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Effect of sweeteners and carbonation on aroma partitioning and release in beverage systems

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ABSTRACT

The effect of monosaccharides (glucose, fructose and galactose) and disaccharides (sucrose and lactose) at 10, 20 and 30 % w/v on the in-vitro aroma partitioning of C_4 – C_{10} aldehydes and ethyl esters, as well as limonene (concentration of aroma compounds at 1 μg mL^{-1}), was studied using atmospheric pressure chemical ionisation–mass spectrometry. An increase in sugar concentration from 0 to 30 % w/v resulted in a significant increase in partitioning under static headspace conditions for the majority of the compounds ($p < 0.05$), an effect generally not observed when 10 % w/v sucrose was substituted with low-calorie sweeteners ($p > 0.05$). The complexity of the system was increased to model a soft drink design – comprising water, sucrose (10, 20 and 30 % w/v), acid (0.15 % w/v), carbonation (~7.2 g/L CO_2) and aroma compounds representative of an apple style flavouring, namely ethyl butanoate and hexanal (10 μg mL^{-1} each). Although the addition of sucrose had no significant in-vivo effect, carbonation significantly decreased breath-by-breath (in-vivo) aroma delivery ($p < 0.05$). To understand the physical mechanisms behind aroma release from the beverage matrix, the effect of sucrose on the kinetics of the matrix components was explored. An increase in sucrose concentration from 0 to 30 % w/v resulted in a significant decrease in water activity ($p < 0.05$), which accounted for the significantly slower rate of self-diffusion of aroma compounds ($p < 0.05$), measured using diffusion-ordered spectroscopy–nuclear magnetic resonance spectroscopy. No significant effect of sucrose on carbon dioxide volume flux was found ($p > 0.05$).

1. Introduction

The Global Action Plan published by the World Health Organisation reiterated that non-communicable diseases, such as obesity, diabetes and cardiovascular diseases, present a global burden and threat to public health and sustainable development (WHO, 2013). Population-based fiscal policy interventions, such as taxes and subsidies, to promote healthy diets and reduce the consumption of calorie-dense foods are recommended to address these challenges. As a result, sugar taxes have gained traction in recent years, with implementation in>50 countries and jurisdictions (World Bank, 2022). In response, many manufacturers have stepped up product reformulation efforts in sugar sweetened beverages, such as carbonated soft drinks, launching products with sugar levels below the taxation threshold.

However, sugar reduction or substitution introduces complex technical and sensory challenges. In a multi-component food matrix, sucrose may enhance or suppress other matrix components and modify aroma availability, the extent of which depends on the physical and chemical properties of the ingredients (Goldfein & Slavin, 2015). This is further complicated during beverage consumption as dilution of aroma compounds occurs with the flow of saliva in the mouth, resulting in a shift in the effective partitioning (van Ruth & Roozen, 2010). Additional

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Table 1
Physicochemical properties of aroma compounds.

Aroma compound	Molecular mass (Da)	Vapour pressure (mm Hg)*	Hydrophobicity, Log P*	Air-water partition coefficient, K_H (atm m^3/mole)*
Butanal	72	108	0.82	1.20 E-4
Hexanal	100	9.57	1.80	2.11 E-4
Octanal	128	1.49	2.78	3.71 E-4
Decanal	156	0.235	3.76	6.54 E-4
Ethyl acetate	88	98.3	0.86	2.33 E-4
Ethyl butanoate	116	14.6	1.85	4.10 E-4
Ethyl hexanoate	144	1.8	2.83	7.23 E-4
Ethyl octanoate	172	0.235	3.81	1.27 E-3
Limonene	136	1.45	4.83	3.80 E-1

* Values are estimates at 25 °C and obtained from *EPI* Suite™ (U.S. Environmental Protection Agency).

dilution occurs as small volumes of air are pumped into the tidal flow of the throat during drinking and even larger volumes are injected into the air stream upon swallowing. This results in a dilution of the aroma compounds released from the food matrix to the gas phase in the magnitude of 10 – 100 fold during the transfer from mouth to nose (Taylor, 2002). Rapid on-line aroma analytical tools that offer suitable sensitivity, such as Atmospheric Pressure Chemical Ionisation–Mass Spectrometry (APCI–MS), are therefore essential tools to explore the impact of matrix composition on aroma delivery in-nose during beverage consumption.

Although the effect of sugar type and concentration on aroma release and perception has been studied previously, most studies have focused on only sucrose (Charles et al., 2015; Nahon et al., 2000; Oliveira et al., 2015; Pfeiffer et al., 2006; Rabe et al., 2003), with fewer investigating other sugars such as glucose (Copolovici & Niinemets, 2007; Hansson et al., 2001; Hewson et al., 2008; Piccone et al., 2012), fructose (Hewson et al., 2008; Piccone et al., 2012) and lactose (Piccone et al., 2012). Moreover, among the variety of beverage models and systems investigated, few studies have incorporated carbonation, which is an essential component of many soft drinks (Hewson et al., 2009; Pozo-Bayón et al., 2009; Saint-Eve et al., 2009) and alcoholic beverages (Clark et al., 2011b). The dearth of information available on aroma-matrix interactions in a complex carbonated system accentuates the need to develop deeper knowledge within the area. The aim of this research was to investigate the effect of sweetener and carbonation on aroma partitioning and release, which were measured using APCI–MS. Initially, an in-vitro technique was employed to mimic the orthonasal aroma perception upon the first opening of a beverage. A range of sweetener types, including monosaccharides, disaccharides, artificial and natural low-calorie sweeteners, was used to examine their effect on the aroma partitioning of C_4 – C_{10} aldehydes and esters, as well as limonene, representing aroma compounds with different levels of hydrophobicity and phase partitioning. Subsequently, the complexity of the system was increased to model a soft drink design with a typical commercial beverage composition. In addition to sucrose and citric acid, ethyl butanoate and hexanal were dosed to create an apple-style flavour while carbonation (~7.2 g/L CO_2) was added to produce fizziness. An in-vivo approach was adopted to simulate the beverage consumption experience, facilitating the study of the effect of sucrose and carbonation on aroma delivery upon consumption. Physicochemical characteristics of the system, specifically the water activity and aroma compound's self-diffusion coefficient, were also explored. An understanding of aroma-matrix interactions within a carbonated beverage system will contribute to the formulation of successful products in the soft drinks industry to meet government tax regulations and cater to the shift in consumer preferences for better-for-you products with low or no sugar and simple, clean-label ingredients without comprising on flavour delivery.

Table 2
Concentrations (% w/v) of low-calorie sweetener (blend) in the system.

Low-calorie sweetener (blend)	Concentration (% w/v)
Saccharin/ Aspartame/ Acesulfame-K blend (3:3:1)	0.0455
Sucralose	0.017
Stevia/ Allulose blend (1:186)	3.52
Stevia	0.025

2. Materials and methods

2.1. Materials

Aldehydes and esters (C_4, C_6, C_8 and C_{10}), limonene, fructose, glucose, galactose, sucrose, lactose, anhydrous citric acid and deuterium oxide were obtained from Sigma-Aldrich (Gillingham, UK) or Fisher Scientific (Loughborough, UK). These chemicals were ≥ 95 % purity. Acesulfame-K, aspartame, saccharin and sucralose were obtained from Blends ltd (Liverpool, UK). Stevia was obtained from Bulk Powders (Colchester, UK). Allulose was obtained from Matsutani Chemical Industry (Hyogo, Japan).

2.2. Model systems

2.2.1. Aroma-sugar system

The aroma-sugar system comprised a single aroma compound from Table 1 (1 μg mL^{-1}), 0.15 % w/v citric acid and 0, 10, 20 or 30 % w/v sugar. The monosaccharides, fructose, galactose, glucose, as well as disaccharides, lactose and sucrose, were used. All the dilutions were made using deionised water and mixed on a roller mixer (SRT9D, Stuart Scientific, Redhill, UK) for 1 h at 60 rpm before equilibration at room temperature for ≥ 2 h. These systems were only used for the in-vitro study.

2.2.2. Aroma-low-calorie sweetener system

Low-calorie sweeteners were used as sugar substitutes in these systems and were added to obtain a final concentration of 10 % w/v perceived sucrose sweetness equivalence, as determined by manufacturer's specification and within the range of sucrose concentrations typically present in soft drinks. Table 2 lists the concentrations of sweeteners and blends used at ratios recommended by the manufacturers. The samples were prepared as in Section 2.2.1 and were only used for the in-vitro study.

2.2.3. Model carbonated beverage

The model carbonated beverage was constructed to mimic the composition of commercial soft drinks and consisted of ethyl butanoate and hexanal (10 μg mL^{-1} each), which produced an apple style flavour, 0.15 % w/v citric acid and 0, 10, 20 or 30 % w/v sucrose. The samples were prepared as in Section 2.2.1 and stored at 4 ± 1 °C. Carbonation was carried out following previously validated procedures (Hewson

et al., 2009) using an in-house batch carbonation apparatus (Medical Engineering Unit, University of Nottingham, UK) to achieve ~ 7.2 g/L CO_2 in the samples at 5 °C, a typical carbonation level found in soft drinks. This model was only used for the in-vivo study.

2.3. In-vitro aroma partitioning

In-vitro aroma partitioning was analysed using APCI–MS, which comprised of a MS Nose interface (Micromass, Manchester, UK) fitted to a Quattro Ultima MS (Micromass). All the samples were contained in 100 mL Schott bottles (Fisher Scientific) fitted with a one-port lid. The headspace above each 50 mL sample was drawn into the ionisation source through the port opening at a flow rate of 5–10 mL min^{-1} for 30 s through a heated and deactivated fused silica capillary (0.6 m length × 0.53 mm I.D.) encased in a copper tubing. The aroma compounds entering the source were ionised by a 3.5 kV corona discharge at a cone voltage of 60 V and the ions formed were monitored at *m/z* corresponding to the protonated molecular ion (MH^+) of the compounds. The APCI–MS was operated in a selected ion mode, with a dwell time of 0.50 s and an interscan delay of 0.02 s. A total of 3 replicates were carried out.

2.4. In-vivo aroma release

The study was approved by the School of Biosciences ethics committee (#SBREC160137A) and written consent was obtained from all 5 panellists prior to their participation.

Both carbonated (~7.2 g/L CO_2) and non-carbonated samples were aliquoted into 15 mL screw-top, glass vials at 4 ± 1 °C. The vials were filled to capacity, tightly capped and sealed with plastic film to minimise volatile and carbon dioxide loss. The samples were refrigerated and served to panellists at 4 ± 1 °C on the day of preparation. A randomised block design was constructed for the measurement of breath-by-breath volatile concentrations of triplicate samples. Each panellist was placed in a separate block to account for oral physiological differences between individuals.

Panellists were instructed to open the sample bottle and take in all the sample while holding their breath and avoiding any liquid or air movement in the mouth. A small disposable plastic tube (40 mm length × 10 mm I.D.), which led to the fused silica capillary tube, was immediately inserted into one nostril before panellists consumed all of the sample in one swallow event and started breathing normally through the nose for 30 s while keeping the mouth closed throughout the sampling period.

Eight samples were consumed during each session with a rest period of at least 1 min in between samples. Mineral water (Evian, France) and water biscuits (Carr's, UK) were provided for palate cleansing and the breath of the panellists was also monitored to ensure that no detectable traces of aroma compounds remained prior to consuming the next sample.

In-vivo aroma delivery was analysed using the APCI–MS parameters as described in Section 2.3. The breath of panellists was drawn into the ionisation source at a flow rate of 35 mL min^{-1} for 30 s. Ethyl butanoate and hexanal were monitored at *m/z* 117 and 83 respectively. The APCI–MS was operated in a selected ion mode, with a dwell time of 0.02 s and an interscan delay of 0.02 s.

2.5. Water activity (a_w)

The water activities of sucrose, which was used in the model carbonated beverage, as well as the monomer units fructose and glucose, in 10, 20 and 30 % w/v solutions were measured using a water activity meter (Aqua Lab 4TE, Decagon Devices Inc., USA) at 25 °C. A total of 3 replicates were carried out.

2.6. Self-diffusion coefficients of aroma compounds (D)

Deuterium oxide was used for the preparation of samples containing ethyl butanoate and hexanal (10 μg mL^{-1} each) in 0, 10, 20 or 30 % w/v sucrose solution. All the samples were mixed on a roller mixer (SRT9D, Stuart Scientific) for 1 h at 60 rpm. An aliquot of 700 μL sample was transferred into 5 mm SampleJet tubes (Bruker, Coventry, UK), which were capped and sealed with POM balls (Bruker).

The measurement of the self-diffusion coefficients of the aroma compounds in sucrose solutions was carried out using ^1H Nuclear Magnetic Resonance (NMR). All spectra were recorded using a 600 MHz spectrometer (Avance 600, Bruker) with a 5 mm z-gradient inverse probe (Bruker) at 25 °C using a Pulsed Gradient Spin Echo (PGSE) sequence with convection compensation from the Bruker standard library. A total of 192 scans was collected using the PGSE sequence with a recycle delay of 10 s. Diffusion measurements were using the delays for big delta (Δ) and small delta (δ) at 200 ms and 2.2 ms respectively. Echo intensity was reduced as a function of gradient strength with delta values optimised for 90 % reduction between the start and end values. A total of 10 values were recorded with signal averaging 64 transients. Diffusion coefficients for each resonance were obtained from optimally fitted decay curves based on the areas of the peaks. All the data were processed using Bruker TopSpin 3.1 v3.5 (Bruker). A total of 3 replicates were carried out.

2.7 vol. flux of dissolved carbon dioxide (VF)

The measurement of carbon dioxide volume flux from sucrose, fructose and glucose solutions in 10 %, 20 % and 30 % w/v was carried out using a precision weighing balance (DV215CD, Ohaus, Leicester, UK) interfaced with a computer, following the procedure of Liger-Belair et al. (2009).

Carbonated samples were removed from refrigeration and allowed to equilibrate at 25 °C for 1 h to negate the influence of condensation on the outer surface of the sample bottle on the mass recorded by the balance. After the lid of the sample bottle was opened, the bottle was immediately placed on the chamber base plate of the balance, which triggered data collection on the laptop PC over a 10 min period at 5 s interval. A total of 3 replicates were carried out.

2.8. Data processing and statistical analysis

2.8.1. In-vitro aroma analysis

The mean relative amounts of each aroma compound were determined by comparison of peak height data obtained from chromatograms integrated using MassLynx software v4.1 (Micromass). A relative index (I/I_0) was obtained by comparing the ratio of mean peak height of each sample (I) in comparison to that of the control sample without sweetener addition (I_0) to understand the effect induced by the presence of the specific sweetener. A value above 1 corresponded to an increase in aroma partitioning – the higher the value, the greater the partitioning. On the other hand, a value below 1 corresponded to a decrease in aroma partitioning and indicated a retention of aroma compounds in the matrix.

2.8.2. In-vivo aroma analysis

The mean relative amounts of each aroma compound were determined by comparison of maximum intensity (I_{max}) and total area under the curve (AUC) data obtained from chromatograms integrated using MassLynx software v4.1 (Micromass). While I_{max} corresponded to the maximum intensity of aroma release, AUC provided an indication of the total aroma released in the nose space.

2.8.3. Statistical analysis

All statistical analyses were performed using SPSS v26 (IBM, New York, USA). Statistical differences between samples were tested using

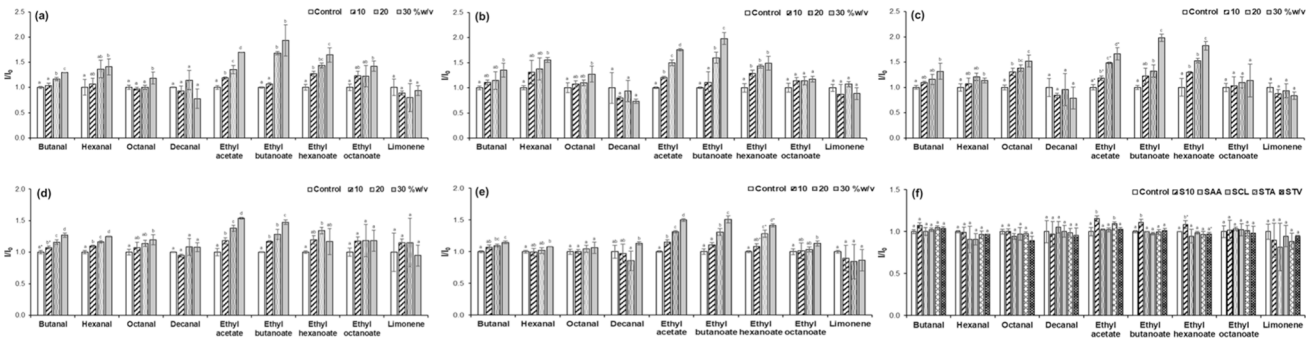

Fig. 1. Normalised mean data (n = 3) for in-vitro partitioning (I/I_0) of aroma compounds in (a) fructose (b) galactose (c) glucose (d) lactose (e) sucrose (f) different sweetener systems (Control = Sucrose, 0 % w/v; S10 = Sucrose, 10 % w/v; SAA = Saccharin/Aspartame/Ace-K blend; SCL = Sucralose; STA = Stevia/Allulose blend; STV = Stevia). Different letters above bar graph indicate significant differences ($p < 0.05$) between samples for each aroma compound. * indicates borderline statistical significance ($p \leq 0.054$).

one-way or multivariate analysis of variance and Tukey's HSD post-hoc tests (where applicable) at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Effect of sweeteners on in-vitro aroma partitioning

3.1.1. Effect of sugar concentration

As sugar concentration increased from 0 to 30 % w/v, there was generally a significant increase in the in-vitro partitioning of aroma compounds ($p < 0.05$, Fig. 1), with the exception of the more hydrophobic compounds – decanal ($p = 0.08$, 0,33, 0.60, 0.17 for fructose, galactose, glucose and lactose respectively; Appendix A1), ethyl octanoate ($p = 0.08$, 0.83, 0.45 for galactose, glucose and lactose respectively; Appendix A1) and limonene ($p = 0.56$, 0.21, 0.27, 0.70, 0.71 for fructose, galactose, glucose, lactose and sucrose respectively; Appendix A1). This is in agreement with previous studies, which attributed the observation to the phenomenon of 'salting-out' (Hansson et al., 2001; Rabe et al., 2003; Tsitlakidou et al., 2019).

In a solvent–solute system, at least three elementary types of molecular associations could occur – solvent–solvent interaction, solvent–solute interaction and solute–solute interaction (Starzak et al., 2000). The active hydroxyl groups of the sugar moieties can establish hydrogen bonds with the hydrogen atoms on the water molecules and the consequence of an increase in sugar concentration is an increase in sugar-water interactions. As sugars are cosmotropes which increase the structural order of water molecules in the system (Baránková & Dohnal, 2016), the addition resulted in a decrease in the volume of free water available for the solubilisation of aroma compounds (Friel et al., 2000). Thus, the effective partition equilibrium of the aroma compounds was shifted towards the gas phase, resulting in the 'salting-out' effect and enhanced aroma partitioning observed.

More interestingly, the significance of the 'salting-out' effect decreased as the alkyl chain length of the aroma compounds within the homologous series increased (Fig. 1a – e). The following comparison is based on the esters in the aroma-sucrose system (Fig. 1e). At the start of the homologous series, each 10 % w/v sucrose addition resulted in a significantly higher aroma partitioning of the ester, namely ethyl acetate ($p < 0.001$; Appendix A2). However, such distinct differences were not observed in systems containing ethyl butanoate and ethyl hexanoate, whereby there was no significant difference between the control and 10 % w/v sucrose solution ($p = 0.25$ and 0.30 respectively; Appendix A2). As for ethyl octanoate, the ester with the longest alkyl chain length in the homologous series, only the addition of sucrose at 30 % w/v resulted in a significantly higher aroma partitioning as compared to the control ($p = 0.04$; Appendix A2) and there was no significant difference between the 10 % v.s. 20 % w/v solutions ($p = 0.96$; Appendix A2) and 20 % v.s. 30 % w/v solutions ($p = 0.14$; Appendix A2). This trend could also be observed within the homologous series of aldehydes and applied to the other sugars.

While the impact of a polar functional group would decrease due to a longer hydrophobic aliphatic chain length (Jeleń & Gracka, 2017), bond rotations leading to changes in distribution of polar and non-polar surfaces on the molecule could also occur in order to achieve more stable conformations, resulting in the shielding of the polar region of the aroma compounds. Thus, larger compounds within the homologous series are less polar and water soluble, thereby actively partitioning into the gas phase, which corresponds to higher log P and K_H values, despite the lower vapour pressure and volatility usually associated with an increase in alkyl chain length due to an increase in molecular size (Belitz & Grosch, 2013). As sugar molecules are highly polar, they compete with the aroma compounds in the formation of hydrogen bonds with water and thus, had a more significant impact on the smaller and more polar aroma compounds within the homologous series (i.e. butanal and hexanal for the aldehydes, as well as ethyl acetate and ethy butanoate for the esters).

As for limonene, the aroma compound with the lowest water solubility and highest air/water partitioning, as indicated by the highest log P and KH values respectively (Table 1), a lack of significant effect of sugar concentration on its release was found. This was also demonstrated by Hansson et al. (2001) and was attributed to the strongly non-polar nature of the compound, while a 'salting-in' effect was observed by Copolovici and Niinemets (2007). It was suggested that the addition of polar solutes such as sugar could increase the hydrophobicity of the solvent character (Nahon et al., 2000), thereby enhancing the aqueous solubility of the less polar aroma compounds and thus, resulting in the lower aroma partitioning observed (Copolovici & Niinemets, 2007).

3.1.2. Effect of sugar type

The effect of sugar type is more complex as each sugar is unique in terms of polarity, molecular conformation and functional groups, eliciting different changes in the properties of the beverage system and thus, favouring the solubility and retention of aroma compounds or vice versa (Piccone et al., 2012). Between the different classes of sugars, there was generally a significantly higher increase in aroma partitioning when a monosaccharide was added to the system as compared to a disaccharide ($p < 0.05$, Fig. 1). As equivalent weight concentrations were used in this study, there was almost twice the number of monosaccharide molecules than that of disaccharides. This would have compensated for the lower specific affinity for water and weaker hydration capacity of monosaccharides as compared to disaccharides, which have more exchangeable hydroxyl groups present.

Within the class of monosaccharides, it was reported that glucose exhibited stronger interactions with water as compared to other monosaccharides due to its higher number of equatorial hydroxyl groups (Aroulmoji et al., 2012). These are hydroxyl groups orientating in the

plane of the six-membered ring of the monosaccharide unit and have a better fit with the quasi-tetrahedral structure of water (Shiraga et al., 2015). However, there was no significant difference between the in-vitro partitioning of the aroma compounds within each class of sugars studied ($p > 0.05$), suggesting that the number of hydrogen bonds had a greater influence on the aroma-matrix interactions than bond strength in this study.

3.1.3. Effect of low-calorie sweeteners

As seen from Fig. 1f, there was no significant difference in the in-vitro partitioning of the majority of the aroma compounds between the sample and control upon the substitution of 10 % w/v sucrose with low-calorie sweeteners ($p > 0.05$), except for ethyl acetate whereby there was a significant difference between the 10 % w/v sucrose solution and those replaced with saccharin/aspartame/acesulfame-k, sucralose and stevia/allulose ($p < 0.005$ for all; Appendix A3). In solutions where a significantly higher aroma partitioning was observed with the addition of 10 % w/v sucrose, it could be attributed to the phenomenon of 'salting-out'. On the other hand, the lower aroma partitioning observed with the addition of low-calorie sweeteners was likely to be due to the much lower quantities added to the solutions to achieve 10 % w/v sucrose equivalence and thus, any alteration in the volume of free water available for the solubilisation of aroma compounds was too little to induce any effect on aroma partitioning.

For ethyl acetate, it was interesting to observe that the incorporation of allulose at a low concentration of 3.5 % w/v in a blend with the intense sweetener stevia, resulted in an enhanced aroma partitioning of similar impact to 10 % w/v sucrose addition ($p = 0.27$). This could be attributed to the fact that allulose, being an epimer of fructose, could cause a 'salting-out' effect, which seems to be limited to aroma compounds with certain physiochemical characteristics such as low logP and K_H values, although butanal was not found to be affected despite having similar properties as ethyl acetate. Nevertheless, this suggests an opportunity to use low-calorie carbohydrate sweeteners, in synergy with intense sweeteners, in a multi-sweetener approach to achieve a similar enhanced aroma partitioning profile to a sugar containing product.

However, intense sweeteners often exhibit undesirable organoleptic properties such as delayed onset of sweetness, lingering aftertaste, narrow taste profile and even metallicity or bitterness (Chattopadhyay et al., 2014) due to different activation mechanisms of the human taste pathways as compared to sucrose (Frank et al., 2008). Thus, data from in to vitro aroma partitioning should be interpreted together with consideration of perceptual and temporal profiles of low-calorie sweeteners.

3.2. Effect of sucrose and carbon dioxide on in-vivo aroma delivery

Building on the investigation of the influence of sweetener concentration and type on headspace aroma partitioning from a closed system (i.e. in-vitro), a model beverage was formulated, comprising water, sucrose, citric acid and a blend of aromas, to study the effect of sucrose concentration (10 % – 30 % w/v) on in-vivo aroma release to mimic a consumption paradigm. In addition, the complexity of the model beverage was increased with the introduction of carbonation, a common factor in commercially available beverages and a possible source of influence on aroma release profiles.

3.2.1. Effect of sucrose

The addition of sucrose from 10 to 30 % w/v had no significant effect on the in-vivo aroma delivery of ethyl butanoate and hexanal ($p = 0.08$; Appendix B). Previous in-vivo studies on beverages have also reported the lack of significance of the effect of sucrose addition to coffee (Charles et al., 2015) and mint-flavoured carbonated drinks (Saint-Eve et al., 2009), although the lower sugar concentrations of 1 – 10 % w/v used in the experiments were suggested to be insufficient to induce differences in the nose space (Saint-Eve et al., 2009).

In comparison to in-vitro data, the greater variation observed in the in-vivo data due to oral physiological differences between individuals could make it more difficult to establish significant differences between the levels of sucrose concentrations. The causes of individual differences include inherent variations in human anatomy and composition, such as relative volumes of the naso-oropharyngeal cavities, velum opening, salivary flow rate and protein composition (Frank et al., 2012), as well as subconscious body functions such as breathing and swallowing patterns (Muñoz-González et al., 2014; Normand et al., 2004), all of which affect the degree of aroma compound partitioning between the liquid and gas phases during in-vivo delivery (Taylor, 2002). Furthermore, it was demonstrated that sucrose intake resulted in a concentration dependent increase in salivary flow, pH and α-amylase activity (Harthoorn et al., 2009), which could in turn lead to the dilution of beverages within the mouth or other complex phenomena associated with the high surface area present in the oral cavity.

The disparity between in and vitro and in-vivo results in terms of direction and magnitude may be inevitable given the vastly different conditions for the measurement of aroma release and have been reported in other studies (Clark et al., 2011a; Saint-Eve et al., 2009). Aroma compounds present at similar concentrations in the food matrix or even equilibrium headspace could be present at substantially different concentrations in the breath following consumption (Buffo et al., 2005; Linforth et al., 2002), highlighting the importance of in-vivo studies, which simulate the real consumption experience. Nevertheless, both in-vitro and in-vivo methods are important as they provide useful insights into the entire beverage consumption experience from the orthonasal aroma perception of the beverage headspace upon its first opening (in-vitro) to the retronasal aroma perception during beverage consumption when the aroma passes back up through the pharynx into the nasal cavity (in-vivo).

3.2.2. Effect of carbon dioxide (CO_2)

The introduction of carbonation resulted in a significant decrease in the in-vivo aroma delivery of ethyl butanoate and hexanal, as measured by I_{max} and AUC ($p < 0.005$; Appendix B)). These results are in contrast with previous in-vivo studies (Clark et al., 2011a; Saint-Eve et al., 2009), which reported an increase in aroma release in the nose space with the introduction of CO_2 in beverages, although the effect was only found in the first swallow breath and not observed to persist. These researchers attributed their observations to the inherent physiochemical properties of the aroma compounds (Clark et al., 2011a), as well as those changes induced by CO_2 addition on the system, such as the aroma stripping and convection phenomena induced by ascendant gas bubble movement (Saint-Eve et al., 2009). The differences observed could arise from differences in methodology of the experiments, such as the consumption protocol adopted by the panellists and the instrument used for in-vivo aroma analysis. In the work of Saint-Eve et al. (2009) on mint-flavoured carbonated beverages dosed with menthol, menthone and (Z)-hex-3-en-ol, the samples were served at a higher temperature of 10 °C and 25 °C (v.s. 4 ± 1 °C in this study), which could have affected CO_2 release and volatile partitioning as CO_2 solubility and retention are inversely related to liquid temperature (Steen, 2006). In fact, it was demonstrated that CO_2 flux was higher at elevated temperatures (Liger-Belair et al., 2009), which could result in a faster rate at which aroma compounds are stripped from the liquid phase and transferred into the gas phase. In addition, the presence of CO_2 could have an influence on the performance of Photon-Transfer-Reaction–Mass Spectrometry (PTR–MS) used in their study in terms of fragmentation pattern and ion mobility, making the accurate quantification of volatile aroma compounds difficult to achieve (Keck et al., 2008).

Meanwhile, in the work of Clark et al. (2011a) on a model beer system, flavoured with ethyl acetate, isoamyl alcohol and phenylethyl alcohol, using a similar sampling protocol and analytical method as in this study, it was suggested that the effect of carbonation was dependent on the physicochemical properties of the aroma compounds. A

Table 3
I_{max} and AUC mean values (n = 3) for the in-vivo release of ethyl butanoate and hexanal from non-carbonated (-) and carbonated (+) samples at different sucrose concentrations (% w/v).

[Sucrose] (% w/v)	CO_2	Ethyl butanoate		Hexanal	
		I_{max} (a.u, E + 6)	AUC (a.u, E + 4)	I_{max} (a.u, E + 6)	AUC (a.u, E + 4)
0	–	12.40 ± 4.79^{a}	20.54 ± 11.40^{a}	5.89 ± 3.02^{a}	9.47 ± 7.03^{a}
	+	6.73 ± 3.24^{b}	12.11 ± 4.82^{b}	3.63 ± 1.99^{b}	5.28 ± 2.57^{b}
10	–	9.81 ± 4.95^{a}	20.94 ± 10.63^{a}	5.19 ± 2.44^{a}	10.14 ± 7.31^{a}
	+	6.63 ± 4.20^{b}	11.66 ± 7.90^{b}	4.14 ± 3.04^{b}	6.20 ± 5.66^{b}
20	–	9.15 ± 3.90^{a}	16.41 ± 9.45^{a}	4.49 ± 3.43^{a}	7.89 ± 6.97^{a}
	+	7.79 ± 4.85^{b}	13.99 ± 8.31^{b}	3.93 ± 2.10^{b}	6.42 ± 4.66^{b}
30	–	11.26 ± 5.09^{a}	23.05 ± 17.38^{a}	6.63 ± 3.55^{a}	8.67 ± 7.42^{a}
	+	8.72 ± 5.40^{b}	14.13 ± 7.42^{b}	4.88 ± 3.46^{b}	5.58 ± 3.69^{b}

Different superscript letters within each column indicate significant differences ($p < 0.05$) between samples.

Table 4
Mean water activity, a_w (n = 3), of solutions at different sugar concentrations (% w/v).

Concentration (% w/v)	Water activity (a_w)		
	Fructose	Glucose	Sucrose
10	0.98 ± 9.24E-4^{ab}	0.98 ± 8.08E-4^{b}	0.98 ± 1.05E-3^{a}
20	0.97 ± 1.00E-3^{c}	0.97 ± 1.72E-3^{c}	0.98 ± 6.56E-4^{b}
30	0.96 ± 1.24E-3^{d}	0.96 ± 1.53E-4^{d}	0.97 ± 1.32E-3^{c}

Different superscript letters within each row and column indicate significant differences ($p < 0.05$) between samples.

relationship between air–water partition coefficient values (K_{aw}) and the effect of carbonation was suggested, whereby the presence of CO_2 had a more significant increase on the activity and release of aroma compounds with higher K_{aw} values, such as ethyl acetate. This was attributed to the faster replenishment of these molecules at the depleted liquid–gas interface by ascendant gas bubble movement promoting the transfer of molecules from the bulk to the interface and thus, enhancing aroma release which would otherwise be limited by the kinetics of aroma diffusion. In comparison to the study by Saint-Eve et al. (2009), similar levels of aroma enhancement were observed between ethyl acetate and menthone, as well as isoamyl alcohol and menthol, upon the introduction of carbonation as these pairs of aroma compounds had similar K_{aw}. However, in this study, although ethyl butanoate and hexanal have similar KH values as ethyl acetate (Table 1), the former compounds have much lower vapour pressures and volatilities due to the longer alkyl carbon chain length. Thus, they partition into the gas phase less readily than ethyl acetate during beverage consumption.

Furthermore, not all of the CO_2 in the oral cavity would escape in the gaseous form due to the rapid interconversion of CO_2 to bicarbonate ions (HCO_3^-) and free protons (H^+) (Dessirier et al., 2000) by carbonic anhydrase on the surface of taste receptor cells. While the extracellular generation of protons serves as the primary stimulus of sour-sensitive taste receptor cells as triggered by the perception of CO_2 (Chandrashekar et al., 2009), the generation of H^+ could potentially increase salivary production, resulting in dilution of the aroma compounds released to the gas phase and exhaled in the breath.

3.3. Effect of sugar on kinetics of water, aroma compounds and carbon dioxide

3.3.1. Water activity (a_w)

As sugar concentration increased from 10 to 30 % w/v, there was a significant decrease in the water activity of the solutions ($p < 0.05$, Table 4), owing to the molecular associations between sugar and water molecules through the formation of hydrogen bonds. In addition, the water activity of the fructose and glucose solutions was lower than that of sucrose solutions at equivalent weight concentrations. Although monosaccharides have a lower number of exchangeable hydroxyl groups to partake in the establishment of hydrogen bonds with water molecules and thus, weaker hydration capacity compared to disaccharides, there was almost twice the number of monosaccharide molecules compared to that of disaccharides at equivalent weight concentrations. Thus, there was a greater number of hydrogen bonds formed, resulting in the lower water activity observed.

As a lower water activity would result in a decrease in the aqueous solubility of aroma compounds (Covarrubias-Cervantes et al., 2005) due to the reduction in the volume of free water available, the partition equilibrium of the aroma compounds would be shifted in favour of the gas phase (de Roos, 2006; Delarue & Giampaoli, 2006), resulting in an enhanced aroma release. Hence, these results support the observation whereby the addition of sugars resulted in an increase in the in-vitro partitioning of the majority of the aroma compounds, with a higher partitioning observed for monosaccharides compared to disaccharides at equivalent weight concentrations (Fig. 1).

3.3.2. Self-diffusion coefficients of aroma compounds (D)

The aroma compounds in a solution are in constant random translational motion and this diffusion behaviour is influenced by both the properties of the molecules, such as size, shape and molecular weight, as well as the surrounding environment (Novoa-carballal et al., 2010), such as sugar concentration. The process can be quantitatively measured using DOSY–NMR spectroscopy and expressed as self-diffusion coefficients.

For ethyl butanoate, 5 peaks corresponding to the proton groups of the molecule were expected to be present in the NMR spectrum as observed in the control (Fig. 2a). However, due to the interference arising from the sucrose molecules which were present at higher concentrations, only 3 peaks within the regions of 0.81 – 0.86 ppm, 1.16 – 1.21 ppm and 1.52 – 1.57 ppm were distinctly observed while the 2 other peaks within the regions of 2.26 – 2.31 ppm and 4.07 – 4.12 ppm were hindered or perturbed (Fig. 2b), as was the case reported by Savary et al. (2006). Similarly for hexanal, 5 peaks corresponding to the proton groups of the molecule were expected as observed in the control (Fig. 2c) but only 3 peaks within the regions of 0.89 – 0.93 ppm, 1.27 – 1.38 ppm, 1.60 – 1.67 ppm were distinctly observed while the other 2 peaks within the regions of 2.40 – 2.44 ppm and 9.76 – 9.78 ppm were obscured (Fig. 2d). The mean self-diffusion coefficients for each aroma compound were calculated from the undisturbed peaks.

As sucrose concentration increased from 0 to 30 % w/v, there was a significant decrease in mean self-diffusion coefficients of both the aroma compounds ($p < 0.05$, Fig. 3). Since it was demonstrated that the self-diffusion of aroma compounds was highly related to the mobility of water molecules (Savary et al., 2006) and observed in Table 4 that a decrease in water activity was a consequence of sucrose addition, a slower rate of self-diffusion of aroma compounds in solutions of higher sucrose concentrations was expected. However, unlike the work of Savary et al. (2006) which reported a drastic decrease of approximately 70 % in the self-diffusion coefficients of aroma compounds at 35 % w/v sucrose, the decreasing trend observed in this study was less, although the molecular diffusion of hexanal decreased at an increasing rate with a 11 %, 13 % and 20 % difference observed upon every 10 % w/v sucrose addition. As it was suggested that the lack of water molecules available for the solubilisation of aroma compounds was the reason for the slower

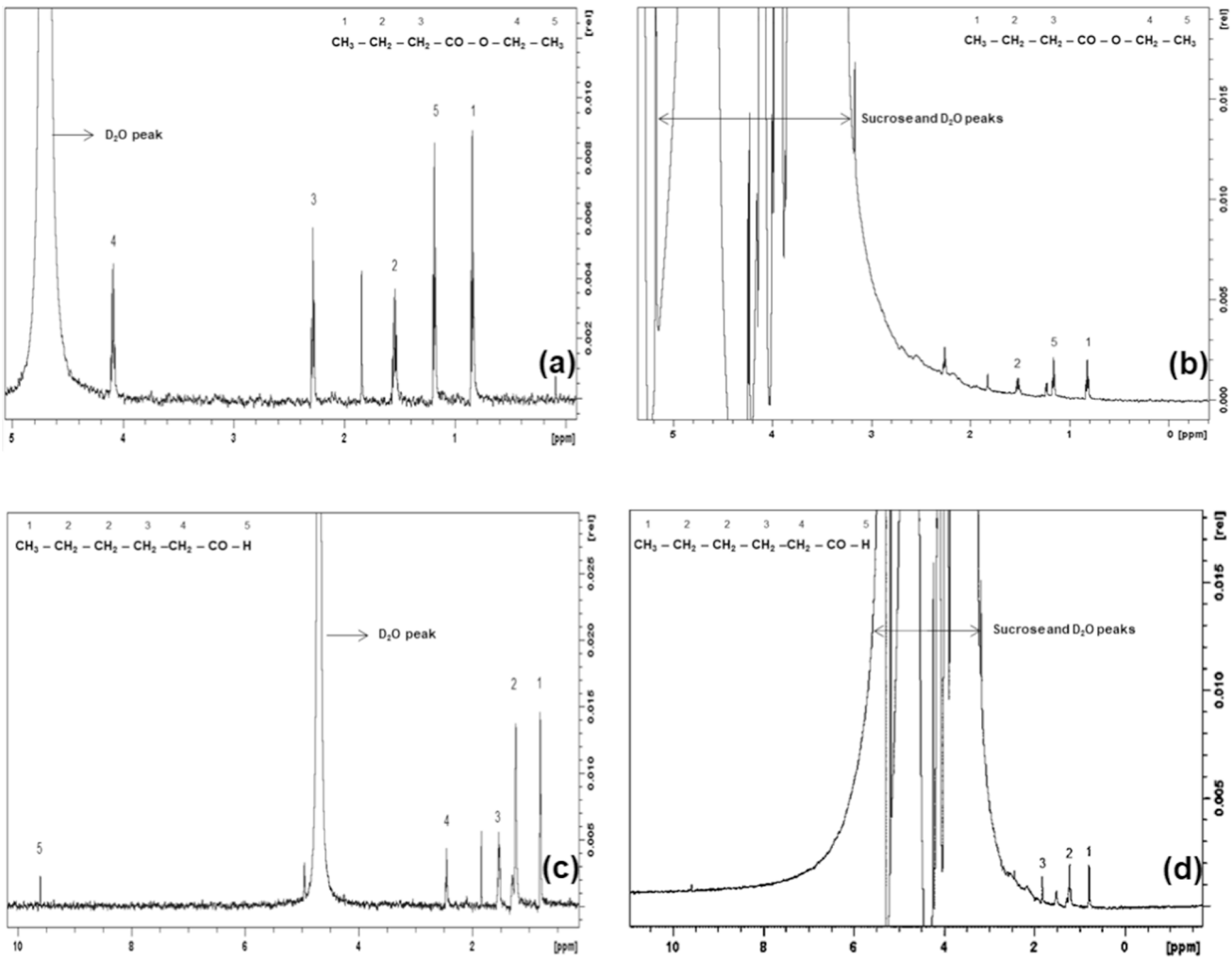

Fig. 2. ^{1}H 1D NMR spectrum of (a) ethyl butanoate in D_2O (b) ethyl butanoate in 30 % w/v sucrose solution (c) hexanal in D_2O (d) hexanal in 30 % w/v sucrose solution.

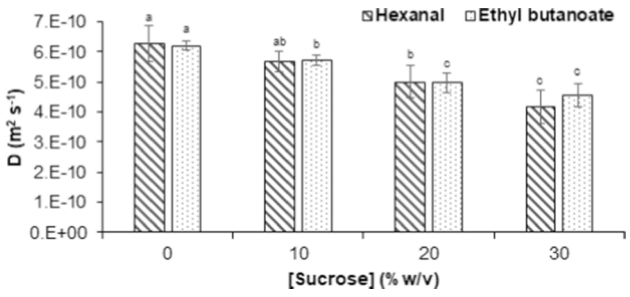

Fig. 3. Mean self-diffusion coefficients, D (m^2/s) of ethyl butanoate and hexanal at different sucrose concentrations (% w/v). Different letters above bar graph indicate significant differences ($p < 0.05$) between samples for each aroma compound.

diffusion, it could be possible that water availability was not a limiting factor for the 10 μg mL^{-1} concentration of aroma compounds used in this study, which was a magnitude lower than the 100 μg mL^{-1} used in Savary's work (2006), suggesting that changes in self-diffusion coefficients of aroma compounds are dependent on the concentration of the volatile aroma compound itself in addition to the concentration dependency of sugars.

3.3.3 vol. flux of dissolved CO_2 (V_F)

When the sample bottle was hermetically sealed, the capacity of a large quantity of CO_2 to remain dissolved in the liquid phase was achieved due to the high pressure of gaseous CO_2 maintained in the headspace. However, when the lid was removed, the thermodynamic equilibrium was disturbed and dissolved CO_2 progressively escaped from the liquid phase in order to establish an equilibrium with the partial pressure of gaseous CO_2 in the atmospheric air. Besides in the form of heterogeneously nucleated bubbles observed in the carbonated beverages, dissolved CO_2 inherently present in liquids could also diffuse from the liquid–gas interface in both carbonated and non-carbonated beverages, contributing to the cumulative mass and volume losses observed in the sample over time. Thus, the progressive release of CO_2 desorbing from the sample bottle could be characterised by the volume

Table 5
Mean cumulative CO_2 volume flux, V_F (cm^3; n = 3) at different sugar concentrations (% w/v).

Concentration (% w/v)	Mean cumulative CO_2 volume flux, V_F (cm^3)		
	Fructose	Glucose	Sucrose
10	7.26 ± 2.32^{a}	7.41 ± 1.66^{a}	7.12 ± 1.75^{a}
20	6.10 ± 1.02^{a}	6.26 ± 2.30^{a}	6.84 ± 1.39^{a}
30	6.61 ± 1.71^{a}	5.78 ± 0.44^{a}	5.33 ± 1.37^{a}

Different superscript letters within each row and column indicate significant differences ($p < 0.05$) between samples.

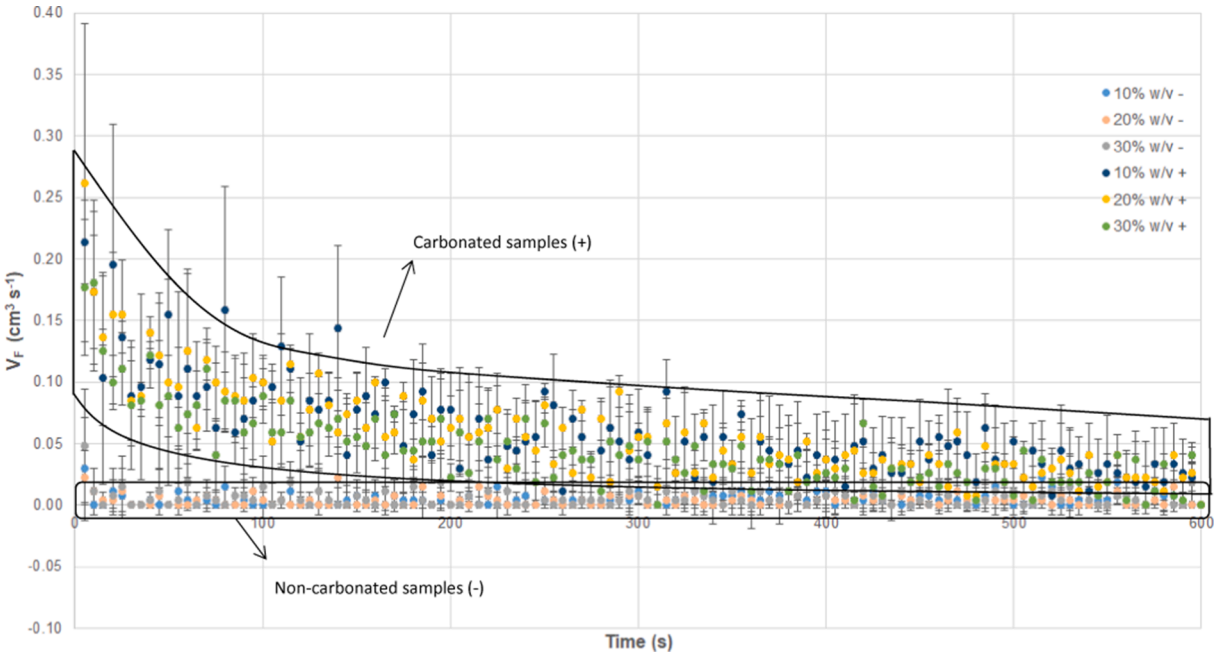

Fig. 4. Mean CO_2 volume flux, V_F (cm^3 s^{-1}, n = 3) in non-carbonated (-) and carbonated (+) sucrose solutions of different concentrations (% w/v) over time (s). Error bars indicate standard deviation at each time point.

flux of CO_2 escaping from the liquid–gas interface (Liger-Belair et al., 2015). The presence of other non-CO_2 dissolved gases should be acknowledged, although the relatively lower concentrations would have a negligible impact on the kinetics of CO_2 studied in the carbonated beverages in this study.

Although there was no significant effect of sugar addition on cumulative CO_2 volume flux ($p > 0.05$, Table 5), the general reduction in cumulative volume flux could be attributed to the slower diffusion of CO_2, which was reported to be affected by the solvent's viscosity, and in turn the number and strength of hydrogen bonds in the aqueous solution (Lv et al., 2018). It could also be observed that the introduction of carbonation to a system resulted in a much higher CO_2 volume flux (Fig. 4) as compared to non-carbonated samples which inherently contained minimal quantities of dissolved CO_2. The difference was especially pronounced at the first instant of opening the lid of the bottle but carried through even up to the end point of sampling at 10 min.

The rate of CO_2 volume flux would inevitably influence the kinetics of aroma release and in turn olfactory perception. It was suggested that the myriad of bubbles nucleating on the liquid wall and travelling through the liquid bulk in carbonated beverages could increase the exchange surface between the liquid and atmosphere during the rise and collapse of bubbles in effervescence (Liger-Belair et al., 2009), radiating a multitude of tiny droplets above the free surface of the liquid and releasing aroma compounds into the headspace (Liger-Belair, 2012).

With the enhanced aroma partitioning in the headspace, orthonasal perception of aroma upon the opening of the lid of a carbonated beverage would be expected to be higher, translating into a stronger first impression of the aroma profile by consumers. However, this could also be at the expense of retronasal olfaction as the aroma compounds were lost to the surroundings, along with the progressive desorption of CO_2 from the liquid surface, even before consumption. Hence, these observations could partly account for the significant reduction in in-vivo aroma delivery in carbonated beverages (Table 3).

4. Conclusion

The addition of monosaccharides and disaccharides resulted in an increase in in-vitro aroma release but the substitution of 10 % w/v sucrose with low-calorie sweeteners did not result in significant differences between the sample and control for the majority of the aroma compounds. Thus, low-calorie sweeteners offer a viable solution for manufacturers when only considering the orthonasal impact of the beverage during the replacement of low concentrations of sucrose. In the soft drink model, carbonation resulted in a significant decrease in in-vivo aroma delivery of hexanal and ethyl butanoate but sucrose had no significant effect. This suggests that a manipulation of carbonation levels could be leveraged to achieve the desired impact of aroma delivery. In addition, since human perception is a complex multi-modal experience, it is worthwhile to extend these findings in future studies to include static and dynamic sensory evaluation to gain further insights. Finally, further work could investigate the impact of other sugar types and mixtures at relevant concentrations on in-vivo aroma delivery as it could be useful for other beverage systems. The exploration of other common beverage components such as acids and salts, as well as active ingredients such as caffeine, nootropics and antioxidants, on aroma partitioning and release, could also be worthwhile to cater to the rapidly growing functional beverage category.

CRediT authorship contribution statement

HuiQi Yeo: Conceptualization, Methodology, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **Robert Linforth:** Supervision, Methodology, Validation. **William MacNaughtan:** Methodology, Investigation, Writing – review & editing. **Huw Williams:** Methodology, Investigation. **Louise Hewson:** Writing – review & editing. **Ian D. Fisk:** Supervision, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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