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18 11 20

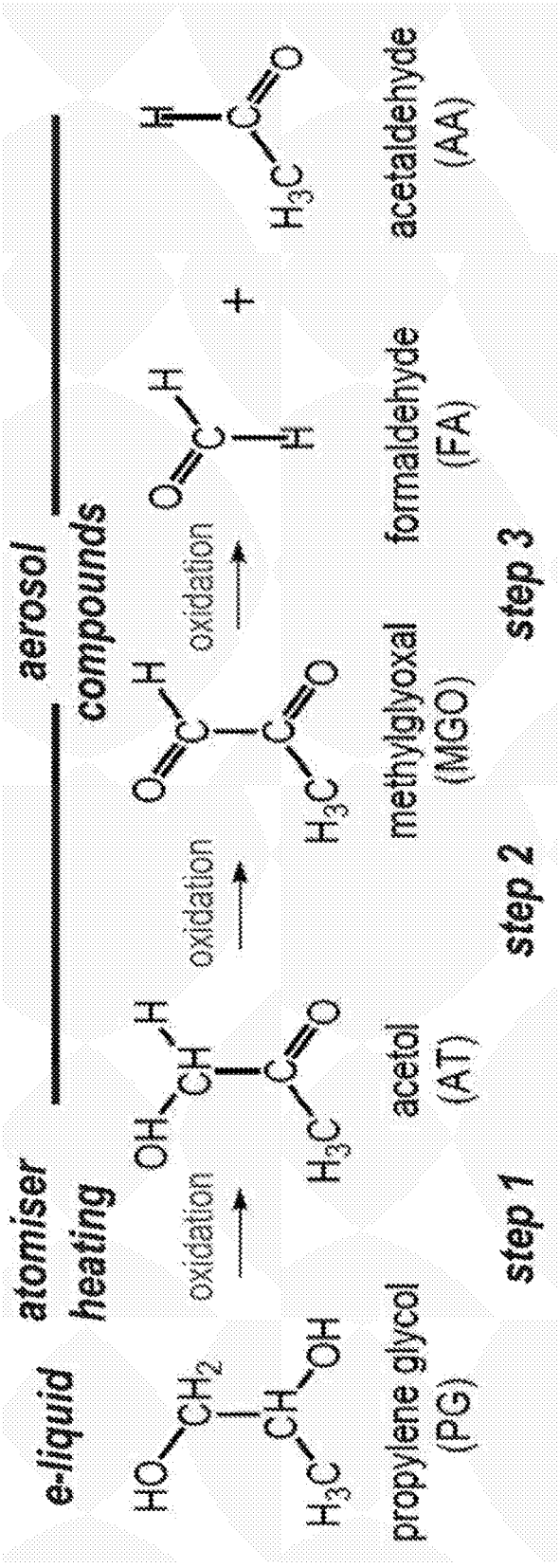

FIGURE 1

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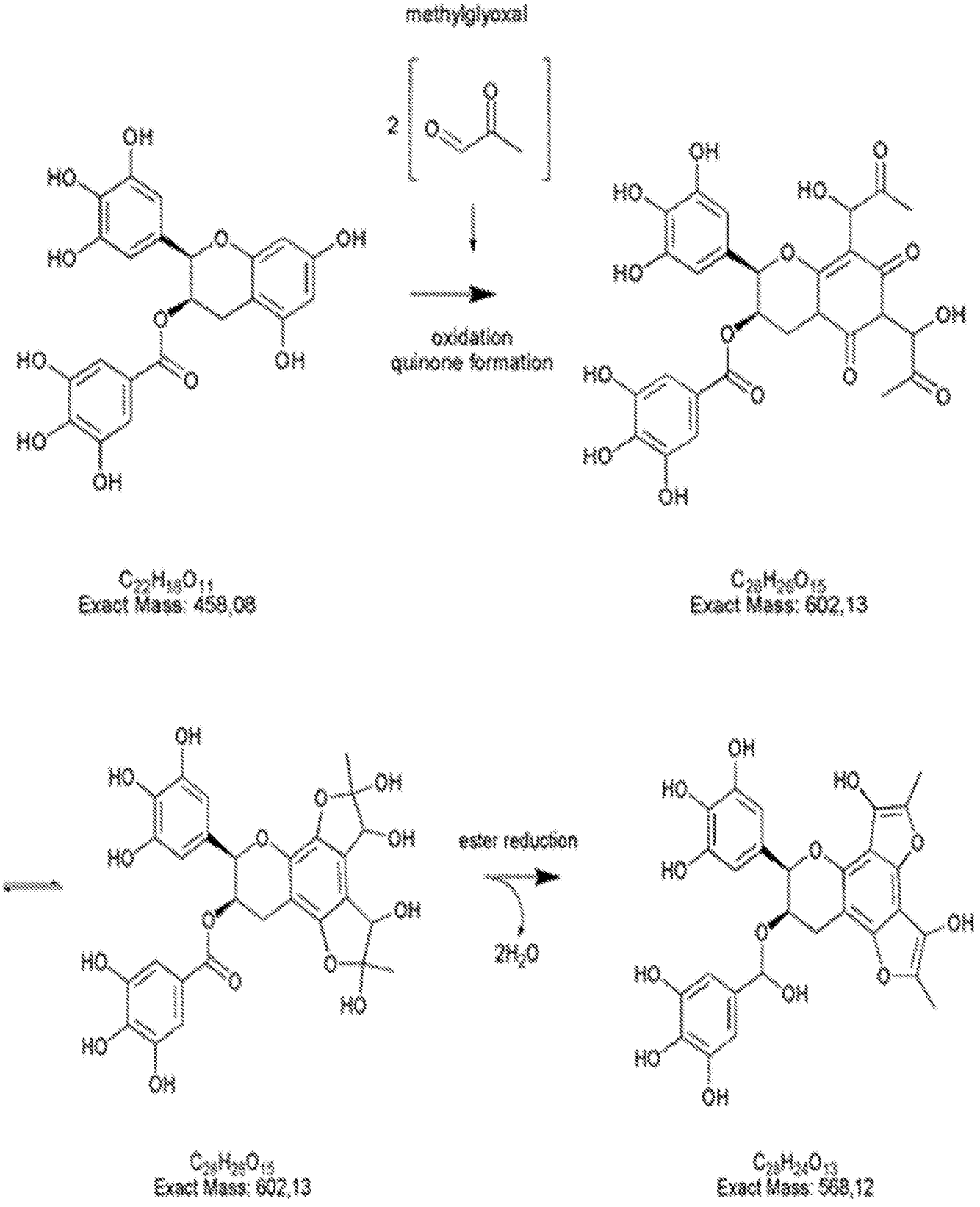

Figure 2

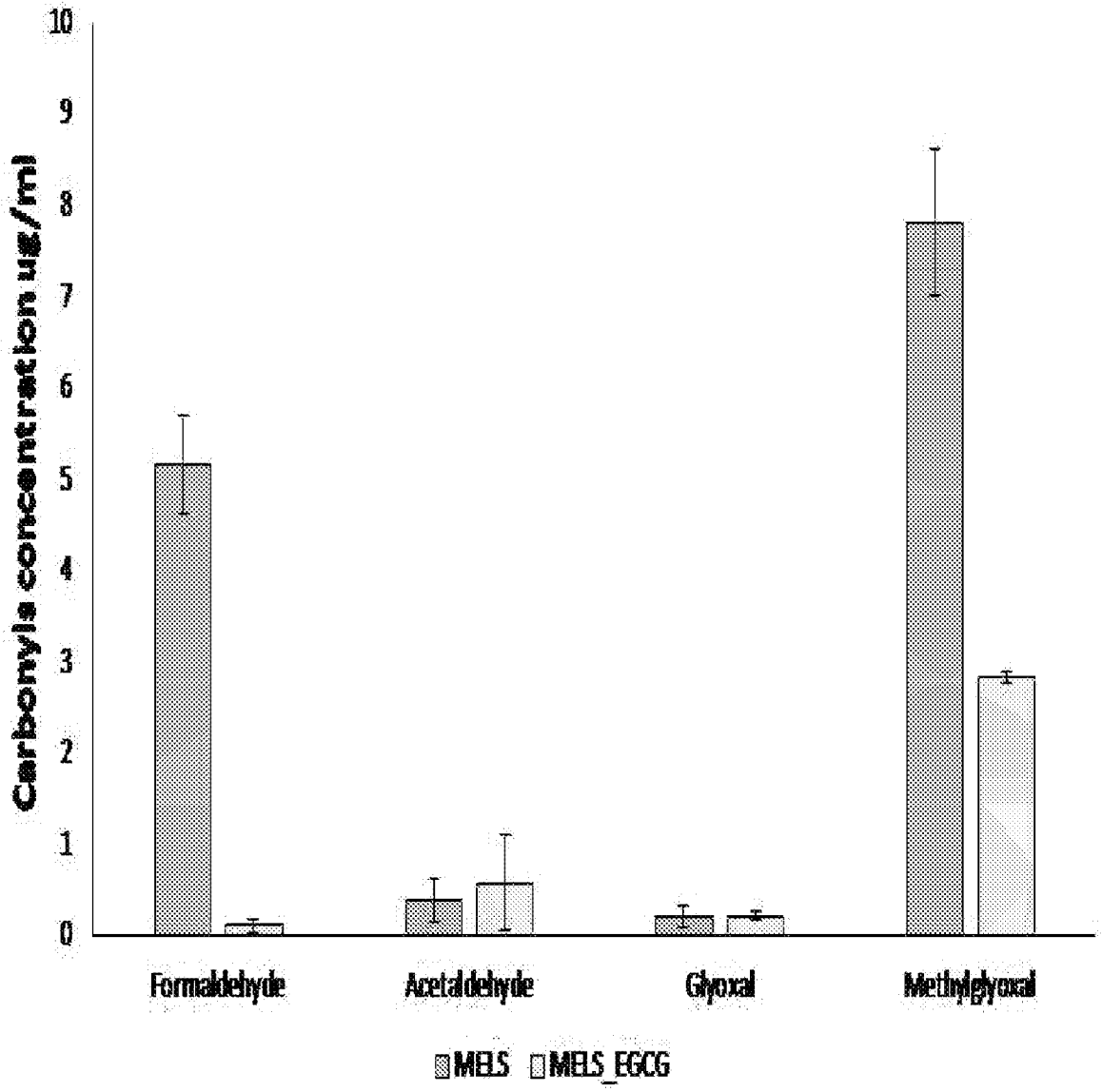

FIGURE 3

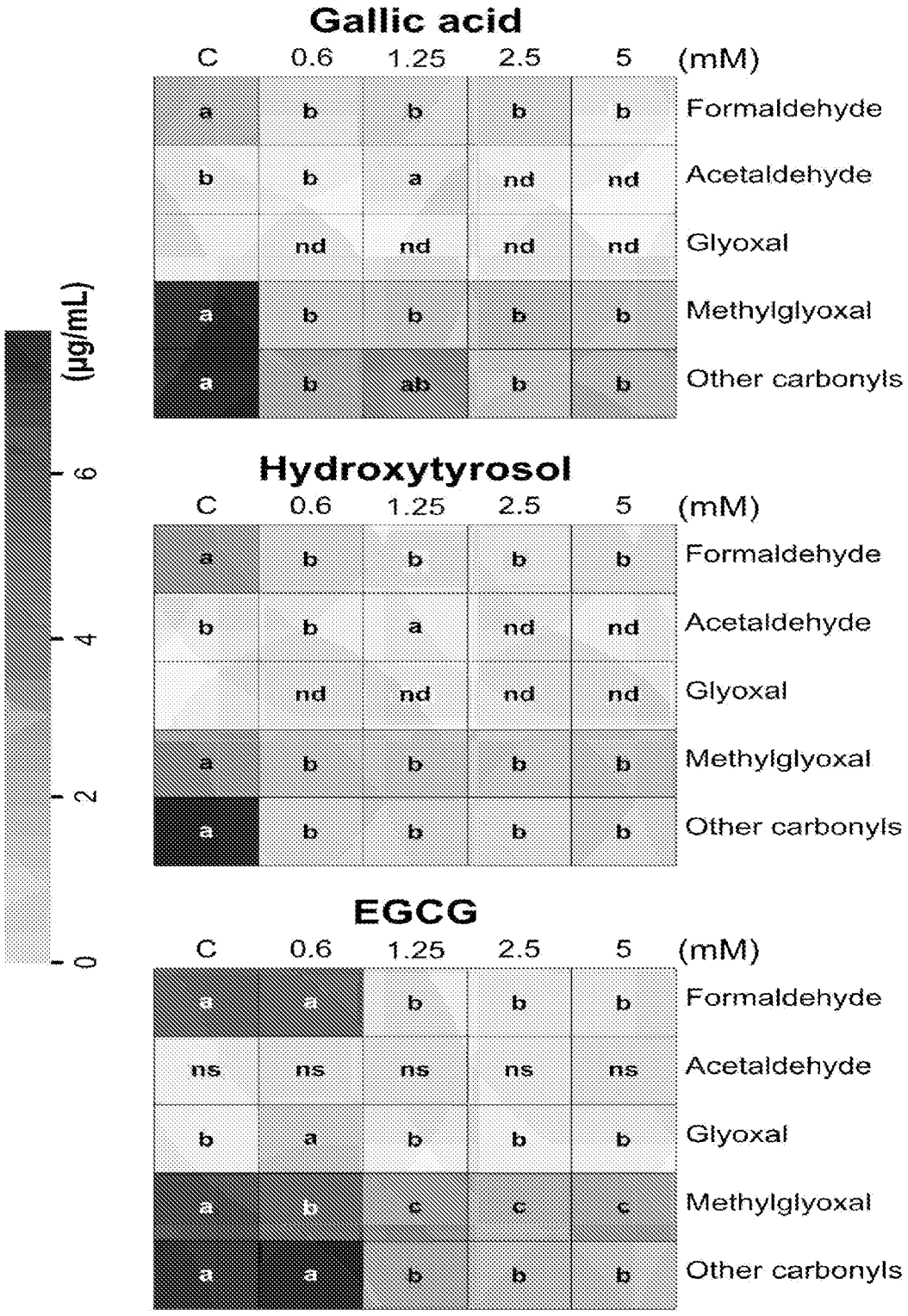

FIGURE 4

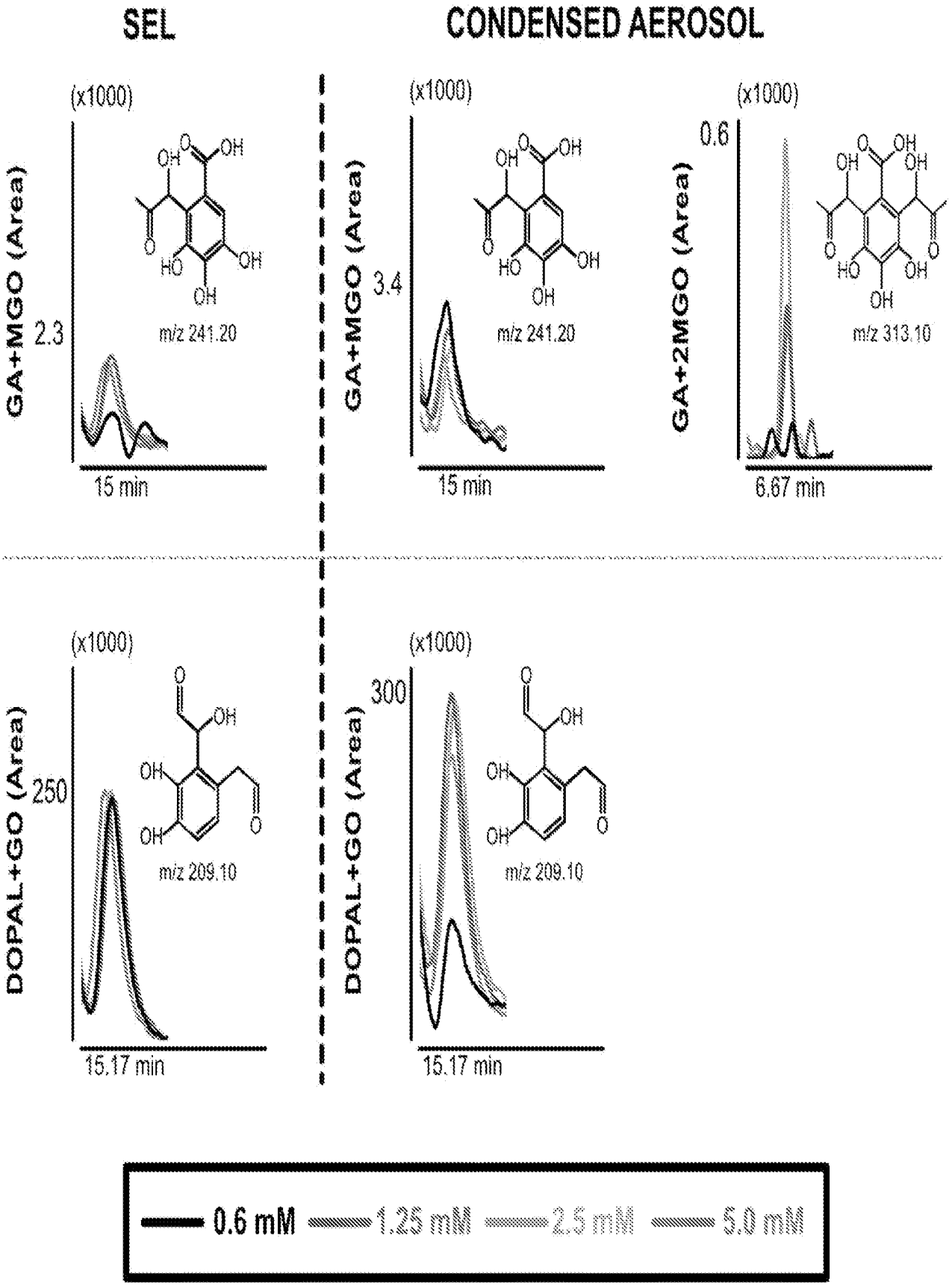

FIGURE 5

E-LIQUID

[001] The present invention relates to an e-liquid for forming an aerosol in a vaping device, such as an e-cigarette.

[002] The World Health Organisation estimates that about half of all lifetime smokers will die of a smoking-related disease, and 91% of these deaths will be directly attributable to cancer, cardiovascular or respiratory disease (Eriksen, M. P.; Mackay, J.; Ross, H. (2012) The Tobacco Atlas. 4th ed.; American Cancer Society; New York, NY (www.TobaccoAtlas.org)). While smoking rates continue at high levels, strategies to alleviate the toll on global health (approximately 6 million deaths/year) must address the toxic components that are implicated in these diseases.

[003] Electronic cigarettes ("e-cigarettes") are promoted as a means of delivering nicotine at similar levels to tobacco combustion while not exposing the user to the same levels of toxic compounds. E-cigarettes are handheld battery-powered vaporizers that simulate smoking but without burning tobacco. Using an e-cigarette is commonly referred to as "vaping", because instead of cigarette smoke the user inhales an aerosol or vapour generated from a liquid solution, commonly referred to as an "e-liquid", which is atomised by a heating element within the e-cigarette.

[004] In the last decade, since gaining a significant foothold in the nicotine delivery market, vaping electronic cigarettes has become accepted in the United Kingdom as a reduced exposure alternative to smoking and a potential route to smoking cessation, including by Public Health

England and the UK National Health Service, as well as almost every major public health stakeholder in the UK.

[005] The available evidence indicates that e-cigarette emissions are highly variable, which in turn leads to variations in modelled health risks. In the case of cancer, it has been shown that the cancer potency of cigarette smoke generated by machine testing of global cigarette brands (under the Health Canada protocol) varies over approximately one order of magnitude, whereas the same measure for e-cigarettes varies over six orders of magnitude (Stephens, W.E. (2018) Comparing the cancer potencies of emissions from vapourised nicotine products including e-cigarettes with those of tobacco smoke. Tobacco Control 27:10-17). Those e-cigarettes at the higher end of the potency distribution have potencies close to tobacco smoke, whereas those at the lower end have cancer potencies similar to nicotine inhalers, medically approved for smoking cessation. Treating e-cigarettes as a single entity in public health policy can thus be misleading.

[006] The risks of long-term vaping are not known, and very few experts believe the risk of physical harm to users is zero. Reducing the quantities of harmful compounds while maintaining the level of nicotine delivery and other sensory compounds that make vaping acceptable to many smokers could lead to products with lower cancer potencies (approaching the low risks of medicinal nicotine inhalers), and reduced potential for cardiovascular and respiratory diseases.

[007] Multiple factors influence the major carcinogens and toxicants contribution to e-cigarette emissions, most

important among which are (a) the device and its components, (b) the e-liquid being vaped, and (c) user topography including device settings (particularly those that determine the power supplied to the heating element) and the way the liquid is vaped. The e-liquid may contain toxicants such as tobacco specific nitrosamines that are residues from the extraction of nicotine from tobacco (synthetic nicotine is more expensive). More importantly, harmful compounds may be created during the process of vaping by oxidation and dehydration processes involving the main excipients of e-liquids: glycerol (GL) and glycols (e.g. propylene glycol (PG), trimethylene glycol polyethylene glycol) nicotine and some flavourings. GL and PG rapidly oxidise on vaping to a range of compounds including the reactive carbonyl species (RCS) formaldehyde and acetaldehyde.

[008] RCS are electrophiles which react with nucleophilic sites in important macromolecules such as DNA and proteins, leading to dysfunction and cytotoxicity. Reducing the RCS content of e-cigarette emissions will thus substantially reduce the long-term risk of cancer, and will also contribute to reducing the risk of cardiovascular disease in which formaldehyde is significantly implicated. There is also evidence that RCS act as irritants and may play significant roles in respiratory disease.

[009] In summary, it is widely accepted that RCS, especially formaldehyde and acetaldehyde, are likely to be among the most potent vaping-related toxicants and carcinogens. Reducing the concentrations of these compounds in e-cigarette emissions is thus important for public health, because smokers who have turned to vaping for health

reasons could reduce their exposure to toxicants and carcinogens still further. Currently, few if any practical solutions to this aspiration exist, with the most effective strategy at present being the advice to users not to vape their e-liquids at high power device settings in order to avoid high formaldehyde emissions. However, for many users this reduces the delivery of nicotine and may not provide the desired sensory effect, and consequently the advice may not be widely heeded. No chemical solution is currently available.

[0010] The present invention seeks to provide a solution to the problem of the reduction of vaping-related toxicants and carcinogens.

[0011] According to the present invention in a first aspect there is thus provided an e-liquid for forming an aerosol in a vaping device which comprises an inhibitor for inhibiting the formation of RCS (reactive carbonyl species) within the e-liquid and/or the aerosol formed therefrom, the inhibitor comprising a polyphenol compound.

[0012] Herein, the term "inhibitor" refers to a substance which slows down or effectively prevents a particular chemical process (i.e. the formation of RCS in the context of the present invention).

[0013] The e-liquid of the present invention can mitigate the risks to the health of vapers attributable to RCS inhaled with vaping device emissions, by inhibiting the formation of these compounds both before and during vaping through the use of a non-toxic inhibitor compound that readily traps these compounds. The e-liquid of the present

invention also has an improved shelf-life over prior art e-liquids, by inhibiting autooxidation of e-liquid components, for example PG, leading to the formation of formaldehyde and acetaldehyde during storage.

[0014] It is to be noted that the e-liquid of the present invention is suitable for use with e-cigarettes and other vaping devices, such as electronic cigars, pipes, shisha sticks and the like.

[0015] The present invention thus provides an e-liquid which comprises an inhibitor for inhibiting the formation of RCS within the e-liquid and/or the aerosol produced therefrom during vaping. Herein, the terms "RCS" and "reactive carbonyl species" refer to molecules comprising at least one carbonyl group (C=O) susceptible to attack by a nucleophile. The RCS may be a dicarbonyl molecule, for example of the chemical formula (I) R^1-(C=O)-(CH_2)n-(C=O)-R^2 wherein R^1 and R^2 are independently hydrogen or a substituted or unsubstituted aliphatic group, and n is 0 or an integer. For example n may be 0 or an integer from 1 to 6, i.e. 1, 2, 3, 4, 5 or 6. R^1 and R^2 may both be hydrogen, or may both be a substituted or unsubstituted aliphatic group. R^1 may be a substituted or unsubstituted aliphatic group and R^2 may be hydrogen. RCS include α-oxoaldehydes, such as glyoxal, methylglyoxal, hydroxypyruvaldehyde, erythrosone, 3-deoxyerythrosone, ribosone, 3-deoxyribosone, glucosone, 1-deoxyglucosone, 3-deoxyglucosone, and 3,4-dideoxyglucosone-3-ene. The inhibitor used in the e-liquid of the present invention preferably inhibits the formation of one or more of the dicarbonyls glyoxal, methylglyoxal and their aldehyde oxidation products formaldehyde and acetaldehyde.

[0016] The inhibitor used in the e-liquid of the present invention to inhibit the formation of the RCS comprises a polyphenol compound.

[0017] Herein the term "polyphenol" refers to a compound having more than one hydroxyl (-OH) group attached to a benzene or other arene ring. Examples of polyphenols thus include gallic acid, pyrogallol, hydroxytyrosol, homovanillic acid, stilbenes, lignans and flavinoids. Natural products which contain polyphenols include teas, such as green tea, coffee, various fruits (including for example apples, plums, kiwi fruit, cherries, berries), nuts, red wine, legumes, vegetables, and olive oil.

[0018] Herein the term "flavinoids" refers to flavinoids, isoflavinoids and neoflavinoids, derived from 2-phenylchromen-4-one (2-phenyl-1,4-benzopyrone), 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) and 4-phenylcoumarin (4-phenyl-1,2-benzopyrone) respectively. Flavinoid subgroups include flavonols, flavanols, flavones, flavanones, isoflavones, anthocyanidins and anthoxanthins. Flavonols include quercetin, kaempferol, rutin, myricetin and isorhamnetin. Flavanols include catechins (compounds derived from a 3-hydroxyl-2-phenylbenzopyrone structure, such as epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate) and theaflavins (including theaflavin, theaflavin-mono-gallate and theaflavin-digallate). Flavones include luteolin and apigenin. Flavanones include hesperetin, naringenin and eriodictyol. Isoflavones include genistein, daidzein and glycitein. Anthocyanidins include cyanidin, delphinidin, malvidin,

pelargonidin, peonidin and petunidin. Anthoxanthins include quercetin.

[0019] Preferred inhibitors for use in the e-liquid of the present invention include gallic acid, pyrogallol, homovanillic acid, hydroxytyrosol and epigallocatechin gallate.

[0020] Preferably, the inhibitor may be present in the e-liquid in a concentration from 0.01 to 1.0mM, for example 0.05 mM to 0.5 mM, including 0.06, 0.125, and 0.25mM.

[0021] The other components making up the e-liquid may be as are conventional in the art. As referred to above, the main excipients of e-liquids are glycerol (GL) and glycols, such as propylene glycol (PG, propane-1,2-diol), trimethylene glycol (propane-1,3-diol), nicotine, flavourings, and water. Typically, the e-liquid comprises approximately 90 wt% or more, for example 90 to 95 wt%, glycol and glycerol (e.g. composed of from 50 to 70 wt% glycol and 25 to 40 wt% glycerol), with the remaining components (e.g. from 5 to 10 wt%) typically being flavourings, nicotine and other additives. E-liquids may contain or not contain nicotine, although the majority of e-liquids currently on the market do contain some level of nicotine. Flavourings may contain menthol, sugars, esters and pyrazines. For example, sugars are used to provide a sweet flavour, diacetyl, acetoin and 2,3-pentadione are used for buttery flavouring, and camphor and cyclohexanone are used for minty flavouring. Many different flavourings are available.

[0022] According to the present invention in a second aspect there is provided a vaping device comprising an e-liquid of

the first aspect of the present invention. As noted above, the vaping device may be an e-cigarette, e-cigar, pipe, shisha stick or the like.

[0023] According to the present invention in a third aspect there is provided a method for inhibiting the formation of RCS in an aerosol produced from an e-liquid in a vaping device, which method comprises adding an inhibitor to the e-liquid, wherein the inhibitor comprises a polyphenol compound. The polyphenol compound used in the method of the third aspect of the present invention is preferably as described herein for the first aspect of the invention.

[0024] According to the present invention in a fourth aspect there is provided an e-liquid according to the first aspect of the present invention for use in the prevention or inhibition of cancer, cardiovascular disease, and/or respiratory disease caused by the inhalation of an aerosol produced from the e-liquid in a vaping device, wherein the e-liquid composition comprises an RCS inhibitor comprising a polyphenol compound.

[0025] Embodiments of the e-liquid of the first aspect of the present invention will now be described in detail with reference to the accompanying drawings, in which:

Figure 1 shows the model pathway from PG in an e-liquid to RCS compounds;

Figure 2 shows the structures of (-)-Epigallocatechin gallate (EGCG), methylglyoxal (MGO), and EGCG-2MGO adduct;

Figure 3 shows the results of measuring RCS concentrations in a model e-liquid system (MELS) with and without the addition of EGCG inhibitor, as described in Example 1;

Figure 4 shows a heatmap of the concentrations of RCS in the aerosols of vaped e-liquid embodiments according to the present invention, as described in Example 2; and

Figure 5 shows the formation of adducts between polyphenol compounds and dicarbonyls in e-liquid and aerosol condensate samples from e-liquid embodiments according to the present invention, as described in Example 2.

[0026] Thus, Figure 1 shows the model pathway from PG in an e-liquid to toxic RCS compounds. On vapourising in the atomiser (step 1) PG oxidises to acetol (AT) which in turn oxidises to the dicarbonyl RCS methylglyoxal (MGO) at step 2. MGO further oxidises to formaldehyde (FA) plus acetaldehyde (AA) at step 3. In this scheme AT, MGO, FA and AA are all components of the aerosol. The dicarbonyl MGO and the compounds with a single carbonyl group (FA and AA) are all associated with toxic effects, FA strongly so.

[0027] In the e-liquid of the present invention, the polyphenol-containing inhibitor diverts the oxidation sequence before step 3 and creates a non-toxic molecule from MGO while preventing the formation of two further toxic molecules (FA and AA). For this reason, the inhibitor is present in the e-liquid and remains available in the aerosol until MGO is formed, with which the inhibitor then forms adducts. It is to be noted that other pathways to RCS involving glycerol (GL) and the dicarbonyl glyoxal (GO) are

not shown in Figure 1 but may also be reduced in concentration by the inhibitor.

[0028] As noted above, the inhibitor used in the e-liquid of the present invention comprises a polyphenol compound, and a preferred compound is (-)-Epigallocatechin gallate (EGCG, UPAC name [(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl) chroman-3-yl] 3,4,5-trihydroxybenzoate). Figure 2 shows the structures of EGCG and MGO. MGO forms adducts with EGCG at the C-6 and C-8 positions of the A ring (Sang, S. et al. (2007) Chem. Res. Toxicol. 20, 1862-1870) resulting in adducts of EGCG-MGO on either C-6 or C-8, as well EGCG-2MGO on both C-6 and C-8 as illustrated. The formation of MGO and GO at step 2 of the pathway shown in Figure 1 may be involved singly or collectively in forming adducts at the two favourable sites (C-6 and C-8 on the A ring). The main adducts are EGCG-MGO, EGCG-2MGO, EGCG-GO, EGCG-2GO, EGCG-MGO-GO. The extent and sites of adduct formation have been shown in the literature to be functions of the composition (ratio of EGCG:MGO/GO) and pH. Other preferred polyphenol compounds for use in the present invention include gallic acid and hydroxytyrosol, as is described further with reference to Example 2 below.

[0029] All materials used in the following Examples were obtained from SigmaAldrich.

Example 1

[0030] A model e-liquid system (MELS) was created based on analyses of commercial products, and comprised 67% PG, 23% GL and 10% water (all % by weight). An e-liquid composition according to the first aspect of the present invention was

also created, in which the water component of MELS was replaced by a dilute solution of EGCG (5 mM) made from pure EGCG and MilliQ deionized water (MELS-EGCG).

[0031] Samples of MELS and MELS-EGCG were vaped on a laboratory vaping simulator under the following conditions. An e-cigarette using a 180 mL syringe pump was vaped using the modified Health Canada protocol (4 second puff of 55 mL every 30 seconds), sampling 50 puffs in total. The emitted aerosol was collected by trapping the condensed fraction of aerosol particulates within a mesh of pure silica fibres ("silica wool") formed into a plug contained within a 10 mL polypropylene syringe. The aerosol condensate was recovered from the silica plug by centrifuging the plug at 4700 rpm, and the recovered condensate was stored at -20°C until ready for analysis. The samples of condensate were analysed by first derivitising the condensate liquid with DNPH (2,4-dinitrophenylhydrazine) for carbonyls and OPD (o-phenylenediamine) for dicarbonyls, and then analysing the carbonyl and dicarbonyl species using HPLC.

[0032] Figure 3 shows the results of the testing. As shown, the condensate of the emission from MELS-EGCG shows a reduction of carbonyls and dicarbonyls compared with the control sample of MELS. In the oxidation model shown in Figure 1, it thus seems that oxidation beyond step 3 has been inhibited to a significant extent.

Example 2

[0033] An e-liquid formulation was prepared by combining propylene glycol, glycerol and Milli-Q water and used as a model e-liquid system. Formulations were gravimetrically

prepared by weighing each component in ratio 70:20:10, respectively. This e-liquid model system was used as control sample and compared to e-liquid formulations in which water was replaced by standard polyphenol-containing solutions. Gallic acid, hydroxytyrosol and epigallocatechin gallate were prepared at four concentrations (0.6, 1.25, 2.5 and 5 mM) in Milli-Q water. Each e-liquid formulation was then vortexed for 1 minute, sonicated for 3 minutes to remove bubbles of air and stored at 4°C until further use.

[0034] Laboratory vaping and aerosol collection was performed as follows. A Subox Mini C device (KangerTech, Shenzhen, China) was used as a recent generation vaping device. This device is popular among vapers and has the advantage that the atomising coil can be easily removed for visual inspection between vaping runs without disturbing the tank containing the e-liquid. The pump, solenoids and atomiser power supply and controller are part of the Gram Universal Vaping Machine package (UVM, Gram Health Inc., USA). The coil received adequate supply of e-liquid throughout the run by creating holes in the cotton wrapping around the coil which was found to reduce the incidence of wick deposits or burning (Stephens, W.E.; de Falco, B.; Fiore, A. A strategy for efficiently collecting aerosol condensate using silica fibres: Application to carbonyl emissions from e-cigarettes. Chem. Res. Toxicol. 2019). Aerosol collection and run conditions were carried out according to the methods used in that paper: the aerosol was trapped in a plug of amorphous silica fibres (0.75 g of 4 µm diameter) within a 10 mL syringe inserted between the e-cigarette mouthpiece and the pump of the vaping machine. The atomiser was vaped with a power of 30W to create high temperatures in the 1.5-ohm coil to generate excess carbonyl production.

Twenty-five puffs of 55 mL were drawn over four seconds and repeated every 30 seconds. Condensate collection from the silica plug was achieved by centrifuging the 10 mL syringe within a 50 mL centrifuge tube at 4700 rpm for five minutes. The condensate was immediately sealed and stored at -20 °C until required. In order to minimize contamination, five coils were used to run experiments, one for all control samples and the others for gallic acid, hydroxytyrosol, and epigallocatechin gallate samples.

[0035] Details of HPLC-UV and HPLC-ESI-MS/MS techniques used to analyse carbonyl and dicarbonyl compounds are given below.

[0036] All samples were analysed in triplicate and results are presented as mean values ± standard deviation. Data were analysed by ANOVA using XLSTAT (version 2014.5.03, Addinsoft, NY). Significant differences between the samples with a confidence interval of 95% were determined using Tukey's multiple range test.

[0037] The polyphenol compounds gallic acid, hydroxytyrosol, and epigallocatechin gallate used in the e-liquid formulations reduced the concentration of RCS in the aerosol condensate, and the results are shown in Figure 4. Figure 4 is a heatmap showing the concentration of carbonyls and dicarbonyls in the aerosols of vaped e-cigarettes after the addition of the polyphenol compounds. The addition of the polyphenol compounds reduces carbonyl and dicarbonyl concentration in the aerosols. Data were subjected to one-way ANOVA and significant differences ($P \leq 0.05$) between means were determined using Tukey's test. In this connection, the different lowercase letters in

Figure 4 indicate whether or not differences between experiments are statistically significant, the use of the same letters between experiments of the same polyphenyl compound and RCS indicating no significant difference when statistical analysis is applied. Each block is the mean value of three replicates ± SE, n=3. (C=control (model e-liquid system); EGCG=epigallocatechin gallate; nd=not detected; ns=not significant).

[0038] Thus, in the gallic acid e-cigarette model system (0.6, 1.25, 2.5 and 5 mM), the concentration of formaldehyde, acetaldehyde, methylglyoxal, and other carbonyls decreased up to 99.6%, 100%, 82.2, and 76%, respectively, compared with the control model system, with similar results shown for the hydroxytyrosol (formaldehyde, methylglyoxal and other carbonyls) and epigallocatechin gallate (formaldehyde, methylglyoxal, and other carbonyls) model systems. Formaldehyde and acetaldehyde were the main carbonyls identified and quantified.

[0039] The formation of adducts between the polyphenol compounds and RCS was also studied, using liquid chromatography tandem mass spectrometry, in particular the formation of adducts between the polyphenol compounds and MGO or GO. Searches were made for putative chemical structures of adducts and obtained structural information starting with selected ion monitoring (SIM). Table 1 below shows mass spectrometric data of the main adducts identified along with their corresponding retention times, as well as precursor and product ions, and Figure 5 shows the abundance of each adduct in model systems.

[0040]

Table 1				
Adduct	RT(min)	$[M-H]^-$(m/z)	Fragment ions(m/z)	Collision energy (V)
Gallic acid (GA)				
GA+MGO	15.11	241.20	209.25	15
GA+2MGO	6.67	313.10	75.05, 91.05	43, 15
Hydroxytyrosol (HT)				
DOPAL+GO	15.17	209.10	153.15, 79.00	14, 22
Epigallocatechin gallate (EGCG)				
EGCG+2MGO	30.66	567.10	299.15	15
	31.16	567.10	299.15	15

[0041] For gallic acid and hydroxytyrosol two adducts and one adduct respectively were identified. For the hydroxybenzoic acid derivative, both mono- and di-methylglyoxal adducts were identified. In the case of mono-methylglyoxal adducts (gallic acid + methylglyoxal), one major peak was identified (Figure 5). The molecular ion was 241 $[M-H]^-$ and the fragment ion 209 $[M-H]^-$, indicating the loss of two hydroxyl groups. The mono-methylglyoxal adduct with gallic acid was evident in both liquid and vaped samples, and the di-methylglyoxal adduct was present in vaped samples (Figure 5). Regarding hydroxytyrosol, the one identified adduct related to its degradation product 3,4-dihydrophenyl-acetaldehyde (DOPAL) (see Table 1), that underwent electrophilic aromatic substitution(see Table 1).

[0042] Regarding epigallocatechin gallate derivatives, a putative precursor ion at m/z $[M-H]^-$ 567 was identified indicating a preliminary adduction with two MGO molecules ($C_{28}H_{26}C_{15}$, exact mass 602.13) followed by oxidation into

quinone and finally an intramolecular aldol condensation yielding the formation of two additional rings, and the final dehydration of two hydroxyl groups to introduce molecular unsaturation (see Figure 2). This precursor ion generated a main fragment at m/z 299 $[M-268-H]^-$, which is fully compatible with the chemical structure hypothesized.

[0043] Relevant components of the model e-liquids (propylene glycol, glycerol and water) degraded prior to vaping and the addition of the polyphenol compounds led to the formation of adducts with dicarbonyls. The same adducts were also identified in the vaped condensate. This suggests that RCS have formed, in part or in whole, in the e-liquid prior to vaping. The addition of appropriate polyphenol compounds to the e-liquid thus limits the production of RCS prior to vaping, during vaping-related heating, and/or in the aerosol.

[0044] The present invention thus demonstrates the ability of polyphenol compounds to trap RCS, and thus reduce the levels of harmful carbonyls to which vapers are exposed, with the consequent potential public health benefit. The e-liquid of the present invention also has an improved shelf-life over prior art e-liquids, by inhibiting autooxidation of e-liquid components, for example PG, leading to the formation of formaldehyde and acetaldehyde during storage.

Method and apparatus - HPLC-UV analysis of carbonyl compounds

[0045] Quantification of carbonyl compounds was carried out according to the industry-standard method (CORESTA method 74) with some modifications (CORESTA, 2018). Both e-liquid

and condensed vapour were diluted in acetonitrile (1:10) and 50 µl of each sample was derivatised with 10 µl of 0.02 M 2,4-dinitrophenylhydrazine (DNPH) for 25 minutes to allow the formation of DNPH-adducts. Samples were stabilized with Trizma® base solution (acetonitrile/aqueous Trizma 80:20) and analysed using high performance liquid chromatography (HPLC) coupled to a UV detector. Chromatographic separations for both carbonyls and dicarbonyls were achieved using a Raptor ARC-18 (150 x 4.6 mm, 2.7 µm; Thames Restek, Saunderton, UK) column set at 40°C. The HPLC-DAD system consisted of a Thermo Scientific Dionex UltiMate 3000 system (Fisher Scientific, Loughborough, UK), composed of a degassing device, an ASI-100 automated sample injector and a PDA-100 photodiode array detector set at 365 nm. Separation of carbonyls was achieved by injecting 5 µl of derivatised sample using a flow rate of 0.6 mL/min and an elution gradient composed of two solvents: ultra-high purity water (A) and acetonitrile mixed with methanol (1:14 v/v) (B): 0 min 70% B; 10 min 75% B; 16 min 90% B; 16.01min 100% B; 17 min 100% B. Limits of quantification for formaldehyde, and acetaldehyde were 0.171 and 0.135 µg/mL, respectively, in accordance to Stephens et al. 2019 (referred to above).

Method and apparatus - HPLC-UV analysis of dicarbonyl compounds

[0046] Dicarbonyl compounds were identified and quantified as described by Hellwig et al., 2010 (Hellwig, M.; Degen, J.; Henle, T. 3-Deoxygalactosone, a "new" 1, 2-dicarbonyl compound in milk products. J. Agric. Food Chem. 2010, 58, 10752-10760) with the following modifications: 100 µl of diluted sample (1:10) was mixed with 30 µl of 0.2% *o*-PD in

9.6 mM EDTA solution and 30 µl of phosphate buffer solution (0.4 M, pH 7.0) in order to derivatise methylglyoxal and glyoxal into 2-methylquinoxaline (2-MQx) and quinoxaline (Qx), respectively. Samples were then incubated at 37°C for 3 hours and analysed. Separation of dicarbonyls was achieved on a Raptor ARC-18 (150 x 4.6 mm, 2.7 µm; Thames Restek, Saunderton, UK) column by injecting 20 µl of derivatised sample using a flow rate of 0.8 mL/min and an elution gradient composed of two solvents: 0.075% acetic acid (A) and acetonitrile (B) in the following order: 5 min 2% B, 22 min 70% B, 25 mir. 70% B, 28 min. All compounds were identified by comparison with pure reference standards. Calibration curves for dicarbonyls were prepared in the range 0.06 - 3.00 µg/mL in acetonitrile. Limits of quantification for methylglyoxal and glyoxal were 0.03 and 0.03 µg/mL respectively. Results were expressed as µg/mL.

Method and apparatus - HPLC-ESI-MS/MS analysis

[0047] High performance liquid chromatography - electrospray ionisation - mass spectrometry (HPLC-ESI-MS/MS) was carried out using a Shimadzu Nexera X2 system coupled to a LCMS 8040 triple-quadrupole mass spectrometer (Shimadzu Europa GmbH, Duisburg, Germany) according to the method described by Navarro and Morales (Navarro, M.; Morales, F.J. Mechanism of reactive carbonyl species trapping by hydroxytyrosol under simulated physiological conditions. Food Chem. 2015, 175, 92-99). Samples were separated on a Raptor ARC-18 column (150 × 4.6 mm, 2.7 µm; Thames Restek Ltd, Saunderton, UK) and the column temperature was set to 40°C. The column was eluted at a flow rate of 0.3 mL/min with a solvent gradient consisting of water acidified with 0.5% acetic acid (solvent A) and methanol (solvent B).

Solvent B was increased linearly from 2% at zero time to 60% at 1.5 min, followed by a further linear ramping to 98% at 30 min, held isocratically for 5 min, brought to initial conditions (2% solvent B) in 1 minute and kept constant for a further 4 minutes to re-equilibrate the column. A 5 µL aliquot of sample or standard compound was injected for each run and elution profiles were detected using multiple reaction monitoring (MRM). For MS detection, ESI was carried out in negative mode and MS settings were optimised through direct infusion of samples using the method optimisation function of the LabSolutions software (Shimadzu Europa GmbH, Duisburg, Germany). Automatic procedure included the following steps: scanning, optimisation and adjustment of precursor ions, autoselection of product ions with consequent optimisation of collision energy (CE) with increments of 5V, and finally optimisation for Q1 and Q3 voltages. The ion source settings were as follows: nebulizing gas flow: 3 L/min; desolvation line (DL) temperature: 250°C; heat block temperature: 400°C; drying gas flow: 15 L/min; while collision-induced dissociation (CID) gas and collision energy were set at 17 kPa and 5-35 V, respectively. Data acquisition and analysis was performed using the LabSolutions software.

[0048] It will be appreciated that the specific embodiments described herein are for illustrative purposes only, and that further modifications and variations of the embodiments are possible without departing from the scope of the present invention as defined by the appended claims.

CLAIMS

1. An e-liquid for forming an aerosol in a vaping device which comprises an inhibitor for inhibiting the formation of reactive carbonyl species within the e-liquid and/or the aerosol formed therefrom, wherein the inhibitor consists of at least one polyphenol compound.

2. An e-liquid according to claim 1 herein the reactive carbonyl species includes at least one of a dicarbonyl compound, formaldehyde and acetaldehyde.

3. An e-liquid according to claim 2 wherein the dicarbonyl compound has the chemical formula (I) $R^1-(C=O)-(CH_2)n-(C=O)-R^2$ wherein R^1 and R^2 are independently hydrogen or a substituted or unsubstituted aliphatic group, and n is 0 or an integer.

4. An e-liquid according to claim 2 or 3 wherein the dicarbonyl compound is an α-oxoaldehyde.

5. An e-liquid according to claim 4 wherein the α-oxoaldehyde includes at least one of glyoxal, methylglyoxal, hydroxypyruvaldehyde, erythrosone, 3-deoxyerythrosone, ribosone, 3-deoxyribosone, glucosone, 1-deoxyglucosone, 3-deoxyglucosone, and 3,4-dideoxyglucosone-3-ene.

6. An e-liquid according to any preceding claim wherein the reactive carbonyl species includes glyoxal and/or methylglyoxal.

7. An e-liquid according to any preceding claim wherein the at least one polyphenol compound includes at least one compound selected from gallic acid, pyrogallol, homovanillic acid, hydroxytyrosol, flavinoids, isoflavinoids and neoflavinoids.

8. An e-liquid according to any preceding claim wherein the at least one polyphenol compound includes at least one compound selected from flavonols, flavanols, flavones, flavanones, isoflavones, anthocyanidins and anthoxanthins.

9. An e-liquid according to any preceding claim wherein the at least one polyphenol compound includes at least one flavonol compound selected from quercetin, kaempferol, rutin, myricetin and isorhamnetin.

10. An e-liquid according to any preceding claim wherein the at least one polyphenol compound includes at least one flavanol compound including at least one catechin selected from epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate and/or theaflavin compound selected from theaflavin, theaflavin-mono-gallate and theaflavin-digallate.

11. An e-liquid according to any preceding claim wherein the at least one polyphenol compound includes at least one flavone compound selected from luteolin and apigenin.

12. An e-liquid according to any preceding claim wherein the at least one polyphenol compound includes at least one flavanone compound selected from hesperetin, naringenin and eriodictyol.

13. An e-liquid according to any preceding claim wherein the at least one polyphenol compound includes at least one isoflavone selected from genistein, daidzein and glycitein.

14. An e-liquid according to any preceding claim wherein the at least one polyphenol compound includes at least one anthocyanidin compound selected from cyanidin, delphinidin,

malvidin, pelargonidin, peonidin and petunidin, and/or the anthoxanthin compound quercetin.

15. An e-liquid according to any preceding claim wherein the at least one polyphenol compound is selected from at least one of gallic acid, pyrogallol, homovanillic acid, hydroxytyrosol and epigallocatechin gallate.

16. An e-liquid according to any preceding claim wherein the at least one polyphenol compound includes epigallocatechin gallate.

17. An e-liquid according to any preceding claim wherein the inhibitor is present in the e-liquid in a concentration from 0.01 to 1.0mM, preferably 0.05 mM to 0.5 mM.

18. An e-liquid according to any preceding claim which comprises glycerol and at least one glycol.

19. An e-liquid according to claim 18 wherein the glycol is selected from at least one of propylene glycol, trimethylene glycol, and polyethylene glycol.

20. An e-liquid according to claim 18 or 19 wherein the at least one glycol and glycerol make up 90 wt% or more of the e-liquid composition.

21. An e-liquid according to any preceding claim for use with e-cigarettes, electronic cigars, pipes, and/or shisha sticks.

22. An e-liquid according to any preceding claim for use with an e-cigarette.

23. A vaping device comprising an e-liquid according to any one of claims 1 to 21.

24. A vaping device according to claim 23 which is an e-cigarette.

25. A method for inhibiting the formation of reactive carbonyl species in an aerosol produced from an e-liquid in a vaping device, and/or in the precursor e-liquid prior to vaping which method comprises adding an inhibitor to the e-liquid, wherein the inhibitor consists of at least one polyphenol compound.

26. A method according to claim 25 wherein at least one polyphenol compound is as defined in any one of claims 7 to 16.

27. An e-liquid according to any one of claims 1 to 22 for use in reducing exposure to compounds associated with cancer, cardiovascular disease and/or respiratory disease caused by the inhalation of an aerosol produced from the e-liquid in a vaping device.