds (~~~70% of the e-liquids tested ~~) of the results (in 11 out of the 16 total chemical comparisons)~~, the heating methods demonstrated a similar trend~~,~~ i.e. ~~(~~increase or decrease of chemical concentration. ~~) while in one-third (~30%) of the results (5 out of 11 comparisons) a different trend was observed between heating methods.~~ The four chemicals implicated ~~when different trends were observed~~in the five differing comparisons were mostly aldehydes (ethyl vanillin (2/11), furfural (1/11), and benzaldehyde (1/11)) except for ethyl maltol (1/11) being an alcohol. In three cases; ~~Three of these five differences (~~ethyl vanillin (2/11) and ethyl maltol (1/11)~~,~~ ~~demonstrated~~ an increase in chemical concentration with the "accelerated" heating method compared to the "typical" method was detected. The observed "increase" with the "accelerated" method can ~~be in fact,~~ be attributed to a loss of aerosol with the "typical" vaping experimental method. Rainout (recondensation of the aerosol as it cools) of the liquid aerosol was observed in the three-way tap system and the very thin tubing (ID ~~<~~< 45 mm) connecting the tap system to the 27 L chamber (Figure 1, a).~~decrease in chemical quantity sampling with the "typical" method: , due to loss of aerosol was lost with our the "typical" vaping experimental method, as evidenced by rainout (recondensation of the aerosol as it cools) of the liquid aerosol in the three-way tap system and very thin tubing (ID < 5 mm) connecting the tap system to the 27 L chamber (Figure 1, a). Rainout is likely to contribute to the reduction, considering that only six of the 13 chemicals tested for were detected at all in the impinger fluid (typical vaping set-up), and these six included the four chemicals where the trend (to increase or decrease from baseline) differed between heating methods (ethyl vanillin, ethyl maltol, benzaldehyde, and furfural), albeit at very low levels. We suspect that with a~~A modifi~~cation of the~~ed design (e.g. larger tubing) ~~might~~would reduce the~~,~~ rainout losses~~ could be reduced or sampled~~, thus increasing the yield from the impinger with the "typical" method ~~and also~~and improving ~~comparability~~reproducibility between methods. It is also possible that the flavor aldehydes were present in their propylene glycol acetal form instead of their aldehyde form, as aldehydes are known to form acetals readily (Erythropel et al., 2019). The inability to fully capture the aerosol from the "typical" heating method due to rainout, meant we are unable to confirm the suitability of our "accelerated" heating method as an impinger for aerosol, but only its validity to compare heating methods. ~~However~~But, it is likely to be suitable considering that the data were consistent ~70% of the time, and differences can be mostly (in 3/5 instances) explained by losses to rainout.~~

The remaining two ~~(out of five)~~ discrepancies involved furfural (1/11) and benzaldehyde (1/11), and these compounds were found in increased quantities with the "typical" method compared to the "accelerated" method. ~~Whilst it is suggested in the literature that the solubility of the flavoring compound in the parent compound, and not the boiling point, is the major indicator of whether or not a compound will be detected in/carried over into an aerosol , lower quantities when using the "accelerated" method may still be partially explained by the low molecular weight and , which volatile low boiling point of these compounds (Erythropel et al., 2019).~~ Because these two discrepancies contain low molecular weight/low boiling point products we suspect they may have evaporated more readily (compared to the "typical" method) and that our "accelerated" method was simply unable to capture compounds with low boiling point that volatise easily (Erythropel et al., 2019). For example, furfural has the lowest boiling point and molecular weight of all chemicals detected (162°C, 96.09 g/mol) ~~and benzaldehyde the second lowest (178.1°C, 106.12 g/mol)~~was detected as decreased from the unheated sample in both "butterscotch" and "tiramisu" flavor,

~~meaning it is plausible they may volatize/form vapor (not aerosol) more readily and be lost as vapor if the wetted wick temperature was different between methods (perhaps increased with accelerated method).~~ While o~~O~~ur study design angled the beaker at 45° to allow re-condensation of any vapor on the beaker wall, ~~however~~ the experiment was carried out in a ventilated fume hood ~~for health and safety~~, which may ~~have assisted vapor removal~~have increased the loss of highly volatile compounds.

In future studies~~y~~, the addition of ~~putting~~ a lid on the angled beaker, ~~and~~as well as monitoring the ~~wetted wick temperature of the coil in both methods (not just coil temperature), the~~ liquid temperature on the wick (or surrounding the wick)~~, or other parameters such as in previous studies~~, ~~would~~may ~~elucidate~~help to reduce discrepancies ~~further mechanisms behind these discrepancies, and~~to allow full validation of the method for detection of aerosols (Li et al., 2021, Palazzolo, Caudill, Baron, & Cooper, 2021, Bitzer et al., 2018). Further studies should also be designed to consider the solubility of the flavoring compound in the base excipients (i.e. propylene glycol or glycerol), and not the boiling point, as this is suggested to be a major indicator of whether a compound will be detected in/carried over into an aerosol (Erythropel et al., 2019).

Undetected chemicals included 4-(4-methoxyphenyl)-2-butanone, ~~thymol~~thymol, and 2-chloro-phenol. Considering no "fruity" flavors were assessed, it is less surprising that 4-(4-methoxyphenyl)-2-butanone was undetected, as it is a raspberry ketone methyl ether – a common flavoring in "berry" flavored e-liquids. Although we were looking for thymol because of its use as a precursor for racemic menthol

Synthetic thymol (produced from m-cresol) its absence may be explained because ~~is used as a precursor to produce racemic menthol, as is~~ pulegone and other terpenoids are also used as the precursor. ~~However, t~~Thymol (a phenol and monoterpenoid) ~~is a flavor of its own and might~~, but synthetic thymol (produced from m-cresol) is also used as a precursor to produce racemic menthol, as is pulegone and other terpenoids, and so perhaps thymol was not the precursor for menthol in those e-liquids and therefore not be a contaminant in some e-liquids~~ (Dylong, Hausoul, Palkovits, & Eisenacher, 2022). Additionally, m~~M~~enthol was not detected in either "tiramisu" or "choc-caramel" flavors so perhaps ~~either~~ synthetic analogues were present (where menthol was not detected) such as N-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (trade name WS-3)~~), or the production method for the menthol was not using thymol as an intermediary.~~ ~~For example, as~~As the demand for menthol increases, alternative methods to produce L-menthol are on the rise, such as from citronellal (Dylong, Hausoul, Palkovits, & Eisenacher, 2022). ~~Additionally, there are synthetic analogues to menthol such as N-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (trade name WS-3).~~

~~Menthol was not detected in either tiramisu or choc-caramel flavors so perhaps either synthetic analogues were present (where menthol was not detected), or the production method for the menthol was not using thymol as an intermediary.~~ Chlorophenols like 2-chloro-phenol have previously been detected in e-liquids probably because they are notorious environmental contaminants. Particularly, ~~–~~ 2-chloro-phenol is a priority contaminant in both the US and EU and has previously been found in e-liquids (Larcombe et al., 2021, Chivers, Janka, Franklin, Mullins, & Larcombe, 2019, Igbinosa et al., 2013). Two-chloro-phenol is used for many applications, predominantly ~~its role~~ as a detergent but also~~, however, other roles for 2-chlorophenol includes its use~~ as an intermediate in the manufacturing of agricultural chemicals, pharmaceuticals, biocides, and dyes~~.,~~ T~~th~~erefore,~~us~~ it is commonly detected in environmental water samples after being discharged from industrial effluents

325 (Igbinosa et al., 2013, Yahaya, Okoh, Agunbiade, & Okoh, 2019). It has ~~been~~ previously been suggested that 2-chloro-phenol may be a contaminant from the glycerin excipient, for two reasons; 1) vegetable glycerin is made from plant crops such as canola and 2-chloro-phenol has been found in canola as a pesticide residue and, 2) glycerin (not from plants) is a by-product of bio-diesel production and biodiesel can be made with canola (Abdel-Gawad, H., & Hegazi, B., 2010, Yahaya, Okoh, Agunbiade, & Okoh, 2019). It is possible that ~~lesser known/detected~~ derivatives of 2-chloro-phenol or other phenolic

330 derivatives known to be priority contaminants were present. However, it was not within the scope of the study to assess these compounds as they are not commonly reported to be found/tested for in e-liquids. Eugenol and trans-cinnamaldehyde were the least detected compounds, they were found in only one flavor e-liquid. ~~Trans-cinnamaldehyde was only detected in a single accelerated sample, which is perhaps expected as cinnamaldehyde is less commonly found.~~, ~~H~~however ~~C~~cinnamaldehyde ~~it could also be absent~~might not be detected ~~because~~as it is known to form propylene glycol acetals ~~(αβ, unsaturated aldehyde)~~

335 ~~the acetal~~and this form is known to be relatively ~~of the parent aldehyde is known to be more~~ reactive ~~(αβ, unsaturated aldehyde) and to degrade to secondary compounds more readily~~ (Erythropel et al., 2019).

4.1 Limitations and future directions

A limitation of this study is that we tested a pre-determined list of chemicals, based on our knowledge of known e-liquid ingredients, available ~~standards~~standards, and available analytical methods, rather than ~~obtaining~~ looking for a complete

340 chemical characterization. This approach allowed us to test a larger range of e-liquids and demonstrate~~s~~ the utility of the "accelerated" aging technique, as per the over-arching goal of the study. However, an "open ended" approach may be useful for future studies if this method is to become standardized. An open-ended approach would allow a more complete comprehension of the aging process and oxidation reactions occurring but would require a broader range of analytical techniques than demonstrated here. Furthermore, whilst we assessed for some ingredients which are now banned (i.e.

345 benzaldehyde and cinnamaldehyde), this study was designed and conducted prior to the enactment of banned ingredients by the Therapeutic Goods Administration in 2021 (Therapeutic Goods Administration, 2021) and expanding future~~the~~ analysis to include the full range of banned~~se~~ chemical products would be helpful ~~in future studies~~. The same comment applies for chemicals banned in other jurisdictions.

The method we describe in this study has many advantages over current methods for testing e-liquids. It is a rapid and

350 inexpensive set-up allowing assessment of the chemical composition of heated e-liquids and~~,~~ ~~potentially~~ with minor modifications, their resultant aerosols. It could be used with any available coil that can be modified and powered as described. Furthermore, the accelerated method is likely to capture aerosol generated from a heated e-liquid in a manner comparable to the "typical user" vaping method as described in CORESTA in terms of both type and quantity of chemicals produced. Our submerged, rapid heating and cooling method ~~is able to~~can economically sample heated liquid and aerosols (but not vapor)

355 within a single sample in two minutes, which may have advantages over some other methods. This method is more representative of what the user inhales as it is testing a heated liquid during exposure to the coil (potentially) catalytic surface, rather than only an unheated e-liquid.

5 Conclusion

In summary, the accelerated method described here is a suitable screening tool for rapid chemical assessment of heated e-liquids and their aerosols, that mimics typical e-cigarette vaping on an accelerated timeframe. ~~It is a fairly recent (2014) recommendation by t~~The EU TPD recommendation is to assay heated e-liquids, ~~and~~ however (to the best our knowledge) there has only been one previously published study on the effects of aging/heating on e-liquids. We propose that this method (with our recommended improvements) can~~to~~ be used as a standardized screening tool for e-liquids, and their aerosols, to identify potentially harmful chemicals, such as those recently banned in Australia or previously banned in Europe and the United Kingdom. With minor modification, this test could be used prior to importation or sale, to ensure that only tested products, containing approved ingredients, reach the consumer. In the absence of an approved therapeutic goods status for e-cigarettes, the type of high-throughput testing described here is necessary as a minimal precaution to assess and reduce the potential harms of a consumer product that is generally accepted in the public to be a less harmful alternative to smoking.

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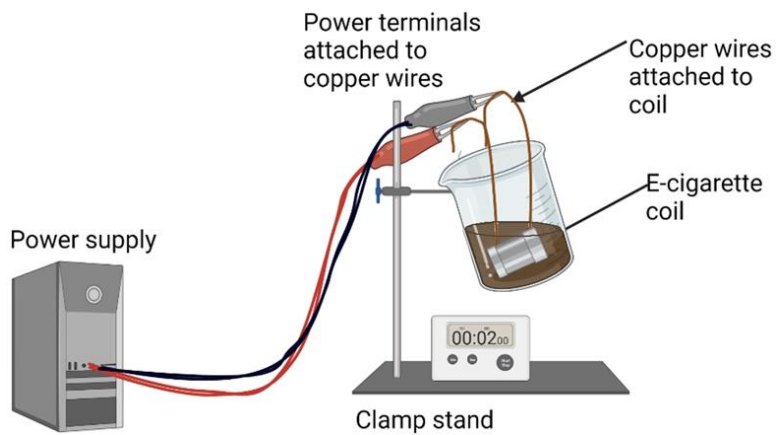
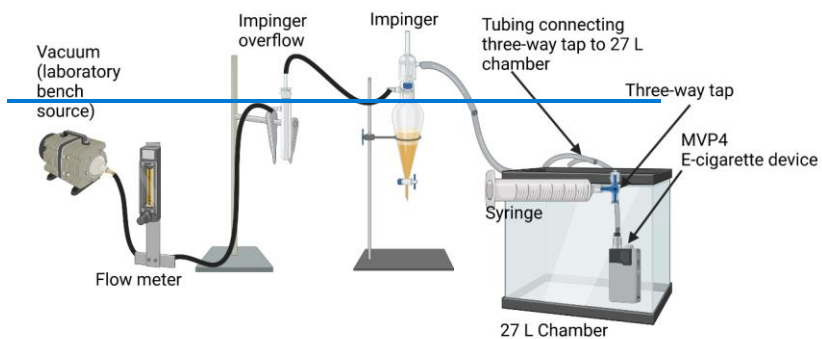
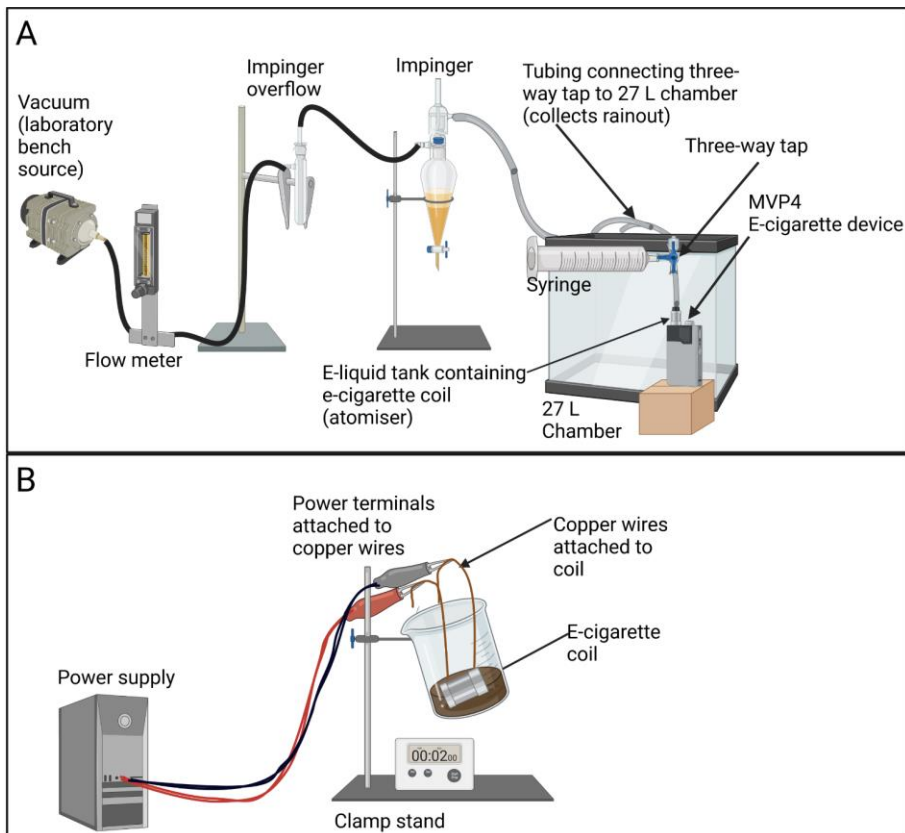
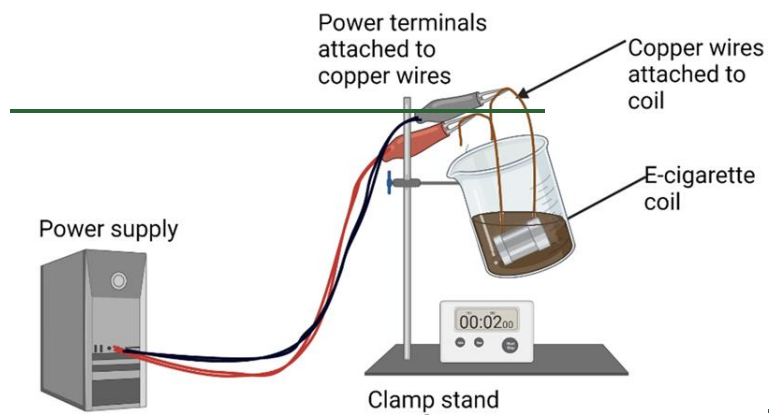


Figure 1. **Vaping set-ups. A. Typical vaping set up.** ~~Typical vaping set-up.~~ A vacuum drew air through the system at ~3 Lpm. The aerosol was drawn into a 60 mL syringe and a three-way tap was turned to allow the syringe to push the aerosol through two ~4 mm ID and ~15 cm tubing lengths and into the 27 L chamber for mixing. Air containing aerosol was drawn first into an impinger with 50:50 propylene glycol:glycerin base liquid. ~~Figure 2.~~B. **Accelerated vaping set-up.** The power supply was attached to copper wires which were attached to an e-cigarette coil (Kanthal BVC) which was ~~fully submerged at all times~~always submerged in e-liquid within a 100 mL beaker. Key differences between methods: 1) Aerosol is allowed to mix in air before capture rather than impinged immediately in liquid, 2) Volume of liquid is different: 3.5 mL is present in atomiser with typical method, compared to ~30 mL in beaker with accelerated method. Key similarities between methods: both methods apply the same amperage to the coil (and therefore heat coil to the same temperature).

525   **Figure 2. Accelerated vaping set up.** The power supply was attached to copper wires which were attached to an e-cigarette coil (Kanthal BVC) which was fully submerged at all times in e-liquid within a 100 mL beaker.

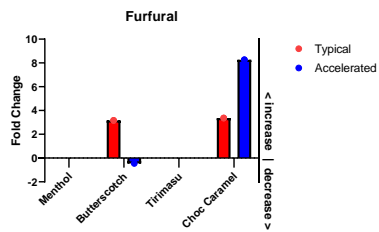
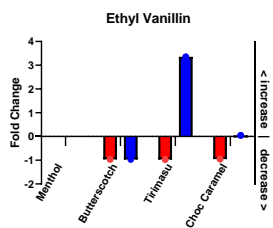
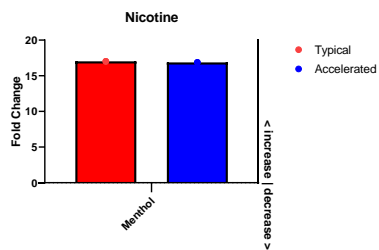
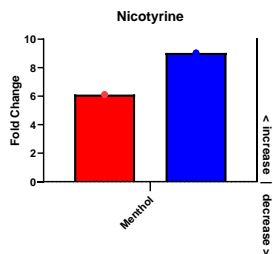
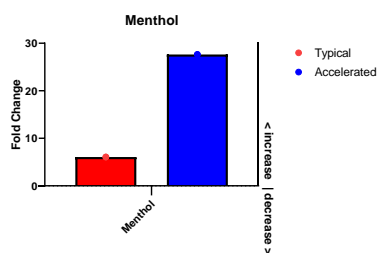
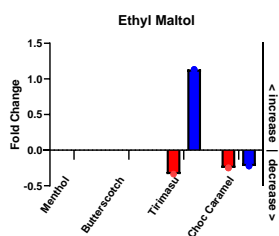
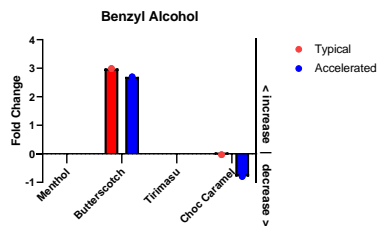
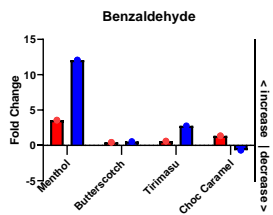
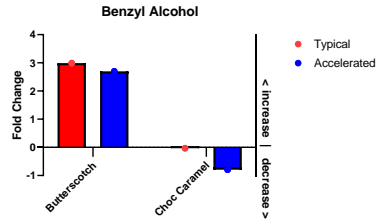
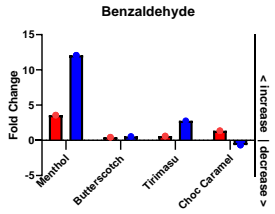
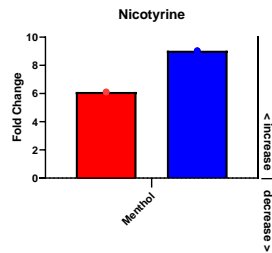
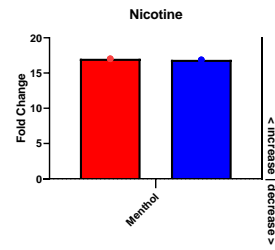
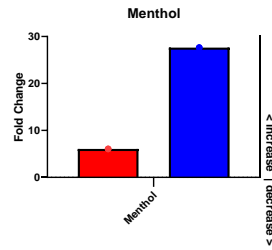
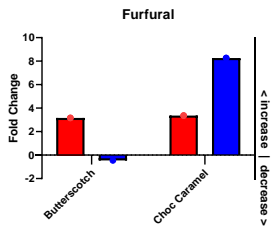
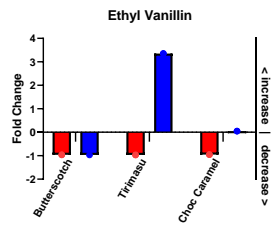
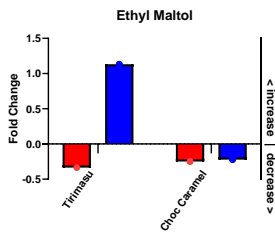



Figure 23. Fold change comparison between heating methods. E-liquid flavors are described on the X axis. Accelerated and Typical methods are indicated by the blue and red respectively. Y axis indicates the fold change compared to unheated i.e. Fold change = (Y-X)/X, where X is the unheated sample (mg/L concentration) and Y is the heated sample (mg/L concentration). Note different scales. Values that fall below the horizontal line at zero indicate a decrease in concentration from unheated sample, values above zero are an increase from unheated. If any chemical was not detected in a particular flavor then those flavors are not shown (e.g. there was no ethyl maltol detected in the menthol or butterscotch flavor).

Table 1. Assessment of 13 different chemical compounds from four e-liquids in both unheated and heated (accelerated and typical vaping methods). Unheated sample is comprised of only e-liquid in "fresh" or un-vaped form. Heated sample for typical method assessed the leftover from the atomiser tank and accelerated method assessed heated e-liquid produced from a coil submerged in e-liquid. Chemicals are listed in alphabetical order. GreyBlue shading = indecrease from unheated sample., Orange shading = increase from unheated sample. Acc = "accelerated" or "aged". Uu = undetected., Italicized results indicate that the compound was not present in one of the heating method samples and therefore fold change analysis was not possible for that chemical in that e-liquid flavor.italicized results indicate presence of an undetected sample and exclusion from analysis. Numbers are accurate to three significant figures (digits) and values less than zero are displayed with logarithmic scale for ease of reading.

Chemical Tested (mg/L)	Menthol unheated	Menthol Heated (acc)	Menthol Heated (typical)	Butter-scotch unheated	Butter-scotch Heated (acc)	Butter-scotch Heated (typical)	Tiramisu unheated	Tiramisu heated (acc)	Tiramisu heated (typical)	Choc Caramel unheated	Choc Caramel heated (acc)	Choc Caramel heated (typical)
Benzaldehyde	9.87×10^{-3}	1.29×10^{-1}	4.51×10^{-2}	1.49	2.33	2.16	6.24×10^{-1}	2.48	1.06	1.49	2.81×10^{-1}	2.05
Benzyl Alcohol	u	u	u	13.40	49.53	53.46	*345*	*u*	*285*	238	49.53	231
Ethyl Maltol	u	u	u	u	u	u	339	723	225	891	693	669
Ethyl Vanillin	u	u	u	45.65	1.46	1.63	747	3250	23.73	1660	1740	76.98
Eugenol	u	u	u	u	u	u	*1.89×10^{-1}*	*u*	*1.25×10^{-1}*	u	u	u
Furfural	u	u	u	1.73×10^{-1}	9.38×10^{-2}	7.21×10^{-1}	6.57×10^{-1}	*u*	*8.68×10^{-1}*	5.16×10^{-1}	4.78	2.25
Menthol	32.54	932	230	*u*	*30.18*	*11.83*	u	u	u	u	u	u
Nicotine	4.77×10^{-1}	8.53	8.60	u	u	u	u	u	u	u	u	u
Nicotyrine	2.70×10^{-4}	2.71×10^{-3}	1.92×10^{-3}	u	u	u	u	u	u	u	u	u
Thymol	u	u	u	u	u	u	u	u	u	u	u	u
Trans-cinnamaldehyde	u	u	u	u	u	u	u	u	u	*u*	*13.39*	*u*
2-chloro-phenol	u	u	u	u	u	u	u	u	u	u	u	u
4-(4-methoxyphenyl)-2-butanone	u	u	u	u	u	u	u	u	u	u	u	u