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Radiofrequency cold plasma – A novel tool for flavour modification in fresh and freeze-dried strawberries

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ABSTRACT

Cold plasma treatment is an emerging non-thermal food processing tool. This study aimed to identify how cold plasma can improve the aroma of a model space food system (freeze-dried strawberries) by mitigating freeze-drying aroma losses. Cold plasma significantly reduced the negative impact of freeze-drying on aroma; hence, it might be useful as a secondary treatment technique for foods that require extended shelf-life with improved aroma, such as in space travel. "Zero-averaged gravity" (0.004 *g*), a proxy for non-terrestrial environments, reduced the abundance of two important volatile aroma compounds (methyl butanoate and methyl hexanoate) in the gas phase above strawberries; this loss was mitigated when treated by cold plasma. The impact of cold plasma on aroma was also shown to be matrix and treatment parameter-dependent, and six cold plasma treatment options were subsequently identified as enabling bespoke aroma modifications that ultimately enhanced strawberry aroma compounds.

1. Introduction

Growing fresh produce (e.g. strawberries) for extended crewed missions in space has been trialled by European (ESA), American (NASA), Canadian (CSA), and Chinese (CNSA) space agencies. Products, such as strawberries, were selected due to their ability to grow in low-light conditions, their nutritional value and psychological benefits (Cooper, Catauro, & Perchonok, 2012; Elaine, 2005; Hava et al., 2020; Wheeler, 2017). Considering NASA's imminently planned Mars mission in the 2030s, sustainable foods must be developed to last more than five years (Douglas, Zwart, & Smith, 2020; Simon et al., 2017) and accommodate variations in astronaut's health and flavour perception (Douglas et al., 2016) (Taylor et al., 2020). This is not yet possible due to weight and shelf-life constraints; therefore, astro-farming (including hydroponics for in-situ space crops, e.g., lettuce) combined with freeze-drying (successfully used on strawberries in analogue space missions) and other shelf-life enhancing treatments such as cold plasma, are required (Carillo, Morrone, Fusco, De Pascale, & Rouphael, 2020; Douglas et al., 2020; Gronwald et al., 2022; Hava et al., 2020).

Freeze-drying has historically been used in food applications for crewed space missions (e.g., Mercury in the 1960s) (Dunbar, 2019). It uses pressure-assisted (cyclical reduction and increase in pressure) sublimation after freezing (−80 °C) to reduce the water content. This reduces weight and microbial growth (through reduced water activity) and increases nutritional density and shelf-life (a great advantage for weight-limited missions) (Alp & Bulantekin, 2021; Jiang, Zhang, Bhandari, & Cao, 2020; Xu et al., 2021). This is particularly important in extended crewed missions (space, marine, submarine, and polar), where

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chemical and physical stability is critical for sustaining life (Gasier et al., 2014; Jiang et al., 2020).

For heat-sensitive products such as fresh fruits, freeze-drying improves the quality compared to thermal processes such as caning and retorting (Coad & Bui, 2020). However, there is still a significant loss of important volatile aroma compounds which contribute to the perceived flavour of the food (Xu et al., 2021). This negatively impacts food quality and acceptability. During extended crewed missions, maintaining sufficient calorie and nutritional intake is critical. However, food boredom and palatability of process-stabilised foods can lead to calorie deficiencies and loss of body mass; hence, improvements in onboard food quality and palatability are urgently required (Douglas et al., 2020; Taylor et al., 2020; Tran et al., 2022).

Cold plasma is formed through low-temperature ionisation of gas with electrical discharge. This generates cations, anions, free and excited electrons and a range of volatile atoms and molecules (Pan, Cheng, & Sun, 2019) that can be applied to the surface of foods. Cold plasma food treatment has previously improved the shelf-life of fruits by decreasing microbial growth and decreasing the activity of cellular degradation enzymes (i.e. fruit rot) (Bußler, Ehlbeck, & Schlüter, 2017; Niemira, 2012; Schnabel, Niquet, Schlüter, Gniffke, & Ehlbeck, 2015; Thomas et al., 2018; Ziuzina, Patil, Cullen, Keener, & Bourke, 2014) and modified brown rice to increase the abundance of volatile esters (Liu et al., 2021). This increase in ester content suggested that, if repeatable, cold plasma could increase fruity and floral aromas in fruits (Du, Plotto, Baldwin, & Rouseff, 2019). Cold plasma has a wide range of other applications for food (e.g., improving flour functionality), farming (e.g., ammonia synthesis), engineering (e.g., hydrophobic surface fabrication), and medicine (e.g., in-situ wound healing and sterilisation) (Bahrami et al., 2016; Dimitrakellis & Gogolides, 2018; Dubey et al., 2022; Mehta & Yadav, 2022; Winter, Ashford, Hong, Murphy, & Chen, 2020) and has a history of use on the ISS for non-food applications.

Cold plasma can be generated in many ways; these include dielectric barrier discharge (DBD) (two oppositely charged plates) and radiofrequency cold plasma (RF) (switches the voltage to alternate the position of the anode and cathode) (Campelo et al., 2020b; Thomas et al., 2018), and can combine with other technologies, such as 3D printing (Lin, 2015) enabling flexibility of application for both product and process (Campelo et al., 2020a; Campelo et al., 2020b; Douglas et al., 2020; Liu et al., 2021; Silveira et al., 2019; Warne et al., 2021). However, its efficacy under microgravity conditions is still unknown. Whilst cold plasma has yet to be approved for use on food (USA, EU, UK), its approval for use in medical surgeries (in the EU) makes future approval more likely (Katsigiannis, Bayliss, & Walsh, 2022).

Successful extended space missions still require new technologies to be developed that address food production, safety, and quality (i.e. aroma) requirements. Combining freeze-drying and cold plasma is proposed to solve, in part, some of the last remaining key issues surrounding food production and stabilisation for space travel. Therefore, this study aimed to determine the impact of cold plasma (RF) in combination with freeze-drying on targeted volatile aroma compounds, in a model food system (strawberries) under simulated microgravity conditions and to identify, through process optimisation, if the aroma profile of freeze-dried strawberries can be positively modified.

2. Materials and methods

2.1. Sample material and preparations

2.1.1. Strawberries

Fresh strawberries (*Fragaria x ananassa*) (F0) were purchased from Co-op Food, Manchester, UK (strawberry leaf heads were cut off). Freeze-dried strawberries (F1) were prepared by freezing fresh strawberries (~6 g to 14 g) at −80 °C for three days, before freeze-drying in a Christ-Alpha 2–4 LD freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for five days. The condenser temperature was set to between −80 °C to −89 °C, and the pressure cycle range was 10 mbar to 75,000 mbar.

Three commercially available freeze-dried strawberry samples were also used: truncated conical strawberries (S1) from Brix (Prievidza, Slovakia), sliced cylindrical strawberries (S2) from Honeyberry (Burnley, UK), and truncated freeze-dried strawberries (S3) from Forager Fruits (Red Hills, Tasmania, Australia).

2.1.2. Volatile compounds

Strawberry volatile compounds were selected based on six previous GC-O and sensory studies on strawberries; compounds that were present in the majority of the samples in each experiment were selected and used as target volatile compounds (Du et al., 2019; Ingham, Linforth, & Taylor, 1995; Komes, Lovrić, Kovačević Ganić, & Gracin, 2003; Prat, Espinoza, Agosin, & Silva, 2014; Ulrich, Komes, Olbricht, & Hoberg, 2007; Xu et al., 2019). Ethyl butanoate (CAS: 105-54-4), mesifuran (4077-47-8), butanoic acid (107-92-6), hexanoic acid (142-62-1), furaneol (658-77-3), γ-decalactone (706-14-9), methyl butanoate (623-42-7), and methyl hexanoate (106-70-7) were obtained from Sigma Aldrich (Merck Life Science UK Ltd., Gillingham, UK) and were of analytical purity. Target volatile compounds were prepared as stock solutions at 0.1%, 0.5%, 0.01%, 0.05%, and 0.001% *w*/w in methanol and 0.1%, 0.5%, 0.01%, 0.05%, and 0.001% w/w in propylene glycol (Sigma Aldrich, Gillingham, UK).

Target volatile compounds were treated on 3 cm aroma paper strips (Premium aroma assessment papers, LS materials, Sudbury, UK) and placed into GC–MS vials (as well as their non-treated counterpart, i.e., C0 or C15). Samples tested on aroma paper strips included 0.01% w/w solutions of ethyl butanoate, mesifuran, butanoic acid, hexanoic acid, furaneol, and γ-decalactone in either methanol (M1) or propylene glycol (PG1), respectively. PG2 was made with 300 μL of 0.05% w/w of ethyl butanoate, mesifuran, butanoic acid, hexanoic acid, furaneol, and γ-decalactone in propylene glycol. Individual samples were prepared in the following way: ethyl butanoate (100 μL of 0.01% *w*/w) in propylene glycol (EbPG1); ethyl butanoate (EbM1) (100 μL of 0.01% w/w), mesifuran (MeM1) (100 μL of 0.01% w/w), butanoic acid (BaM1) (300 μL of 0.05% w/w), hexanoic acid (HaM1) (300 μL of 0.05% w/w), furaneol (FuM1) (100 μL of 0.05% w/w), or γ-decalactone (GdM1) (300 μL of 0.05% w/w) in methanol.

2.2. Cold plasma treatments

Cold plasma treatments were applied to fresh strawberries, freeze-dried strawberries, and the targeted strawberry volatile compounds. All cold plasma (CP) treatments were applied in a fume hood at room temperature (18 °C to 22 °C), relative humidity (RH) (36.5% to 38.0%), and atmospheric pressure. Temperatures were measured using a CHY 110 infrared thermometer (Jutech Electric & Electronic Pte Ltd., Singapore). Relative humidity was measured using the Rotronic hygrometer A1 (Rotronic Instrument Corp., New York, USA).

All freeze-dried strawberry samples were stored at room temperature. Fresh strawberries (F0) were stored at 4 °C, while fresh strawberries that were freeze-dried (F1) were stored at room temperature. Aroma compounds and paper strips were kept at 4 °C in small glass vials (see Table 1 for dimensions). "C0" denotes the control (i.e., samples not cold plasma treated), which were freshly prepared for each experiment. Unless otherwise specified, treatments were applied to 5 replicate samples ($n = 5$) (Table 1).

RF plasmas (experiments 1–10) were chosen based on their historical use on the ISS (although there are minor differences in configurations) (Thomas et al., 2018).

2.2.1. Atomflo 250 RF Cold plasma

The Surf Atomflo250 (Surfx Technologies LLC, Redondo Beach, CA, USA) is a semi-direct radiofrequency-based cold plasma that was used to generate plasma by applying 110 W, 30 L min^{-1} helium, and 1 L min^{-1}

Table 1
Cold plasma treatments and identification codes for each experiment and sample.

Experiment and sample codes (n)[1]	Cold plasma treatment	Plasma source	Container[2] and delivery	Exposure time and plasma to source distance	Power (W) and voltage (V, AC, Frequency) output[3]	Gas used and flow rate
All	C0	N/A	Varied	0 s; N/A	N/A	N/A
1–1 g of S1 (5)	C1	Surfx Atomflo-250	PP	30 s; 3 cm	110 W, AC, 27.12 MHz	He (30 L min^{-1}) and O_2 (1 L min^{-1})
	C2			60 s; 3 cm		
	C3			90 s; 3 cm		
2–1 g of S1 (5)	C4	kINPen IND-x	PP	30 s; 3 cm	3.5 W, 2–3 kV AC, 1 MHz	4 L min^{-1} N_2
	C5			45 s; 3 cm		
	C6			60 s; 3 cm		
	C7			75 s; 3 cm		
	C8			90 s; 3 cm		
3–1 g of S1	C9	kINPen IND-x	Glass	30 s; 5 cm	3.5 W, 2–3 kV AC, 1 MHz	4 L min^{-1} N_2
	C10			60 s; 5 cm		
	C11			90 s; 5 cm		
	C12			120 s; 5 cm		
4–2 g of S2	C13	kINPen IND-x	PP	120 s; 3 cm	3.5 W, 2–3 kV AC, 1 MHz	4 L min^{-1} Air
	C14					4 L min^{-1} N_2
5–300 μL of M1	C15	kINPen IND-x	PP-paper	60 s; 2–5 cm	N/A	4 L min^{-1} Air
	C16				3.5 W, 2–3 kV AC, 1 MHz	
6–1 g of S1	C15	kINPen IND-x	PP	60 s; 3 cm	N/A	4 L min^{-1} Air
	C17				3.5 W, 2–3 kV AC, 1 MHz	
	C18		Glass	60 s; 5 cm		
	C19		PP			
7–100 μL of individual compounds[4]	C20	kINPen IND-x	Glass	60 s; 2–5 cm	3.5 W, 2–3 kV AC, 1 MHz	4 L min^{-1} Air
8–100 μL of M1	C20	kINPen IND-x	Glass - liquid	60 s; 2–5 cm	3.5 W, 2–3 kV AC, 1 MHz	4 L min^{-1} Air
	C21				3.5 W, 2–3 kV AC, 1 MHz	
	C22			300 s; 2–5 cm	(Pulsed)	
	C23				3.5 W, 2–3 kV AC, 1 MHz	
9–100 μL of PG2	C24	kINPen IND-x	Glass-liquid	60 s; 2–5 cm	3.5 W, 2–3 kV AC, 1 MHz	5 L min^{-1} Air
	C25				3.5 W, 2–3 kV AC, 1 MHz[5]	
	C26				3.5 W, 2–3 kV AC, 1 MHz	4.5 L min^{-1} Air
	C20					4 L min^{-1} Air
	C27				3.5 W, 2–3 kV AC, 1 MHz[5]	4 L min^{-1} Air
10–100 μL of PG2	C28	kINPen IND-x	Glass-paper	60 s; 2–5 cm	3.5 W, 2–3 kV AC, 1 MHz	4 L min^{-1} Ar
	C29					4 L min^{-1} N_2
	C20					4 L min^{-1} Air
11–15 g S3	C30	Rotary plasma reactor	Glass	300 s; 0 cm	50 W, AC, 13.56 MHz	Low-pressure (60 mTorr) air

[1] Number of replicates shown as *"n"*.
[2] PP – Polypropylene.
[3] Dissipated power and voltage output were only listed if displayed by the manufacturer. The exact voltage output for kINPen IND-x could not be obtained.
[4] EbPG1, EbM1, MeM1, BaM1, HaM1, FuM1, & GdM1.
[5] Input voltage was set as 70 V (all other kINPen IND/IND-x inputs were set at 65 V).

oxygen to samples positioned 3 cm away from the plasma source in a polypropylene petri dish. For experiment 1, S1 (1 g, $n = 4$) was exposed to Surfx Atomflo250 radiofrequency-based cold plasma treatments at 0 s, 30 s, 60 s, and 90 s (C0, C1, C2, & C3) (Table 1). Before each experiment, the gas (no plasma) was purged for 20 s, and then the plasma was purged for 5 min with a 10 s gap between each treatment. Sample temperatures were maintained at 20 ± 2 °C (hence, there should be no thermal impact from the plasma). HS-SPME GCMS was used to analyse 0.5 g of each replicate.

2.2.2. kINPen IND RF Cold plasma

kINPen IND-x Cold plasma (CP) is a direct radiofrequency-based ionisation cold plasma source which operates with an input (adaptable) from 45 V to 75 V (DC; <50 W), causing an output voltage of 2 kV to 3 kV (AC; 1 MHz) (~3.5 W of power output) (exact output cannot be measured; proportional scaling is assumed) (Reuter, Von Woedtke, & Weltmann, 2018). kINPen IND-x was connected to gas mixing unit II-2 (GMU II-2) and controlled using the gas mixing unit software (kINPen_GMUII) (sourced from Neoplas GmbH, Greifswald, Germany). kINPen IND-x was applied in experiments 2 to 10 (Table 1). Sample temperatures ranged from 19.1 °C to 28.7 °C, and plasma humidity was 22.5% RH. Before each experiment, the kINPen was plasma purged for 15 min, and a 10 s gap between replicates was used. HS-SPME GCMS was used to analyse 0.5 g of each replicate. Unless otherwise stated, all samples were CP treated at 65 V input (2 kV to 3 kV at 1 MHz AC output) with 4 L min^{-1} air-based cold plasma in 5 replicates and analysed once per replicate.

2.2.3. Rotary plasma reactor (RPR)

Whole freeze-dried strawberries (S3) (Forager Fruits, Red Hills, Tasmania, Australia) were treated using a rotary plasma reactor (RPR) (Fig. 1). This was a RF plasma generated inside a rotating round bottom glass at 50 W (~25 °C) and 60 mTorr (low-pressure air) (described in further detail by Michl, Coad, Hüsler, Vasilev, and Griesser (2015)). 15 g (a whole pack) was treated for 5 mins, and 2 g was added to each of the three GCMS vials (Supplementary Fig. 2). This was repeated with and without plasma and with and without "zero-averaged" gravity (0.004 *g*; 0.2618 rad s^{-1}). Sample S3 (20 mm) was packed within glass wool (10 mm) to restrict free movement (Supplementary Fig. 2). Two packs (one with and one without CP) were applied in this way on a random positioning machine (Airbus Netherlands, Leiden, Netherlands) to generate 0.004 *g* (averaged) with a 0.2618 rad s^{-1} tumbling rate for 1 h, with the exposed SPME fibre positioned 1 cm away from the strawberries. The same conditions (same positioning as Supplementary Fig. 2 but kept upright and static) were used for the other two packs (with and without CP) without "zero-averaged" gravity (1 *g*). "Zero-averaged" gravity was the closest possible simulation (without sending this configuration into space) of the extreme microgravity experienced on board the ISS (and other future space stations and exploration vessels). Limitations include temporary acceleration beyond 1 *g*; however, aroma partitioning occurs

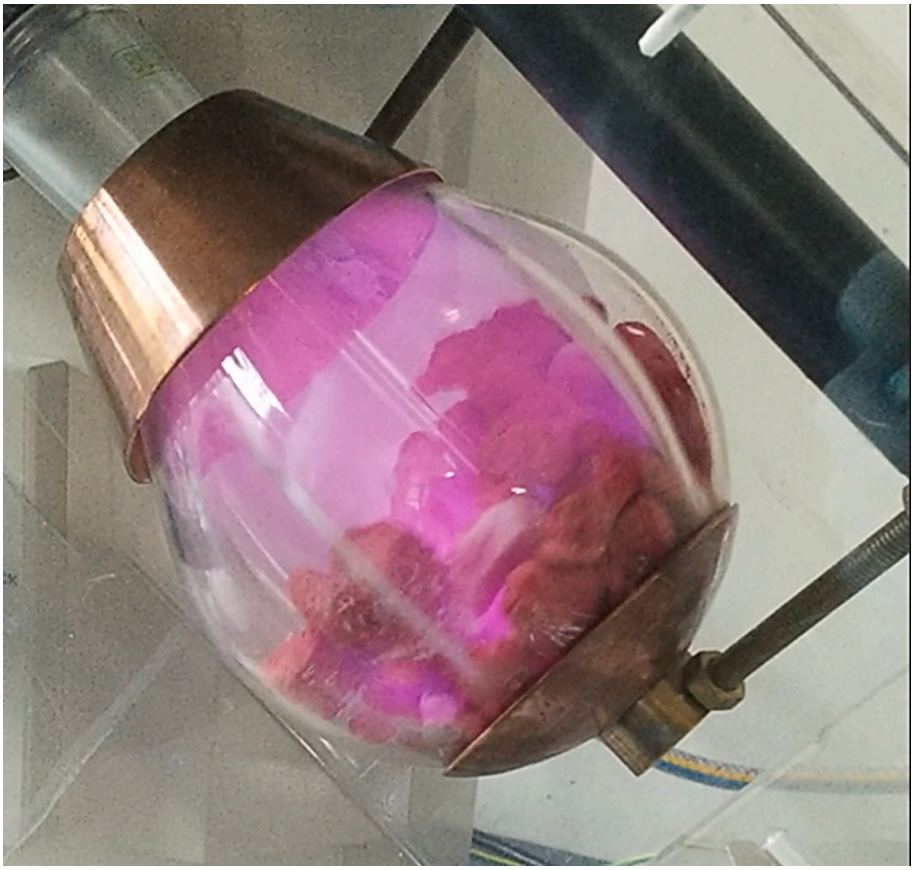

Fig. 1. Rotary Plasma reactor set-up from a modified rotary evaporator developed by Michl et al. (2015).

much slower than this temporary acceleration. Therefore, gravity can be assumed to be "zero-averaged" (Kiss, Millar, & Edelmann, 2012). Multiple studies have used this "zero-averaged" gravity approach to generate analogous microgravity (Wuest, Richard, Kopp, Grimm, & Egli, 2015).

2.3. Cold plasma-APCI-MS

Real-time volatile analysis was conducted during air-based cold plasma treatment (4 L min^{-1}) at 65 V input (2 kV to 3 kV at 1 MHz AC output, C20) using Atmospheric Pressure Chemical Ionisation mass spectroscopy-MS nose (APCI-MS nose) samples consisted of aroma paper with 300 μL of 0.01% *w*/w solutions of ethyl butanoate in glass vials (Table 1) (see Section 2.4.2 for analysis details). Samples were treated and analysed according to the setup in Supplementary Fig. 1 (processing controls were included (C0)).

2.4. Analysis

Analytical measurements were conducted within a week of treatment. To account for any impact from the dates of the experiments and analytical measurements, control samples (5 replicates) without cold plasma treatment were also freshly extracted and analysed for each experiment (Table 1).

2.4.1. Gas-chromatography mass-spectroscopy

Headspace solid-phase microextraction (HS-SPME) of samples in 20 mL amber-tinted vials (with crimped cap) was performed by gas-chromatography (Trace 1300 Gas chromatograph, Thermo Fisher Scientific Inc., Waltham, USA) mass-spectroscopy (ISQ single quadrupole Mass Spectrometer, Thermo Fisher Scientific Inc., Waltham, USA) (GCMS) using an adapted method from Lester et al. (2021a). Empty vials (20 mL) were used for background subtraction. Briefly, samples were incubated for 10 min at 37.5 °C using the same HS SPME fibre (DVB/CAR/PDMS) and then extracted onto the fibre for 20 min. The column (ZB-Wax) (30 m × 0.25 mm I.D.) temperature was held at 40 °C for 2 min, then increased at six °C min^{-1} to 240 °C, and held at 240 °C for 5 min. Helium was the carrier gas with a constant splitless flow pressure of 18 psi. MS spectra were obtained for the full mass range of the MS (35 Da to 300 Da).

TraceFinder 5.1 with the Deconvolution Plugin 1.2 (Thermo Fisher Scientific Inc., Waltham, USA) and the Xcalibur Qual browser (Thermo Xcalibur 2.2.42 Qual browser, Thermo Fisher Scientific Inc., Waltham, USA) were used to process and identify compounds with NIST 17 (National Institute of Standards and Technology, Gaithersburg, USA) and a calibration curve (Supplementary Fig. 1).

2.4.2. Gas-chromatography mass-spectroscopy – solid-phase microextraction

Manual SPME (2 cm; DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) was carried out for FDS3 in 20 mL amber-tinted vials (with crimped cap). Fibres were preconditioned (30 min) and inserted (4 cm) into the vial so that it was 1 cm away from the strawberry layer of samples, and three fibres were exposed for 1 h ($n = 3$). Fibres were injected into the GCMS (8890 GC, 7010B TQ MS) (Agilent, Santa Clara, USA) for 15 mins (desorption time). The column (DB-Wax) (60 m × 0.25 mm I.D.) temperature was held at 40 °C for 2 min, then increased at 6 °C / min to 240 °C, and then held at 240 °C for 5 min. Helium was the carrier gas with a constant splitless flow pressure of 27 psi. Auto sampling was also performed using the PAL RTC 120. MS spectra were obtained for the full mass range of the MS (35–300 Da). NIST 20 library was used with Mass Hunter Unknowns Analysis (9.0.647.0) (Agilent) and a 75% library match or above with peak deconvolution (signal-to-noise ratio of 2 or higher).

2.4.3. Atmospheric pressure chemical ionisation – MS-nose

Atmospheric Pressure Chemical Ionisation-MS (APCI-MS) was applied (APCI Quattro Ultima, Micromass, Wythenshawe, UK) using an adapted method of Lester et al. (2021b) to determine if cold plasma affects the release of volatile aroma compounds from the filter paper in real-time. Briefly, a heated (120 °C) silica tube attachment ("MS nose") and negative pressure flow (30 mL min^{-1} nitrogen (140 °C)) were used to draw ambient air and volatile compounds through to the MS-MS of the APCI. Ethyl butanoate was monitored in its protonated form in SIR mode at 117 *m/z* using the following software and settings: Mass lynx 4.1 (Waters Corporation, Milford, USA), ES+ ionisation source, cone voltage 30 V, capillary voltage 8 eV, dwell time 0.02 s, source temperature 60 °C, and desolvation temperature 20 °C. (see Section 2.3 for processing details).

2.5. Statistical analysis

Data were analysed by analysis of variance (ANOVA), with either Games Howell or Tukey post hoc tests applied using SPSS (SPSS v27.0.1.0 64-bit edition for Windows, IMB Corporation, New York, USA). Post hoc tests were based on the results of the homogeneity of variance tests (if $P > 0.01$, then the Tukey test was performed). Where post hoc tests were not performed (samples with <3 variables), Welch's and Brown Forsythe tests were conducted (SPSS) to compare treatment devices (Supplementary Table 2). Microsoft Excel 365 (Microsoft Corporation, Redmond, USA) was used for data treatment. $P < 0.05$ were considered significant. Unless otherwise stated, all samples were treated five times and a double Grubbs test was used to identify outliers ($P < 0.001$) (XLSTAT 2021.1.1, Addinsoft, New York, USA). Detected outliers were removed if they increased the coefficient of variation (%) to >20% (Supplementary Table 3).

3. Results and discussion

This study sought to identify how cold plasma could enhance the aroma quality of a model space food (freeze-dried strawberries). Positively associated aroma compounds (ethyl butyrate, methyl butanoate, methyl hexanoate, furaneol, mesifuran and γ-decalactone) and negatively associated aroma compounds (butanoic acid and hexanoic acid) were evaluated after freeze-drying and cold plasma treatment under a proxy ("zero-averaged gravity") for microgravity environments found during space travel. The positively associated aromas are strawberry-like, floral, fruity, caramel, whereas the negatively associated aromas have previously been described as sweaty and cheesy (Du et al., 2019; Fukuhara, Li, Okamura, Nakahara, & Hayata, 2005; Gomes da Silva & Chaves das Neves, 1999; Ingham et al., 1995; Komes et al., 2003; Prat et al., 2014; Ulrich et al., 2007; Xu et al., 2019). All six compounds of interest were found in S1 (conical strawberry), and all but ethyl butanoate were found in S2 (flat strawberry) (Table 2). Only ethyl butanoate (fruity and sweet aroma), methyl butanoate (fruity and sweet aroma) and methyl hexanoate (pineapple-like and fruity aroma) were found in F0 (fresh strawberry) and F1 (freeze-dried strawberry) (Du et al., 2019; Ulrich et al., 2007) (Table 2).

Variations in cold plasma treatments chosen are intended to showcase how different plasma systems could be industrially applied: i.e., the impact of different container materials (process flexibility), distance of the plasma from the food (product flexibility and sample size limits), gas types (gas sustainability and process flexibility), flow rate, time (i.e. processing speed) and plasma energy output (power and voltage) (i.e. peak energy requirements).

3.1. Effects of freeze-drying and cold plasma treatments on key esters

Fresh strawberries were cold plasma treated and then freeze-dried (Fig. 2). Of the targeted strawberry compounds, ethyl butanoate, methyl butanoate and methyl butanoate were identified. Freeze-drying significantly ($P < 0.05$) negatively impacted the positively associated aroma compounds: methyl butanoate, ethyl butanoate, and methyl hexanoate. Surprisingly, cold plasma significantly reduced the negative impact of freeze-drying for all three compounds ($P < 0.05$) (Fig. 2). Liu et al. (2021) observed that cold plasma treatments increased esters in rice, but this has not been trialled in volatile systems such as fruits. The negative impact of freeze-drying on flavour is well known (Xu et al., 2021) and is normally attributed to the loss of volatile compounds as water evaporates. However, cold plasma increased all three esters after freeze-drying and further increased methyl hexanoate in the fresh strawberries, so it is unlikely that the changes are associated with moisture sublimation. This surprising result shows that cold plasma enhanced the aroma of freeze-dried fruits typically used in space travel and could improve the acceptability of space food and mitigate astronaut food aversion (Douglas et al., 2016; Douglas et al., 2020).

3.2. Impact of "zero-averaged" gravity and a rotary plasma reactor on target volatile aroma compounds

Previous studies have shown changes in flavour perception in space (Taylor et al., 2020). These may be due to a combination of environmental factors (e.g., background aroma, humidity differences), changes in the physics of flavour (e.g., convection) and the human body (e.g., changes in saliva physics). To determine if changes in the gravitation field could impact volatile aroma compounds, a random positioning machine was used as a proxy for microgravity ("zero-averaged" gravity) to generate 0.004 *g*. The rotational properties of the RPR RF plasma provide uniform treatment of the samples, demonstrating a good way of treating whole foods (Fig. 1).

Applying 0.004 g "zero-averaged" gravity condition reduced (below detectable limits) methyl butanoate and methyl hexanoate in the gaseous phase above freeze-dried strawberries (Fig. 3a and b). Applying 0.004 g "zero-averaged" gravity had no statistically significant impact on mesifuran, butanoic acid, and hexanoic acid (Fig. 3c, d, and e). The rotary plasma reactor significantly increased mesifuran (+308.2%; $P < 0.05$) and seemingly increased (above detectable limits) methyl butanoate and methyl hexanoate under "zero-averaged" gravity conditions; these all increased when plasma treated above the concentrations observed under standard gravity (Fig. 3a, b, and c). However, the changes were not significant under standard gravity (+57.9, +105, and − 63.4%, respectively; $P > 0.05$). The headspace abundance of mesifuran and butanoic acid increased significantly by cold plasma under "zero-averaged" gravity, suggesting an interaction effect (Fig. 3c and d).

This is the first time an interaction effect of cold plasma and "zero-averaged" gravity has been demonstrated; it may indicate a change in mass transfer due to microgravity or the "zero-averaged" gravity process.

3.3. Effects of semi-direct vs. direct plasma treatments on key aroma volatiles

The effects of two devices: the semi-direct RF plasma (Atomflo 250, uses helium and oxygen in a high flow rate and high power system) and the direct RF plasma (kINPen IND-x, low flow rate and low power system that can use argon, nitrogen, and oxygen) was evaluated on the target aroma compounds (ethyl butanoate, mesifuran, butanoic acid, hexanoic acid, furaneol, and γ-decalactone) in freeze-dried strawberries. The semi-direct cold plasma treatment significantly increased ethyl butanoate, mesifuran, and furaneol after 30 s ($P < 0.05$) (Fig. 4). Therefore, 30 s was selected as the optimal semi-direct RF plasma condition (increased the concentration of two positively associated aroma compounds without increasing butanoic and hexanoic acids). This result agreed with Silveira et al.'s (2019) findings that these acids were not significantly affected by nitrogen-based single electrode (400 W) cold plasma.

In the semi-direct RF plasma, the direct plasma significantly

Table 2
Percentage change (% δC) in concentration of ethyl butanoate, mesifuran, butanoic acid, hexanoic acid, furaneol, and γ-decalactone, in different samples and cold plasma treatments, compared to their corresponding control treatments (i.e., just gas (C15: used as a control for C16, C17, C18, & C19 only) or no treatment (C0: used as a control for all other treatments)); these are represented as no significant difference (yellow), a statistically significant increase (green), or a statistically significant reduction (red).

C1	S1	3	194.8±52.6	262.8±51.5	20.14±3.8	16.61±3.27	98.6±25.06	-44.9±23.73
C2	S1	3	-1.472±0.65	-1.961±1.89	-10.75±3.54	-2.11±0.429	4.567±2.768	-22.96±5.4
C3	S1	3	38.48±5.48	-54.15±7.58	-25.13±1.18	-4.112±0.769	-48.42±8.81	15.6±2.38
C4	S1	3 + S3	7.769±4.342	60.91±22.77	39.1±10.58	85.33±25.8	54.22±20.1	9.713±4.281
C5	S1	3 + S3	-8.803±3.97	37.36±6.65	24.2±4.16	39.26±7.72	20.54±9.16	-3.185±0.47
C6	S1	3 + S3	10.53±4.49	71.15±24.12	61.64±5.41	57.53±14.4	-11.28±1.99	-2.778±0.526
C7	S1	3 + S3	44.4±15.79	9.778±1.233	31.25±6.67	29.25±8.07	-37.19±13.18	-7.961±1.626
C8	S1	3 + S3	-2.944±0.796	105±28.2	87.87±15.08	102.9±24.7	32.02±10.52	44.9±12.72
C9	S1	S3	15.26±2.77	105.2±21	48.9±10.37	120.8±18	-46.76±10.55	100.5±10.5
C10	S1	S3	8.151±1.518	116.7±17.6	111.9±17.2	137.5±28.7	-31.76±3.07	103.2±11.6
C11	S1	S3	-13.2±4.62	81.27±10.53	102.7±21.2	114.8±11.5	-40.27±5.92	104.9±9.6
C12	S1	S3	42.07±5.94	213.7±28.6	112.3±21.5	113±21.2	18.31±2.83	97.09±13.92
C13	S2	S4	N/A	13.3±4.49	-15.25±2.68	58.01±11.89	49.62±19.26	20.04±2.37
C14	S2	S4	N/A	-56.92±23.19	-54.94±16.69	-21.47±5.84	15.24±6.98	16.76±2.55
C16	M1	4	-24.26±0.95	65.98±2.82	185.4±16.6	251±17.5	-67.02±39.58	303.4±8
C17	S1	4	-64.62±32.95	72.35±7.04	15.44±1.85	13.12±0.69	48.9±2.01	80.94±7.79
C18	S1	4	-54.09±45.41	-36.99±34.28	-0.986±0.137	-2.58±0.907	2.876±1.926	285.7±95.3
C19	S1	4	-75.03±28.24	43.48±3.32	33.61±2.5	14.65±0.46	25.2±1.13	325.8±45.1
C20	EbM1	5	36.48±0.88	N/A	N/A	N/A	N/A	N/A
C20	EbPG1	5	-17.23±4.43	N/A	N/A	N/A	N/A	N/A
C20	MeM1	5	N/A	171.8±30.3	N/A	N/A	N/A	N/A
C20	BaM1	NA	N/A	N/A	1037.9±137.6	N/A	N/A	N/A
C20	HaM1	NA	N/A	N/A	N/A	880.4±161.4	N/A	N/A
C20	FuM1	5	N/A	N/A	N/A	N/A	-9.764±1.39	N/A
C20	GdM1	5	N/A	N/A	N/A	N/A	N/A	9.842±1.252
C20	M1	S5	N/A	479.5±251.8	7,305.4±1,683.4	82,447±16,756	N/A	12,793.2±581.9
C21	M1	S5	11.13±1.32	146.6±19.8	N/A	N/A	N/A	462±82.2
C22	M1	S5	-82.71±10.68	-74.54±16.06	1208.8±188.6	899.6±185	N/A	809.9±144.6
C23	M1	S5	-84.79±25.28	-74.96±21.27	1105.5±170.4	1082.4±249.7	N/A	862.1±214.2
C24	PG2	S6	19.49±3.71	18.58±0.66	75.14±2.41	96.49±19.65	-59.45±49.01	29.4±11.58
C25	PG2	S6	33.21±1.32	-6.513±0.15	24.78±1.58	23.49±2.34	-75.66±30.88	7.292±0.367
C26	PG2	S6	30.71±1.55	-9.133±0.449	14.01±1.1	15.81±1.94	-72.25±37.41	7.034±0.718
C20	PG2	S6	34.7±0.9	-10.05±0.73	13.64±0.22	13.57±2.29	-50.33±9.88	2.406±0.396
C27	PG2	S6	-27.5±3.43	0.442±0.021	49.0±3.0	66.09±6.8	-77.76±15.28	26.75±2.68
C28	PG2	NA	11.37±2.08	15.63±0.46	-6.909±0.409	-9.275±0.679	N/A	4.227±0.257
C29	PG2	NA	-6.194±0.986	1.678±0.017	6.734±0.278	17.51±0.39	N/A	1.35±0.017
C20	PG2	NA	56.08±2.62	-2.801±0.077	9.969±0.299	24.82±0.86	N/A	1.868±0.089

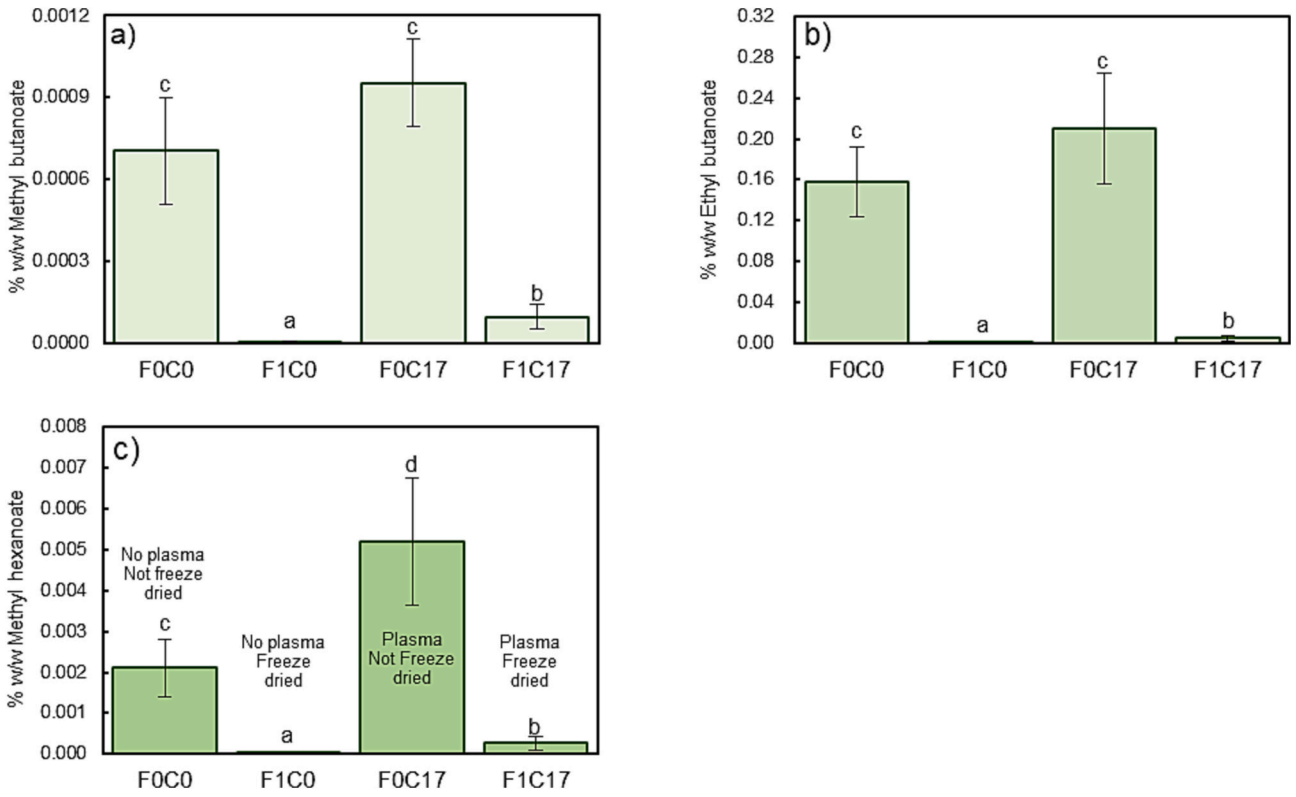

Fig. 2. Mean concentrations (% *w*/w) ($n = 4$–5) (± s.d.) of (a) methyl butanoate (MB), (b) ethyl butanoate (EB), and (c) methyl hexanoate (MH) in fresh strawberries (F0), with (F1) or without (F0) freeze drying and with (C17) or without direct RF (C0) air-based plasma treatment.

increased hexanoic acid at 30 s and 90 s and butanoic acid at 60 s and 90 s (Tukey's HSD) (Fig. 4), both associated with negative quality traits.

3.4. Effects of cold plasma treatments on different sample matrices

To understand the effects of plasma-matrix interaction, volatile compounds in strawberries were compared with those aliquoted onto aroma strips to form a simplified matrix model with a fixed exposed surface area and surface topology.

Ethyl butanoate was significantly diminished, and γ-decalactone was significantly enhanced after cold plasma treatment of the filter paper; this was also seen in the freeze-dried strawberry matrix (Fig. 5). Mesifuran, butanoic acid and hexanoic acid concentrations were significantly increased in the filter paper after cold plasma treatment but not in the freeze-dried strawberry matrix (Fig. 5). In contrast, furaneol was significantly increased only in the strawberry matrix. This suggests a matrix-dependent interaction that is impacted by the variable nature of the strawberry matrix (Liang et al., 2022; Ulrich, Kecke, & Olbricht, 2018; Xiao, Jiang, & Niu, 2022). Except for furaneol, the distance between the sample and source (3 cm vs. 5 cm) had no significant effect on any key compounds (Fig. 5). The container material had no significant impact (Fig. 5).

3.5. Effect of gas type on key aroma volatiles

To understand how aroma can change with different plasma gas sources, plasma generated with argon, nitrogen, helium, or air was applied to strawberries and filter papers infused with the target volatile aroma compounds. Furaneol and γ-decalactone were not significantly affected by compressed air or nitrogen-based treatments (C13 & C14) of freeze-dried strawberries (S2) (Table 2; Supplementary Fig. 4). However, nitrogen (C14) significantly decreased mesifuran and butanoic acid concentrations, compared to no treatment (C0) and air (C13), and nitrogen also decreased hexanoic acid concentrations, compared to air (C13). Propylene glycol did not affect butanoic acid and γ-decalactone, irrespective of the gas used (C20, C28, & C29) (Table 2). However, gas type significantly affected ethyl butanoate, mesifuran, and hexanoic acid concentrations, which increased after air (C20), argon (C28), or air and nitrogen (C20 & C29), respectively ($P < 0.05$) (Table 2).

3.6. Effects of direct CP treatments on individual volatile compounds

Plasma treatments were also applied to compounds individually to ensure that cold plasma-induced changes were not competing with or obscuring other reactions. Ethyl butanoate significantly increased when cold plasma was applied to samples in methanol but not propylene glycol (Fig. 6a). This suggests a matrix-dependent effect, where propylene glycol limits the abundance of ethyl butanoate, presumably due to a change in volatility. γ-Decalactone and furaneol were not significantly affected by cold plasma, but mesifuran was significantly increased (Fig. 6b and c). Overall, cold plasma increased the aroma concentration in the gas phase, which was retained over an extended period (note that GCMS analysis was performed a few days after cold plasma treatment). Observing shelf-life changes to aroma would be interesting to determine if these effects are still apparent after extended periods.

3.7. Effect of cold plasma voltage input and flow rate on key aroma volatiles

In previous studies, cold plasma decreased microbial growth, with increased power and flow rate directly impacting efficacy (Niemira, 2012; Schnabel et al., 2015; Ziuzina et al., 2014). In this study, the effects on aroma were assessed. 4 L min^{-1} or 4.5 L min^{-1} of air plasma did not significantly affect aroma compounds; However, 5 L min^{-1} of air plasma significantly increased mesifuran, butanoic acid, and hexanoic acid at 65 DC V input (2–3 kV at 1 MHz AC output) (Table 2; Supplementary Fig. 6). There was a significant loss of these three compounds

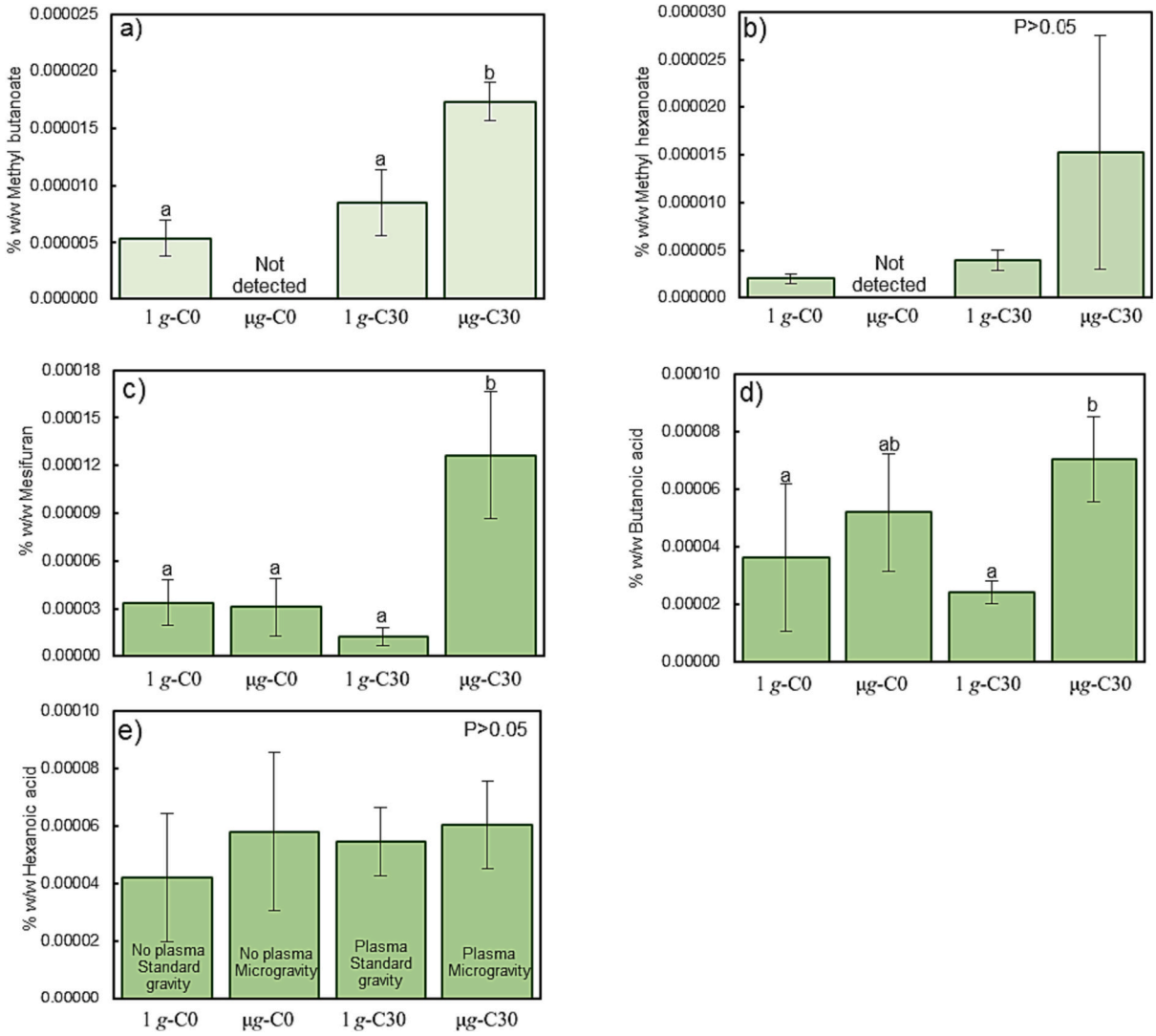

Fig. 3. Mean concentrations (% w/w) ($n = 3$) (± s.d.) of methyl butanoate (a), methyl hexanoate (b), mesifuran (c), butanoic acid (d), hexanoic acid (e) in freeze-dried strawberries (S3), with (C30) or without (C0) RPR RF plasma and with "zero-averaged" gravity (0.004 g) ("µg") or standard gravity (1 g).

when changing from 65 V to 70 V input at 5 L min^{-1}, but a significant increase when changing from 65 V to 70 V input at 4 L min^{-1}. Additionally, plasma at 4 L min^{-1} and 65 V (input) did not significantly affect butanoic acid, hexanoic acid, or γ-decalactone. This agrees with the propylene glycol experiment (Table 2) (Supplementary Fig. 6). Propylene glycol also mitigated the impact of evaporation from cold plasma treatments, which was attributed to its higher vapour pressure relative to methanol. It was also noted by Campelo et al. (2020a), that flow rate had a greater effect on ethyl butanoate concentration than the duration of treatment.

3.8. Real-time analysis of the impact of cold plasma on ethyl butanoate using MS-nose-APCI-MSMS

Unlike the single-point aroma analysis conducted using the GCMS, APCI-MS MS-nose is a unique approach that enables real-time qualitative analysis of aroma compounds during treatment; for this study, ethyl butanoate was used (Fig. 7). Whilst both treated and non-treated ethyl butanoate decreased in intensity over time (significant negative Pearson's correlation (−0.644; $P < 0.001$)), cold plasma treated samples retained the headspace abundance to a greater degree than the non-treated samples ($P < 0.001$) indicating that cold plasma treatment enhances the abundance or increases the replenishment rate of ethyl butyrate (Fig. 7).

3.9. Proposed mechanistic interactions with plasma ions and eight key aroma compounds

To understand why the eight key aroma compounds (methyl butanoate, methyl hexanoate, ethyl butanoate, mesifuran, butanoic acid, hexanoic acid, furaneol, γ-decalactone) are affected by cold plasma, there are many factors to consider. In strawberries, this is compounded by the complexity of the matrix, with the presence of internally bound compounds, surface cellular trapped compounds, and glycosidically bound compounds, which together affect the exposure, release and potential reactivity of volatile compounds (Liang et al., 2022; Ulrich et al., 2018; Xiao et al., 2022). Furthermore, the interaction between plasma and additional compounds within the strawberry matrix could lead to a change in local microchemistries and a subsequent change in the volatility of aroma compounds.

The kINPen IND (an older model of the IND-x without adjustable voltage input) has previously been shown to produce OH, NH, N_2, C⩵N, O^+, O^-, H_α, H_β, H_γ, and Ar in argon-based plasma (Pho et al., 2023). Pandiyaraj et al. (2020) also found NO, and O_1 in argon-based plasma. Lotfy (2020), used 14 L min^{-1} of helium with 0.2% O_2 and found He, OH, O_1, O_2, N_2, N_2^+, N_2^-, N_2^{2+}. This was similar to the conditions used in

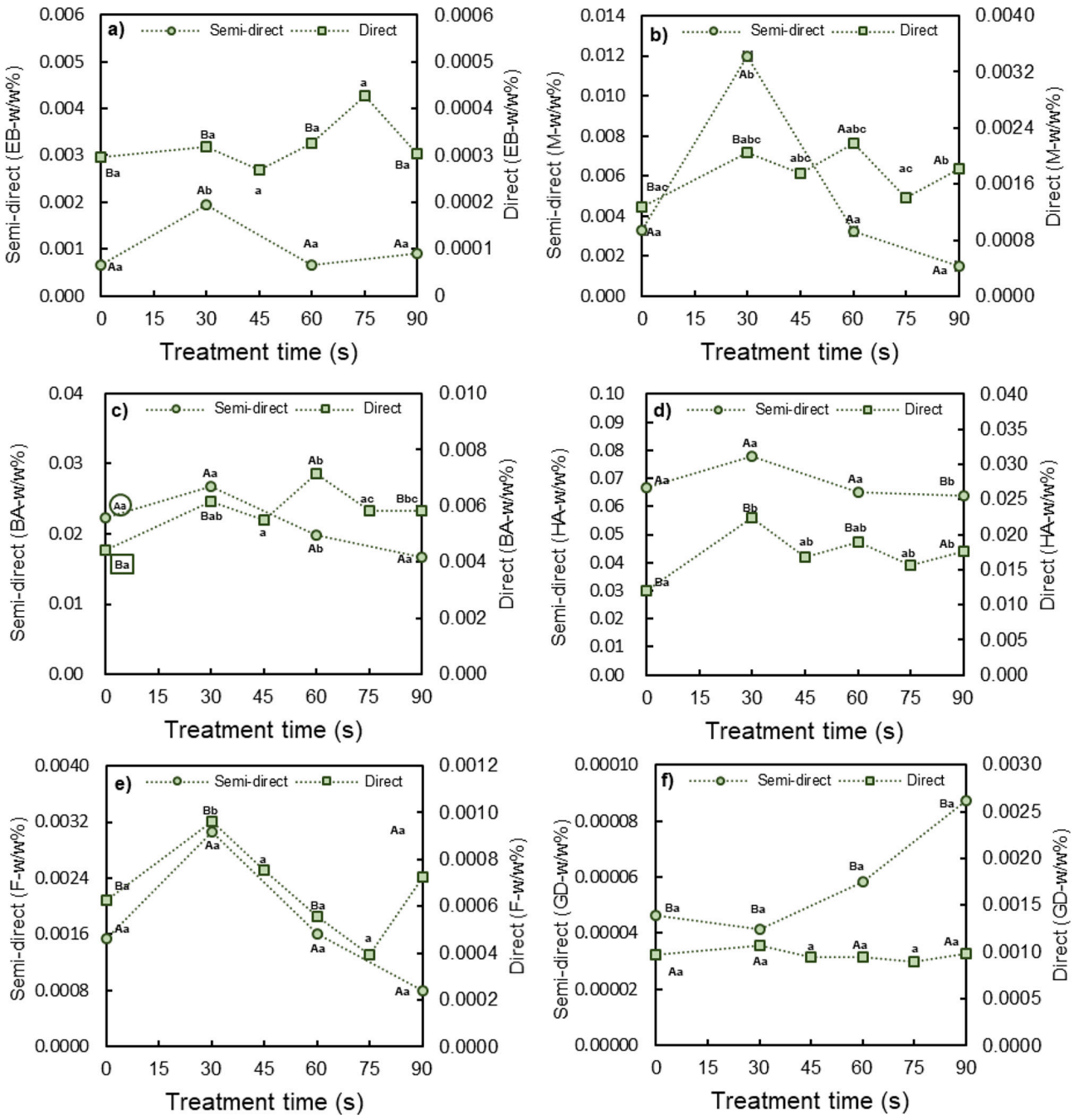

Fig. 4. Mean concentrations (% *w*/w) ($n = 5$) of ethyl butanoate (EB) (a), mesifuran (M) (b), butanoic acid (BA) (c), hexanoic acid (HA) (d), furaneol (F) (e), and γ-decalactone (GD) in freeze-dried strawberries (S1) after semi-direct RF helium-based plasma (120 W) (at 0, 30, 60, or 90 s (C0, C1, C2, or C3, respectively)) or direct RF nitrogen-based plasma (2–3 kV at 1 MHz AC output) (at 0, 30, 45, 60, 75, or 90 s (C0, C4, C5, C6, C7, or C8 respectively)). Lower case letters show significant differences between treatment times; upper case letters show significant differences between plasma types.

the semi-direct plasma (Atomflo-250), so it was considered to have a similar atomic output. For Air-based plasmas, the atomic and ionic output is estimated to be closer to those found by Bao, Reddivari, and Huang (2020) and Roy, Talukder, and Chowdhury (2017): N_2, N_2^+, O_1, H_α, $H\gamma$ and OH. Nitrogen-based plasmas are similar but have an increased prevalence of N_2 (Bao et al., 2020). At 75 W, Sarapirom and Yu (2021) found OH, CH, O_1, H_γ, & H_β present in a low-pressure argon plasma system. Similar plasma species should be present in the air plasma in the rotary plasma reactor (50 W), although their study appeared to show reduced power decreased the presence of these excited species (Sarapirom & Yu, 2021).

All of the volatile aroma compounds found within this study are naturally formed within the strawberry by a variety of mechanisms; for example, long-chain free fatty acids (oleic acid, linoleic acid and linolenic acid) can be naturally cleaved through enzymatic oxidation to form carboxylic acids such as butanoic acid and hexanoic acid (Hu et al., 2018; Bajramova & Spégel, 2022). Campelo et al. (2020a) speculated that plasma might oxidise and split linoleic acid to form aldehyde compounds which could have then been further reduced to form hex-3-en-1-ol and hexan-1-ol. The also suggested activation of the LOX enzymatic pathway might be responsible for production of the alcohols. Either way, this finding shows that hexanoic acid (the third product of linoleic acid) could also be produced by cleavage at the C6 of linoleic acid (Fig. 8a). Enzymatic processes or plasma cleavage may also be

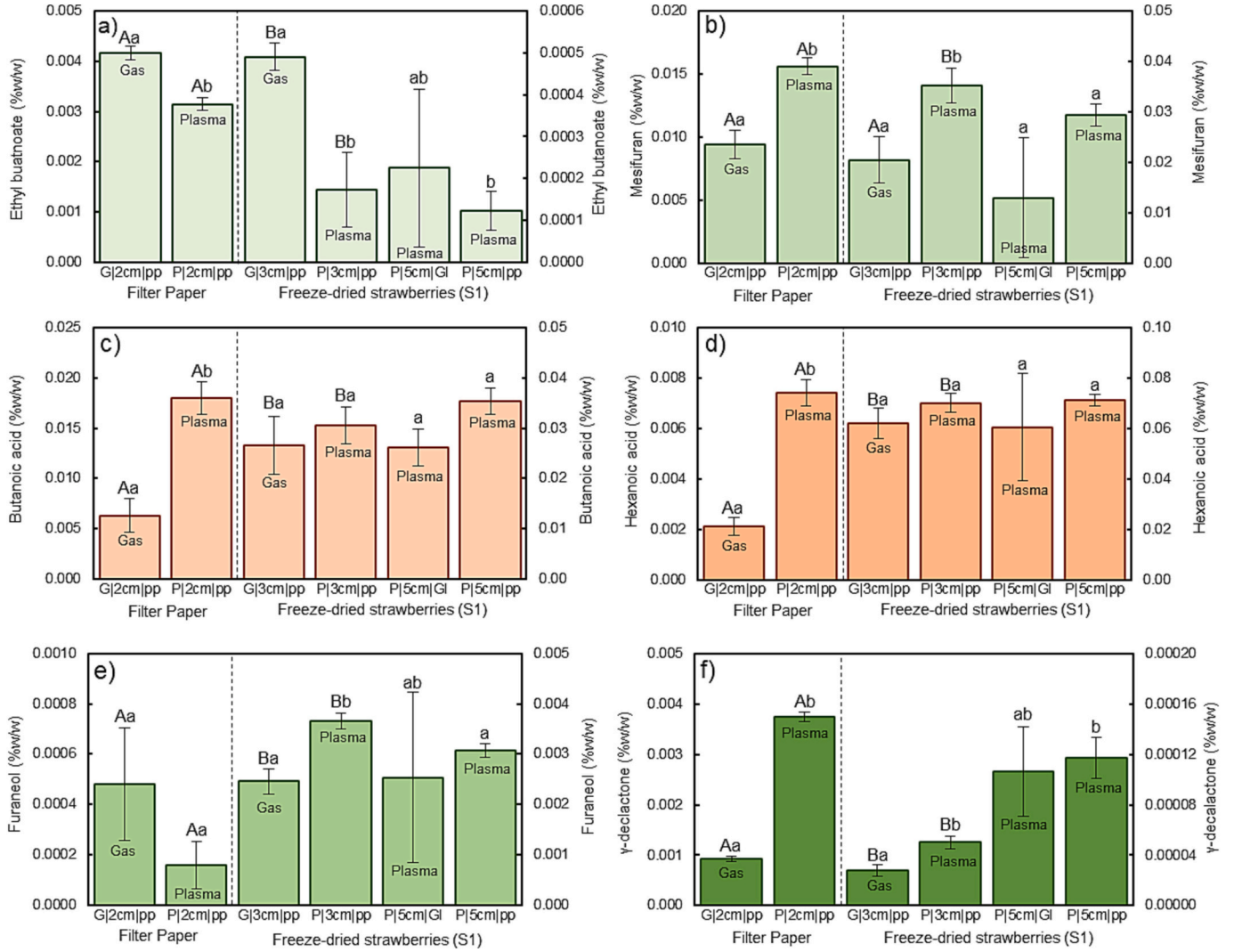

Fig. 5. Average concentrations (% *w*/w) (n = 3 to 5) (± s.d.) of ethyl butanoate (a), mesifuran (b), butanoic acid (c), hexanoic acid (d), furaneol (e), and γ-decalactone (f) in either the M1 solution (mix of these volatiles on a 3 cm aroma paper strip) (Y1 axis) or in freeze-dried strawberries (S1) (Y2 axis). These were treated with air-based RF plasma with ("P") or without (just air) ("G") ionisation at 65 V input (2–3 kV at 1 MHz AC output) for 60 s. Strawberries were placed either 3 cm or 5 cm away (axially) from the plasma source. Paper strips were kept 2 cm away axially from the tip (5 cm to the base) to the plasma source. Lowercase letters indicate significant differences between treatment types. Uppercase letters indicate significant differences between matrices.

responsible for generation of butanoic acid and hexanoic acid (Fig. 8a) but the pathway for doing this is currently unknown. Further investigations should seek to understand the significant butanoic and hexanoic acid changes when treated with air- and N_2-based direct plasma (kINPen IND-x) (Supplementary Fig. 4). Despite this, when treating strawberries (S1 and S3) using the air-based RF rotary plasma reactor (low pressure) (C30) and the semi-direct RF plasma (Atomflo) (helium and oxygen) (C1-C3), hexanoic acid and butanoic acid were not observed to increase, suggesting that different pathways may be operating (Table 2). Furthermore, the increase in butanoic acid and hexanoic acid observed in direct RF plasma treatment (C20) of the individual compounds (BaM1 and HaM1, respectively) could be caused by the re-oxidation of butanoic and hexanoic acids from 1-butanol and 1-hexanol (Duignan, Parsons, & Ninham, 2015; Hernández-Montelongo, García-Sandoval, & Aguilar-Garnica, 2015) (Fig. 8a).

There could be a similar mechanism for mesifuran and furaneol, which undergo reversible methylation and protonation, respectively (Duignan, Parsons, & Ninham, 2015; Hernández-Montelongo, García--Sandoval, & Aguilar-Garnica (2015); Pho et al., 2023; Zhang et al., 2018). Many instances were observed in strawberries (C8-C12) and as compounds (C16, C17, C20), where mesifuran was significantly increased, and furaneol was not significantly affected by cold plasma treatments. This could be caused by promoting the methylation of ethanol and furaneol in a condensation reaction (Fig. 8b) via the hydroxyl group of furaneol H+ ions produced by the plasma-induced dissociative excitation of water (Duignan et al., 2015; Hernández--Montelongo et al., 2015; Pho et al., 2023; Zhang et al., 2018).

Based on the data from Leonardou et al. (2021), Oh et al. (2021), and Silva et al. (2021), γ-decalactone can be synthesised naturally in strawberries through the hydroxylation of oleic and linoleic fatty acids into carboxylic acids such as decanoic acid, that are then lactonised into γ-decalactone (Fig. 8c). In plasma systems, it could be suggested that linoleic acid can be cleaved through ion interaction, although the nature of γ-decalactone synthesis from decanoic acid remains unknown (Leonardou et al., 2021; Oh et al., 2021; Silva et al., 2021). Therefore, the proposed mechanism may be that the free hydroxyl ions produced induce this self-esterification of decanoic acid at C4. However, since OH could cause this at any carbon on the chain, it is unclear why C4 would be more impacted. To address this, a future experiment could use plasma to treat linoleic acid and use both SPME and liquid extraction to identify the output of γ-decalactone and decanoic acid.

It is clear from previous studies that different cold plasma conditions

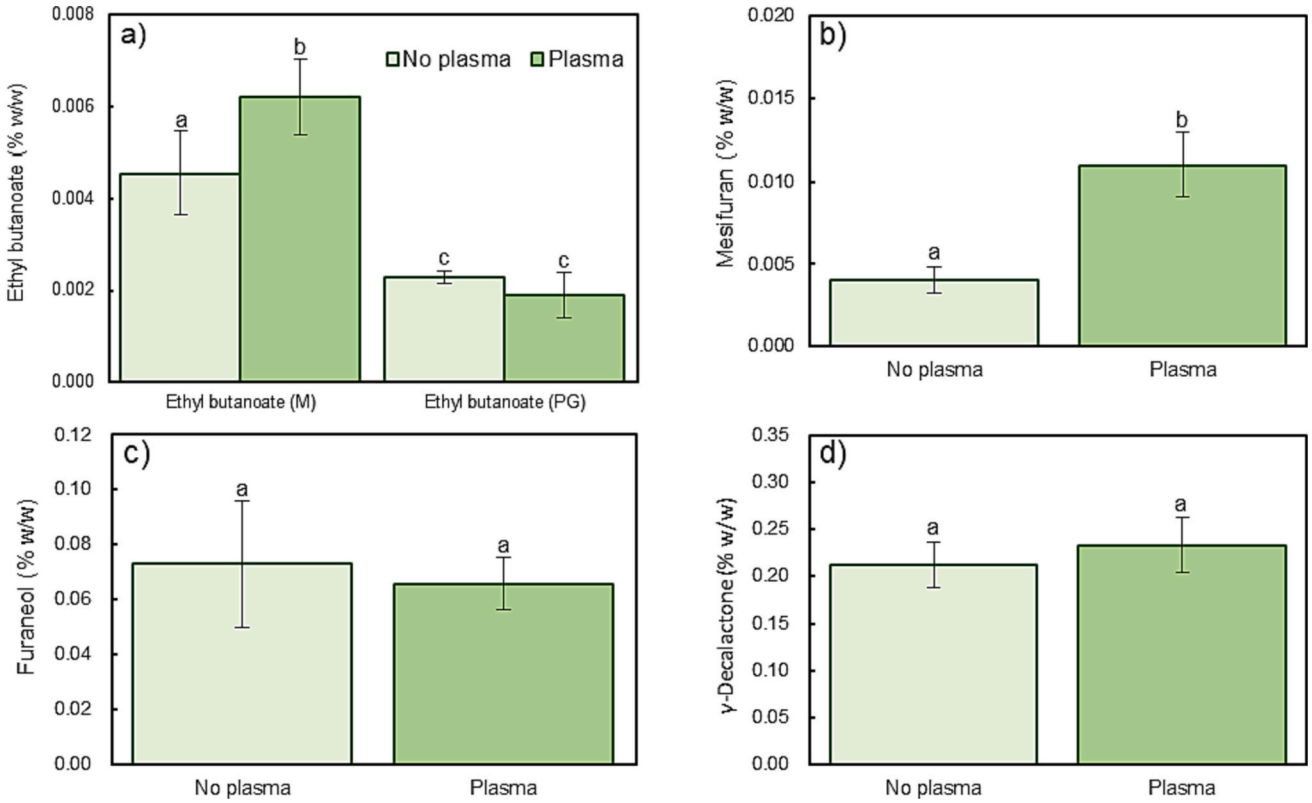

Fig. 6. Mean calculated concentrations (n = 3–5) (± s.d.) of ethyl butanoate (a), mesifuran (b), furaneol (c), and γ-decalactone (d) in aroma paper that contained methanol (M) or polypropylene (PG) solutions of the following respective compounds: ethyl butanoate in PG or M (100 μL at 0.01% *w*/w) (a), mesifuran in M (100 μL at 0.01% w/w) (b), furaneol in M (100 μL at 0.05% w/w) (c), and γ-decalactone in M (300 μL at 0.05% w/w) (d). These samples were analysed with (C or without (C0) Air-based CP treatment. The CP treatments were all conducted for 60 s at 65 DC V input (2 kV to 3 kV at 1 MHz AC output), 4 L min^{-1}, no pulse, and a sample-to-source gap of 2–5 cm from the plasma tip to the base of the filter paper.

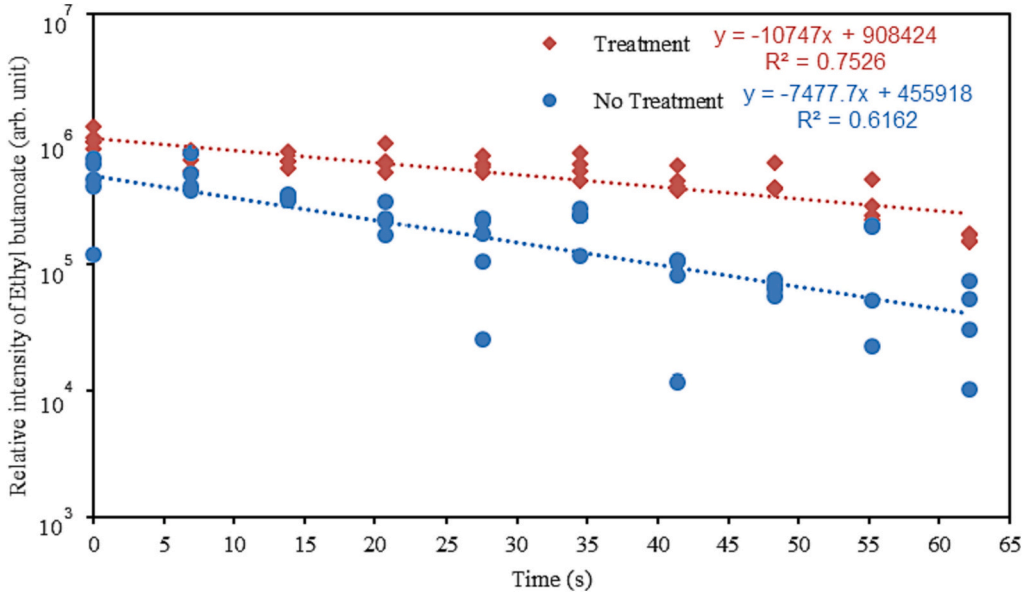

Fig. 7. Mean relative intensity of the nearest apex peaks (n = 4 to 6) (± s.d.) of ethyl butanoate from aroma paper infused with 300 μL of 0.01% w/w solutions of ethyl butanoate in methanol. These strips were treated with 4 L min^{-1} of compressed air with ionisation at 65 DC V input (2 kV to 3 kV at 1 MHz AC output) for 60 s (C20) with the kINPen IND-x. Aroma paper strips were treated 2 to 5 cm (tip to the base of the strips, respectively) away in small glass vials. Samples were analysed in real-time with six replicates using the APCI-MS.

produce various excited, ionic (e.g., O+), and neutral (e.g., OH) species that will ultimately impact their reactivity and interactions with compounds (Bao et al., 2020; Pandiyaraj et al., 2020; Pho et al., 2023; Roy et al., 2017; Sarapirom & Yu, 2021; Zaplotnik, Bišćan, Krstulović, Popović, & Milošević, 2015). Hence, while the reactive species produced by the plasmas in this study can be roughly approximated based on

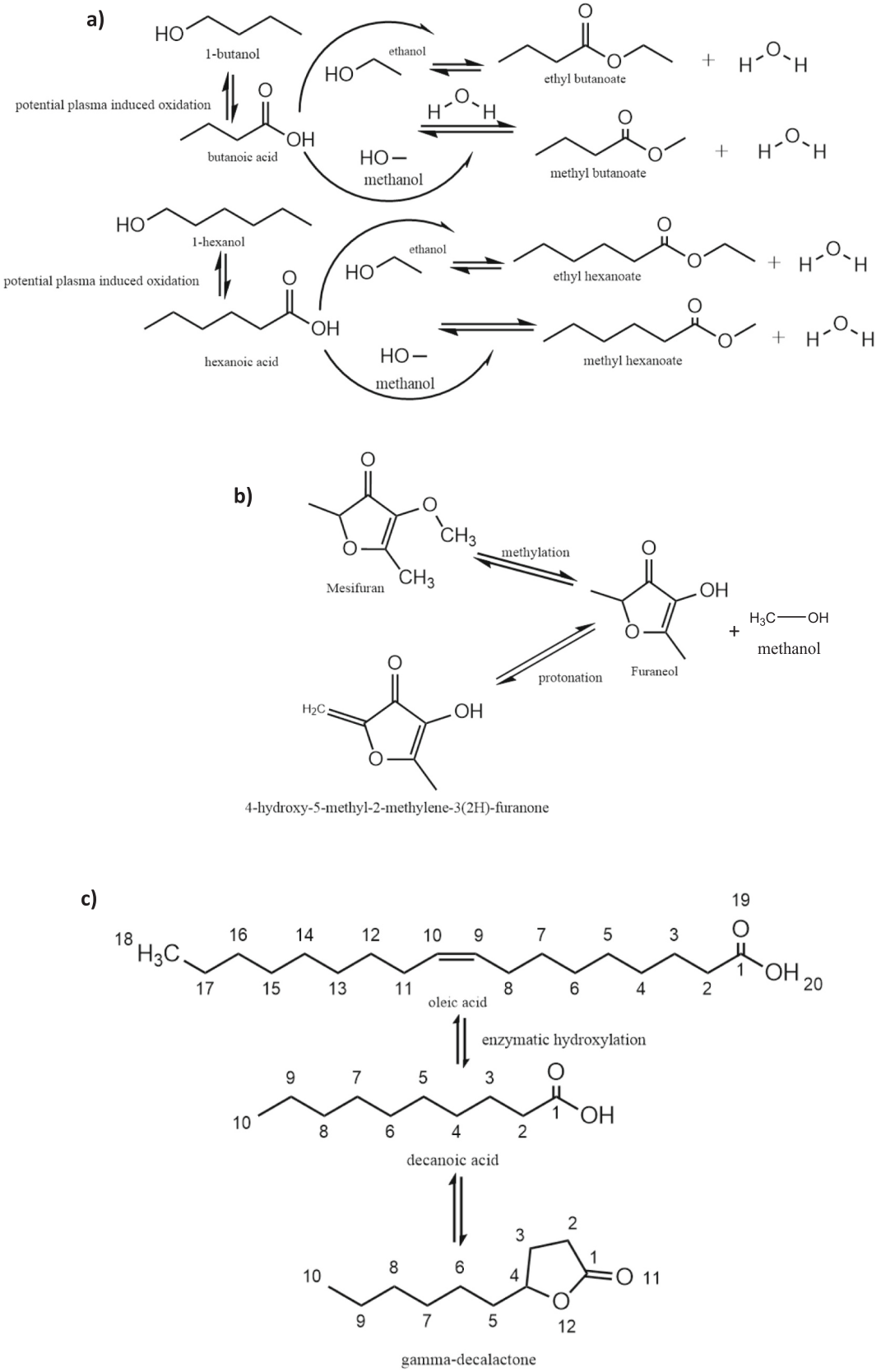

Fig. 8. Proposed chemical precursors and products of ethyl butanoate (a), methyl butanoate (a), methyl hexanoate (a), butanoic acid (a), hexanoic acid (a), mesifuran (b), furaneol (b), and γ-decalactone (c) in strawberries based on studies by Campelo et al. (2020a), Hu et al. (2018), Leonardou et al. (2021), Oh et al. (2021), Silva, Coelho, Aguiar, and Domingues (2021), Xu, Zang, Sun and Li (2022), and Zhang et al. (2018).

previous OES data, it is still a qualitative assessment. Future studies may benefit from bespoke OES analysis of each plasma used and from UV (UV-OES) and infrared (laser-induced fluorescence) analysis for other ionic and neutral species, or by using cavity ring-down spectroscopy (Zaplotnik et al., 2015). Despite the diversity of plasma devices used and their resultant ionic output, key reactive oxygen and nitrogen species appear to be consistently present across different devices and conditions, such as O_1, OH, and H_γ, which are arguably the key contributors to the mechanisms proposed by this study (Bao et al., 2020; Pandiyaraj et al., 2020; Pho et al., 2023; Roy et al., 2017; Sarapirom & Yu, 2021).

3.10. Outlook: flavour in space

This study showed that cold plasma could impact aroma and, therefore, could enhance the impact of complementary technologies such as sonic seasoning (matches audio to the aroma), which improved food and beverage perception on passenger aircraft flights (Spence, 2014; Victor, 2014), and hence potentially space flights (Obrist, Tu, Yao, & Velasco, 2019). However, it is not yet fully understood how humans will perceive these plasma-based aroma changes in space. A report by NASA (Douglas et al., 2016) has found that astronauts perceive flavour differently on the ISS compared to Earth. These differences reduce food intake, which significantly reduces nutritional intake (Douglas et al., 2020). Hence, improving these aspects in a holistically integrated research approach (i.e., flavour, agriculture, nutrition, medicines, and health) will be necessary for future advances in space travel (Hava et al., 2020; Tran et al., 2022; Hessel et al., 2022; Sawyers et al., 2022). There could be numerous influences on this, including changes in nasal physiology, a shift of body fluids to the head, or the absence of natural convection in microgravity, reducing aroma release (Inglesby et al., 2020; Mutabazi et al., 2016; Taylor et al., 2020). Sensory studies (in microgravity) are difficult to conduct with a limited sample size (small number of astronauts). So, aroma-based studies (non-human based) in microgravity are more likely to facilitate this understanding (Taylor et al., 2020). While this study used the closest possible microgravity simulation on earth for aroma studies, future microgravity studies could improve on the current study by repeating this study on the ISS, a CubeSat, or ESA's Zero-G flight; this can be done by modifying the SPME fibres in a similar set-up (Supplementary Fig. 2).

4. Conclusion

This study found that the fruity esters in strawberries (ethyl butanoate, methyl butanoate, and methyl hexanoate) were significantly reduced during freeze-drying. Cold plasma significantly mitigated this loss, validating its use as a complementary processing technique. This study also highlights the plasma conditions that could provide optimal aroma profiles in fresh and freeze-dried strawberries, including beneficial or neutral changes. Although treatment types influence the volatile aroma compounds, there are no direct correlations between increasing flow, power, or distance and resultant aroma changes. The type of gas used impacted aroma: nitrogen, for example, significantly reduced butanoic and hexanoic acids in methanol compared to compressed air. Matrix and cold plasma parameter effects were observed for ethyl butanoate, the most beneficial of which are C1, C20, C25, and C26. Six cold plasma treatments were identified as providing an optimal aroma: C1, C17, C19, C20, C21, and C28. Optimal aromas include increased fruity aromas (ethyl butanoate, mesifuran, furaneol, and γ-decalactone) and decreased cheesy or unripe aromas (butanoic acid and hexanoic acid). Mesifuran was increased by various cold plasma treatments (C1, C8-C12, C16, C20, C21, C24, and C28). However, butanoic acid and hexanoic acid both tended to either be unaffected by cold plasma or to significantly increase, the most beneficial of these being treatment C14 (as it either reduces or maintains the carboxylic acid concentrations). Furaneol was only increased by C1 & C17, so it could be worth using this to treat other volatile compounds in future research. γ-decalactone was the only compound to benefit from each cold plasma treatment in that it either increased or maintained its initial concentration. "Zero-averaged gravity" impacted the release of methyl butanoate and methyl hexanoate, showing that both are affected by simulated changes in gravitation forces and that cold plasma reduced this negative impact.

This study demonstrated that RF cold plasma can target specific aroma profiles and potentially improve overall quality and acceptability for foods destined for space. Furthermore, the technology has already been used on the ISS, so there is considerable potential for its use on space food. Further research could include applying the optimal parameter conditions and monitoring the effects on microbial growth to determine if it can simultaneously improve the quality and safety of the food with those treatment conditions, thereby inhibiting food spoilage in space.

CRediT authorship contribution statement

George R. Warne: Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft, Writing - review & editing. **Mui Lim:** Methodology, Writing – review & editing. **Kerry Wilkinson:** Writing – review & editing. **Volker Hessel:** Writing – review & editing. **Philip M. Williams:** Supervision, Funding acquisition, Writing - review & editing. **Bryan Coad:** Methodology - Development or design of methodology. **Ian D. Fisk:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2023.103497.

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