Alterations of pulmonary surfactant function by e-cigarette vapour

Emma Graham[1,2*], Lynda McCaig[1,2], Gloria Shui-Kei Lau[1], Akash Tejura[1], Anne Cao[1], Yi Y. Zuo[3,4], Ruud Veldhuizen[1,2,5*]

[1]Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada

[2]Lawson Health Research Institute, London Health Sciences Centre, London, Ontario, Canada

[3]Department of Mechanical Engineering, University of Hawaii at Manoa, Honolulu, Hawaii, United States of America

[4]Department of Pediatrics, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, United States of America

[5]Department of Medicine, University of Western Ontario, London, Ontario, Canada

* Emma Graham
E-mail: egraha59@uwo.ca (EG)

*Ruud Veldhuizen
E-mail: rvelhui@uwo.ca (RV)

Abstract

E-cigarette (EC) and vaping use continue to remain popular amongst teenage and young adult populations, despite several reports of vaping associated lung injury. This popularity is due in part to the vast variety of appealing flavours and nicotine concentrations easily accessible on the market. One of the first compounds that EC vapour comes into contact within the lungs during a deep inhalation is pulmonary surfactant. This lipid protein mixture lines the alveoli, reducing surface tension and preventing alveolar collapse. Impairment of surfactant's critical surface tension reducing activity can contribute to lung dysfunction. Currently, information on how EC vapour impacts pulmonary surfactant remains limited. We hypothesized that exposure to EC vapour impairs the surface tension reducing ability of surfactant. BLES was used as a model surfactant in a direct exposure syringe system. BLES (2ml) was placed in a syringe (30ml) attached to an EC. Vapour was drawn into the syringe and then expelled, repeated 30 times. Biophysical analysis after exposure was completed using a constrained drop surfactometer (CDS). Minimum surface tensions increased after exposure to vapour. Variation in device used, addition of nicotine, or temperature of the vapour had no additional effect. Two e-liquid flavours, menthol and red wedding, had further detrimental effects, resulting in higher surface tension than the vehicle exposed BLES. Alteration of surfactant properties through interaction with vapour was observed with a basic e-liquid vehicle, however additional compounds produced by added flavourings appeared to be able to increase inhibition, however the exact mechanism remains unclear. In conclusion, EC vapour alters surfactant function through increases in minimum surface tension. This impairment can contribute to lung dysfunction and susceptibility to further injury.

53

54 # Introduction

55 Vaping and the use of e-cigarettes (ECs) has seen a marked increase over the last decade,
56 particularly amongst youth and young adults (1-3). Although originally regarded as a popular
57 smoking cessation tool, it is becoming increasingly clear that the target demographic for EC
58 marketing has shifted from smokers to teenagers and young adults (3). Most of this population
59 are not regular smokers, but instead are drawn to the attraction of efficient nicotine delivery and
60 the large variety of appealing flavours available for recreational use (1, 3). Unfortunately,
61 previous studies have indicated negative impacts of vaping (4). For example, in 2019 an outbreak
62 of e-cigarette or vaping associated lung injury (EVALI), resulting in over 2000 hospitalizations
63 and 68 deaths, was reported (5-7). Although studies associated the addition of vitamin E acetate
64 with the incidences of EVALI, vaping without the compound has still been linked to lung injury,
65 and there is not enough evidence to suggest vitamin E acetate to be the sole cause of injury (6, 8-
66 10). Clearly, more information regarding the impact of vaping on the lung is required.

67

68 The process of vaping involves an EC device that uses a battery powered coil (atomizer) which
69 heats an e-liquid comprised of solvents propylene glycol (PG) and vegetable glycerine (VG) to
70 produce an inhalable vapour. Complexity in studying the effects of this habit arise from the
71 multitude of vaping options. There are presently over 7000 commercially available e-liquid
72 flavours and almost 500 brands of ECs currently on the market (11, 12). Adding to this
73 complexity is the fact that many users make modifications to their device and/or generate their
74 own e-liquid. Furthermore, the harmful effects of vaping are not solely due to the impact of the
75 ingredients of the e-liquid since it has been demonstrated that the generation of the aerosol

76 results in the formation of a multitude of new products including formaldehyde and acetaldehyde
77 (13, 14). This effect may vary among different devices, with particulate matter reported to
78 increase with the evolution of some of the newer designs, thereby potentially increasing
79 hazardous effects to the health of vapers (4, 15). Thus, although the general concept of vaping is
80 relatively straightforward, complications in studying its effects arise from the variability and
81 customization of the vaping process.
82
83 From a respiratory perspective, one of the first substances that EC vapour encounters in the lungs
84 is pulmonary surfactant. Surfactant is made up of 85-90% by weight phospholipids, 5-10%
85 neutral lipids, mainly cholesterol, and 10% surfactant proteins, designated SP-A, SP-B, SP-C,
86 and SP-D (16, 17) . The primary function of surfactant is to reduce surface tension in the lung.
87 To achieve this, surfactant rapidly absorbs to the surface forming the surface film, to reach a
88 surface tension of ~23 mN/m, and subsequently reducing surface tension to ~0 mN/m upon
89 compression (18, 19). This function is essential for breathing as it allows the lung to inflate and
90 deflate with minimal changes in pressure, i.e., it maintains lung compliance, and prevents
91 atelectasis (17, 20). As can be deduced from its function, absence or alterations to pulmonary
92 surfactant can contribute to lung dysfunction. Previous studies have demonstrated that some
93 ingredients of e-liquids can interfere with the function of model systems of surfactant (21-23).
94
95 Based on the above information, the objective was to investigate the impact that e-cigarette
96 vapour has on the function of surfactant. We initially focused on the impact of the common
97 ingredients of vapour, VG and PG, prior to testing the impact of selected additives. Using *in*
98 *vitro* methods to replicate the dynamic compression and expansion of surfactant during

99 respiration, this study focused on the biophysical changes to surfactant after direct exposure of a
100 modified natural surfactant preparation to EC vapour. It was hypothesized that EC vapour
101 inhibits surfactant's ability to effectively reduce surface tension.
102

103 Methods

104 Materials

105 Bovine lipid extract surfactant (BLES) at 27mg/ml phospholipid concentration was obtained
106 from BLES biochemicals inc (London, ON) and was diluted to 2mg/ml in a buffer containing
107 0.9% NaCl, 1.5mM $CaCl_2$, and 2.5mM HEPES (pH 7.4). Three common vaping devices were
108 used. The Aspire Pockex with 0.6 ohm coil, and Vaporesso Gen with GT4 0.15 ohm coil, were
109 purchased from Vapour North (Pickering, ON). The Uwell Caliburn A2 with 0.9 ohm pre-
110 installed mesh coil was purchased from Fogged Up Vape Shop (London, ON). All e-liquids were
111 purchased from Vapour North.
112

113 Exposure to vapour

114 To expose surfactant to vapour, 2ml of BLES was placed in a 30ml syringe connected to an EC,
115 via silicone tubing. Vapour was drawn into the syringe and the syringe was placed on a rocker
116 set to rotate 15-20 times/min for a 30 second exposure period. The vapour was then expelled, and
117 this procedure was repeated 30 times to simulate 30 vapour inhalations. The control group
118 consisted of BLES (2ml at 2mg/ml) placed in a 30ml syringe exposed only to air, no vapour, and
119 placed on the rocker for the same amount of time as the vaped samples. The exposed BLES was

120 either used immediately for the analysis of surface tension reduction, or frozen at -20°C for
121 subsequent analysis.
122

123 Biophysical analysis of surfactant

124 A constrained drop surfactometer (CDS) was used to assess the surface tension reducing
125 properties of the BLES as previously described (24, 25). Briefly, an 8.5μL drop of the 2mg/ml
126 surfactant sample was placed on the pedestal in an environmentally controlled chamber at 37 ^{o}C.
127 A small capillary in the middle of the pedestal was attached to a computer controlled 2.5ml
128 Hamilton syringe (Hamilton Co., Reno, NV) filled with distilled water. Two minutes after
129 placing the BLES on the pedestal, the surface film of the drop was compressed and expanded via
130 the controlled movement of the water in and out of the pedestal via the syringe. A total of 20
131 compression/expansion cycles were completed at a frequency of 1 cycle/ 1.5 seconds, with an
132 area compression of approximately 20%. Aligned with the horizontal plane of the pedestal was a
133 camera which took rapid pictures of the samples (15 fps) as they underwent dynamic changes.
134 The images were then analysed by the Axisymmetric Drop Shape Analysis (ADSA) computer
135 software to obtain precise measurements of the surface tension and surface area of the samples
136 calculated based on the shape of the drop after the 2 minutes of adsorption as well as throughout
137 the repeated compression/expansion cycles (26).
138

139 Experimental design

140 Using the above procedure, a variety of exposure conditions were applied to surfactant followed
141 by biophysical analysis. Initial experiments tested the e-liquid vehicle composed of unflavoured,
142 nicotine free, e-liquid with a 50:50 VG:PG ratio using the Aspire Pockex. Subsequently, the

143 50:50 VG:PG liquid was tested in different devices, as well, other vehicle compositions (70:30
144 VG:PG and Max VG) tested with the Aspire Pockex device. The Vaporesso GEN EC was used
145 to examine the effects of temperature through adjustment of the wattage in the device settings,
146 with low (30W), medium (55W), and high (80W) temperatures. Of the three devices used,
147 resistance varied between models (0.6ohm, 0.9ohm, 0.15ohm), and due to the simplicity of the
148 Pockex and Caliburn models, atomizer temperature (through wattage) was only able to be
149 assessed with the Vaporesso device.
150
151 To determine the impact of vapourization, vehicle 50:50 VG:PG e-liquid was mixed directly
152 with BLES at 10 and 20% volumes, followed by analysis on the CDS.
153
154 To assess the impact of additives such as flavouring and nicotine to the vehicle, ten common
155 vapour e-liquids were tested: Vehicle + 18mg/ml nicotine, Juicy Burst + 3 mg/ml nicotine, RY4
156 Double (tobacco) + 18 mg/ml nicotine, RY4 Double (tobacco), Cigar Passion, Caramel
157 Macchiato, Pink Lemonade, Red Wedding, Cinnaswirl, Menthol, Wintergreen, and Cherry.
158 Experiments were performed in groups with multiple flavours, with each day of vapour
159 exposures including separate unexposed and vehicle exposed BLES controls.
160

161 Statistics analysis

162 Data was expressed as the mean ± standard error of the mean (SEM) with a minimum of three
163 independent replicates. Statistical analysis was performed using Graphpad Prism 9 software.
164 Comparison of surface tensions across compression/expansion cycles for each sample were
165 assessed using a two-way repeated measure analysis of variance (ANOVA) with a Bonferoni's

post hoc test. No significant differences between cycles were seen in samples except for the red wedding flavoured vapour. Minimum surface tensions at the twentieth compression cycle were compared between groups using an ordinary one-way ANOVA, or two tailed unpaired t-test (air vs vehicle). For red wedding and menthol vapours, minimum surface tensions at the first, fifth, tenth, and twentieth cycles were compared to vehicle and air control using an ordinary one-way ANOVA. P values less than or equal to 0.05 were considered statistically significant.

Results

Effect of vehicle vapour on surfactant function

Using the above protocol, BLES was exposed to vehicle (50:50 VG:PG) vapour and analyzed on the CDS. Representative surface tension versus relative area isotherms for the fifth and tenth compression/expansion cycle are shown in Figs 1A and 1B. At the fifth compression/expansion cycle, the air-exposed BLES sample shows a reduction in surface tension by compression to values below 5mN/m (lower curve) with subsequent expansion leading to an increase in surface tension with relative low amount of hysteresis (upper curve). In contrast, the vehicle vapour exposed group had higher surface tension after compression (Fig 1A). Similar results were observed at the tenth cycle (Fig 1B). The results of the analysis of the minimum and maximum surface tension of all compression/expansion cycles are shown in Figs 1C and 1D. The air control BLES sample demonstrates low minimal surface tensions starting at the first compression that decrease further over the first few cycles to values below 5mN/m. Compared to these low values observed in the air control group, minimal surface tension in the vehicle exposed BLES group were significantly higher, reaching minimal values of approximately 10mN/m. Maximum

188 surface tension of the control sample was approximately 37mN/m (Fig 1D), these values were
189 not significantly different than those observed in the vehicle exposed group.
190 Additional data obtained from this analysis is shown in Table 1. The surface tension after two
191 minutes of adsorption was not significantly different between the two groups. Furthermore, the
192 calculated values for the maximum area compression, as well as the area compression required to
193 reach minimum surface tension were also not significantly different between the two groups.
194
195 **Fig 1. Vehicle compression/expansion.** BLES exposed to vehicle (■) compared to air control
196 (○). **A.** Representative surface tension vs area isotherm at the fifth compression/expansion cycle.
197 **B.** Representative surface tension vs area isotherm at the tenth compression/expansion cycle. **C.**
198 Minimum surface tensions across 20 compression/expansion cycles. **D.** Maximum surface
199 tensions across 20 compression/expansion cycles. *P<0.05 compared to air control.
200
201 **Table 1. Adsorption and compression data**

	Air Control	Vehicle Exposed
Adsorption (mN/m)	24.11 ±0.11	24.57±0.17
Maximum Area Compression (%)	20.33±0.21	20.65±0.73
Area Compression at Min Surface Tension (%)	19.27±0.31	19.41±0.90

202 Average adsorption and percent compression for air control and vehicle exposed groups
203 After establishing surfactant inhibition by the vehicle vapour using a single experimental
204 condition, this observation was further explored by examining two additional vehicle
205 compositions, testing two other e-cigarette devices, examining three different wattages (i.e.,
206 temperatures) for vapour generation, and mixing with non-vapourized vehicle e-liquid. Fig 2A

207 displays minimum surface tension trends (mN/m ± SEM) for BLES exposed to vapour with each
208 of the three different ratios of vehicle composition. Compared to air control samples, which
209 reached low minimum surface tension values, 50:50 VG:PG vehicle achieved significantly
210 higher minimum surface tensions. 70:30 VG/PG and max VG exposed samples resulted in higher
211 minimum surface tensions than air control, however results were not significant. Minimum
212 surface tensions obtained with vapour from three different devices are shown in Fig 2B. Values
213 for each of the devices were significantly higher than the air control. Comparison among the
214 three EC devices revealed no statistically significant differences. A similar pattern was observed
215 when comparing vapour generated at three different wattages, as seen in Fig 2C. These wattages
216 impact the temperature of the generated vapour. The results show no difference among the
217 varying wattages, with all three vapour exposed samples resulting in significantly higher
218 minimum surface tension than the air control. After mixing with e-liquid volumes up to 20%, no
219 significant difference in minimum surface tension was observed compared to control, shown in
220 Fig 2D. Surface tensions after two minutes of absorption, maximum surface tensions, and
221 maximum area compression values were not significantly different between groups (data not
222 shown).
223
224 **Fig 2. Minimum surface tensions of experimental variables.** Minimum surface tensions (±
225 SEM) across 20 compression/expansion cycles for BLES exposed to three experimental
226 variables, compared to air control. **A.** Three different vehicle compositions with increasing VG
227 content. **B**. Three different EC devices. **C.** Three different wattages set on a programmable EC to
228 alter vapour temperature. **D.** BLES mixed with three different volumes of vehicle e-liquid. N=3
229 *P<0.05 compared to air control.

230

231 ## Effect of commercially available e-liquid vapours

232 Having established the effects of the vehicle vapour on surfactant, our subsequent experiment
233 evaluated the effect of vapour from a variety of e-liquids containing flavourings and/or nicotine.
234 Ten popular commercially available e-liquids were tested. Fig 3 summarizes the data by
235 displaying the minimum surface tension values obtained for the fifth compression cycle as
236 compared to its vehicle and air control. The values for each of the 20 compression/expansion
237 cycles are shown in the supplementary data (S1 Fig. A-I). Consistent with the above
238 experiments, in each of the tests the vehicle vapour exposure led to higher minimum surface
239 tension as compared to air-exposed BLES (Fig 3). With the exception of two of the e-liquids, all
240 flavours resulted in minimum surface tension that were not significantly different to the
241 unflavoured vehicle. This included samples with nicotine additives. The two exceptions to this
242 pattern were menthol and red wedding flavoured vapour, which resulted in significantly
243 increased minimum surface tension compared to both air control and the unflavoured vehicle
244 control.

245

246 **Fig 3. Minimum surface tensions of flavoured e-liquids.** BLES exposed to vapours
247 containing nicotine and/or flavouring additives compared to air control and unflavoured
248 vehicle at the fifth compression/expansion cycle (± SEM). N=3-4 *P<0.05 compared to
249 air control.

250

251 A further exploration of the data obtained from menthol and red wedding e-liquids is shown in
252 Fig 4 (Menthol) and Fig 5 (Red Wedding). Isotherms for the fifth (Fig 4A) and tenth (Fig 4B)

253 cycles show menthol exposed BLES to result in minimum surface tension at ~20 mN/m, higher
254 than the vehicle exposed BLES which achieved minimum surface tension of ~10 mN/m.
255 Menthol exposure resulted in significantly higher minimum surface tension than both
256 unflavoured vehicle and air control groups at all 20 compression/expansion cycles (Fig 4C).
257 Maximum surface tension remained consistent between groups, at ~35 mN/m. Isotherms at the
258 fifth cycle for red wedding exposed BLES, also show significant impairment, with minimum
259 surface tension at ~20 mN/m, higher than both the vehicle and air exposed groups (Fig 5A). At
260 the tenth cycle however, the red wedding flavour exposed group achieves minimum surface
261 tension similar to that of the vehicle exposed group (Fig 5B). A steep decline on a cycle-by-cycle
262 basis can be observed for the red wedding exposed group, until approx. the tenth cycle, where
263 surface tension remains similar to the vehicle exposed (Fig 5C). Both flavoured and vehicle
264 exposed BLES showed higher minimum surface tension than the air control. Red wedding
265 further appeared to have lower maximum surface tension during the first ten cycles, maintaining
266 values ~30 mN/m, whereas air control groups had an average maximum surface tension of ~40
267 mN/m (Fig 5D). The absorption and area compression required to reach minimum surface
268 tension was not significantly different among groups (data not shown).
269
270 **Fig 4. Menthol compression/expansion data**. BLES exposed to menthol flavoured
271 vapour (▲) compared to vehicle (■) and air control (○). **A.** Representative surface
272 tension vs area isotherm at the fifth compression/expansion cycle. **B.** Representative
273 surface tension vs area isotherm at the tenth compression/expansion cycle. **C.** Minimum
274 surface tensions (± SEM) across 20 compression/expansion cycles. **D.** Maximum surface

tensions (± SEM) across 20 compression/expansion cycles. (N=4) *P<0.05 compared to air control, #P<0.05 compared to vehicle.

Fig 5. Red wedding compression/expansion data. BLES exposed to red wedding flavoured vapour (▲) compared to vehicle (■) and air control (○). **A.** Representative surface tension vs area isotherm at the fifth compression/expansion cycle. **B.** Representative surface tension vs area isotherm at the tenth compression/expansion cycle. **C.** Minimum surface tensions (± SEM) across 20 compression/expansion cycles. **D.** Maximum surface tensions (± SEM) across 20 compression/expansion cycles. (N=3) *P<0.05 compared to air control.

Discussion

This study tested the hypothesis that EC vapour inhibits surfactant's ability to effectively reduce surface tension. Results supported this hypothesis as exposure to EC vapour resulted in impaired surfactant function, in particular its ability to reduce surface tension to near-zero values. All vapour exposed samples, regardless of additive, device, or vehicle composition resulted in higher minimum surface tensions than air exposed samples. Furthermore, surfactant dysfunction was observed in all cases of vapour exposure with different additives, and most of the flavours tested resulted in similar inhibition and minimum surface tensions to those of the vehicle. Interestingly, two flavours, menthol and red wedding, resulted in significantly higher minimum surface tensions compared to the vehicle. It is concluded that vapour exposure can lead to surfactant impairment which, *in vivo*, could impact lung function. This effect of vapour can be augmented due to certain flavour additives.

298

299 Previous studies have examined the effects of e-liquid aerosols on surfactant, with a variety of
300 experimental approaches. Distinctions in the type of surfactants used, with several studies using
301 surfactant-like lipid mixtures, as well as differences in exposure method including mixing of
302 surfactant with isolated components of the e-liquid directly, have been reported (21, 27, 28).
303 Furthermore, a variety of analyses, including structural and functional assessments have been
304 reported (23, 29). Building on these studies we used a modified natural surfactant, exposed the
305 samples to vapour, and focused on the main function of surfactant by measuring surface tension
306 reduction. Specifically, we used BLES, a well-established commonly prescribed exogenous
307 surfactant composed of the surfactant lipids and the hydrophobic proteins B and C (30). Vapour
308 exposure was performed in a syringe containing a surfactant suspension allowing direct exposure
309 of surfactant to the vapour. Analysis was done using the CDS, which was able to provide
310 extremely accurate measurements of changes in surface tension and droplet area during
311 consistent dynamic cycling with compression maintained within physiological levels (18). To
312 provide a broad assessment of the impact of vapour on surfactant, we used several commercially
313 available EC devices and multiple e-liquids to accurately reflect the vaping tools on the market
314 today. Overall, we deemed that this approach allowed for effective testing of our hypothesis.
315

316 One of the main goals of our study was to examine the effect of the base vehicle of e-liquid, VG
317 and PG, since these compounds are universally utilized during vaping. Although both
318 compounds are safe and frequently used humectants in a wide range of foods and consumer
319 cosmetic products (31-33), research on the effects of vapourization and inhalation of these
320 compounds is limited. Focusing on the effect of surfactant specifically, it has been previously

321 shown that EC vapour did not induce harmful effects on surfactant interfacial properties but
322 caused minor alterations in lateral structure arrangement after exposure (23). In this case
323 however, surfactant (Infasurf) was not directly exposed to the vapour, but rather the vapour was
324 bubbled into the subphase in a Langmuir-Blodgett trough before addition of the surfactant,
325 without measurement of aerosol concentrations. Alterations observed to the lateral structure are
326 nonetheless important to note, as proper arrangement of the surface film phospholipids are
327 essential to reaching near zero surface tensions upon compression (34). In two other studies,
328 impairment of surfactant function was observed. Using a lipid mixture as a model system of
329 surfactant it was observed that exposure to EC vapour resulted in lower maximum surface
330 pressures at any given area (higher minimum surface tensions) and altered compressibility on a
331 Langmuir balance (27). Further, direct mixing of non-aerosolized PG and VG with surfactant,
332 resulted in impaired surfactant function (increased surface tension amplitude, mean surface
333 tensions, and surface elasticity), particularly in ultra-high concentrations of PG dominant
334 mixtures (28). The current study builds upon these findings, by exposing a natural surfactant to
335 vapourized VG/PG and dynamic measurement of surfactant function. Furthermore, we showed
336 that the VG/PG induced reduction in surface activity was consistent among different devices and
337 different wattages for vapour generation. Together, these findings support a modification in
338 dynamic surface-active properties of surfactant due to vehicle vapour exposure.
339
340 Having established a general, albeit mild, inhibition of surfactant by EC vapour in general, our
341 second goal was to examine the effect of different flavored e-liquids. Rationally selecting
342 specific e-liquids among the 7000 flavours currently available is difficult, however, we picked
343 popular e-liquids in different categories (tobacco alternatives, drinks, candy, and dessert).

344 Interestingly, whereas most e-liquids showed similar results to vehicle, menthol and red wedding
345 resulted in further inhibition of surfactant function. The mechanism of inhibition of surfactant by
346 menthol has been recently studied and indicates a direct interaction of this flavouring with
347 surfactant phospholipids and proteins thereby impacting the surface tension reducing surface
348 film (29). The specific process by which red wedding flavouring impacts surfactant is not clear,
349 however, the pattern of inhibition in which high surface tensions are only observed in the early
350 compression/expansion cycles would suggest that chemicals are inserted into the surface film but
351 are removed, or squeezed out, during repeated compression and expansion (18, 35). In contrast,
352 it appears as if surfactant remains inhibited by menthol exposure across compression/expansion
353 cycles, suggesting a more permanent alteration of the surface film. Regardless of the specific
354 mechanism, the data clearly indicate that variability exist between the impact of vapours from
355 different e-liquids. Unlike in food and beverages, the chemicals in e-liquids are not highly
356 regulated, and not all the specific ingredients are disclosed. For example, a study examining the
357 chemical concentrations in JUUL pod-based e-liquids identified 59 chemicals, with menthol,
358 vanillin, and ethyl maltol having the highest concentrations, with the remaining 56 chemicals
359 having concentrations of <1.0mg/mL (36). Considering our data demonstrated enhanced
360 surfactant inhibition with 2 out of 12 e-liquid samples selected, we speculate that a substantial
361 fraction of the commercially available e-liquid flavours available have a negative impact on
362 surfactant function.
363
364 Further complicating the study of vapour interactions with the surfactant system is the generation
365 of various compounds during the vapourization process. For example, flavour aldehydes have
366 been shown to react causing the formation of PG acetals (37). Benzaldehyde (cherry flavouring),

367 ethylvanillin, vanillin, cinnamaldehyde, and citral were examined to determine reaction products
368 after vaping and were shown to produce PG acetals that remained stable for hours (37). It has
369 also been reported that vapourization, and subsequent degradation of VG/PG can lead to the
370 generation of harmful chemicals, including carbonyl compounds such as formaldehyde,
371 acetaldehyde, and acrolein, as well as volatile organic compounds, tobacco-specific
372 nitrosamines, metals, free radicals, and reactive oxygen species in EC aerosols (14, 38-40). In a
373 non-targeted study, over 115 volatile organic compounds were found in a single puff of vapour
374 (41). Further, compared to the composition of the non-aerosolized liquid, vapourization produced
375 more than 80 additional compounds beyond the 60 identified within the liquid itself (41). It has
376 also been reported that higher power EC devices increase reactive oxygen species production
377 (42). However, it should be noted that in the current study, no differences were observed
378 between three devices, as well as no significant differences between power levels (wattage) on
379 our highest power device (Vaporesso), suggesting that either the lowest wattage was able to
380 maximally effect the surface tension of the surfactant, or that reactive oxygen species production
381 was not a major contributor to the observed surfactant inhibition. Furthermore, no significant
382 differences were seen when mixing the non-vapourized e-liquid directly with BLES, indicating
383 the importance of vapourization for inhibition. Overall, the published data suggest that the
384 inhibition of surfactant by vapour may not only be the results of the incorporation of e-liquid
385 components but also due to additional components generated during vapourization. This includes
386 those generated from flavour additives, which can either insert into the surfactant and/or
387 chemically react with surfactant lipids and proteins. As such, it is important that future
388 regulations regarding vaping and e-liquid composition should base recommendations on studies
389 that have evaluated the health impact after vapour generation.

390

391 Although the current study examined the effects of vapour on surfactant *in vitro*, the rationale for
392 our experiments was the interaction of vapour with surfactant in the lung, thus it is important to
393 extrapolate our findings to the *in vivo* situation. Although we observed a slight decrease in
394 surface tension reducing ability after vapour exposure, such minor surfactant dysfunction may
395 not have a major impact *in vivo*. Nevertheless, a clinical study by Dr. Vardavas and colleagues
396 demonstrated that using an EC device for only five minutes led to an immediate, subtle, decrease
397 in lung mechanics in a group of volunteers (43). Considering the rapid nature of this effect on
398 lung mechanics, it would be consistent with a rapid direct interaction of the vapour with
399 surfactant, rather than, for example, a cellular mechanism. A second potential consequence of
400 altered surfactant function may be a susceptibility to lung injury. Thus, although vaping induced
401 alterations to surfactant may not be sufficient to cause lung dysfunction, in scenarios where the
402 surfactant is further challenged it maybe become more impaired and ultimately affect lung
403 function. For example, it has been shown that surfactant that contains lyso-phospholipids was
404 more susceptible to inhibition by serum proteins as compared to control (44). Future studies
405 examining the acute impact of vapour on surfactant *in vivo*, as well as susceptibility of vapour
406 exposed surfactant to further inhibition, are warranted.

407

408 Our study has several limitations. First the current study uses an exogenous surfactant, BLES, to
409 test the impact of vapour. All studies on the effect of vaping have utilized either exogenous
410 surfactant or an even more limited, lipid model system. The rationale for using exogenous
411 surfactant is that it is contains all surfactant lipids and the hydrophobic proteins, SP-B and C, and
412 has consistent functional properties. Nevertheless, future studies with natural surfactants from

413 animal and/or humans would provide additional information of the impact of the hydrophilic
414 surfactant protein A, as well as other components that co-isolate with surfactant obtained from
415 lung lavage. A second limitation is that only the effects of acute exposure on surfactant were
416 tested. People that vape will do so in a prolonged fashion which may have additional
417 consequences. For example, previous *in vivo* work on mice exposed daily to vapour for several
418 weeks showed altered lipid homeostasis in alveolar macrophages, as well as accumulation of
419 phospholipids in bronchoalveolar lavage fluid (22). The impact of vapour on surfactant *in vivo*
420 may thus be affected by acute direct effect as observed in the current experiments, as well as
421 prolonged effects related to the impact on surfactant synthesis and metabolism.
422

423 Conclusion

424 This study demonstrated that the vapour from e-liquid vehicle (VG/PG) was able to alter
425 surfactant function independent of nicotine or device style, with some flavourings providing
426 further inhibition. From these results we conclude that acute vapour exposure leads to
427 impairment of the pulmonary surfactant system through increases in minimum surface tension.
428 We speculate that this has potential detrimental effects in the *in vivo* scenario by contributing to
429 lung dysfunction and making the lung more susceptible to further injury.
430

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435 # References

436 1. Canada S. Canadian Student Tobacco, Alochol and Drugs Survey Published online 2019:
437 Dly; 2018-2019 [Available from: https://www.canada.ca/en/health-canada/services/canadian-
438 student-tobacco-alcohol-drugs-survey/2018-2019-detailed-tables.html.
439 2. Canada S. Canadian Tobacco and Nicotine Survey Published online 2020:19-22: Dly;
440 2019 [Available from: https://www.canada.ca/en/health-canada/services/canadian-tobacco-
441 nicotine-survey/2019-summary/2019-detailed-tables.html.
442 3. Lee SJ, Rees VW, Yossefy N, Emmons KM, Tan ASL. Youth and Young Adult Use of Pod-
443 Based Electronic Cigarettes From 2015 to 2019: A Systematic Review. JAMA Pediatr.
444 2020;174(7):714-20.
445 4. Miyashita L, Foley G. E-cigarettes and respiratory health: the latest evidence. J Physiol.
446 2020;598(22):5027-38.
447 5. Cao DJ, Aldy K, Hsu S, McGetrick M, Verbeck G, De Silva I, et al. Review of Health
448 Consequences of Electronic Cigarettes and the Outbreak of Electronic Cigarette, or Vaping,
449 Product Use-Associated Lung Injury. J Med Toxicol. 2020;16(3):295-310.
450 6. CDC. Outbreak of Lung Injury Associated with the USe of E-Cigarette, or Vaping,
451 Products [Available from: https://www.cdc.gov/tobacco/basic_information/e-
452 cigarettes/severe-lung-disease.html.
453 7. Landman ST, Dhaliwal I, Mackenzie CA, Martinu T, Steele A, Bosma KJ. Life-threatening
454 bronchiolitis related to electronic cigarette use in a Canadian youth. Cmaj. 2019;191(48):E1321-
455 e31.

456 8. DiPasquale M, Gbadamosi O, Nguyen MHL, Castillo SR, Rickeard BW, Kelley EG, et al. A
457 Mechanical Mechanism for Vitamin E Acetate in E-cigarette/Vaping-Associated Lung Injury.
458 Chem Res Toxicol. 2020;33(9):2432-40.
459 9. Xantus G, Anna Gyarmathy V, Johnson CA, Sanghera P, Zavori L, Kanizsai PL. The role of
460 vitamin E acetate (VEA) and its derivatives in the vaping associated lung injury: systematic
461 review of evidence. Crit Rev Toxicol. 2021;51(1):15-23.
462 10. Ponzoni L, Moretti M, Sala M, Fasoli F, Mucchietto V, Lucini V, et al. Different
463 physiological and behavioural effects of e-cigarette vapour and cigarette smoke in mice. Eur
464 Neuropsychopharmacol. 2015;25(10):1775-86.
465 11. Zhu S-H, Sun JY, Bonnevie E, Cummins SE, Gamst A, Yin L, et al. Four hundred and sixty
466 brands of e-cigarettes and counting: implications for product regulation. Tobacco Control.
467 2014;23(suppl 3):iii3-iii9.
468 12. Hsu G, Sun JY, Zhu S-H. Evolution of Electronic Cigarette Brands From 2013-2014 to
469 2016-2017: Analysis of Brand Websites. J Med Internet Res. 2018;20(3):e80.
470 13. Jensen RP, Luo W, Pankow JF, Strongin RM, Peyton DH. Hidden formaldehyde in e-
471 cigarette aerosols. N Engl J Med. 2015;372(4):392-4.
472 14. Kosmider L, Sobczak A, Fik M, Knysak J, Zaciera M, Kurek J, et al. Carbonyl compounds in
473 electronic cigarette vapors: effects of nicotine solvent and battery output voltage. Nicotine Tob
474 Res. 2014;16(10):1319-26.
475 15. Protano C, Avino P, Manigrasso M, Vivaldi V, Perna F, Valeriani F, et al. Environmental
476 Electronic Vape Exposure from Four Different Generations of Electronic Cigarettes: Airborne
477 Particulate Matter Levels. Int J Environ Res Public Health. 2018;15(10):2172.

478 16. Possmayer F. A proposed nomenclature for pulmonary surfactant-associated proteins.
479 Am Rev Respir Dis. 1988;138(4):990-8.
480 17. Goerke J. Pulmonary surfactant: functions and molecular composition. Biochim Biophys
481 Acta. 1998;1408(2-3):79-89.
482 18. Zuo YY, Veldhuizen RA, Neumann AW, Petersen NO, Possmayer F. Current perspectives
483 in pulmonary surfactant--inhibition, enhancement and evaluation. Biochim Biophys Acta.
484 2008;1778(10):1947-77.
485 19. Keating E, Zuo YY, Tadayyon SM, Petersen NO, Possmayer F, Veldhuizen RAW. A
486 modified squeeze-out mechanism for generating high surface pressures with pulmonary
487 surfactant. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2012;1818(5):1225-34.
488 20. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane
489 disease. AMA J Dis Child. 1959;97(5, Part 1):517-23.
490 21. Hayeck N, Zoghzoghi C, Karam E, Salman R, Karaoghlanian N, Shihadeh A, et al. Carrier
491 Solvents of Electronic Nicotine Delivery Systems Alter Pulmonary Surfactant. Chem Res Toxicol.
492 2021;34(6):1572-7.
493 22. Madison MC, Landers CT, Gu BH, Chang CY, Tung HY, You R, et al. Electronic cigarettes
494 disrupt lung lipid homeostasis and innate immunity independent of nicotine. J Clin Invest.
495 2019;129(10):4290-304.
496 23. Przybyla RJ, Wright J, Parthiban R, Nazemidashtarjandi S, Kaya S, Farnoud AM. Electronic
497 cigarette vapor alters the lateral structure but not tensiometric properties of calf lung
498 surfactant. Respir Res. 2017;18(1):193.

499 24. Valle RP, Wu T, Zuo YY. Biophysical Influence of Airborne Carbon Nanomaterials on
500 Natural Pulmonary Surfactant. ACS Nano. 2015;9(5):5413-21.
501 25. Milos S, Khazaee R, McCaig LA, Nygard K, Gardiner RB, Zuo YY, et al. Impact of
502 ventilation-induced lung injury on the structure and function of lamellar bodies. American
503 Journal of Physiology-Lung Cellular and Molecular Physiology. 2017;313(3):L524-L33.
504 26. Yang J, Yu K, Zuo YY. Accuracy of Axisymmetric Drop Shape Analysis in Determining
505 Surface and Interfacial Tensions. Langmuir. 2017;33(36):8914-23.
506 27. Davies MJ, Birkett JW, Kotwa M, Tomlinson L, Woldetinsae R. The impact of cigarette/e-
507 cigarette vapour on simulated pulmonary surfactant monolayers under physiologically relevant
508 conditions. Surface and Interface Analysis. 2017;49(7):654-65.
509 28. Sosnowski TR, Jabłczyńska K, Odziomek M, Schlage WK, Kuczaj AK. Physicochemical
510 studies of direct interactions between lung surfactant and components of electronic cigarettes
511 liquid mixtures. Inhal Toxicol. 2018;30(4-5):159-68.
512 29. Xu L, Yang Y, Simien JM, Kang C, Li G, Xu X, et al. Menthol in Electronic Cigarettes Causes
513 Biophysical Inhibition of Pulmonary Surfactant. Am J Physiol Lung Cell Mol Physiol. 2022.
514 30. Stockley E, Valotaire R, Miller M, Da Silva O. Effects of bovine lipid extract surfactant
515 administration in preterm infants treated for respiratory distress syndrome. Health Science
516 Reports. 2018;1(4):e34.
517 31. Fiume MM, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler D, et al. Safety
518 assessment of propylene glycol, tripropylene glycol, and PPGs as used in cosmetics. Int J Toxicol.
519 2012;31(5 Suppl):245s-60s.
520 32. C.F.R. Code of Federal Regulations 21CFR 182.1320, April, 1990 (2014) [

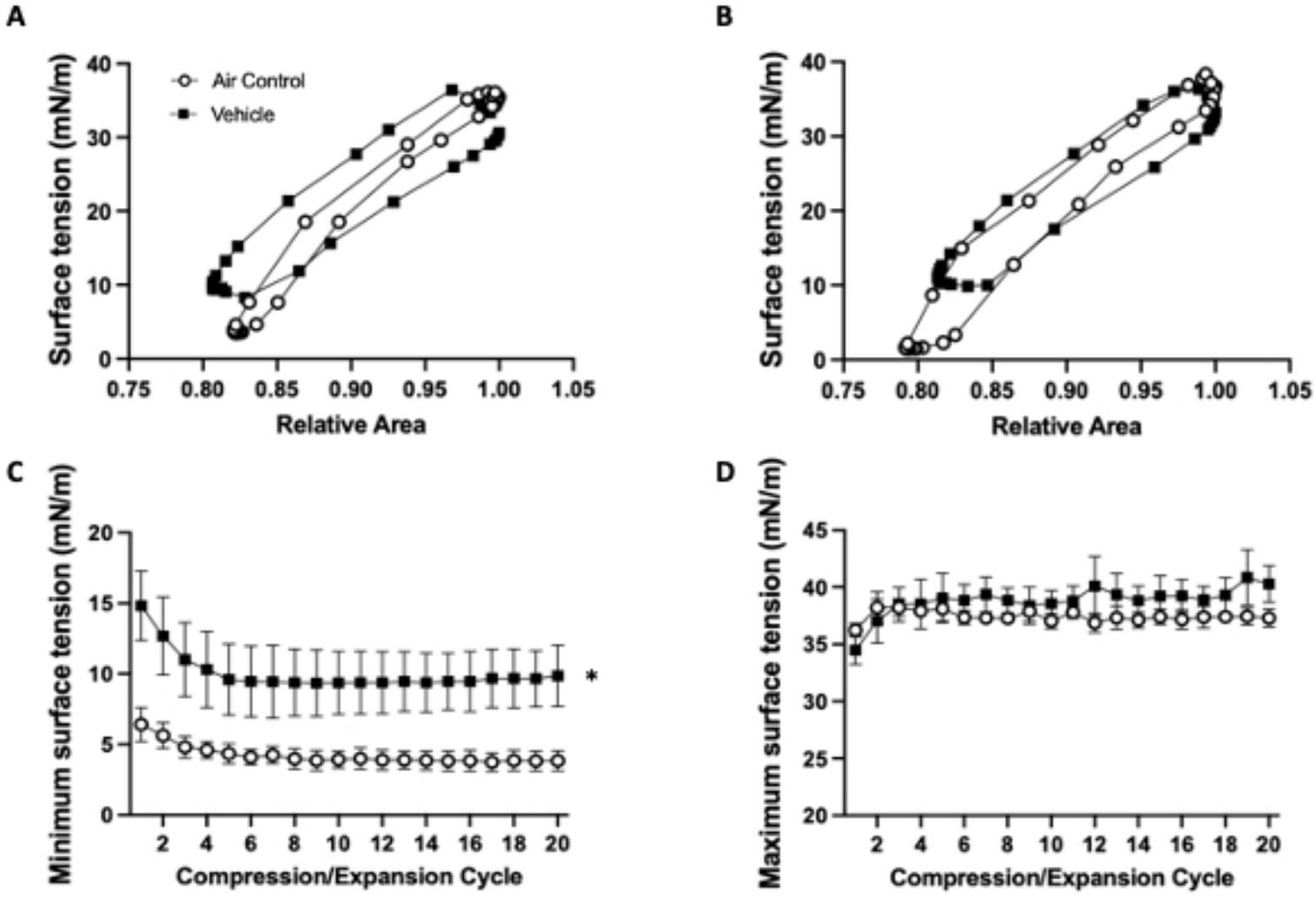
521 33. Phillips B, Titz B, Kogel U, Sharma D, Leroy P, Xiang Y, et al. Toxicity of the main
522 electronic cigarette components, propylene glycol, glycerin, and nicotine, in Sprague-Dawley
523 rats in a 90-day OECD inhalation study complemented by molecular endpoints. Food and
524 Chemical Toxicology. 2017;109:315-32.
525 34. Zhang H, Wang YE, Fan Q, Zuo YY. On the Low Surface Tension of Lung Surfactant.
526 Langmuir. 2011;27(13):8351-8.
527 35. Veldhuizen EJ, Haagsman HP. Role of pulmonary surfactant components in surface film
528 formation and dynamics. Biochim Biophys Acta. 2000;1467(2):255-70.
529 36. Omaiye EE, McWhirter KJ, Luo W, Pankow JF, Talbot P. High-Nicotine Electronic
530 Cigarette Products: Toxicity of JUUL Fluids and Aerosols Correlates Strongly with Nicotine and
531 Some Flavor Chemical Concentrations. Chemical Research in Toxicology. 2019;32(6):1058-69.
532 37. Erythropel HC, Jabba SV, DeWinter TM, Mendizabal M, Anastas PT, Jordt SE, et al.
533 Formation of flavorant-propylene Glycol Adducts With Novel Toxicological Properties in
534 Chemically Unstable E-Cigarette Liquids. Nicotine Tob Res. 2019;21(9):1248-58.
535 38. Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J, et al. Levels of
536 selected carcinogens and toxicants in vapour from electronic cigarettes. Tobacco Control.
537 2014;23(2):133-9.
538 39. Díaz E, Sad ME, Iglesia E. Homogeneous oxidation reactions of propanediols at low
539 temperatures. ChemSusChem. 2010;3(9):1063-70.
540 40. Lim HH, Shin HS. Determination of volatile organic compounds including alcohols in refill
541 fluids and cartridges of electronic cigarettes by headspace solid-phase micro extraction and gas
542 chromatography-mass spectrometry. Anal Bioanal Chem. 2017;409(5):1247-56.

543 41. Herrington JS, Myers C. Electronic cigarette solutions and resultant aerosol profiles. J
544 Chromatogr A. 2015;1418:192-9.
545 42. Haddad C, Salman R, El-Hellani A, Talih S, Shihadeh A, Saliba NA. Reactive Oxygen
546 Species Emissions from Supra- and Sub-Ohm Electronic Cigarettes. J Anal Toxicol.
547 2019;43(1):45-50.
548 43. Vardavas CI, Anagnostopoulos N, Kougias M, Evangelopoulou V, Connolly GN, Behrakis
549 PK. Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow
550 resistance, impedance, and exhaled nitric oxide. Chest. 2012;141(6):1400-6.
551 44. Cockshutt AM, Possmayer F. Lysophosphatidylcholine sensitizes lipid extracts of
552 pulmonary surfactant to inhibition by serum proteins. Biochim Biophys Acta. 1991;1086(1):63-
553 71.
554

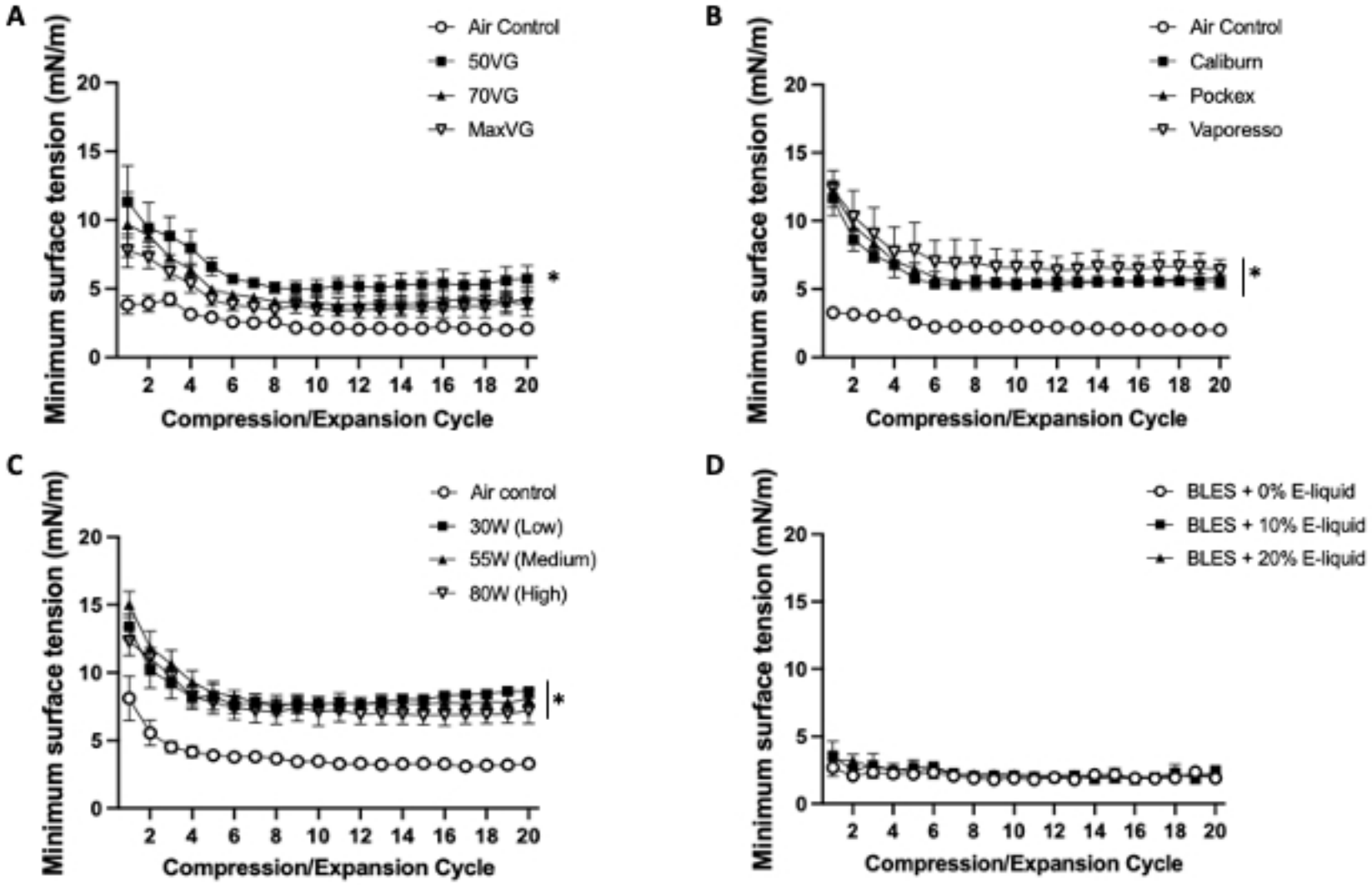
555 Supporting information

556 **S1 Fig. A-I.** Minimum surface tensions (± SEM) across 20 compression/expansion cycles for
557 BLES exposed to nine flavourings/additives compared to unflavoured vehicle control. N=3
558 *P<0.05 compared to air control.

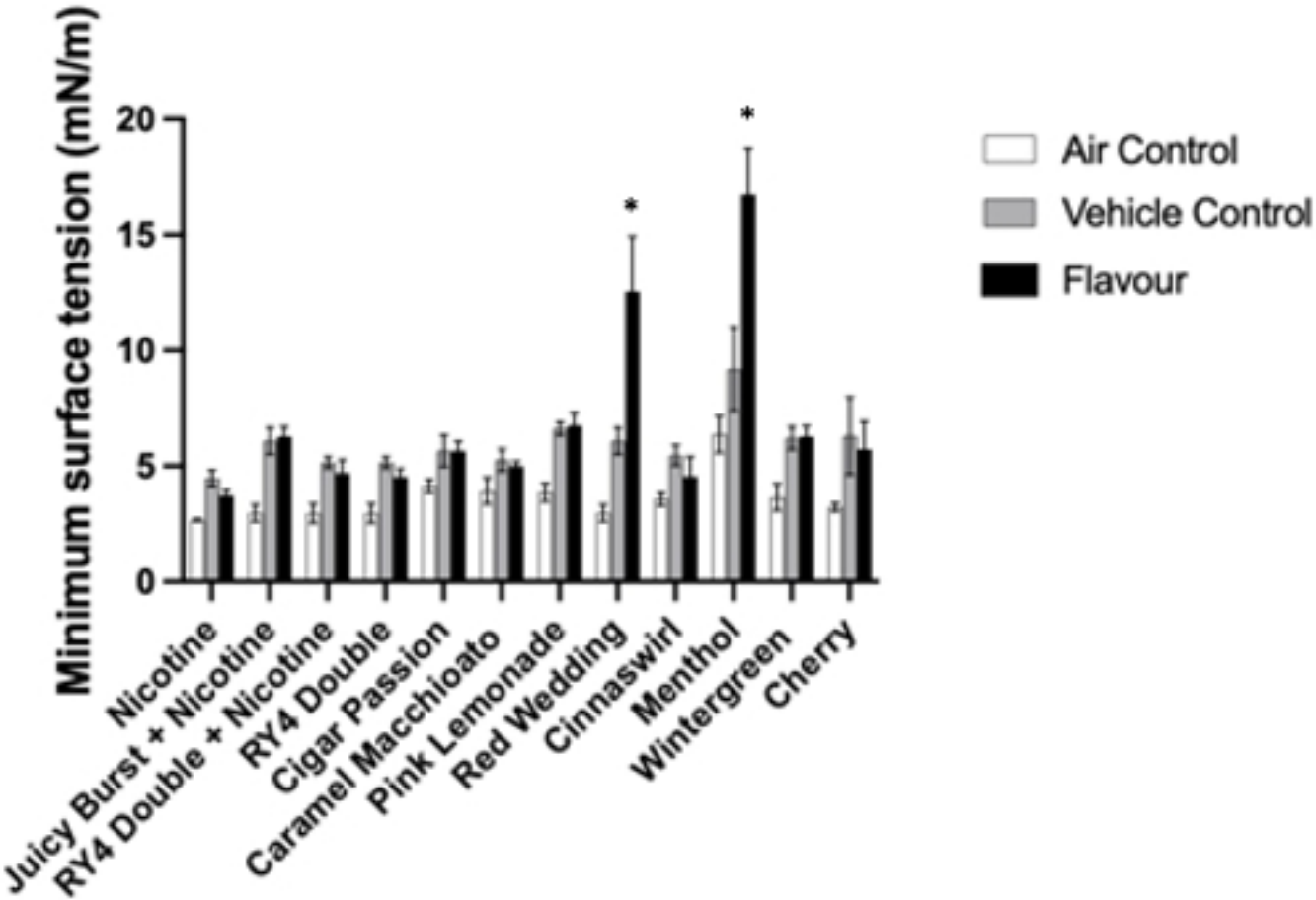
559


A
Air Control
Vehicle
Surface tension (mN/m)
Relative Area
B
Surface tension (mN/m)
Relative Area
C
Minimum surface tension (mN/m)
Compression/Expansion Cycle
*
D
Maximum surface tension (mN/m)
Compression/Expansion Cycle

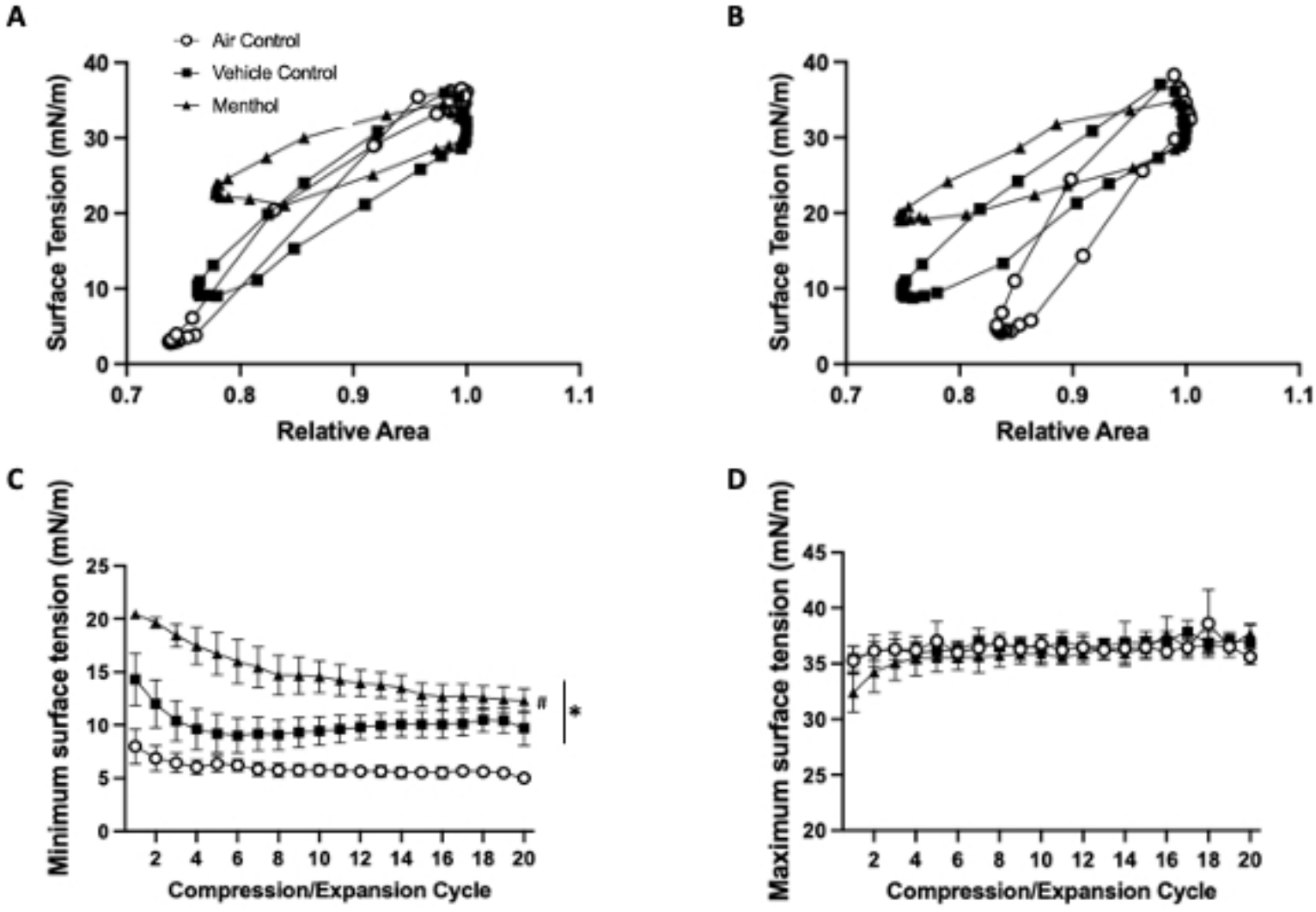
Figure


A
Minimum surface tension (mN/m)
Compression/Expansion Cycle
Air Control
50VG
70VG
MaxVG
*
B
Minimum surface tension (mN/m)
Compression/Expansion Cycle
Air Control
Caliburn
Pockex
Vaporesso
*
C
Minimum surface tension (mN/m)
Compression/Expansion Cycle
Air control
30W (Low)
55W (Medium)
80W (High)
*
D
Minimum surface tension (mN/m)
Compression/Expansion Cycle
BLES + 0% E-liquid
BLES + 10% E-liquid
BLES + 20% E-liquid

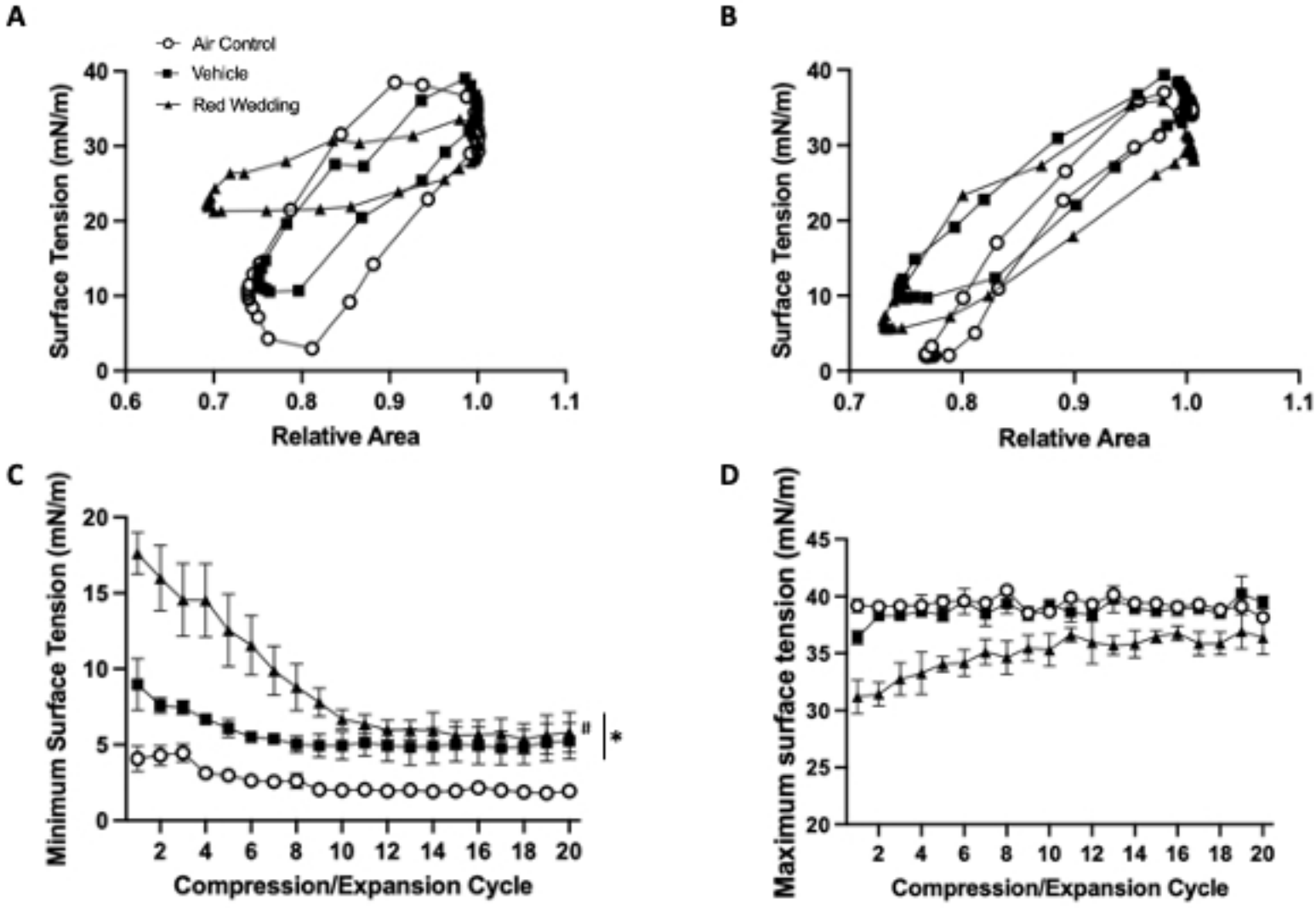
Figure


Minimum surface tension (mN/m)
20
15
10
5
0
*
*
Air Control
Vehicle Control
Flavour
Nicotine
Juicy Burst + Nicotine
RY4 Double + Nicotine
RY4 Double
Cigar Passion
Caramel Macchioato
Pink Lemonade
Red Wedding
Cinnaswirl
Menthol
Wintergreen
Cherry

Figure


A
Air Control
Vehicle Control
Menthol
Surface Tension (mN/m)
Relative Area
B
Surface Tension (mN/m)
Relative Area
C
Minimum surface tension (mN/m)
Compression/Expansion Cycle
*
D
Maximum surface tension (mN/m)
Compression/Expansion Cycle

Figure


A
Air Control
Vehicle
Red Wedding
Surface Tension (mN/m)
Relative Area
B
Surface Tension (mN/m)
Relative Area
C
Minimum Surface Tension (mN/m)
Compression/Expansion Cycle
D
Maximum surface tension (mN/m)
Compression/Expansion Cycle

Figure