Aroma Release from Carbonated Beverages

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To the end of a chapter and the beginning of another.

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ABBREVIATIONS AND SYMBOLS

Abbreviation/ Symbol		Unit
ANOVA	Analysis of Variance	-
APCI	Atmospheric Pressure Chemical Ionisation	-
AUC	Area Under Curve	arbitrary unit (a.u)
a_w	Water activity	unitless
CO_2	Carbon dioxide	-
D	Self-diffusion coefficient	$m^2\ s^{-1}$
D_2O	Deuterium oxide	-
DHS	Dynamic Head Space	-
DOSY	Diffusion-Ordered SpectroscopY	-
EB	Ethyl Butanoate	-
EIMS	Electron Impact Mass Spectrometry	-
FID	Flame Ionisation Detection	-
GC	Gas Chromatography	-
H	Hexanal	-
I.D.	Internal Diameter	mm
I_{max}	Maximum intensity	arbitrary unit (a.u)
K_{aw}	Air/water partition coefficient	unitless
K_H	Henry's law constant	$atm\ m^3\ mol^{-1}$
Log P	Logarithim of octanol/water partition coefficient	unitless
m/z	Mass-charge ratio	unitless
M_F	Mass flux	$g\ s^{-1}$
MS	Mass Spectrometry	-
MW	Molecular Weight	$g\ mol^{-1}$
NMR	Nuclear Magnetic Resonance	-

Abbreviation/ Symbol		Unit
NS	Nose Space	-
PTR	Proton Transfer Reaction	-
QDA	Quantitative Descriptive Analysis	-
SD	Standard Deviation	-
SHS	Static Head Space	-
SPME	Solid Phase Micro-Extraction	-
TCATA	Temporal Check All That Apply	-
TDS	Thermodesorption	-
TDS	Temporal Dominance of Sensations	-
TI	Time-Intensity	-
ToF	Time of Flight	-
V_F	Volume flux	$cm^3\ s^{-1}$

ABSTRACT

The effect of monosaccharides (glucose, fructose and galactose) and disaccharides (sucrose and lactose) at different concentrations (10, 20 and 30% w/v) on the static headspace *in-vitro* release of C4 – C10 aldehydes, ethyl esters and limonene was studied using Atmospheric Pressure Chemical Ionisation–Mass Spectrometry (APCI–MS). An increase in sugar concentration from 0 – 30% w/v resulted in a significant increase in aroma release under static headspace conditions for the majority of the compounds ($p < 0.05$).

This initial study formed the basis for the design of a soft drink model – a system comprised of water, sucrose, acid and aroma compounds representative of an apple style flavouring, namely ethyl butanoate and hexanal. However, the introduction of carbonation to the soft drink model not only added the characteristic fizziness, but also conferred complexity to the system as the diffusion of carbon dioxide from the liquid-gas interface and the formation of effervescence could affect aroma release under the dynamic conditions of beverage consumption. In fact, it was found that the introduction of carbonation resulted in a significant decrease in *in-vivo* aroma delivery during breath-by-breath analysis ($p < 0.05$).

To understand the physical mechanisms behind aroma release from the beverage matrix, the effect of sugar on the kinetics of the matrix components, namely water, aroma compounds and carbon dioxide, was explored. An increase in sugar concentration from 0 – 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 (DOSY)–Nuclear Magnetic Resonance (NMR) spectroscopy. No significant effect of sugar on carbon dioxide volume flux was found ($p > 0.05$).

1 INTRODUCTION

1.1 Trends and Challenges in the Soft Drinks Industry

Carbonated soft drinks are a class of beverages which are generally manufactured by the addition of sweeteners, flavourings, acidulants and chemical preservatives with the artificial impregnation of gaseous carbon dioxide (Taylor, 2006, Potter and Hotchkiss, 2012).

Accounting for 38% of the market share, carbonated soft drinks comprise the largest category in the industry (British Soft Drinks Association, 2016). Within the category, regular calorie soft drinks are still the most frequently consumed. However, non- or low-caloric options are fast gaining popularity due to the burgeoning trend of consumer health awareness and calorie reduction initiatives by the industry, which has even pledged to 20% calorie reduction in its products by 2020 (British Soft Drinks Association, 2016). In addition, the future implementation of a soft drinks industry levy by the government targeting producers and importers of soft drinks containing added sugar is a further incentive for manufacturers to step up product reformulation efforts. Under the levy, a main rate charge will be imposed on drinks with total sugar content above 5 g per 100 mL and a surcharge for those above 8 g per 100 mL (HM Treasury, 2016).

However, sugar reduction or substitution across the entire range of soft drink products, including regular calorie varieties, introduces complex technical and sensory challenges. Sucrose has conventionally been a common source of sweetener used at 6 – 12% w/v to impart sweetness (Taylor, 2006, Burgos et al., 2016). It remains the benchmark despite the advent of alternative sweeteners as no other sweetener has been discovered or developed to replicate most, if not all, of the functional properties of sucrose (Goldfein and Slavin, 2015). These functional properties are derived from the sensory and physicochemical properties of sucrose, as well as its many reactions and interactions with other components in the food matrix (Cooper, 2006).

The relative sweetness purity of sucrose, without any unpleasant aftertaste or undesirable secondary reaction, has been known to synergistically complement the traditionally popular flavours of soft drinks, such as fruity and caramel flavours in juice and cola beverages respectively, providing a balanced flavour profile in addition to the reduction of perceived acidity (Cooper, 2006). In a low pH environment typical of soft

drinks, sucrose undergoes gradual inversion and splits into its component monosaccharides glucose and fructose in a ‘maturing’ process, which rounds off the flavour of the beverage (Matheis, 2007).

In a multi-component food matrix, the role of sucrose in the modification of aroma availability involves its physical and chemical properties to either enhance or suppress the other matrix components (Goldfein and Slavin, 2015). Being a small disaccharide molecule with multiple hydroxyl groups, sucrose is highly soluble in water and interacts strongly with water molecules, which can affect the liquid-gas partitioning of aroma compounds (Delarue and Giampaoli, 2006). Furthermore, sucrose contributes to the viscosity and mouthfeel of drinks, providing stability to the clouds and pulps added to drinks as visual appeal (Cooper, 2006).

Moving forward, soft drink manufacturers need to actively engage in new product development and creative recipe changes to achieve sugar and calorie reduction in their products. Thus, deeper knowledge of the interactions between matrix components within a beverage system will be essential for the formulation of successful products without compromising flavour delivery to meet consumer expectations.

1.2 Aroma Release

Aroma compounds exist as volatile, odorous organic compounds at atmospheric pressure and are characterised by a diverse range of structural features in terms of molecular size, shape and functional group, as well as physicochemical properties such as volatility, hydrophobicity, chemical reactivity, vapour pressure, activity and partition coefficients (Fisk, 2015, Voilley and Souchon, 2006). Depending on the nature of food matrix and class of aroma compound, one or several properties may appear to be more dominant and thus, aroma release is a complex process depending on several factors.

Aroma release is governed by both thermodynamic factors, which are mainly influenced by the physicochemical properties of the aroma compounds, as well as kinetic factors, which are primarily dependent on the concentration gradient and mass diffusivity of the aroma compounds within the food matrix (van Ruth and Roozen, 2010, Voilley and Souchon, 2006). Knowledge of the degree and rate of partitioning of aroma compounds between different phases and their binding behaviour with matrix components is of practical importance during product formulation. A timely and targeted release of aroma

compounds improves the effectiveness of food flavours, broadens the application range and ensures optimal dosage, thereby ensuring cost effectiveness for food manufacturers (van Ruth and Roozen, 2010).

1.2.1 Equilibrium conditions

Within a static closed vessel, aroma compounds in a food matrix not only partition into the gaseous headspace depending on their volatility, but also within different phases of the matrix depending on their hydrophobicity.

In a beverage system, the volatility of aroma compounds can be expressed by the air/water partition coefficient (K_{aw}, unitless, Equation 1.1*)*, which describes the directly proportional relationship between the concentrations of an aroma compound in the air (C_a, unit = mol m^{-3}) above the beverage matrix and the solvent phase, water, (C_w, unit = mol m^{-3}) under equilibrium conditions.

$$K_{aw} = \frac{C_a}{C_w}$$

Equation 1.1 Air/water partition coefficient (K_{aw})

K_{aw} can be further defined by Henry's law constant (K_H, unit = atm m^3 mol^{-1}, Equation 1.2), which states the ratio between the partial pressure of an aroma compound in the gas phase (*p*, unit = atm) and its solubility in the aqueous water phase (C_w, unit = mol m^{-3}) under equilibrium conditions.

$$K_H = \frac{p}{C_w}$$

Equation 1.2 Henry's law constant (K_H)

Given the diversity of aroma compounds, a wide range of Henry's law constants and partitioning occur across food matrices. Generally, hydrocarbons have higher K_H values as compared to other classes of compounds such as aldehydes, alcohols and esters, indicating that these aroma compounds are present in higher concentrations in the gas phase than aqueous phase under equilibrium. Despite the assumptions of Henry's Law constant for ideal compounds in infinite solutions at constant temperature and equilibrium, knowledge of partition coefficients can be useful for the estimation of the

maximum volatile concentration which may occur above the food matrix under defined temperature and pressure conditions (Linforth, 2010).

Meanwhile, the hydrophobicity of aroma compounds can be expressed by the logarithmic value of the octanol/water partition coefficient (Log P, unitless, Equation 1.3), which describes the relative concentrations of an aroma compound between the octanol phase (C_o, unit = mol m^{-3}) and water phase (C_w, unit = mol m^{-3}) under equilibrium conditions.

$$\mathrm{Log\ P} = \mathrm{Log}\ \frac{\mathrm{C_o}}{\mathrm{C_w}}$$

Equation 1.3 Logarithim of the octanol/water partition coefficient (Log P)

As the octanol/water partition closely resembles that between water and biological membranes, the form of many lipids in food, it is an adequate estimation of the distribution of aroma compounds between lipid and aqueous phases within a food matrix and hence, an indication of their hydrophilicity or hydrophobicity (Taylor, 2002).

Although lipids rarely constitute the bulk phase of food matrices, they may be present homogenously or non-homogenously across aqueous continuous systems in microscopic or macroscopic regions (Fisk, 2015). As the majority of aroma compounds are hydrophobic and demonstrate preferential solubility in the lipid phase (van Ruth and Roozen, 2010), the presence of lipids affects physical partitioning of aroma compounds within the matrix, which in turn affects release of the compounds into the headspace.

Similar to Henry's law constants, aroma compounds exhibit a range of log P values. However, as the scale is logarithmic, small differences augment major changes in the availability of the aroma compounds in the aqueous phase and thus, release into the gas phase (Fisk, 2015).

1.2.2 Non-equilibrium conditions

While aroma release is chiefly controlled by thermodynamic factors in an equilibrium system, disturbances to the phase equilibria such as the introduction of air sweeping across the surface of the product exemplify and amplify the importance of kinetic factors, which determine the rate at which aroma compounds are replenished in the headspace in order to re-establish an equilibrium (Fisk, 2015, de Roos, 2006).

In a beverage system, the driving force for aroma transfer across the liquid-gas interface to replenish the headspace is the concentration difference between the beverage matrix and gas phase. Thus, the rate of unidirectional diffusion is dependent on the concentration gradient and mass transfer coefficient of the aroma compounds in the respective phases (van Ruth and Roozen, 2010).

The transport of aroma compounds occurs through two mechanisms – static diffusion and convective diffusion. Static diffusion occurs as a result of random molecular motion of the aroma compounds and is a relatively slow process, while convective diffusion is the more important transport mechanism under non-equilibrium conditions as the movement of fluid eddies from one location to another contributes to the transport of aroma compounds (de Roos, 2006, van Ruth and Roozen, 2010). However, due to the assumption of rapid diffusion of aroma compounds in the gas phase, the concentration gradient in the gas phase is usually neglected and depends on the depletion of the aroma compounds at the liquid-gas interface (van Ruth and Roozen, 2010).

The mass transfer coefficient (k) is a measure for the velocity at which the solute diffuses through the phase and can be explained by Fick's law of diffusion (Equation 1.4), where the diffusive flux or mass transfer rate, mass (m, unit = g) per unit time (t, unit = s) is proportional to the concentration (C, unit = g m^{-3}) gradient over a set distance (x, unit = m) per unit cross-sectional area (A) and the diffusion coefficient (D, unit = m^2 s^{-1}) (Fisk, 2015).

$$\frac{dm}{dt} = -AD\frac{dC}{dx}$$

Equation 1.4 Fick's law of diffusion

To control the retention of aroma compounds in food products or their release from food products, it is necessary to understand their mass transport during food preparation (effect of formulation and/or process), storage (interactions with packaging materials and/or with non-volatile compounds) and consumption.

1.3 Aroma Delivery

Following aroma release from the food matrix, a sufficiently high concentration of aroma compounds has to be delivered to the olfactory receptors in the main olfactory epithelium located in the nasal cavity in order for the stimulation of the olfactory system and the

elicitation of a response to occur (van Ruth and Roozen, 2010). While the aroma concentration in the headspace above a food product may be a good indicator of the proximal stimulus reaching the olfactory receptors (Taylor and Hort, 2004), it does not take into account the non-equilibrium conditions and complexities of olfaction and oral processing.

Flavour perception begins with the release of aroma compounds from the food matrix to the saliva phase, subsequent transport to the distinct gustatory, olfactory and trigeminal systems and finally, activation and interaction of the site receptors to elicit an integrated chemosensory perception (Holley, 2006, Taylor, 2002). The flavour release and transport processes involved are governed by thermodynamic and kinetic parameters, which are in turn dependent on the nature of the food matrix, physicochemical properties of the aroma compounds and physiological conditions during consumption (Taylor, 2002, Voilley and Souchon, 2006). Hence, scientific understanding of the contribution of solvent-solute-aroma interactions in the food matrix to aroma release and delivery can be translated into enhanced flavour perception during beverage design by soft drink manufacturers.

1.3.1 Olfaction

Aroma compounds reach the olfactory receptors in the nasal epithelium through two routes – orthonasal or retronasal (Figure 1.1) , and such ortho-retronasal duality in aroma perception is unique to mammals (Rowe and Shepherd, 2016). In orthonasal olfaction, volatile aroma compounds from the external environment travel directly through the anterior nares of the nostrils towards the olfactory epithelium during nasal inhalation (Negoias et al., 2008). In retronasal olfaction, the aroma compounds, which are released from the product matrix upon consumption, pass back up through the pharynx into the nasal cavity to stimulate the olfactory receptors during and after the mastication and swallowing process (Taylor and Hort, 2004). Thus, this mode of olfaction carries an additional domain of information about aroma molecules, which are released in the oral cavity during the breakdown of food through mastication, salivary interactions and actions of the tongue, thereby evoking a different set of responses (Rowe and Shepherd, 2016).

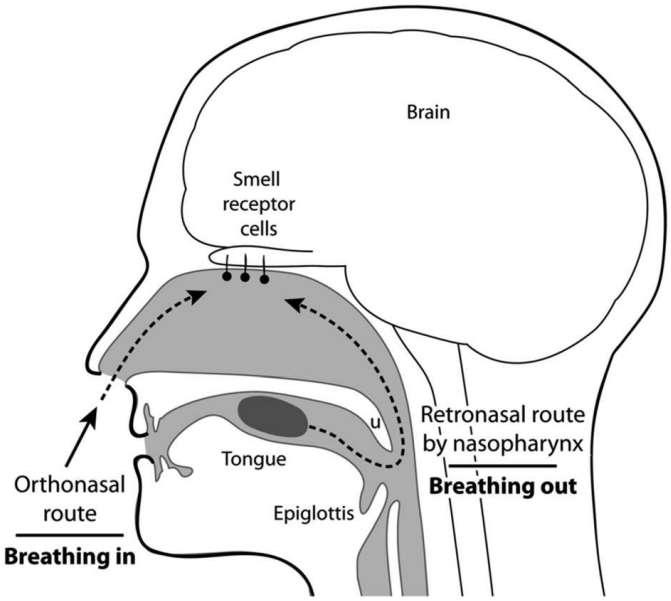


Figure 1.1 Orthonasal and retronasal olfaction routes (Shepherd, 2013)

1.3.2 Oral processing

During consumption, aroma compounds are released from the food matrix into the liquid phase within the mouth, where further partitioning into the gas phase occurs, and portions of the buccal gas phase are transferred to the throat during swallowing (Wright et al., 2003).

Dilution of aroma compounds occurs due to the flow of saliva in the mouth, typically at a rate of 2 – 5 mL min^{-1}, but varying between individuals and depending on the presence of salivary stimulants such as food acids (Taylor and Hort, 2004). Saliva introduction not only causes a shift in the effective partitioning of aroma compounds, but also the reversible or non-reversible binding equilibria with other matrix components, thereby affecting a change in the release kinetics of aroma compounds (van Ruth and Roozen, 2010). Moreover, foods can undergo temperature changes upon introduction into the mouth, with a higher temperature causing a greater partitioning of volatiles in favour of the gas phase (Boelrijk et al., 2006). Further 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).

Beverage consumption is a relatively fast process as individuals swallow the liquid almost immediately after taking the product into their mouth, allowing only a short time

frame for aroma release from the product while the liquid is briefly kept in the mouth. Thus, small irregularities in drinking patterns arising from swallowing, depth of breath, jaw and tongue movements, as well as saliva flow can result in a huge impact on the process of aroma release (Boelrijk et al., 2006). Meanwhile, solid food consumption is further complicated by the mastication process causing significant changes to the food in terms of surface area, hydration and time in mouth, which in turn affect the mass transfer processes involved in aroma release and delivery while the solid food resides in the mouth (Taylor and Hort, 2004).

1.4 Effect of Matrix Components

1.4.1 Effect of sugars

The effect of sugar type and concentration on aroma release and perception has been extensively investigated in a myriad of beverage models and systems. Although sucrose has been the focus of the majority of the studies, monosaccharides such as fructose and galactose, as well as disaccharides such as lactose, have also garnered interest. Table 1.1 lists some of the recent research which highlights the importance of these interactions from both academic and industrial perspectives.

Table 1.1 Summary of papers investigating the effect of sweeteners on flavour release and/or perception in beverage models and systems

Reference	Matrix	Sweeteners	Odourants	Instrumental method	Sensory method	Results
Nahon et al. (2000)	Aroma-sugar solutions	Sucrose	Ethyl acetate, ethyl butanoate, methyl butanoate, hexanal, octanal	SHS–GC–FID	None	Increasing sucrose concentration could increase or decrease partition coefficient of volatile compounds
Hansson et al. (2001)	Model soft drink	Sucrose, invert sugar, glucose syrup	Tutti-frutti flavour (24 molecules)	SHS–GC–FID–MS	None	Addition of sucrose (20–60%), invert sugar (20–60%) and glucose syrup (60%) significantly increased release of the most polar volatiles
Rabe et al. (2003)	Aroma-sugar solutions	Sucrose	Esters, alcohols, pyrazine, pyridine, thiazole, lactone	DHS–TDS[a]–GC–FID	None	Increasing sucrose concentration predominantly increased flavour release (partly significant trend for 6 out of 13 aroma compounds)

Reference	Matrix	Sweeteners	Odourants	Instrumental method	Sensory method	Results
Pfeiffer et al. (2006)	Model soft drink	Sucrose	Strawberry flavour (ethyl acetate, ethyl butyrate, ethyl caproate)	NS–APCI–MS	Sensory evaluation by trained panel using magnitude estimation with reference modulus (ISO 11056)	Sucrose addition did not significantly change aroma release but significantly increased intensity of strawberry flavour perceived
Copolovici and Niinemets (2007)	Aroma-sugar solutions	Glucose, sucrose	Limonene and linalool	SHS–GC–FID	None	Sugar addition resulted in salting in of aroma compounds
Hewson et al. (2008)	Model soft drink	Glucose, fructose	Citrus flavour (citral and limonene)	SHS–APCI–MS	Sensory evaluation by trained panel using magnitude estimation with reference modulus	Sugar addition resulted in a concentration-dependent enhancement of citrus flavour intensity
Piccone et al. (2012)	Model coffee; diluted espresso coffee beverages	Glucose, fructose, sucrose, lactose	Diacetyl, 2,3-pentanedione, ethylpyrazine and hexanal	SHS–SPME–GC–MS	None	Sugar addition significantly increased release of the more polar volatiles and vice versa

Reference	Matrix	Sweeteners	Odourants	Instrumental method	Sensory method	Results
Charles et al. (2015)	Espresso coffee beverage	Sucrose	Light and dark roast Arabica coffee	NS–PTR–ToF–MS	Sensory evaluation by trained panel using TDS	Sugar addition did not significantly affect aroma release but modified sensory perception
Oliveira et al. (2015)	Probiotic chocolate-flavoured milk	Commercial sugar	Alkaline cocoa powder, artificial vanilla flavour	None	Sensory evaluation by trained panel using TCATA; consumer study	Sugar reduction did not significantly affect citation proportions for chocolate flavour but mainly influenced sweetness, bitterness and thickness

APCI *= Atmospheric Pressure Chemical Ionisation;* ***DHS*** *= Dynamic headspace;* ***FID*** *= Flame Ionisation Detector;* ***GC*** *= Gas Chromatography;* ***MS*** *= Mass Spectrometry;* ***NS*** *= Nose space;* ***SHS*** *= Static headspace;* ***SPME*** *= Solid Phase Micro Extraction;* ***TCATA*** *= Temporal Check All That Apply;* ***TDS[a]*** *= Thermodesorption;* ***TDS[b]*** *= Temporal Dominance of Sensations;* ***ToF*** *= Time of Flight*

A general agreement exists among the studies that an increase in sugar concentration results in an increase in aroma release. This is attributed to the 'salting out' phenomenon (Figure 1.2b), whereby the strong interaction between the active polar groups on sugar moieties and water molecules reduces the volume of free water available for the solubilisation of aroma compounds, which in turn shifts the partition equilibrium of the aroma compounds in favour of the gas phase (Delarue and Giampaoli, 2006, de Roos, 2006). However, due to the complexity of solvent-solute-aroma interactions, this phenomenon may not always be observed and the opposite 'salting in' effect can also occur (Figure 1.2c). It was suggested that sucrose addition increased the hydrophobicity of the solvent character, resulting in higher gas/liquid partition coefficients of more polar aroma compounds ('salting out') and vice versa for less polar compounds ('salting in') (Nahon et al., 2000). Hence, depending on the properties of each matrix component, the overall effect of solute addition may be an increase, decrease or no change in the headspace concentration of the volatiles (Friel et al., 2000).

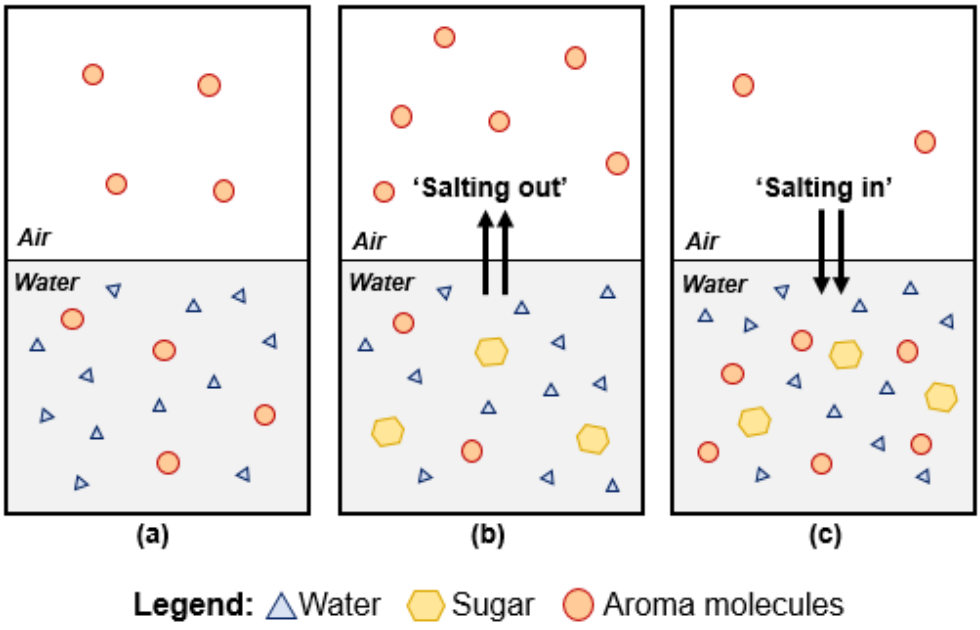


Figure 1.2 *In-vitro* schematic diagram of aroma compounds (a) at equilibrium in water (b) 'salting- out' (c) 'salting-in' due to solute addition

However, no consensus has been reached regarding the range of sugar concentrations over which the enhancement of aroma release and perception takes place or the extent to which the effect occurs. This is in part due to the disparity in types and concentrations of sugars relevant to specific beverage systems, which is further complicated by the

presence of other chemical ingredients unique to each matrix. Since changes in sugar type and concentration exert different effects on the solubility and volatility of aroma compounds, modifications in flavour profile and delivery in the beverage matrices will vary accordingly (Paquin, 2009). Furthermore, a variety of research tools have been employed in the studies, ranging from instrumental methods for measurement of aroma release, such as atmosphere pressurised chemical ionisation–mass spectroscopy (APCI–MS) and gas chromatography–mass spectroscopy (GC–MS), to sensory methods, such as magnitude estimation and temporal check all that apply (TCATA), for understanding of aroma perception. Thus, a fair comparison may not be achieved based on conclusions drawn from studies using different methods of analysis.

1.4.2 Effect of alternative sweeteners

In comparison to studies on the effect of sugar on aroma release and perception, fewer attempts have been made to examine the effect of intense sweeteners, which have a high potency sweetness relative to sucrose and are used at such low concentrations in the product that they are considered non-caloric (The British Dietetic Association, 2016). Although blends of intense sweeteners and low levels of carbohydrate sweeteners can be experimented with during product formulation, sugar reduction or substitution with alternative sweeteners results in a decrease in total soluble solids and consequently, a thinner mouthfeel may be perceived in the beverage (Cooper, 2012), which is an unpalatable effect for regular consumers of full calorie products. Moreover, alterations in the temporal delivery of sweetness and distortions to the volatility of aroma compounds may lead to an unbalanced flavour profile of the final product (Paquin, 2009). It was demonstrated that although the addition of acesulfame potassium, aspartame and sucralose at equisweet levels compared to sucrose had no significant impact on *in-vivo* aroma delivery, there were significant differences in the aroma perception of the lemon flavoured beverages reported by panellists during sensory evaluation (Itobe and Kumazawa, 2017).

1.4.3 Effect of carbon dioxide (CO_2)

Many theories about multisensory integration have been conceived to elucidate the mechanisms of trigeminal stimulation. However, literature exploring carbonation as a chemesthetic stimulus in beverage models and systems is limited despite the sensory properties elicited by carbonation playing a major contribution to consumer choices and

preferences. In addition, most studies investigate the effect of carbonation on human perception in terms of gustatory, olfactory and trigeminal modalities, which are usually limited to sensory methods, such as through visual observation by bubble number (Saint-Eve et al., 2010) or in-mouth sensations such as tingling and fizziness (Hewson et al., 2009). Nevertheless, the use of instrumental methods for the study of carbonation, such as real-time aroma analysis using APCI–MS (Clark et al., 2011a) or PTR–MS (Saint-Eve et al., 2009, Pozo-Bayón et al., 2009), micro-GC for the quantification of gaseous CO_2 concentration in beverage headspace and infrared imaging technique for the visualisation of the flow of gaseous CO_2 desorption from the beverage (Liger-Belair et al., 2012), has proven to be useful and can be more widely adopted. Table 1.2 lists some of the recent research which seeks to characterise the effect of carbonation on aroma perception.

Table 1.2 Summary of papers investigating the effect of carbonation on flavour release and perception in beverage models and systems

Reference	Matrix	Tastants	Odourants	Carbonation method	CO_2 level	Research method	Results
McLellan et al. (1984)	Apple juice	Sucrose	Filtered apple juice	Pilot scale carbonation tank	0–4 volumes	QDA by trained panel using 6 attributes	CO_2 did not significantly affect aroma intensity
Yau et al. (1989)	Blueberry flavoured milk	Sucrose, high fructose corn syrup, aspartame, pear concentrate	Blueberry concentrate, blueberry flavour with other natural flavourings	Pilot-scale carbonation tank	20–22 psi	Sensory evaluation by trained panel using magnitude estimation; consumer study using hedonic and 'just right' rating scale	CO_2 significantly increased perception of overall intensity, sweetness and blueberry flavour but had no effect on viscosity
Yau and McDaniel (1992)	Model carbonated system	Sucrose, aspartame; citric acid, phosphoric acid	None	Stainless steel carbonator	0, 2 and 3 volumes	Sensory evaluation by trained panel using magnitude estimation	CO_2 had little effect on sweetness but increased sourness rating at low acid concentrations

Reference	Matrix	Tastants	Odourants	Carbonation method	CO_2 level	Research method	Results
Hewson et al. (2009)	Model carbonated beverage	Glucose, fructose; citric acid	Orange flavour (citral, limonene)	In-house laboratory carbonation apparatus using CO_2 cylinder	0, 1.5 and 3.6 volumes	Sensory profiling by trained panel using 10 attributes	CO_2 increased sour intensity, supressed sweetness and caused bitter aftertaste
Pozo-Bayón et al. (2009)	Model carbonated system	None	Benzaldehyde, ethyl butyrate, ethyl propionate, isoamyl acetate, limonene and 2-nonanone	Perrier sparkling natural mineral water	NA	SHS-PTR–MS	CO_2 increased the release of aroma compounds to the headspace and effect was higher when compounds were added in mixture
Saint-Eve et al. (2009)	Model carbonated beverage	Sucrose	Mint flavour (*Z*-hex-3-en-1-ol, menthol and menthone)	Benchtop carbonator	0 and 5 g L^{-1}	NS-PTR–MS; sensory evaluation by trained panel using discontinuous dynamic sensory procedure	CO_2 significantly increased gas-to-product partition coefficients

Reference	Matrix	Tastants	Odourants	Carbonation method	CO_2 level	Research method	Results
Saint-Eve et al. (2010)	Model carbonated beverage	Sucrose	Mint flavour (*Z*-hex-3-en-1-ol, menthol and menthone)	Benchtop carbonator	0 and 5 g L^{-1}	Sensory profiling by trained panel using 14 attributes	CO_2 increased sour intensity and intensity of green note perceived
Clark et al. (2011b)	Model beer	Hop acids	Beer flavour (ethyl acetate, isoamyl acetate, dimethyl sulphide, phenethyl alcohol and isoamyl alcohol)	In-house carbonation apparatus using CO_2 cylinder	0, 2 and 3.6 volumes	QDA by trained panel using 8 attributes	CO_2 interaction with other matrix components had a significant impact on all attributes, including suppression of sweetness and modification of bitterness
Symoneaux et al. (2015)	Model cider	Fructose; malic acid; procyanidins	None	Device consisting tank, pump and venturi	0 and 5 g L^{-1}	Sensory evaluation by trained panel using 4 attributes	CO_2 decreased sweetness intensity and increased sour intensity and astringency but had no effect on bitterness

***MS** = Mass Spectrometry; **NS** = Nose space; **PTR** = Proton Transfer Reaction **QDA** = Quantitative Descriptive Analysis; **SHS** = Static headspace*

A general agreement exists among the studies that the introduction of carbonation exerts a suppressive influence on sweetness perception but enhances sourness perception. In addition, bitterness and astringency were among the aftertastes observed, attributed to the dissociation of carbon dioxide to carbonate, bicarbonate and carbonic acid at equilibrium (Hewson et al., 2009, Symoneaux et al., 2015). More interestingly, functional magnetic resonance imaging had illustrated that the presence of carbonation reduced the neural processing of sweetness-related signals of sucrose more than that of artificial sweeteners (Di Salle et al., 2013), potentially providing insights on the effective use of carbonation and artificial sweeteners during product formulation to bridge the gap between the perception of non-caloric and caloric sweeteners.

In terms of the effect on olfactory perception, it was demonstrated that carbonation increased aroma intensity perceived for green and fresh notes in mint-flavoured beverages (Saint-Eve et al., 2010). The diffusion of carbon dioxide from the liquid-gas interface, as well as bubble formation and collapse during effervescence, could strip aroma compounds from the liquid phase and accelerate their transfer into the gas phase, thereby accounting for the enhanced flavour perception (Liger-Belair, 2012). However, in the greater space of time when an equilibrium would eventually be established, the primary influence of carbonation on volatile release was proposed to be its effect on the gas/liquid partition coefficient of aroma compounds, with an increased release observed in the more volatile and hydrophobic compounds (Pozo-Bayón et al., 2009, Saint-Eve et al., 2009). Moreover, the interaction of carbonation with other tastants, such as sugars and acids, in the matrix could positively or negatively influence perception of flavour intensity, depending on the concentration of tastants present (Hewson et al., 2009). For instance, it was suggested that glucose was able to suppress attributes hypothesised to be chemogenic trigeminal responses, such as tingling and irritant (Hewson et al., 2009), possibly due to the mediated release of endogenous opioids, which act centrally as analgesics (Kracke et al., 2005).

1.5 Real-Time Aroma Analysis

Conventional techniques in aroma analysis involve the use of gas chromatography–electron impact mass spectrometry (GC–EIMS) whereby aroma compounds are first separated by a capillary column before ionisation and partial fragmentation to produce characteristic spectra for identification and quantification (Taylor et al., 2000). Although the customary use of MS is to complement the resolving power of GC for the separation

of complex aroma mixtures, direct MS techniques may be employed as rapid on-line tools for aroma analysis.

Atmospheric chemical pressure ionisation–mass spectrometry (APCI–MS) is a straightforward approach, which produces a simple mass spectrum for the assignment of aroma compounds to ions. In this system (Figure 1.3), volatile aroma molecules (M) are drawn into the ionisation source, usually a corona discharge produced at atmospheric pressure, by a Venturi effect created by a high nitrogen source gas flow before ionisation via a proton transfer reaction using the hydronium ion ($H3O^+$) as the reactant ion ($H_3O^+ + M \rightarrow H_2O + MH^+$).The protonated molecular ions (MH^+) formed are sampled into a standard quadrupole MS maintained under vacuum and can be monitored in either full scan or selective ion mode.

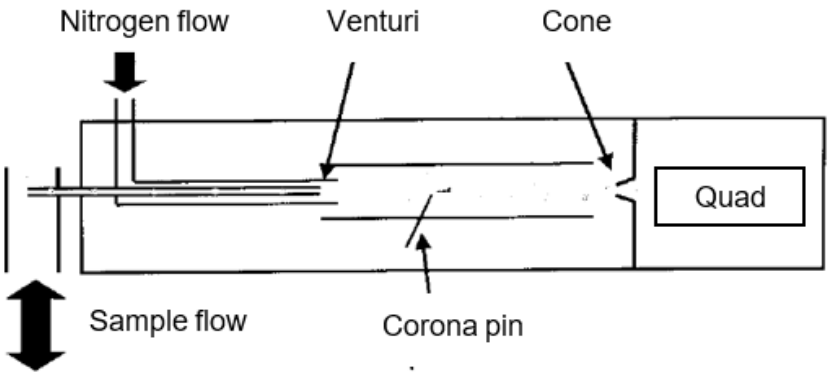


Figure 1.3 Schematic diagram of APCI–MS (Taylor et al., 2000)

Water serves as a suitable reactant molecule as its proton affinity lies above that of the main components of air but below most volatile organic compounds. Since the H_3O^+ ion will only transfer its charge to molecules with higher proton affinities, only the aroma compounds are susceptible to ionisation but not the nitrogen, oxygen or carbon dioxide gases present in air (Taylor and Linforth, 2010). In addition, unlike electron impact, chemical ionisation is a 'soft' process operating at lower chemical ionisation energies, which results in the ionisation of intact molecules with limited fragmentation. However, some classes of compounds such as alcohols and aldehydes undergo dehydration and manipulation of instrument settings, such as cone voltage, may be necessary to optimise the degree of fragmentation and ion intensity for discrimination between compounds with identical molecular mass (Taylor et al., 2000).

1.5.1 Static headspace analysis (in-vitro)

Static headspace techniques are a direct measure of the concentration of volatile aroma compounds present in the gas phase above a solid or liquid sample contained in a sealed vessel under controlled equilibrium conditions (Kolb and Ettre, 2006). While they provide an indication of what the olfactory receptors perceive (Reineccius, 2010), these equilibrium techniques are limited to an aliquot of headspace above the sample and are highly dependent on the partition coefficients of the aroma compounds between the headspace and sample matrix as the volatiles are not exhaustively removed from the sample (Ross, 2012). Thus, the primary limitation is its inadequate sensitivity as aroma compounds present in trace quantities or with very low vapour pressures are usually not detected (Reineccius, 2006).

1.5.2 In-nose breath analysis (in-vivo)

As human physiological processes are rapid events, multiple data points and fast sampling times are required during data collection to obtain accurate determination and adequate resolution of peak heights and areas, which are indicative of the concentration of aroma compounds detected in individual breaths (Taylor and Linforth, 2010). The use of APCI–MS in such *in-vivo* applications has gained popularity as direct MS techniques offer both the sensitivity and speed necessary for monitoring aroma profiles delivered to the olfactory receptors during food consumption.

During in-nose breath sampling, the highest aroma release is usually found in the first exhalation after swallowing (Linforth et al., 2002) as the brief opening of the velum-tongue barrier during the swallowing action facilitates the transfer of aroma compounds from the oral cavity to the nose cavity (Buettner et al., 2001). Thus, most studies adopted a methodological approach using the 'swallow breath' as a measurement of aroma release, whereby the relative concentration of aroma compounds is determined from the first peak in the release signal after swallowing (Boelrijk et al., 2006). Comparison of the maximum intensities of the aroma compounds across different products can provide valuable information for manufacturers seeking to achieve the same aroma impact after reformulation.

To ensure that panellists breathe through their nose during food consumption, and to check the regularity of their breathing pattern, acetone is routinely monitored during

breath sampling. Endogenous acetone, which is a metabolic by-product of fatty acid metabolism in the liver, is transported from blood, into lung air and exhaled breath due to its small molecular weight (Anderson et al., 2006). Examination of breath-by-breath traces by making use of acetone as a biomarker for exhalation and retronasal olfaction can provide an indication of aroma compounds reaching the olfactory receptors through the retronasal route, thereby giving an estimation of the relative importance of retro- and ortho- nasal routes. This is notably useful since volatile aroma compounds can enter the nose orthonasally from the headspace above the beverage, as well as from the liquid in mouth via the retronasal route (Taylor et al., 2000).

1.6 Aim and Objectives

Although there is a plethora of literature on the effect of sugar type and concentration on aroma release and perception, the majority of the studies focused on sucrose as it has conventionally been the common source of sweetener. Moreover, among the variety of beverage models and systems investigated, few studies incorporated the carbonation component of soft drinks. The dearth of information available on aroma-matrix interactions in a carbonated system accentuates the need to develop deeper knowledge within the area.

The aim of this research was to investigate the effect of sugar and carbonation on real-time aroma release using APCI–MS. An understanding of aroma-matrix interactions would contribute to the timely and targeted release of aroma compounds for cost effective formulation efforts to manufacture low-sugar and low-calorie products, in response to the impending sugar tax to be levied on the soft drinks industry.

The first objective was to evaluate the effect of different sugar types and concentrations on the static headspace *in-vitro* release of aroma compounds with a range of physicochemical properties under static headspace conditions. This formed the basis for the design of a soft drink model – a system comprised of water, sugar, acid and aroma compounds representative of a fruity style flavouring. Thus, the second objective was to investigate the effect of sugar concentration and carbonation on the *in-vivo* release of aroma compounds during beverage consumption by recruiting panellists for breath-by-breath analysis. Finally, the last objective sought to employ tools of physical chemistry to understand the physicochemical mechanisms which influence aroma release from the

beverage matrix from a molecular perspective, exploring the effect of sugar on the kinetics of the matrix components, namely water, aroma compounds and carbon dioxide.

2 MATERIALS AND METHODS

2.1 Experiment 1: Effect of sweeteners on static headspace *in-vitro* aroma release

2.1.1 Preparation of volatile stock solutions

Table 2.1 lists the volatile aroma compounds used and their key physicochemical properties. All of the chemicals, obtained from Sigma-Aldrich, Gillingham, UK, were food grade and ≥ 95% in purity.

Table 2.1 Physicochemical properties of volatile aroma compounds

Class	Compound	Formula	MW (g mol^{-1})	Vapour pressure (mm Hg)	Water solubility (mg L^{-1})	Log P (unitless)	K_H (atm m^3 mol^{-1})
				MPBWIN v1.42 est	WSKOW v1.41 est	KOWWIN v1.67 est	HENRYWIN v3.10 (Bond est)
Aldehyde	Butanal	C_4H_8O	72.11	108.00	2.39E+04	0.82	1.20E-04
	Hexanal	$C_6H_{12}O$	100.16	9.57	3.53E+03	1.80	2.11E-04
	Octanal	$C_8H_{16}O$	128.21	1.49	3.94E+02	2.78	3.71E-04
	Decanal	$C_{10}H_{20}O$	156.27	0.24	4.35E+01	3.76	6.54E-04
Ester	Ethyl acetate	$C_4H_8O_2$	88.11	98.30	2.99E+04	0.86	2.33E-04
	Ethyl butanoate	$C_6H_{12}O_2$	116.16	14.60	2.75E+03	1.85	4.10E-04
	Ethyl hexanoate	$C_8H_{16}O_2$	144.21	1.80	3.09E+02	2.83	7.23E-04
	Ethyl octanoate	$C_{10}H_{20}O_2$	172.27	0.24	3.34E+01	3.81	1.27E-03
Terpene	Limonene	$C_{10}H_{16}$	136.23	1.45	4.58	4.83	0.38

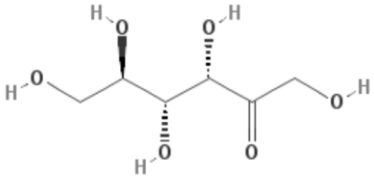
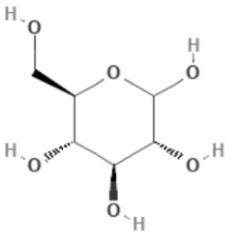
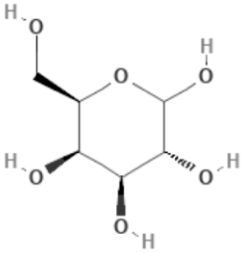
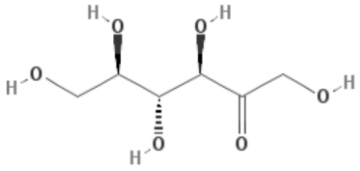
Values obtained from EPI-Suite v4 (Environmental Protection Agency, USA).

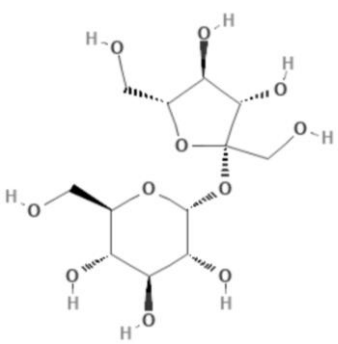
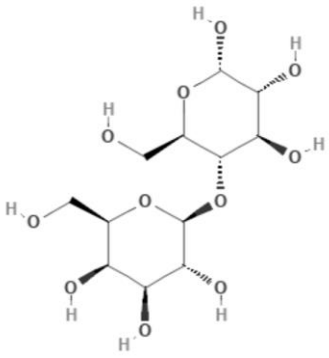
A 25 ppm solution was prepared in deionised water for each aroma compound, with the exception of limonene which was added to propylene glycol due to its relatively poor water solubility. All the stock solutions were mixed on a roller mixer (SRT9D, Stuart Scientific, Redhill, UK) for 3 h at 60 rpm to ensure complete solubilisation and kept in refrigerated storage at 4 ± 1 °C.

2.1.2 Preparation of non-volatile stock solutions

Tables 2.2 and 2.3 list the non-volatile compounds used and their properties. All of the common monosaccharide and disaccharide sugars, obtained from Sigma-Aldrich, Gillingham, UK or Fisher Scientific, Loughborough, UK, were analytical grade and ≥ 99% in purity. Anhydrous citric acid was obtained from Sigma-Aldrich, Gillingham, UK. Allulose (a rare monosaccharide and fructose isomer) was obtained from Matsutani Chemical Industry, Hyogo, Japan. Stevia was obtained from Bulk Powders, Colchester, UK. Acesulfame-K, aspartame, saccharin and sucralose were obtained from Blends Ltd, Liverpool, UK. All of the chemicals were food grade and ≥ 97% purity, where specified.

Table 2.2 Properties of sugars

Compound	Formula	Structure	MW	Relative sweetness (Sucrose = 1)
Fructose	$C_6H_{12}O_6$		180.16	1.21
Glucose	$C_6H_{12}O_6$		180.16	0.64
Galactose	$C_6H_{12}O_6$		180.16	0.50
Allulose	$C_6H_{12}O_6$		180.16	0.70

Sucrose	$C_{12}H_{22}O_{11}$		342.30	1.00
Lactose	$C_{12}H_{22}O_{11}$		342.30	0.33

Relative sweetness of allulose is obtained from Matsutani Chemical Industry Co. Ltd. (2015)
All other values are obtained from Wrolstad (2012)

Table 2.3 Properties of intense sweeteners and others

Compound	**MW**	**Relative sweetness** (Sucrose = 1)
Stevia	804.87	400
Acesulfame potassium (Ace-K)	201.24	200
Aspartame	294.30	180
Saccharin	183.18	400
Sucralose	297.63	600
Citric acid	192.12	-

Values of relative sweetness are obtained from Wrolstad (2012)

A 50% w/v solution was prepared in deionised water for each sugar and stirred with a magnetic stirrer for ≥ 2 h to ensure complete dissolution. A 15% w/v citric acid solution and 0.5% w/v solutions for each intense sweetener or sweetener blend were prepared. These stock solutions were stirred with a magnetic stirrer for ≥ 0.5 h to ensure complete dissolution. All of the stock solutions were kept in refrigerated storage at 4 ± 1 °C.

2.1.3 Mixing of final solutions

In the aroma-sugar systems, an aliquot of volatile stock solution was added to the non-volatile stock solutions to obtain 50 mL samples with a final concentration of 1 ppm volatile, 0.15% w/v citric acid and 0, 10, 20 or 30% w/v sugar. For example, 2 mL of

volatile stock solution was added to 37.5 mL of fructose stock solution and 0.5 mL of citric acid stock solution to obtain a sample with 10% w/v fructose.

In the aroma-alternative sweetener systems, the alternative sweeteners were added to the samples to obtain a final concentration equivalent to 10% w/v sucrose equivalence, which is within the range of sucrose concentrations typically present in soft drinks. For example, 2.5 mL of stevia stock solution was added to obtain a 50 mL sample with a final concentration of 0.025% w/v stevia, an intense sweetener which is 400 times sweeter than sucrose. Table 2.4 lists the final concentrations of alternative sweeteners and sweetener blends (at a ratio recommended by the manufacturer) in the system.

Table 2.4 Final concentrations (% w/v) of alternative sweetener/ sweetener blend in the system

Alternative sweetener/ sweetener blend	Concentration (% w/v)
Saccharin/ Aspartame/ Ace-K blend (3:3:1)	0.0455
Sucralose	0.017
Stevia/ allulose blend (1:186)	3.52
Stevia	0.025

All of the dilutions were made using deionised water. All of the samples were mixed on a roller mixer (SRT9D, Stuart Scientific, Redhill, UK) for 1 h at 60 rpm to ensure homogeneity before equilibration at room temperature for ≥ 2 h.

2.1.4 Static headspace *in-vitro* aroma *analysis*

A randomised block design was constructed for the measurement of the static headspace above triplicate samples (Appendix A). Each aroma compound was placed in a separate block to account for potential fluctuations in instrument sensitivity over the time course of the experiment.

In-vitro aroma release was analysed using APCI–MS, which comprised of a MS Nose interface (Micromass, Manchester, UK) fitted to a Quattro Ultima MS (Micromass, Manchester, UK). All of the samples were contained in 100 mL Schott bottles (Fisher Scientific, Loughborough, UK) fitted with a one-port lid (Figure 2.1). The headspace above each 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 x 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 introduced into the high vacuum region of the MS where they were separated and 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.

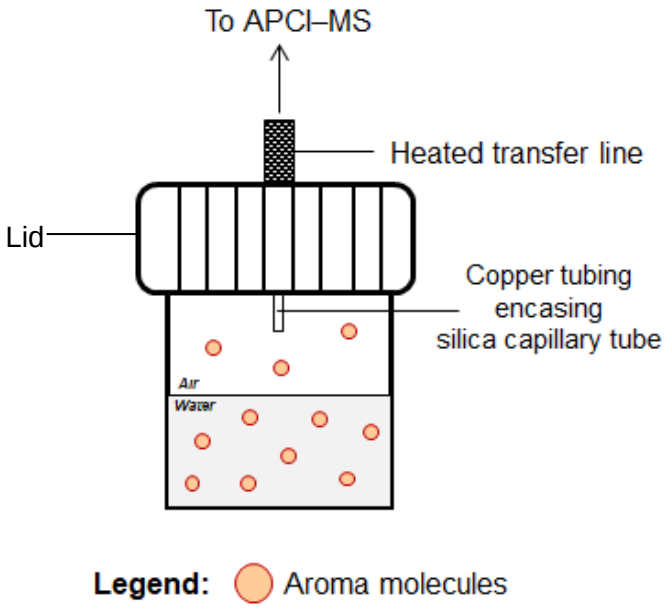


Figure 2.1 Schematic diagram of the *in-vitro* set-up

2.1.5 Data processing and statistical analysis

The output generated a chromatogram trace of the intensity of the monitored ions, which was recorded as peak height ion counts, and analysed using MassLynx v4.1 (Micromass, Manchester, UK). The peak height of each sample provided an indication of the number of ions formed during ionisation and thus the concentration of aroma compounds in the static headspace.

For each aroma compound, the ratio of mean peak height of each sample (I) in comparison to that of the control sample without sugar addition (I_0) was computed to obtain a relative index (I/I_0) to understand the effect induced by the presence of the specific sugar. A value above 1 corresponded to an increase in aroma release – the higher the value, the greater the release. On the other hand, a value below 1 corresponded to a decrease in aroma release and indicated a retention of aroma compounds in the matrix.

The results are reported as normalised means and standard deviations. ANOVA was performed using Design Expert v6.0.11 (Stat-Ease Inc., Minneapolis, USA) to evaluate the main and interaction effects of sugar concentration and type on *in-vitro* aroma release at a significance level of $p < 0.05$.

2.2 Experiment 2: Effect of sucrose and carbonation on *in-vivo* aroma delivery

2.2.1 Soft drink model

Volatile and non-volatile stock solutions were prepared as described in Section 2.1. Ethyl butanoate and hexanal were the volatile aroma compounds used in combination to produce an apple style flavouring for the model beverage system.

Aliquots of volatile stock solutions were added to the non-volatile stock solutions to obtain samples with a final concentration of 10 ppm of each volatile, 0.15% w/v citric acid and 0, 10, 20 or 30% w/v sucrose. All of the dilutions were made using distilled water. All of the samples were mixed on a roller mixer (SRT9D, Stuart Scientific, Redhill, UK) for 1 h at 60 rpm to ensure homogeneity and kept in refrigerated storage at 4 ± 1 °C.

2.2.2 Carbonation system

A schematic diagram of the batch carbonation apparatus, which was developed and manufactured in-house (Medical Engineering Unit, University of Nottingham, UK), is illustrated in Figure 2.2.

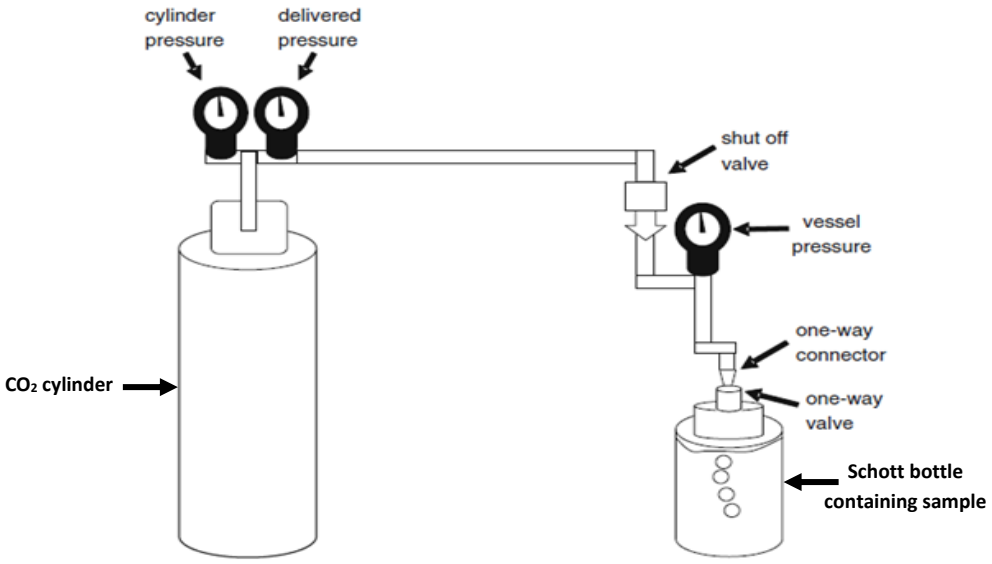


Figure 2.2 Schematic diagram of batch carbonation apparatus (Clark et al., 2011b)

Samples were prepared in Schott bottles (Fisher Scientific, Loughborough, UK) fitted with modified caps incorporating a one-way connecting valve (RS Components, Corby, UK) and tightly secured with a silicone sealing ring (RS Components, Corby, UK).

Food grade CO_2 (BOC, Surrey, UK) was delivered directly from the gas cylinder to the sample bottle through a regulator, which was set to the desired level of gas pressure for CO_2 delivery based on a forced-carbonation table (Appendix B). To achieve the level of carbonation typically present in soft drinks at ~3.6 volumes of CO_2, a gas pressure of 25 psi had to be achieved in the samples at 5 °C.

The sample bottle was connected to the regulator and the isolation switch was opened to allow the flow of CO_2 into the vessel. During the process of carbonation, the sample bottle was gently shaken to facilitate the dispersion of CO_2 into the liquid. Once equilibrium was achieved, as indicated by the cessation of gas bubbles entering the liquid, the shut-off valve was closed to isolate the sample bottle and the pressure within was monitored using a second pressure gauge to ensure that the desired level had been attained. The sample bottle was disconnected from the carbonation apparatus and kept in refrigerated storage at 4 ± 1 °C.

2.2.3 In-vivo aroma analysis

Ethics approval (#SBREC160137A, Appendix C) for the experiment was granted by the School of Biosciences ethics committee (School of Biosciences, University of Nottingham, UK) and written consent was obtained from all 5 panellists, who were recruited from the student population.

Both carbonated and non-carbonated samples were aliquoted into 15 mL screw-top, glass vials under refrigerated conditions. The vials were filled to the brim before being tightly capped and sealed with plastic film to minimise volatile and CO_2 loss. Samples were kept in refrigerated storage and served to panellists at 5 °C on the day of preparation. A randomised block design was constructed for the measurement of breath by breath volatile concentrations of triplicate samples (Appendix A). 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 15 mL of the sample while holding their breath and avoiding any liquid or air movement in the mouth. A small disposable plastic tube (40 mm length x 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 (Figure 2.3). On each inhalation and exhalation, the plastic tube was filled with surrounding air from the laboratory and expired air from the panellists respectively, allowing the tidal flow of respiration to be monitored and breath by breath volatile concentrations to be determined.

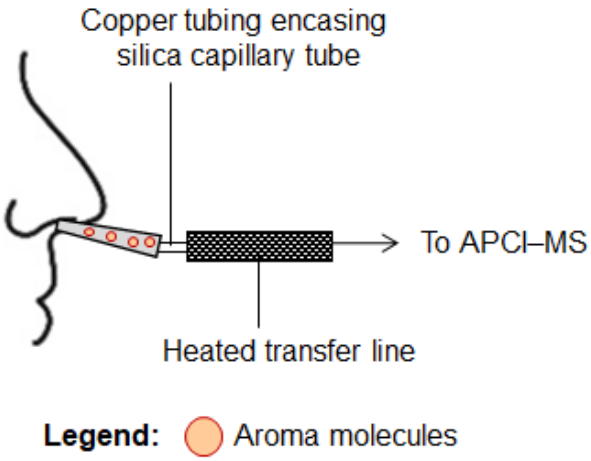


Figure 2.3 Schematic diagram of the *in-vivo* set-up

Eight samples were consumed during each session with a rest period of at least 1 min in between samples. Water and crackers were provided for palate cleansing to avoid desensitisation of oral and olfactory receptors, as well as carry-over of aroma compounds from the previous sample. In addition, the breath of the panellists was monitored to ensure that there were no detectable traces of aroma compounds persistent in the breath and that all compounds had returned to baseline levels prior to consumption of samples. The controlled protocol (Appendix C) adopted served to minimise idiosyncratic differences for the evaluation of the induced effects on sucrose and carbonation on *in-vivo* aroma delivery.

In-vivo aroma delivery was analysed using the APCI–MS parameters as described in Section 2.1.4. 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 corresponding to their protonated molecular ion (MH^+) and dehydrated molecular ion [(MH^+)-H_2O] respectively. In addition, acetone was monitored at m/z = 59. 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.

2.2.4 Data processing and statistical analysis

The output generated a chromatogram trace of a series of peaks and troughs, which corresponded to exhalations and inhalations over the breath sampling period. The chromatogram was integrated using MassLynx v4.1 (Micromass, Manchester, UK) to obtain the maximum intensity (I_{max}) and total area under the curve (AUC) parameters in

terms of arbitrary units. While I_{max} corresponded to the maximum intensity of aroma release, AUC provided an indication of the total aroma released in the nose space.

The results are reported as calculated means and standard deviations. ANOVA was performed using Design Expert v6.0.11 (Stat-Ease Inc., Minneapolis, USA) to evaluate the main and interaction effects of sucrose concentration and carbonation on *in-vivo* aroma delivery at a significance level of $p < 0.05$.

2.3 Experiment 3: Effect of sugar on kinetics of water, aroma compounds and carbon dioxide

2.3.1 Water activity (a_w)

A randomised design was constructed for the measurement of the water activity of triplicate fructose, glucose and sucrose solutions at 10%, 20% and 30% w/v concentrations using a water activity meter (Aqua Lab 4TE, Decagon Devices Inc., USA) at 25 °C (Appendix A). The results are reported as calculated means and standard deviations. One-way ANOVA followed by Tukey HSD post hoc test were performed using SPSS v23 (IBM, New York, USA) to evaluate the effect of sugar concentration on water activity at a significance level of $p < 0.05$.

2.3.2 Self-diffusion coefficients of aroma compounds (D)

Analytical grade deuterium oxide (D_2O) was obtained from Sigma-Aldrich, Gillingham, UK for the preparation of stock solutions. A 250 ppm stock solution was prepared for each of the volatile aroma compound used in the soft drink model, namely ethyl butanoate and hexanal. The stock solutions were mixed on a roller mixer (SRT9D, Stuart Scientific, Redhill, UK) for 3 h at 60 rpm to ensure complete solubilisation. A 50% w/v sucrose solution was prepared and stirred with a magnetic stirrer for ≥ 2 h to ensure complete dissolution. All the stock solutions were kept in refrigerated storage at 4 ± 1 °C.

To obtain the final sample, an aliquot of volatile stock solution was added to the sucrose stock solution to obtain 2 mL samples with a final concentration of 10 ppm volatile and 0, 10, 20 or 30% w/v sucrose. All the dilutions were made using D_2O. All the samples were mixed on a roller mixer (SRT9D, Stuart Scientific, Redhill, UK) for 1 h at 60 rpm to ensure homogeneity. 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, Coventry, UK).

A randomised design was constructed for the measurement of the self-diffusion coefficients of the aroma compounds in triplicate sucrose solutions (Appendix A). All ^{1}H Nuclear Magnetic Resonance (NMR) spectra were recorded using a 600 MHz spectrometer (Avance 600, Bruker, Coventry, UK) with a 5 mm z-gradient inverse probe (Bruker, Coventry, UK) 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.

A schematic diagram of a PGSE NMR diffusion experiment is illustrated in Figure 2.4. The process begins with an excitation phase, whereby a net magnetisation is placed in the *xy* axes and the phase of the spins is coherent. After excitation, a gradient pulse labels the position of the spins with a position-dependent phase angle, in that each plane of the sample perpendicular to the *z* plane contains spins which will be uniformly affected by the gradient pulse. However, as the spins undergo constant random translational motion in the solution, their position along the *z* axis changes, resulting in the 180° rotation of the spin magnetisation by a single or a series of radiofrequency pulse(s). When sufficient time has been allocated for the observation of the translational displacement of spins, a gradient pulse identical to the first is performed to refocus the signals. A maximum signal will be obtained when no diffusion has occurred. On the other hand, if diffusion has taken place, some spins are no longer in the same position along the *z* axis during the second gradient pulse and thus, their phase component imposed by the first gradient will not be cancelled by the second gradient, resulting in signal attenuation. Over the course of the experiment, a series of PGSE NMR spectra is recorded with increasing gradient pulse strength, resulting in signals decaying at rates determined by their diffusion properties (Antalek, 2002).

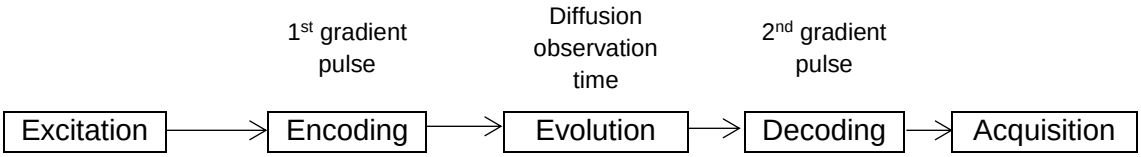


Figure 2.4 Schematic diagram of PGSE NMR diffusion experiment (Antalek, 2002)

All the data were processed using Bruker TopSpin 3.1 v3.5 (Bruker, Coventry, UK). For each aroma compound, several peaks were present in the DOSY spectrum corresponding to each proton group in the molecule and proton peaks were selected to calculate the mean self-diffusion coefficient from the diffusion value of each peak. The results are reported as calculated means and standard deviations. One-way ANOVA followed by Tukey HSD post hoc test was performed using SPSS v23 (IBM, New York, USA) to evaluate the effect of sucrose concentration on the self-diffusion coefficients of aroma compounds at a significance level of $p < 0.05$.

2.3.3 Volume flux of dissolved CO_2 (V_F)

Carbonated samples were prepared as described in Section 2.2.2. A randomised design was constructed for the measurement of CO_2 volume flux from triplicate fructose, glucose and sucrose solutions at 10%, 20% and 30% w/v concentrations using a precision weighing balance (DV215CD, Ohaus, Leicester, UK) interfaced with a computer (Appendix A).

Samples were removed from refrigerated storage and allowed to equilibrate at room temperature 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.

The cumulative mass loss recorded was a result of the progressive desorption of dissolved CO_2 from the liquid phase, as well as the evaporation of volatile aroma compounds and water. Thus, to obtain the cumulative mass loss in a sample solely due to CO_2, the mass loss attributed to evaporation was determined from a control sample containing only water and aroma compounds before subtraction from the total cumulative mass loss.

From a cumulative mass loss-time curve, the mass flux of CO_2 (M_F, unit = g s^{-1}, Equation 2.1) desorbing from the liquid surface could be experimentally deduced by dividing the difference in mass (Δm, unit = g) by the time interval (Δt, unit = s) between two data recordings.

$$M_F = \frac{\Delta m}{\Delta t}$$

Equation 2.1 Mass flux of CO_2 (M_F)

The mass flux could further be converted into volume flux (V_F, Equation 2.2) based on the assumption of ideal gas behaviour of CO_2.

$$V_F = 10^6 \left(\frac{RT}{MP}\right)\frac{\Delta m}{\Delta t}$$

Equation 2.2 Volume flux of CO_2 (V_{F-})

where V_F = CO_2 volume flux (cm^3 s^{-1}), R = ideal gas constant (8.31 J K^{-1} mol^{-1}); T = beverage temperature (K); M = molar mass of CO_2 (44 g mol^{-1}); P = atmospheric pressure (10^5 N m^{-2}); Δm = loss of mass between two successive data records (g) and Δt = time interval between two data recordings (5 s).

The results are reported as calculated means and standard deviations of cumulative volume flux. One-way ANOVA followed by Tukey HSD post hoc test was performed using SPSS v23 (IBM, New York, USA) to evaluate the effect of sugar concentration on cumulative CO_2 volume flux at a significance level of $p < 0.05$.

3 RESULTS AND DISCUSSION

3.1 Experiment 1: Effect of sweeteners on static headspace *in-vitro* aroma release

3.1.1 Static headspace *in-vitro* sampling of aroma compounds

During headspace sampling, there was a rapid increase in signal and a plateau was established over the sampling period before the signal returned to baseline when the sample was removed and thereby forming a peak on the chromatogram (Figure 3.1). The mean peak height of samples could thus be used as a comparison for the elucidation of trends and differences between sweetener type and concentration.

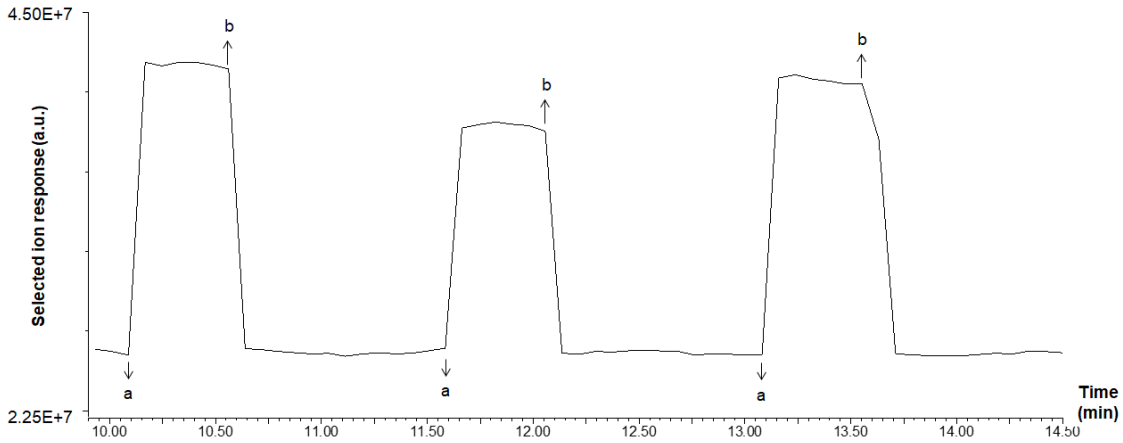


Figure 3.1 Example of APCI–MS chromatogram of ethyl acetate (m/z=89) monitored *in-vitro* under static headspace conditions. Points a and b on the chromatogram indicate the start and end of sampling in different samples respectively.

3.1.2 Effect of sugar concentration

As sugar concentration increased from 0 – 30% w/v, there was a significant increase in the *in-vitro* release of aroma compounds ($p < 0.05$, Table 3.1), with the exception of the more hydrophobic compounds – decanal ($p > 0.05$) and limonene ($p > 0.05$). This is in agreement with many of the previous studies (Table 1.1), which attributed the observation to the phenomenon of ‘salting out’.

Table 3.1 Normalised data for *in-vitro* release (I/I_0) of aroma compounds at different sugar concentrations (% w/v)

Aroma compound	Control (No sugar)	[Fructose] (% w/v)			[Galactose] (% w/v)			[Glucose] (% w/v)		
		10	20	30	10	20	30	10	20	30
Butanal	1.00 ± *0.03*^{a}	1.05 ± *0.05*^{b}	1.18 ± *0.03*^{c}	1.31 ± *0.00*^{d}	1.09 ± *0.05*^{b}	1.13 ± *0.17*^{c}	1.33 ± *0.14*^{d}	1.10 ± *0.02*^{b}	1.16 ± *0.10*^{c}	1.32 ± *0.16*^{d}
Hexanal	1.00 ± *0.14*^{a}	1.06 ± *0.12*^{b}	1.36 ± *0.17*^{c}	1.40 ± *0.16*^{d}	1.15 ± *0.21*^{b}	1.20 ± *0.19*^{c}	1.36 ± *0.04*^{d}	1.21 ± *0.13*^{b}	1.37 ± *0.08*^{c}	1.28 ± *0.05*^{d}
Octanal	1.00 ± *0.11*^{a}	1.08 ± *0.03*^{b}	1.11 ± *0.04*^{c}	1.31 ± *0.13*^{d}	1.09 ± *0.06*^{b}	1.10 ± *0.06*^{c}	1.28 ± *0.16*^{d}	1.15 ± *0.06*^{b}	1.22 ± *0.06*^{c}	1.34 ± *0.11*^{d}
Decanal	1.00 ± *0.20*^{a}	0.84 ± *0.09*^{a}	1.04 ± *0.17*^{a}	0.71 ± *0.18*^{a}	0.85 ± *0.04*^{a}	0.99 ± *0.22*^{a}	0.78 ± *0.04*^{a}	0.86 ± *0.04*^{a}	0.99 ± *0.32*^{a}	0.81 ± *0.22*^{a}
Ethyl acetate	1.00 ± *0.03*^{a}	1.19 ± *0.02*^{b}	1.36 ± *0.08*^{c}	1.70 ± *0.00*^{d}	1.21 ± *0.03*^{b}	1.51 ± *0.06*^{c}	1.77 ± *0.03*^{d}	1.17 ± *0.04*^{b}	1.47 ± *0.02*^{c}	1.65 ± *0.12*^{d}
Ethyl butanoate	1.00 ± *0.03*^{a}	1.04 ± *0.03*^{b}	1.63 ± *0.03*^{c}	1.88 ± *0.30*^{d}	1.13 ± *0.22*^{b}	1.63 ± *0.11*^{c}	2.02 ± *0.12*^{d}	1.24 ± *0.15*^{b}	1.33 ± *0.13*^{c}	1.99 ± *0.07*^{d}
Ethyl hexanoate	1.00 ± *0.10*^{a}	1.29 ± *0.05*^{b}	1.46 ± *0.05*^{c}	1.67 ± *0.14*^{d}	1.29 ± *0.06*^{b}	1.43 ± *0.04*^{c}	1.49 ± *0.14*^{d}	1.27 ± *0.02*^{b}	1.50 ± *0.04*^{c}	1.79 ± *0.08*^{d}
Ethyl octanoate	1.00 ± *0.08*^{a}	1.17 ± *0.08*^{b}	1.16 ± *0.20*^{c}	1.35 ± *0.10*^{d}	1.17 ± *0.06*^{b}	1.17 ± *0.09*^{c}	1.20 ± *0.06*^{d}	1.06 ± *0.19*^{b}	1.13 ± *0.11*^{c}	1.17 ± *0.34*^{d}
Limonene	1.00 ± *0.12*^{a}	0.91 ± *0.04*^{a}	0.83 ± *0.29*^{a}	0.97 ± *0.10*^{a}	0.81 ± *0.18*^{a}	1.00 ± *0.05*^{a}	0.82 ± *0.11*^{a}	0.91 ± *0.08*^{a}	0.97 ± *0.14*^{a}	0.87 ± *0.08*^{a}

Values are reported as mean ± SD for 9 replicates of control and 3 replicates of samples. Samples assigned different superscript letters within the same row are significantly different ($p < 0.05$).

Aroma compound	Control (No sugar)	[Lactose] (% w/v)			[Sucrose] (% w/v)		
		10	20	30	10	20	30
Butanal	1.00 ± *0.02*^{a}	1.06 ± *0.02*^{b}	1.15 ± *0.03*^{c}	1.26 ± *0.03*^{d}	1.05 ± *0.02*^{b}	1.11 ± *0.02*^{c}	1.16 ± *0.02*^{d}
Hexanal	1.00 ± *0.03*^{a}	1.08 ± *0.01*^{b}	1.15 ± *0.02*^{c}	1.23 ± *0.01*^{d}	1.03 ± *0.01*^{b}	1.03 ± *0.05*^{c}	1.10 ± *0.00*^{d}
Octanal	1.00 ± *0.05*^{a}	1.10 ± *0.09*^{b}	1.17 ± *0.05*^{c}	1.22 ± *0.08*^{d}	1.06 ± *0.01*^{b}	1.02 ± *0.05*^{c}	1.04 ± *0.09*^{d}
Decanal	1.00 ± *0.07*^{a}	0.97 ± *0.02*^{a}	1.11 ± *0.14*^{a}	1.11 ± *0.07*^{a}	1.00 ± *0.03*^{a}	0.83 ± *0.15*^{a}	1.11 ± *0.03*^{a}
Ethyl acetate	1.00 ± *0.04*^{a}	1.17 ± *0.05*^{b}	1.36 ± *0.05*^{c}	1.52 ± *0.01*^{d}	1.14 ± *0.03*^{b}	1.33 ± *0.02*^{c}	1.51 ± *0.02*^{d}
Ethyl butanoate	1.00 ± *0.04*^{a}	1.19 ± *0.02*^{b}	1.31 ± *0.08*^{c}	1.50 ± *0.04*^{d}	1.08 ± *0.08*^{b}	1.28 ± *0.05*^{c}	1.48 ± *0.06*^{d}
Ethyl hexanoate	1.00 ± *0.02*^{a}	1.20 ± *0.07*^{b}	1.35 ± *0.05*^{c}	1.17 ± *0.21*^{d}	1.15 ± *0.19*^{b}	1.28 ± *0.06*^{c}	1.41 ± *0.03*^{d}
Ethyl octanoate	1.00 ± *0.06*^{a}	1.16 ± *0.06*^{b}	1.16 ± *0.25*^{c}	1.16 ± *0.17*^{d}	1.07 ± *0.06*^{b}	1.05 ± *0.04*^{c}	1.15 ± *0.04*^{d}
Limonene	1.00 ± *0.23*^{a}	0.98 ± *0.05*^{a}	0.99 ± *0.33*^{a}	0.81 ± *0.14*^{a}	0.98 ± *0.21*^{a}	0.96 ± *0.31*^{a}	0.99 ± *0.19*^{a}

Values are reported as sample mean ± SD for 6 replicates of control and 3 replicates of samples. Samples assigned different superscript letters within the same row are significantly different ($p < 0.05$).

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 thus, 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 and 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 (de Roos, 2006, Delarue and Giampaoli, 2006, Rabe et al., 2003), resulting in the 'salting out' effect and enhanced aroma release 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. While the impact of a polar functional group would decrease due to a longer hydrophobic aliphatic chain length (Jeleń and 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 and 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.

As for limonene, the aroma compound with the lowest water solubility and highest gas/liquid partitioning, as indicated by the highest log P and K_H values respectively, the lack of significant effect of sugar concentration on its release was also demonstrated in other studies and was attributed to the strongly non-polar nature of the compound (Hansson et al., 2001). Unlike the other aroma compounds, a 'salting in' effect was observed for limonene in the experiment, as was the case reported by Copolovici and Niinemets (2007), together with other non-polar compounds such as linalool. 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 release observed (Copolovici and Niinemets, 2007).

3.1.3 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 a significantly higher increase in aroma release when a monosaccharide – fructose, galactose or glucose – was added to the system as compared to a disaccharide – lactose or sucrose ($p < 0.05$, Table 3.2).

Table 3.2 Normalised data for *in-vitro* release (I/I_0) of aroma compounds at different mono- and di- saccharide concentrations (% w/v)

Aroma compound	Control (No Sugar)		[Monosaccharide] (% w/v) 10		20		30	
Butanal	1.00	$\pm 0.01^a$	1.08	$\pm 0.03^b$	1.16	$\pm 0.03^c$	1.32	$\pm 0.01^d$
Hexanal	1.00	$\pm 0.13^a$	1.14	$\pm 0.08^b$	1.31	$\pm 0.09^c$	1.35	$\pm 0.06^d$
Octanal	1.00	$\pm 0.12^a$	1.11	$\pm 0.04^b$	1.14	$\pm 0.07^c$	1.31	$\pm 0.03^d$
Decanal	1.00	$\pm 0.08^a$	0.85	$\pm 0.01^a$	1.01	$\pm 0.03^a$	0.76	$\pm 0.05^a$
Ethyl acetate	1.00	$\pm 0.01^a$	1.19	$\pm 0.02^b$	1.45	$\pm 0.08^c$	1.71	$\pm 0.06^d$
Ethyl butanoate	1.00	$\pm 0.03^a$	1.13	$\pm 0.10^b$	1.53	$\pm 0.17^c$	1.96	$\pm 0.07^d$
Ethyl hexanoate	1.00	$\pm 0.02^a$	1.28	$\pm 0.01^b$	1.46	$\pm 0.04^c$	1.65	$\pm 0.15^d$
Ethyl octanoate	1.00	$\pm 0.04^a$	1.13	$\pm 0.06^b$	1.15	$\pm 0.02^c$	1.24	$\pm 0.10^d$
Limonene	1.00	$\pm 0.06^a$	0.88	$\pm 0.06^a$	0.93	$\pm 0.09^a$	0.89	$\pm 0.07^a$

Values are reported as sample mean ± SD for 9 replicates of control and 9 replicates of monosaccharides (3 replicates each for fructose, galactose and glucose). Samples assigned different superscript letters for each aroma compound are significantly different ($p < 0.05$).

Aroma compound	Control (No Sugar)		[Disaccharide] (% w/v) 10		20		30	
Butanal	1.00	$\pm 0.01^a$	1.06	$\pm 0.01^e$	1.13	$\pm 0.03^f$	1.21	$\pm 0.07^g$
Hexanal	1.00	$\pm 0.01^a$	1.06	$\pm 0.03^e$	1.09	$\pm 0.08^f$	1.16	$\pm 0.10^g$
Octanal	1.00	$\pm 0.03^a$	1.08	$\pm 0.03^e$	1.09	$\pm 0.10^f$	1.13	$\pm 0.13^g$
Decanal	1.00	$\pm 0.04^a$	0.99	$\pm 0.02^a$	0.97	$\pm 0.19^a$	1.11	$\pm 0.00^a$
Ethyl acetate	1.00	$\pm 0.01^a$	1.16	$\pm 0.02^e$	1.35	$\pm 0.02^f$	1.52	$\pm 0.01^g$
Ethyl butanoate	1.00	$\pm 0.03^a$	1.14	$\pm 0.08^e$	1.30	$\pm 0.02^f$	1.49	$\pm 0.02^g$
Ethyl hexanoate	1.00	$\pm 0.00^a$	1.18	$\pm 0.03^e$	1.31	$\pm 0.05^f$	1.29	$\pm 0.17^g$
Ethyl octanoate	1.00	$\pm 0.03^a$	1.12	$\pm 0.06^e$	1.11	$\pm 0.08^f$	1.16	$\pm 0.01^g$
Limonene	1.00	$\pm 0.20^a$	0.98	$\pm 0.00^a$	0.98	$\pm 0.02^a$	0.90	$\pm 0.13^a$

Values are reported as sample mean ± SD for 9 replicates of control and 6 replicates of disaccharides (3 replicates each for lactose and sucrose). Samples assigned different superscript letters for each aroma compound are significantly different ($p < 0.05$).

At first glance, the observation might be counter-intuitive as disaccharides display a stronger ability to bind water than monosaccharides given the higher number of exchangeable hydroxyl groups present. These active polar groups not only contribute to

stronger interactions with water molecules through the formation of intermolecular sucrose-water hydrogen bonds (Aroulmoji et al., 2012), but also affect the number and strength of intramolecular water-water hydrogen bonds (Starzak et al., 2000). Thus, higher values of hydration number had generally been reported for disaccharides, although values available in literature span over a wide range owing to different methods of measurement (Burakowski and Gliński, 2012).

However, equivalent weight concentrations were used in this experiment to simulate practical beverage manufacturing conditions and different molarities of the solutions have to be acknowledged (Table 3.3). Thus, the observation was within expectation as there was almost twice the number of monosaccharide molecules than that of disaccharides at equivalent weight concentrations, which compensated for the lower specific affinity for water and weaker hydration capacity of monosaccharides as compared to disaccharides.

Table 3.3 Molarity of mono- and di- saccharides at equivalent weight concentrations

	Monosaccharides (MW = 180.16 g mol^{-1})			**Disaccharides** (MW = 324.30 g mol^{-1})		
[Sugar] (% w/v)	10	20	30	10	20	30
Molarity (M)	0.555	1.110	1.665	0.308	0.617	0.925

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), which 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 statistically significant difference between the *in-vitro* release of the aroma compounds within each class of sugars studied in this experiment ($p > 0.05$), suggesting that the number of hydrogen bonds had a greater influence on the aroma-matrix interactions than bond strength in this experiment.

3.1.4 Effect of alternative sweeteners

There was no significant difference in the *in-vitro* release of the majority of the aroma compounds upon the substitution of 10% w/v sucrose with alternative sweeteners ($p > 0.05$, Table 3.4), except for the C4 – C8 ethyl esters ($p < 0.05$, Table 3.4). In solutions where a significantly higher aroma release 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 release observed with the addition of alternative 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 release.

Table 3.4 Normalised data for *in-vitro* release (I/I_0) of aroma compounds in different sweetener solutions at 10% w/v sucrose equivalence

Aroma compound	S0		S10		SAA		SCL		STA		STV	
Butanal	1.00	±*0.02*^{a}	1.07	±0.04^{a}	1.00	±0.04^{a}	1.02	±0.02^{a}	1.05	±0.03^{a}	1.04	±0.02^{a}
Hexanal	1.00	±*0.01*^{a}	0.99	±0.06^{a}	0.91	±0.14^{a}	0.91	±0.06^{a}	0.96	±0.04^{a}	0.97	±0.00^{a}
Octanal	1.00	±*0.03*^{a}	1.00	±0.03^{a}	0.94	±0.03^{a}	0.97	±0.07^{a}	0.98	±0.02^{a}	0.89	±0.05^{a}
Decanal	1.00	±*0.13*^{a}	0.97	±0.15^{a}	1.05	±0.07^{a}	1.00	±0.07^{a}	0.99	±0.05^{a}	0.96	±0.08^{a}
Ethyl acetate	1.00	±*0.05*^{a}	1.15	±0.04^{c}	1.03	±0.01^{ab}	1.02	±0.02^{ab}	1.10	±0.02^{bc}	1.03	±0.02^{ab}
Ethyl butanoate	1.00	±*0.01*^{a}	1.11	±0.04^{b}	1.00	±0.00^{a}	0.98	±0.02^{a}	1.00	±0.03^{a}	1.01	±0.03^{a}
Ethyl hexanoate	1.00	±*0.00*^{ab}	1.08	±0.05^{b}	0.95	±0.08^{a}	0.99	±0.02^{ab}	0.97	±0.03^{a}	0.97	±0.02^{ab}
Ethyl octanoate	1.00	±*0.07*^{a}	1.02	±0.12^{a}	1.03	±0.02^{a}	1.02	±0.07^{a}	1.01	±0.06^{a}	0.98	±0.08^{a}
Limonene	1.00	±*0.09*^{a}	0.90	±0.18^{a}	0.82	±0.23^{a}	0.95	±0.12^{a}	0.88	±0.09^{a}	0.95	±0.01^{a}

Values are reported as sample mean ± SD for triplicate control and samples.Samples assigned different superscript letters within the same row are significantly different ($p < 0.05$).
***S0**=Control; **S10**=Sucrose (10% w/v); **SAA**=Saccharin/Aspartame/Ace-K blend (0.0455% w/v); **SCL**=Sucralose (0.017% w/v); **STA**=Stevia/Allulose blend (3.52% w/v); **STV**=Stevia (0.025% w/v)*

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, could result in an enhanced aroma release of similar impact to 10% w/v sucrose addition. This gave rise to the possibility of using low calorie carbohydrate sweeteners in synergy with intense sweeteners in a multi-sweetener approach to achieve a similar aroma impact to the regular product.

However, it has to be acknowledged that intense sweeteners, could exhibit undesirable organoleptic properties such as delayed onset of sweetness, lingering aftertaste, narrow taste profile and even metallicity or bitterness (Bakal, 2012) due to different activation mechanisms of the human taste pathways as compared to sucrose (Frank et al., 2008). Thus, data from *in-vitro* aroma release have to be interpreted with caution and considerations taken into account for the different temporal profiles of alternative sweeteners, either through *in-vivo* experiments or sensory studies.

3.2 Experiment 2: Effect of sucrose and carbonation on *in-vivo* aroma delivery

3.2.1 In-vivo aroma delivery of ethyl butanoate (EB) and hexanal (H) in carbonated and non-carbonated beverages with apple style flavouring

During in-nose breath sampling, two distinct regimes in the aroma release profile could usually be identified – a high concentration of aroma compounds could be observed in the first exhalation upon swallowing, followed by much lower concentrations in subsequent exhalations (Figure 3.2).

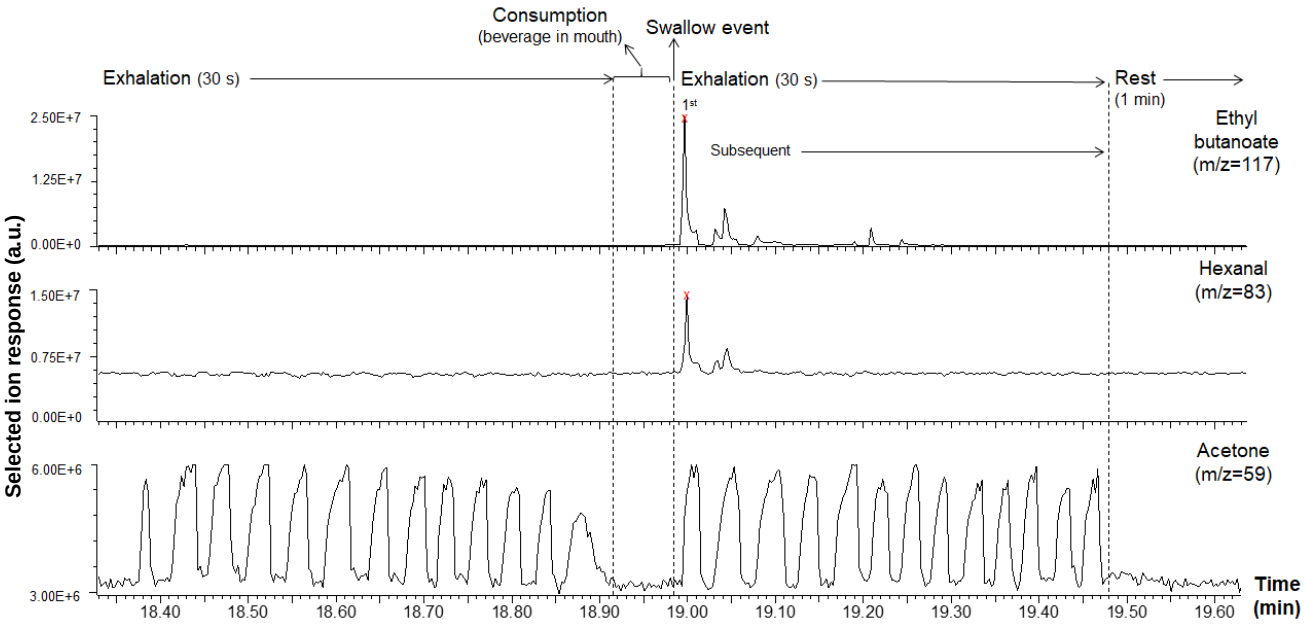


Figure 3.2 Example of APCI–MS chromatogram of ethyl butanoate (m/z=117), hexanal (m/z=83) and acetone (m/z=59) monitored *in-vivo* in the breath of one panellist. The marker 'x' and the shaded areas represent the I_{max} and AUC parameters obtained from the chromatogram respectively.

An initial burst of aroma release immediately follows the swallowing action due to the rapid, direct transfer of aroma compounds as a plug of volatile-laden gas from the pharynx to the nasal cavity during the first exhalation and is commonly referred to as the 'swallow breath' (Normand et al., 2004, Linforth et al., 2002). Meanwhile, a thin film of viscous salivary coating can form on the surface of the throat, pharynx and nasal mucosa and serve as a potential odourant deposit for aroma release during subsequent exhalations (Buettner et al., 2001, Linforth and Taylor, 2000). It involves a slower partition of the aroma compounds from the liquid phase in the thin film into the gas phase of the

tidal air stream before delivery to the olfactory receptors (Linforth et al., 2002, Hodgson et al., 2005, Normand et al., 2004). In addition to being a kinetically controlled process, aroma release in subsequent exhalations is subject to dilution in the upper airway as the thin film is exposed to relatively large airflows during exhalation, resulting in the lower concentrations of aroma compounds observed in successive exhalations (de Roos, 2006).

The persistence of aroma compounds in the breath after consumption is dependent on their physicochemical properties. The more polar aroma compounds with a higher water solubility and lower volatility, as indicated by lower log P values and vapour pressures respectively, have a stronger likelihood to partition into the mucous epithelia of the upper airways, collecting as a reservoir for gradual desorption over subsequent exhalations and resulting in more persistent aroma release. On the other hand, the more non-polar compounds with a higher log P and higher vapour pressure are less likely to be absorbed into the mucous layers, passing through the upper airway quickly as a plug of gas with little reservoir available for replenishment and resulting in less persistent aroma release (Linforth and Taylor, 2000). Hence, characteristic breath-by-breath peaks can be obtained on the chromatogram.

Based on the peaks observed in the chromatogram (Figure 3.2), both ethyl butanoate and hexanal would be considered as less persistent aroma compounds as they displayed a sharp initial peak at the start of exhalation followed by a small shoulder at the base of the peak, which was consistent with previous findings (Linforth and Taylor, 2000, Hodgson et al., 2005). On the other hand, the more persistent aroma compounds would be expected to have wider swallow breath peaks and smaller subsequent breath peaks declining over time, due to the continuous absorption and desorption of aroma compounds between the air and nasal mucosa during exhalation (Linforth and Taylor, 2000). Since both ethyl butanoate and hexanal were not persistent in aroma delivery, only I_{max} and AUC parameters were considered in this experiment.

In addition, the higher signal intensity observed for ethyl butanoate, which was manifested as higher peaks on the chromatogram, indicated a higher aroma release as compared to hexanal despite similar concentrations in the system. Although both aroma compounds have similar log P values (EB = 1.85; H = 1.80), the former has a higher K_H value (EB = 4.10×10^{-4}; H = 2.11×10^{-4}) and vapour pressure (EB = 14.6; H = 9.57), thereby partitioning into the gas phase more readily during beverage consumption.

Furthermore, it is possible for aldehydes to undergo dehydration during the chemical ionisation process (Taylor et al., 2000) and thus, the dehydrated molecular ion [(MH^+)-H_2O] of hexanal was monitored in this experiment due to a relatively stronger signal intensity recorded on the chromatogram as compared to the protonated ion (MH^+).

3.2.2 Effect of sucrose

The addition of sucrose from 10 – 30% w/v had no significant effect on the *in-vivo* aroma delivery of ethyl butanoate and hexanal ($p > 0.05$, Table 3.5). Previous *in-vivo* studies on beverages had 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).

Table 3.5 I_{max} and AUC values for *in-vivo* release of ethyl butanoate and hexanal for non-carbonated (-) and carbonated (+) samples at different concentrations of sucrose (% w/v)

[Sucrose] (% w/v)	CO_2	Ethyl butanoate		Hexanal	
		I_{max} (a.u)	AUC (a.u)	I_{max} (a.u)	AUC (a.u)
0	-	1.24E+07 ± *4.79E+06*^{a}	2.05E+05 ± *1.14E+05*^{a}	5.89E+06 ± 3.02E+06^a	9.47E+04 ± 7.03E+04^a
	+	6.73E+06 ± *3.24E+06*^{b}	1.21E+05 ± *4.82E+04*^{b}	3.63E+06 ± 1.99E+06^b	5.28E+04 ± 2.57E+04^b
10	-	9.81E+06 ± *4.95E+06*^{a}	2.09E+05 ± *1.06E+05*^{a}	5.19E+06 ± 2.44E+06^a	1.01E+05 ± 7.31E+04^a
	+	6.63E+06 ± *4.20E+06*^{b}	1.17E+05 ± *7.90E+04*^{b}	4.14E+06 ± 3.04E+06^b	6.20E+04 ± 5.66E+04^b
20	-	9.15E+06 ± *3.90E+06*^{a}	1.64E+05 ± *9.45E+04*^{a}	4.49E+06 ± 3.43E+06^a	7.89E+04 ± 6.97E+04^a
	+	7.79E+06 ± *4.85E+06*^{b}	1.40E+05 ± *8.31E+04*^{b}	3.93E+06 ± 2.10E+06^b	6.42E+04 ± 4.66E+04^b
30	-	1.13E+07 ± *5.09E+06*^{a}	2.31E+05 ± *1.74E+05*^{a}	6.63E+06 ± 3.55E+06^a	8.67E+04 ± 7.42E+04^a
	+	8.72E+06 ± *5.40E+06*^{b}	1.41E+05 ± *7.42E+04*^{b}	4.88E+06 ± 3.46E+06^b	5.58E+04 ± 3.69E+04^b

Values are reported as sample mean ± SD for triplicate control and samples consumed by 5 panellists. Samples assigned different superscript letters within each column are significantly different ($p < 0.05$).

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. These 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 dilution of the liquid and gas phases during the *in-vivo* delivery of aroma compounds (Taylor, 2002). Furthermore, it was demonstrated that sucrose intake

showed a concentration dependent increase in salivary flow, pH and α-amylase activity (Harthoorn et al., 2009), which could in turn affect aroma compound partitioning between gas and aqueous phases.

Although this is in contrast with the results obtained from static headspace measurements (Table 3.1), disparity between *in-vitro* and *in-vivo* results in terms of direction and magnitude would be inevitable given the vastly different conditions for the measurement of aroma release and have been reported in other studies (Saint-Eve et al., 2009, Clark et al., 2011a). 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 (Linforth et al., 2002, Buffo et al., 2005), highlighting the importance of *in-vivo* studies, which simulate the real consumption experience.

3.2.3 Effect of carbonation

The introduction of carbonation resulted in a significant decrease in the *in-vivo* aroma delivery of ethyl butanoate and hexanal, in terms of the I_{max} and AUC parameters ($p < 0.05$, Table 3.5). More interestingly, it could be observed that the difference in aroma delivery between non-carbonated and carbonated samples generally became smaller as sucrose concentration increased (Figure 3.3) and this was reflected in the results from ANOVA, which indicated a borderline significance ($p < 0.05$) of the interaction effect between sucrose concentration and carbonation for the I_{max} values of ethyl butanoate (Appendix E).

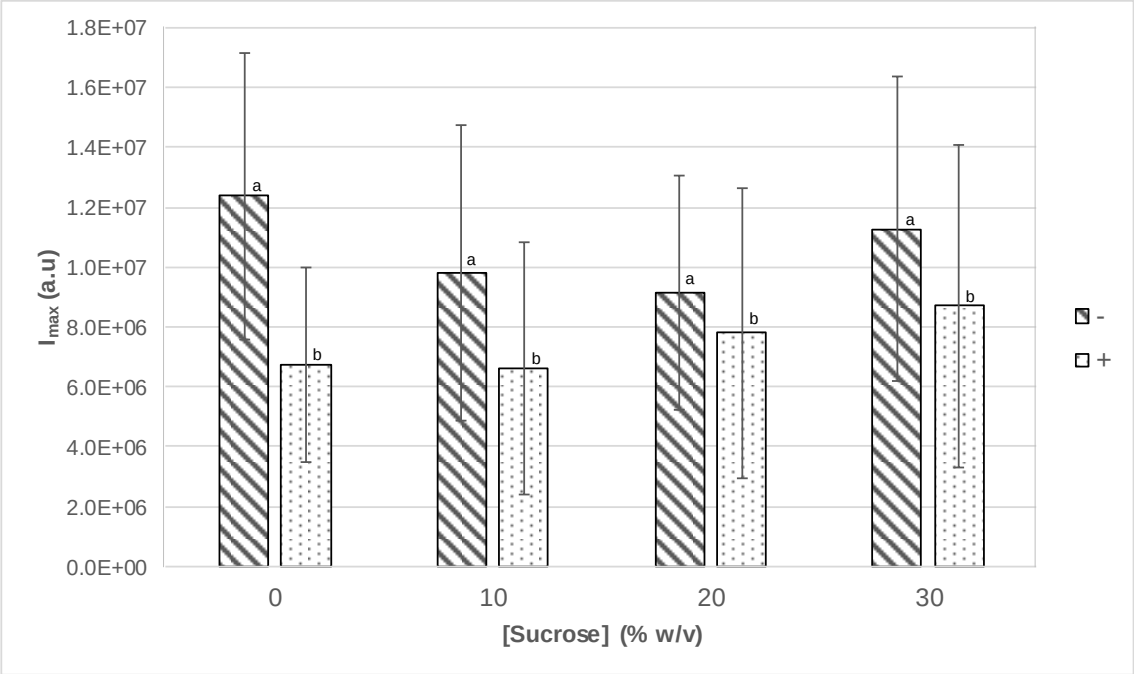


Figure 3.3 I_{max} values for *in-vivo* release of ethyl butanoate for non-carbonated (-) and carbonated (+) samples of different sucrose concentrations (% w/v). Values are reported as mean ± SD for triplicate control and samples consumed by 5 panellists with different letters within each group indicating significant differences ($p < 0.05$).

However, these results are in contrast with previous *in-vivo* studies (Table 1.2), 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 (Saint-Eve et al., 2009, Clark et al., 2011a). The enhanced aroma release was attributed to the aroma stripping and convection phenomena induced by ascendant gas bubble movement, resulting in a higher concentration of aroma compounds reaching the olfactory receptors in the nasal cavity (Saint-Eve et al., 2009).

The discrepancies 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, samples were served at a higher temperature of 10 °C, which could have affected CO_2 release and volatile partitioning, since it is known that 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 PTR–MS 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 using a similar sampling protocol and analytical method as in this experiment, it was suggested that the effect of carbonation was dependent on the physicochemical properties of the aroma compounds. A relationship between 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. Although ethyl butanoate and hexanal have similar K_{aw} values as ethyl acetate, these compounds have much lower vapour pressures and volatilities due to the longer alkyl carbon chain length. Thus, these compounds 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 enzymes anchored on the surface of taste receptor cells. While the extracellular generation of protons serves as the primary stimulus of sour-sensitive taste receptor cells to trigger 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 Experiment 3: Effect of sugar on kinetics of water, aroma compounds and carbon dioxide

3.3.1 Water activity (a_w)

Water activity is a measure of the amount of free water available in the system. As sugar concentration increased from 10 – 30% w/v, there was a significant decrease in water activity of the solutions ($p < 0.05$, Figure 3.4), 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.

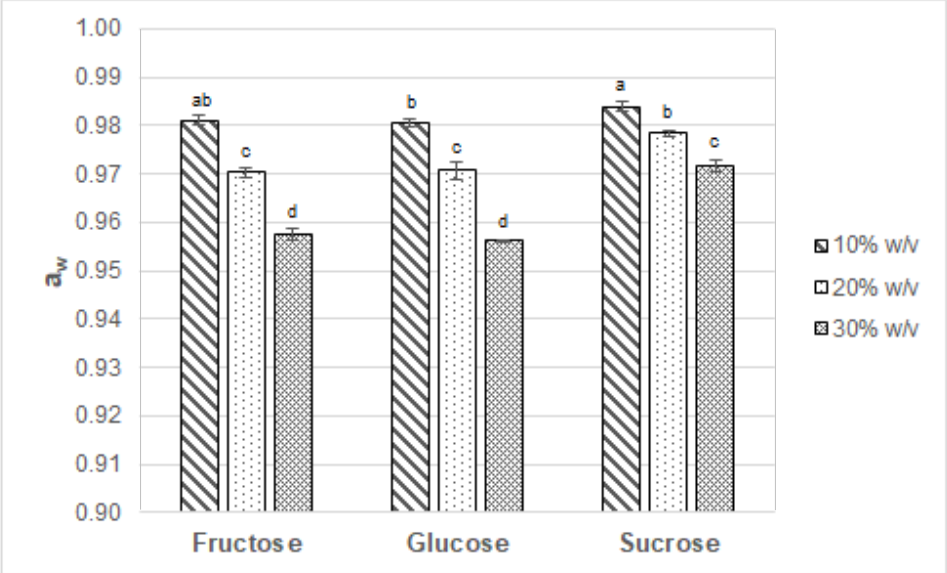


Figure 3.4 Water activity, a_w, of solutions at different sugar concentrations (% w/v). Values are reported as mean ± SD for triplicate samples with different letters indicating significant differences ($p < 0.05$).

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 (Delarue and Giampaoli, 2006, de Roos, 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* release of the majority of the aroma compounds (Table 3.1), with a higher release observed for monosaccharides compared to disaccharides at equivalent weight concentrations (Table 3.2).

3.3.2 Self-diffusion coefficients of aroma compounds (D)

The aroma compounds in a solution are in constant random translational motion (Figure 3.5), which is the self-diffusion of the molecules within the three-dimensional space along

the *x*-, *y*- and *z*- axes (Pfennig, 2015). 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, such as sugar concentration in this experiment (Novoa-carballal et al., 2010). The process can be quantitatively measured using DOSY–NMR spectroscopy and expressed as self-diffusion coefficients.

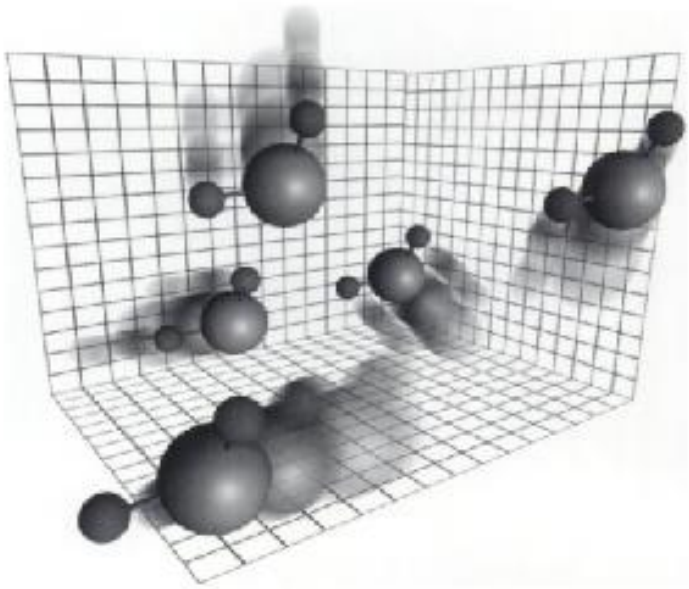


Figure 3.5 Schematic diagram of translational motion of a molecule (Schmidt, 2004)

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 (Figure 3.6). 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 (Figure 3.7), as was the case reported by Savary et al. (2006). Thus, mean self-diffusion coefficients were calculated from these 3 peaks.

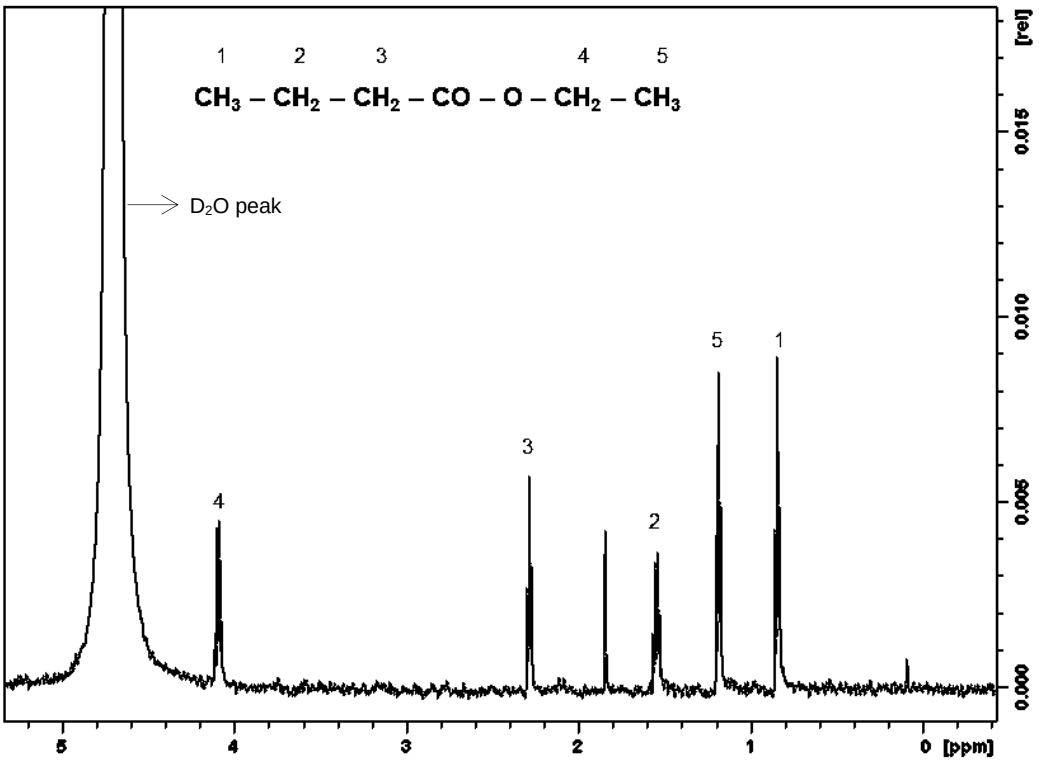


Figure 3.6 ^{1}H 1D NMR spectrum of ethyl butanoate in D_2O (Control)

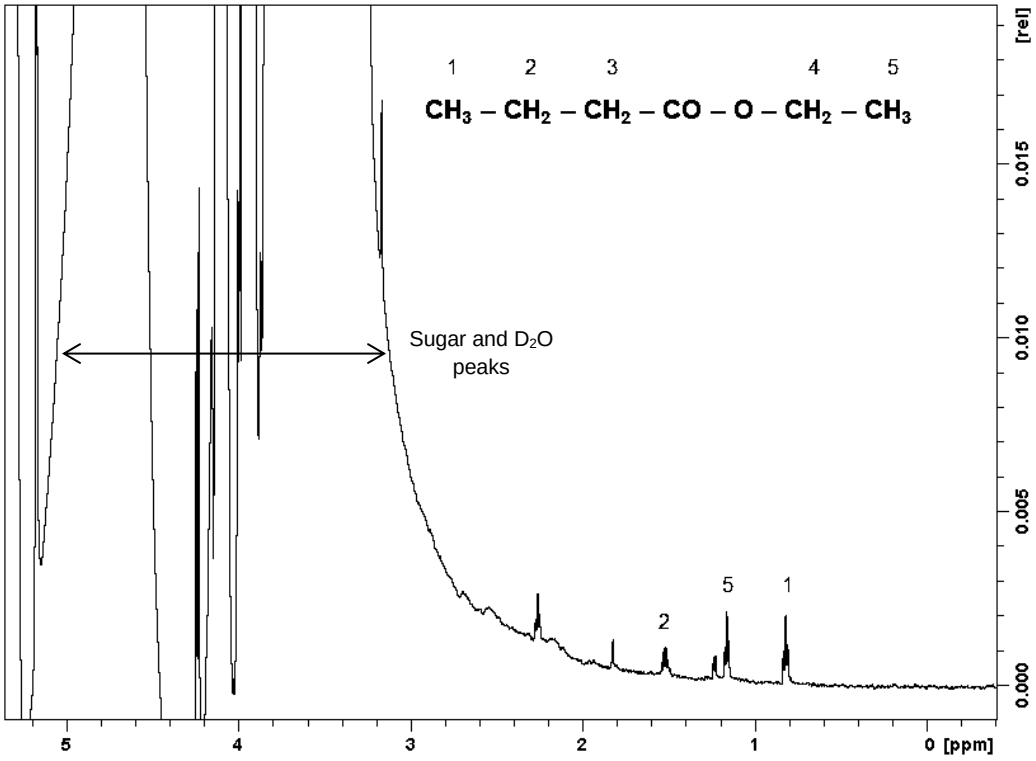


Figure 3.7 ^{1}H 1D NMR spectrum of ethyl butanoate in 30% w/v sucrose

Similarly for hexanal, 5 peaks corresponding to the proton groups of the molecule were expected as observed in the control (Figure 3.8) 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 (Figure 3.9). Thus, mean self-diffusion coefficients were calculated from these 3 peaks.

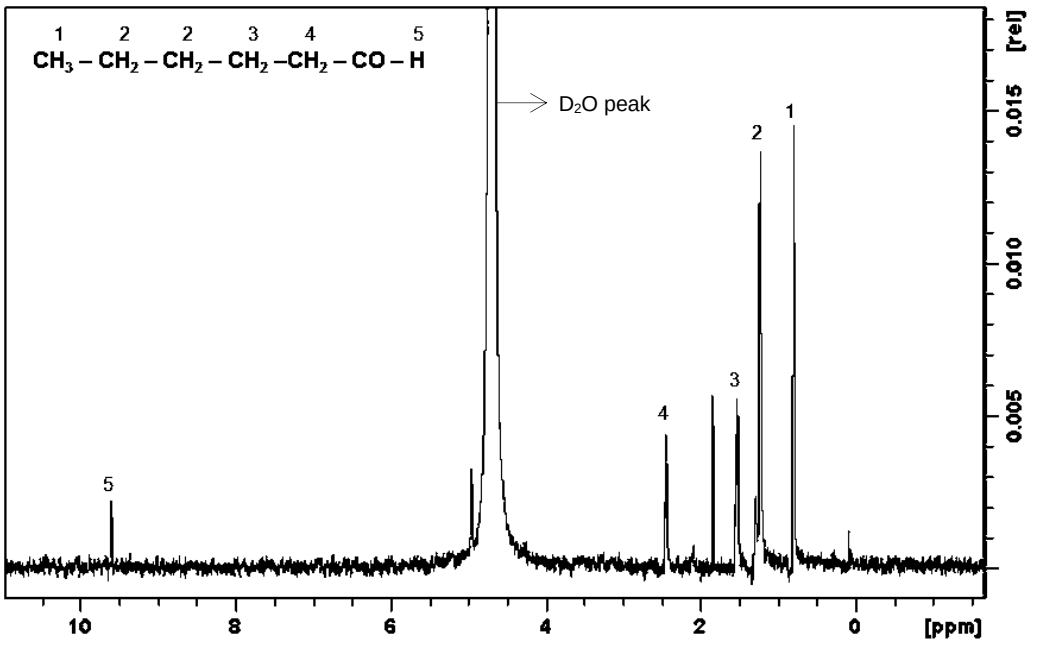


Figure 3.8 ^{1}H 1D NMR spectrum of hexanal in D_2O (Control)

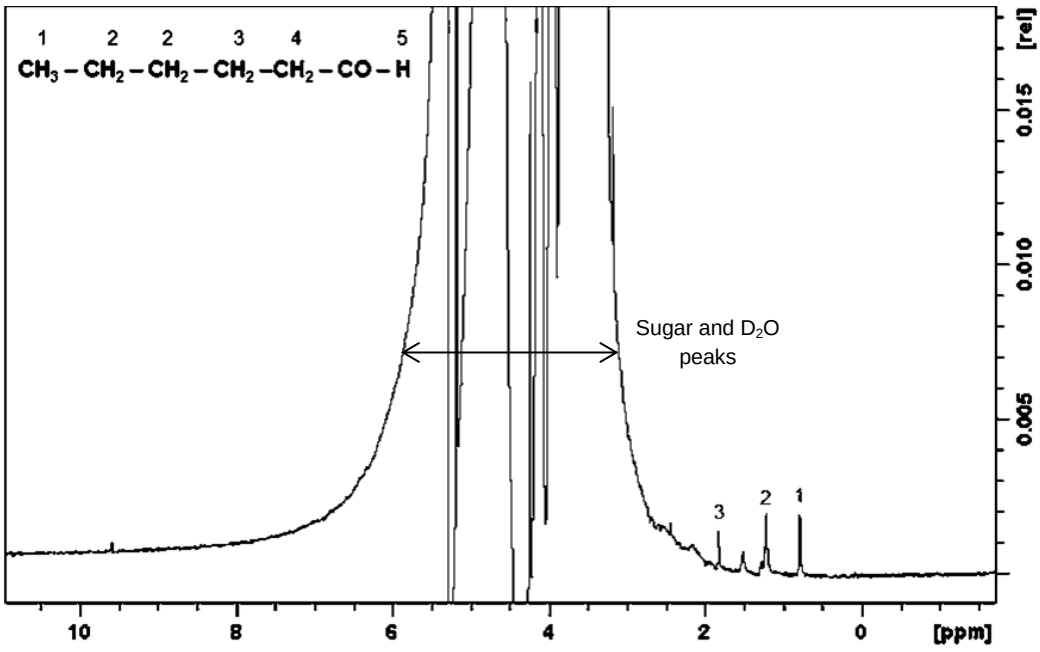


Figure 3.9 ^{1}H 1D NMR spectrum of hexanal in 30% w/v sucrose

Nevertheless, for both aroma compounds, as sucrose concentration increased from 0 – 30% w/v, there was a significant decrease in mean self-diffusion coefficients ($p < 0.05$, Figure 3.10). 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 Figure 3.4 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 within expectation.

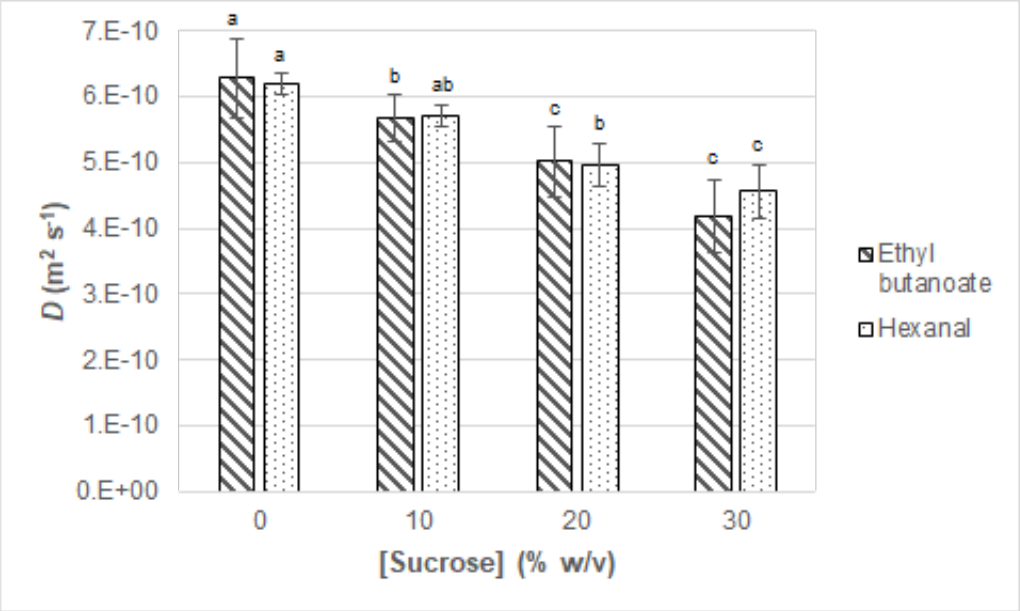


Figure 3.10 Self-diffusion coefficients, D (m^2 s^{-1}) of ethyl butanoate and hexanal at different sucrose concentrations (% w/v). Values are reported as mean ± SD for triplicate samples with different letters indicating significant differences ($p < 0.05$).

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 experiment was of a gradual magnitude, 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 diffusion, it could be possible that water availability was not a limiting factor for the 10 ppm concentration of aroma compounds used in this experiment, which was a magnitude lower than the 100 ppm used in Savary's work (2006).

3.3.3 Volume flux of dissolved CO_2 (V_F)

Henry's Law states that the amount of gas dissolved in a given volume of solvent is proportional to the pressure of the gas with which the solvent is in equilibrium. Thus, 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 thus, 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 (Steen, 2006, Liger-Belair et al., 2015). Besides in the form of heterogeneously nucleated bubbles observed in the carbonated beverages, dissolved CO_2 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 flux of CO_2 escaping from the liquid-gas interface (Liger-Belair et al., 2015). However, the presence of other non-CO_2 dissolved gases had to be acknowledged although the relatively lower concentrations would have a negligible impact on the kinetics of CO_2 studied in carbonated beverages in this experiment.

Although there was no significant effect of sugar addition on cumulative CO_2 volume flux ($p > 0.05$, Figure 3.11), it could be observed that the introduction of carbonation to a system resulted in a much higher CO_2 volume flux (Figure 3.12) 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.

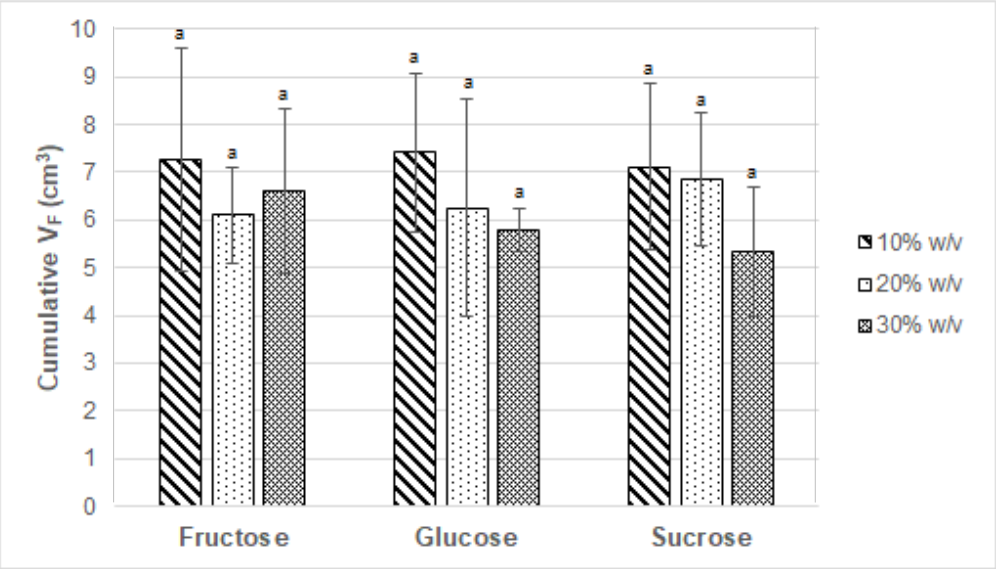


Figure 3.11 Cumulative CO_2 volume flux, V_F (cm^3) at different sugar concentrations (% w/v). Values are reported as mean ± SD for triplicate samples with different letters indicating significant differences ($p < 0.05$)

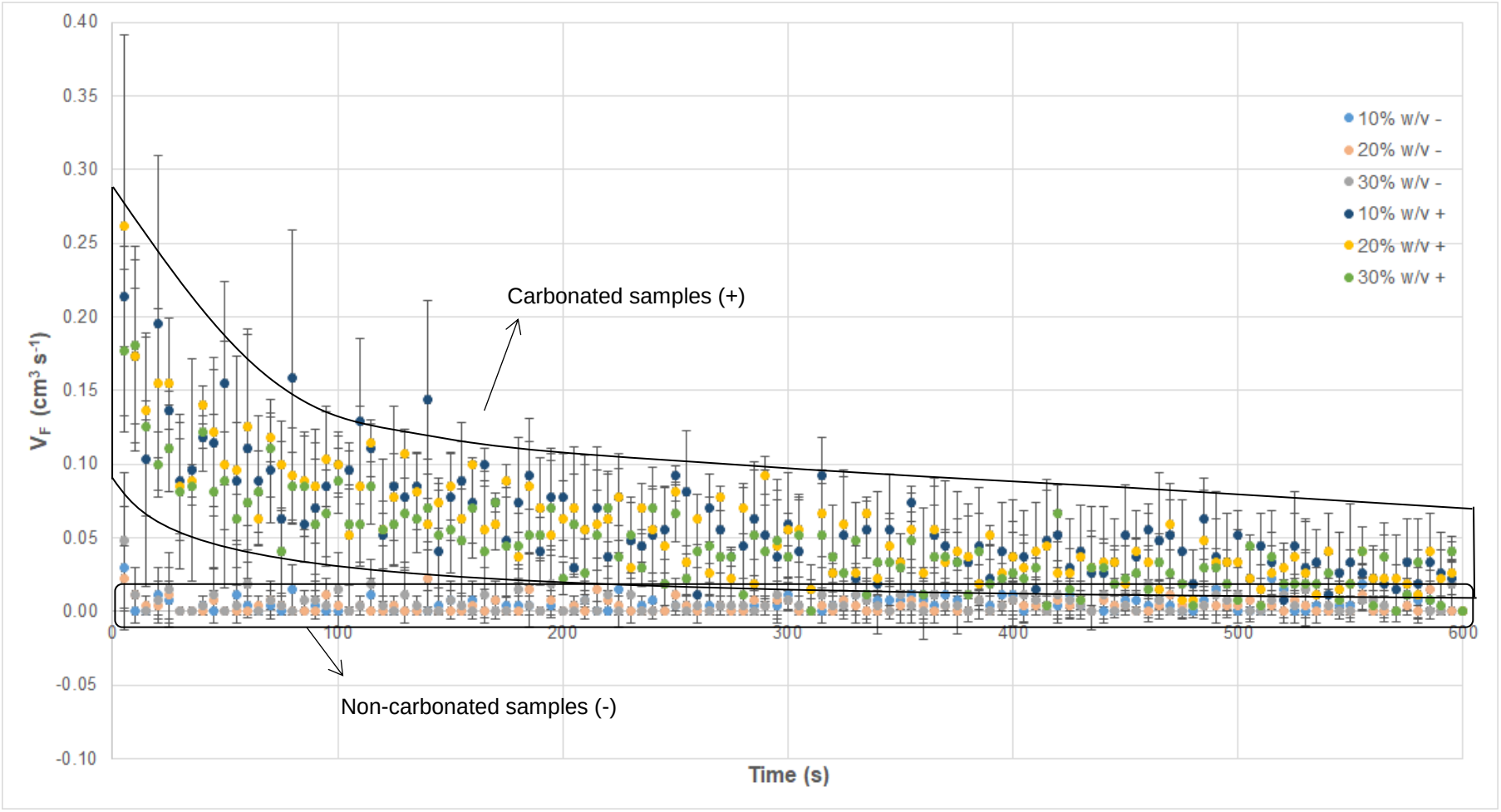


Figure 3.12 CO_2 volume flux, V_F (cm^3 s^{-1}) in non-carbonated (-) and carbonated (+) sucrose solutions of different concentrations (% w/v) over time (s). Values are reported as triplicate mean ± SD.

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 release in the headspace, orthonasal perception of aroma upon the opening of the lid of a carbonated beverage would be expected to be higher. However, this could also be at the expense of retronasal olfaction as the aroma compounds were lost to the surrounding, 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 3.5).

4 CONCLUSIONS AND FUTURE WORK

4.1 Conclusions

The aim of this research was to gain an understanding of aroma-matrix interactions in the context of carbonated beverages by investigating the effect of sugar and carbonation on aroma release using APCI–MS. These findings would be valuable to soft drinks manufacturers during product development and reformulation efforts in response to the impending sugar tax to be levied on the industry.

The results showed that an increase in sugar concentration from 0 – 30% w/v resulted in a significant increase in *in-vitro* aroma release in the static headspace under equilibrium conditions for the majority of the compounds ($p < 0.05$), owing to the phenomenon of 'salting out' as the reduction in the volume of free water available for the solubilisation of aroma compounds shifted the partition equilibrium towards the gas phase. However, the addition of polar solutes such as sugar could also increase the hydrophobicity of the solvent character, resulting in the enhanced aqueous solubility of the less polar aroma compounds and in turn, the lower aroma release observed. Meanwhile, although disaccharides have a stronger hydration capacity as compared to monosaccharides, there was a significantly higher increase in aroma release when a monosaccharide was added to the system ($p < 0.05$) as there was almost twice the number of monosaccharide molecules than that of disaccharides at equivalent weight concentrations. Hence, the overall effect of sugar addition on aroma release was a complex interplay between the various factors influencing the solubilisation of aroma compounds, depending on the water binding capacity of the specific sugar as well as the physicochemical properties of the aroma compounds. It is therefore pertinent for manufacturers to select a range of representative aroma compounds and a suitable beverage matrix during their reformulation instead of assuming additive effects of aroma release.

On the other hand, there was no significant effect of sucrose on the *in-vivo* aroma delivery in the breath of individuals during the consumption of beverages from the soft drink model ($p > 0.05$), while the introduction of carbonation to the soft drink model resulted in a significant decrease in *in-vivo* aroma delivery during breath-by-breath analysis ($p < 0.05$). The disparity between *in-vitro* and *in-vivo* results highlights the importance of *in-vivo* studies, which simulate the real consumption experience,

especially since beverage consumption is a fast and dynamic event whereby aroma compounds do not reach an equilibrium. Manufacturers therefore need to be cautious about solely relying on results from *in-vitro* analyses, be it static or dynamic headspace, and it will be in their interest to carry out *in-vivo* experiments to monitor aroma release. In the case of a lack of access to sophisticated equipment, sensory studies by a consumer or trained panel can also be designed to validate their findings.

To understand the physicochemical mechanisms behind aroma release from the beverage matrix, the effect of sugar on the kinetics of the matrix components, namely water, aroma compounds and carbon dioxide, was explored. The results showed that an increase in sugar concentration from 0 – 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 ($p < 0.05$). No significant effect of sugar on carbon dioxide volume flux was found ($p > 0.05$).

These experiments demonstrated the feasibility of utilising tools of physical chemistry in the study of the kinetics of aroma compounds and other matrix components. Advanced techniques such as DOSY–NMR spectroscopy could be employed to probe microscopic displacements covered by aroma compounds present at low concentrations. The comparison of self-diffusion coefficients of aroma compounds alone and in the presence of other matrix components could provide information on aroma-matrix interactions at the molecular level. Even simple methods such as the measurement of mass flux in the system using a precision balance could also be devised for experimental observations and theoretical developments to be made in a quantitative attempt to understand the process of progressive CO_2 desorption from the liquid surface of the beverage.

While this research had provided insights on aroma-matrix interactions in carbonated systems through *in-vitro* and *in-vivo* aroma analyses, as well as an investigation of the physical mechanisms at the molecular level, the variety and complexity of mechanisms involved continue to limit our understanding of aroma release, delivery and perception. Nevertheless, the increasing consumer health awareness and impending sugar tax implementation continue to be a major driving force behind product reformulation in the soft drinks industry, mandating the need for manufacturers to gain a deeper understanding of the interactions between gustatory, olfactory and trigeminal stimuli within the beverage matrix.

4.2 Future work

Aroma perception is a multi-modal and cross-modal experience derived from complex stimulus-response interactions between the food matrix and human sensory, perceptual and cognitive processes (Keast et al., 2004). Thus, an integrated approach combining instrumental techniques and sensory methods could be adopted to investigate the effect of sweetener and carbonation on aroma release, delivery and perception during beverage consumption.

Although real-time aroma analysis using APCI–MS was a useful approach in monitoring aroma release under the dynamic conditions of food consumption, the value of APCI–MS would be enhanced when coupled with time-intensity (TI) sensory evaluation. As TI measurements establish the pattern of development and decline of specified sensory attributes under study, a wealth of detailed information could be collected, such as the rate of onset of stimulation, maximum intensity perceived, rate of decay of perceived intensity and the total duration of the sensation (Lawless and Heymann, 2010). This information could be correlated with parameters obtained from the APCI–MS chromatograms to provide quantitative insights on temporal changes in perceived sensations from onset through extinction. In addition, this would be relevant in the context of intense sweeteners, which are often added to beverages in quantities too negligible to induce significant differences in *in-vitro* aroma release, yet the chemical interactions with aroma compounds could modify the intensity of flavour attributes, resulting in differences in temporal profiles which could be easily detected by the sensitive human sensory receptors.

Due to the scant literature investigating the physicochemical effects of carbonation on aroma release and perception in beverage systems, it could be worth further exploring the kinetics of dissolved CO_2 using ^{13}C DOSY–NMR spectroscopy. The diffusion coefficients obtained could provide an understanding of the influence of matrix components on CO_2 at the molecular level, which could in turn affect aroma release as the diffusion of CO_2 from the liquid-gas interface, as well as bubble formation and collapse during effervescence, could strip aroma compounds from the liquid phase and accelerate their transfer into the gas phase (Liger-Belair, 2012).

Hence, further research would be useful in the development of a rational approach during product reformulation by manufacturers and address the sensory and technical challenges associated with sugar and calorie reduction.

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6 APPENDICES

A. Experimental design

Experimental design for *in-vitro* experiments (monosaccharides)

Limonene	Butanal	Hexanal	Octanal	Decanal	Ethyl acetate	Ethyl butanoate	Ethyl hexanoate	Ethyl octanoate
GA10	GL30	GL30	F10	F0	GL0	GA10	GA20	GA30
F0	F30	F0	GL10	F10	GA10	GA30	GL20	F10
GA20	F20	F0	F10	GA0	GL0	GL10	GL10	F20
GL30	F20	GA10	GA0	GA10	GA20	GA30	GA10	GA20
GL30	F20	GA30	F30	F20	GL20	GA30	GL10	GA30
GA0	F0	GL30	GL10	GA20	GL10	GA0	GL0	F0
F0	GA20	F10	GL30	GA30	GA20	F30	GL30	GA20
F10	GA0	F10	F0	F10	GL10	GA10	GL0	F20
GA30	GA0	GA0	GA0	GL10	GL10	F20	GA0	F0
F10	GA20	F20	GL30	GL10	GA30	GA0	F0	F20
GA10	F10	GA20	GL20	F10	F30	F10	F0	GA0
GL10	GL0	GL20	GL10	GA20	F10	GA10	F10	GA0
F30	GL20	F30	GA30	GL30	GA30	F0	F30	F10
F20	F0	F30	F0	F0	F20	F30	GL30	GL30
GL0	F0	GL0	GA30	GL10	F10	F20	GA30	F0
GA20	GA30	GA20	F30	GL20	GL0	F30	F30	GL20
GA0	GL20	GL0	F0	GL0	GA0	GL10	F30	GL0
GL30	GA30	GL30	GA10	F0	GL30	GA20	GL20	GL10
GL0	GA0	GA0	F10	GA30	F0	GL30	GA10	GA10
GL20	F30	GL10	GA20	GA10	GA10	F0	GL0	F10
F10	F10	GA30	F20	GA10	GL20	GL20	GA20	GL20

GA30	GA10	GA0	GA30	F20	F20	F20	GL30	GL30
GA0	GA30	GA10	GL0	GA0	GL30	GL10	GL20	GL10
GL20	GA10	F20	GA0	F30	F0	GL0	F10	F30
GA20	F30	F0	GL30	GL30	GL20	GL20	GA30	GA10
GL0	F10	GA20	GA10	F30	F30	GA0	GA30	GL10
F20	GL10	F10	GL0	GA30	GA20	GL30	F0	GL30
F20	GL0	GA30	F30	F30	GL30	GL0	GL10	GA20
GA10	GL30	GL10	GA20	GL20	GA30	GA20	GA0	F30
F30	GL0	GL0	F20	GL30	GA0	GA20	GA20	GL20
F0	GL30	F20	F20	GL0	GA10	GL30	F20	F30
GL10	GA10	F30	GL0	GA0	F30	F0	GA10	GL0
GL10	GA20	GL20	GA20	GL0	GA0	GL20	F20	GA10
GL20	GL20	GA10	GL20	F20	F10	F10	F20	GL0
F30	GL10	GL10	GA10	GA20	F0	F10	F10	GA30
GA30	GL10	GL20	GL20	GL20	F20	GL0	GA0	GA0

F=*Fructose;* ***GA****=Galactose;* ***GL****=Galactose*

0=*Control (No sugar);* ***10****=10% w/v sugar;* ***20****=20% w/v sugar;* ***30****=30% w/v sugar*

Experimental design for in-vitro experiments (disaccharides)

Limonene	Butanal	Hexanal	Octanal	Decanal	Ethyl acetate	Ethyl butanoate	Ethyl hexanoate	Ethyl octanoate
L0	L30	S0	L20	S10	L0	S0	S20	S0
L30	S10	S10	L10	S10	S10	L10	S30	S20
S30	L10	L20	S10	L10	L10	L0	L0	L0
S20	L0	L30	S20	L0	S30	S20	L10	L30
S10	L20	S20	L20	L0	S0	L0	L20	L10
L30	S20	L0	S20	S30	L30	S20	S20	S0
L0	L0	L10	L30	S10	S10	L0	L10	S10
S0	L30	S30	S30	S20	S20	L30	S0	L30
L10	S0	S30	S30	S30	L10	L20	S0	S0
L20	S20	L20	L20	L20	L20	S10	L20	S30
S0	S30	L0	S20	S0	S20	S10	S30	S20
S20	S10	L30	L0	L10	S30	S30	S10	L20
S10	L10	S20	S0	L30	L10	L30	L30	S10
L20	L0	L10	L0	S30	S0	S0	S20	L20
S30	L30	S10	S0	S0	L0	S30	S0	L20
L20	S0	L0	S10	L20	L20	S20	L30	L0
L10	S30	S10	L10	L0	S10	L10	L30	S30
L30	L10	S0	L0	S20	S20	S0	L0	S10
S20	S0	L10	L30	L30	L0	L20	S10	L30
S0	L20	S30	S10	S20	L30	L10	L10	L10
S10	S10	L20	L30	L20	L20	L30	L0	S20
L10	S20	S20	L10	S0	S0	S10	S30	L10
L0	L20	L30	S30	L30	L30	L20	L20	S30
S30	S30	S0	S0	L10	S30	S30	S10	L0

***L**=Lactose; **S**=Sucrose*

***0**=Control (No sugar); **10**=10% w/v sugar; **20**=20% w/v sugar; **30**=30% w/v sugar*

Experimental design for *in-vitro* experiments (alternative sweeteners)

Limonene	Butanal	Hexanal	Octanal	Decanal	Ethyl acetate	Ethyl butanoate	Ethyl hexanoate	Ethyl octanoate
STA	STV	S0	S10	STV	STA	STV	STV	AAS
AAS	S10	S10	S10	SCL	S10	S0	AAS	S10
S0	STA	S0	AAS	S0	AAS	AAS	S0	STV
STV	SCL	S10	SCL	S0	STA	SCL	S10	S0
SCL	S10	STA	S0	AAS	SCL	AAS	STV	STA
SCL	AAS	AAS	S0	STA	S0	SCL	S0	S0
S0	STA	STA	AAS	STA	STA	S10	SCL	STV
S10	AAS	S0	STA	STV	STV	STA	STA	S0
S0	AAS	SCL	SCL	AAS	S0	SCL	AAS	AAS
S10	SCL	AAS	STA	S10	S10	STV	SCL	STA
STA	S0	AAS	SCL	SCL	S0	STA	STA	STV
STV	SCL	SCL	STV	SCL	S10	AAS	SCL	S10
S10	STV	STA	S10	AAS	STV	STA	S10	AAS
SCL	S0	STV	STV	S0	STV	STV	S0	SCL
STA	S10	S10	S0	S10	AAS	S10	STV	STA
AAS	STV	SCL	STV	S10	SCL	S0	AAS	SCL
STV	S0	STV	AAS	STV	AAS	S0	S10	S10
AAS	STA	STV	STA	STA	SCL	S10	STA	SCL

***S0**=Control (No sugar); **S10**=Sucrose (10% w/v); **SAA**=Saccharin/Aspartame/Ace-K blend (0.0455% w/v); **SCL**=Sucralose (0.017% w/v); **STA**=Stevia/Allulose blend (3.52% w/v); **STV**=Stevia (0.025% w/v)*

Experimental design for *in-vivo* experiments (5 panellists)

Set 1	S20c	S0	S30	S10c	S20	S0c	S30c	S10
Set 2	S10c	S30c	S20	S0c	S0	S30	S20c	S10
Set 3	S0	S30c	S10	S10c	S0c	S20	S30	S20c

S0=Control (No sugar); ***S10***=Sucrose *(10% w/v)*; ***S20***=Sucrose *(20% w/v)*; ***S30***=Sucrose *(30% w/v)*; ***c***=*Carbonated*

Experimental design for measurement of water activity

No.	Sample	No.	Sample	No.	Sample	No.	Sample
1	G35	10	F20	19	S20	28	F10
2	S20	11	S35	20	G30	29	S35
3	G20	12	F30	21	G20	30	S30
4	F20	13	S10	22	F30	31	G20
5	S30	14	S20	23	S30	32	S10
6	G10	15	G30	24	S10	33	G35
7	G10	16	F35	25	F35	34	G35
8	F30	17	F35	26	S35	35	F20
9	G30	18	F10	27	G10	36	F10

F=*Fructose;* ***G***=*Glucose;* ***S***=*Sucrose*

10=*10% w/v;* ***20***=*20% w/v;* ***30***=*30% w/v*

Experimental design for NMR measurement of self-diffusion coefficient of aroma compounds

Set 1	S20H	S30	S0H	S20EB	S10H	S30H	S30EB	S20	S0EB	S10	S10EB
Set 2	S10H	S10EB	S20H	S20EB	S30H	S30EB					
Set 3	S10EB	S20H	S30EB	S30H	S10H	S20EB					

S0=*Control (No sucrose);* ***S10***=*10% w/v sucrose;* ***S20***=*20% w/v sucrose;* ***S 30***=*30% w/v sucrose*

EB=Ethyl butanoate; ***EH***=Ethyl hexanoate; ***H***=Hexanal, ***O***=Octana

Experimental design for measurement of CO_2 volume flux

Set 1	Set 2	Set 3
F30	F20	S10
B	S30	S10c
S10c	F20c	F30
F10c	F30c	F10
S20c	F30	Bc
Bc	S20	S30
GL10	GL10c	F10c
GL20c	GL20c	GL10
F20c	Bc	S20c
S20	GL30c	B
GL30c	S10c	S20
S10	GL10	F30c
S30	F10c	GL20c
F10	B	F20c
F20	S30c	GL20
GL10c	F10	GL30
F30c	GL20	GL30c
GL20	S20c	GL10c
S30c	GL30	S30c
GL30	S10	F20

B=Blank (H_2O); ***S10***=Sucrose *(10% w/v)*; ***S20***=Sucrose *(20% w/v)*; ***S30***=Sucrose *(30% w/v);* ***c***=*Carbonated*

B. Forced-carbonation table

Obtained from (UK Craft Beer Network, 2017)

Temp (°C)	Gauge pressure (psi)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Vol of CO_2														
0.0	1.75	1.85	1.95	2.05	2.15	2.27	2.38	2.48	2.59	2.70	2.80	2.90	3.00	3.11	3.21
0.5	1.71	1.81	1.91	2.01	2.10	2.23	2.33	2.43	2.53	2.63	2.74	2.84	2.96	3.06	3.15
1.1	1.68	1.78	1.86	1.97	2.06	2.18	2.28	2.38	2.48	2.58	2.69	2.79	2.90	3.00	3.09
1.7	1.63	1.73	1.83	1.93	2.02	2.14	2.24	2.34	2.43	2.52	2.63	2.73	2.83	2.93	3.02
2.2	1.60	1.69	1.79	1.88	1.98	2.09	2.19	2.29	2.38	2.47	2.57	2.67	2.77	2.86	2.96
2.8	1.55	1.65	1.74	1.84	1.94	2.04	2.14	2.24	2.33	2.42	2.52	2.62	2.71	2.80	2.90
3.3	1.52	1.61	1.71	1.80	1.90	2.00	2.10	2.20	2.29	2.39	2.48	2.57	2.66	2.75	2.85
3.9	1.49	1.58	1.67	1.77	1.86	1.96	2.06	2.15	2.25	2.34	2.43	2.52	2.61	2.70	2.80
4.4	1.47	1.56	1.65	1.74	1.83	1.92	2.01	2.10	2.20	2.30	2.39	2.47	2.56	2.65	2.75
5.0	1.43	1.52	1.61	1.70	1.79	1.88	1.97	2.06	2.16	2.25	2.34	2.43	2.52	2.60	2.70
5.5	1.39	1.48	1.57	1.66	1.75	1.85	1.94	2.02	2.12	2.21	2.30	2.39	2.48	2.56	2.65
6.1	1.37	1.46	1.54	1.63	1.72	1.81	1.90	1.99	2.08	2.17	2.26	2.34	2.43	2.52	2.61
6.7	1.35	1.43	1.52	1.60	1.69	1.78	1.87	1.95	2.04	2.13	2.22	2.30	2.39	2.47	2.56
7.2	1.32	1.41	1.49	1.58	1.66	1.75	1.84	1.91	2.00	2.08	2.17	2.26	2.34	2.42	2.51
7.8	1.28	1.37	1.45	1.54	1.62	1.71	1.80	1.88	1.96	2.04	2.13	2.22	2.30	2.38	2.47
8.3	1.26	1.34	1.42	1.51	1.59	1.68	1.76	1.84	1.92	2.00	2.09	2.18	2.26	2.34	2.42
8.9	1.23	1.31	1.39	1.48	1.56	1.65	1.73	1.81	1.89	1.96	2.05	2.14	2.22	2.30	2.38
9.4	1.21	1.29	1.37	1.45	1.53	1.62	1.70	1.79	1.86	1.93	2.01	2.10	2.18	2.25	2.34
10.0	1.18	1.26	1.34	1.42	1.50	1.59	1.66	1.74	1.82	1.90	1.98	2.06	2.14	2.21	2.30

Temp (°C)	Gauge pressure (psi)														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	Vol of CO_2														
0.0	3.31	3.42	3.52	3.63	3.73	3.84	3.94	4.04	4.15	4.25	4.36	4.46	4.57	4.67	4.77
0.5	3.25	3.35	3.46	3.56	3.66	3.76	3.87	3.97	4.07	4.18	4.28	4.38	4.48	4.59	4.69
1.1	3.19	3.29	3.39	3.49	3.59	3.69	3.79	3.90	4.00	4.10	4.20	4.30	4.40	4.50	4.60
1.7	3.12	3.22	3.32	3.42	3.52	3.62	3.72	3.82	3.92	4.01	4.11	4.21	4.31	4.41	4.51
2.2	3.05	3.15	3.24	3.34	3.43	3.53	3.63	3.72	3.82	3.92	4.01	4.11	4.21	4.30	4.40
2.8	3.00	3.09	3.18	3.27	3.37	3.46	3.56	3.65	3.75	3.84	3.94	4.03	4.13	4.22	4.32
3.3	2.94	3.03	3.12	3.21	3.30	3.40	3.49	3.59	3.68	3.77	3.87	3.96	4.06	4.15	4.24
3.9	2.89	2.99	3.07	3.16	3.25	3.34	3.44	3.53	3.62	3.71	3.81	3.90	3.99	4.08	4.18
4.4	2.84	2.93	3.01	3.10	3.19	3.28	3.37	3.46	3.55	3.64	3.73	3.82	3.91	4.01	4.10
5.0	2.79	2.88	2.96	3.05	3.14	3.23	3.32	3.42	3.50	**3.59**	3.68	3.77	3.86	3.95	4.04
5.5	2.74	2.83	2.91	3.00	3.09	3.18	3.26	3.35	3.44	3.53	3.62	3.70	3.79	3.88	3.97
6.1	2.69	2.78	2.86	2.95	3.04	3.13	3.21	3.30	3.39	3.47	3.56	3.65	3.74	3.82	3.91
6.7	2.64	2.73	2.81	2.90	2.99	3.07	3.10	3.24	3.33	3.41	3.50	3.58	3.67	3.76	3.84
7.2	2.60	2.69	2.77	2.86	2.94	3.02	3.11	3.19	3.28	3.36	3.45	3.53	3.62	3.70	3.79
7.8	2.55	2.64	2.72	2.81	2.89	2.98	3.06	3.15	3.23	3.31	3.40	3.48	3.57	3.65	3.74
8.3	2.50	2.59	2.67	2.76	2.84	2.93	3.02	3.09	3.18	3.26	3.35	3.43	3.51	3.60	3.68
8.9	2.46	2.54	2.62	2.71	2.79	2.88	2.96	3.04	3.13	3.21	3.30	3.38	3.46	3.54	3.63
9.4	2.42	2.50	2.58	2.67	2.75	2.83	2.91	3.00	3.07	3.15	3.23	3.31	3.39	3.47	3.56
10.0	2.38	2.46	2.54	2.62	2.70	2.78	2.86	2.94	3.02	3.10	3.17	3.25	3.33	3.41	3.49

C. Ethics assessment and participant information sheet (#SBREC160137A)

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UG / PGT/ PGR for the School of Biosciences
(Modified from School of Sociology & Social Policy for Staff and Students)

Research ethics checklist for students

The University of Nottingham's Guidance on Ethical Review states: "Ethical review (and approval) is required for all projects where the research involves participation of human subjects, their data and/or their tissue (even where the applicant indicates that there is only minimal risk)."

This form must be therefore be completed for all research projects, research assignments or dissertations/theses which are conducted within the School and involve human participants or data that are sensitive or protected. You must not begin data collection or approach potential research participants until you have completed this form, received ethical clearance, and submitted this form for retention with the appropriate staff.

If the study is based only on a review of documentary sources already in the public domain and involves NO fieldwork of any sort, then this form does not need to be completed.

Completing the form includes providing a brief summary of the research in Section 2 and ticking some boxes in Section 4. Ticking a shaded box in Section 4 indicates that the study is above minimal risk and requires further action by the researcher. Two things need to be stressed:

- Ticking one or more shaded boxes does not mean that you cannot conduct your research as currently anticipated; however, it does mean that further questions will need to be asked and addressed, further discussions will need to take place, and alternatives may need to be considered or additional actions undertaken.
- Avoiding the shaded boxes does not mean that ethical considerations can subsequently be 'forgotten'; on the contrary, research ethics need to be informed - for everyone and in every project – an ongoing process of reflection and debate.

The following checklist is a starting point for an ongoing process of reflection about the ethical issues concerning your study.

For further information on ethical issues, please consult the University pages or the School of Sociology and Social Policy's Ethics webpage: http://www.nottingham.ac.uk/sociology/research/research-ethics.aspx or the School of Biosciences Research Ethics Officer (Dr Kate Millar).

SECTION 1: THE RESEARCHER(S)

To be completed in all cases

Title of project:

Name of principal researcher:

Status:
- ☐ Undergraduate student
- ☐ Postgraduate taught student
- √ Postgraduate research student
- ☐ Staff

Email address: stxhqy@nottingham.ac.uk

Names of other project members: Dr Ian Fisk

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To be completed by students only:

HUI QI YEO

Student ID number: 4285167

Degree programme: MRes Food Science and Engineering

Module name/number: Thesis

Supervisor/module leader or tutor: Dr Ian Fisk

SECTION 2: RESEARCH WITHIN OR INVOLVING THE NHS OR SOCIAL CARE

Does this research involve the recruitment of patients, staff, records or other data through the NHS or involve NHS sites or other property?

□ Yes

√ No

If you have answered **YES** to the above question, ethical approval **MUST** be sought from the relevant NHS research ethics committee. Evidence of approval from such a committee **MUST** be lodged with the School office prior to the commencement of data collection.

Does this research involve the recruitment of users, staff, records or other data through social service authorities (children and adult services) or involve social service sites or other property?

□ Yes

√ No

If you have answered **YES** to the above question, then you must check whether or not the relevant social service authority has its own ethical scrutiny procedures. If appropriate, evidence of approval from such an authority **MUST** be lodged with the School office prior to the commencement of data collection.

Even where external ethical approval has been obtained from an NHS committee or social service authority, completion of this form is mandatory.

SECTION 3: THE RESEARCH

Please provide brief details (100-200 words) about your proposed research, as indicated in each section

1. Research question(s) or aim(s)

This study is part of the research investigating the effect of sugar reduction on aroma release in carbonated soft drinks. In order to monitor real-time aroma release during beverage consumption, in-vivo experiments involving human subjects will need to be carried out for the collection of data for breath-by-breath analysis. The data will be useful in the understanding of aroma-matrix interactions for cost effective formulation efforts to manufacture low-sugar and low-calorie products, in response to the impending sugar tax to be levied on the beverage industry.

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2. Method(s) of data collection

In-vivo data collection using atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS) has been established as a simple and non-invasive sampling method involving human subjects.

During the study, participants will be required to consume a range of beverages. Each beverage will contain fructose, glucose or sucrose (0, 10, 20 or 30% w/v), citric acid (0.15% w/v), hexanal (10 or 25 ppm) and ethyl butyrate (10 or 25 ppm) which is representative of an apple style flavouring, with or without carbonation (~3.6 vol). Chemicals used are obtained from Sigma Aldrich and are food grade. Carbonation will be carried out manually using a food grade carbon dioxide gas cylinder. Beverage preparation will be carried out in a food grade environment (Food Hall in Bioenergy and Brewing Science building). Participants will consume a total of 72 samples (10–20 mL each) with a rest period of 30 s in between during which they can cleanse their palate with water. A longer rest period will be accommodated at the participants' request.

After each beverage consumption, air will be sampled from the nostril of the participants through small, disposable plastic tubings (approx. 10 mm diameter and 40–50 mm length), such that they can still breathe, drink and eat normally, into a 0.53 mm diameter deactivated fused silica capillary tube at a flow rate of 30–50 mL/min for a period of 30 s.

Before data collection, participants will be required to read through the Information Sheet and sign the Consent Form. Data of participants will be stored anonymously in that each participant will be coded by consecutively running alphabets and their names stored separately from the data.

3. Proposed site(s) of data collection

Data collection will be carried out in the Flavour Laboratory in the Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, within office working hours between 8.30am and 5.30pm. Participants will be advised to be in Personal Protective Equipment (laboratory coat and safety goggles) and thus, there are no safety issues associated with the site of collection.

4. How will access to participants be gained?

Participants will be recruited from the student population from the University of Nottingham. A small fee for compensation of time and inconvenience will be provided. In addition, participants will be made fully aware that data will only be used by the Flavour Research Group in the Division of Food Sciences, School of Biosciences.

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SECTION 4: ETHICAL CONSIDERATIONS

Please answer each question by ticking the appropriate box. All questions in section 4 **must** be answered.

4.1 General issues

	Yes	No
Will this research involve any participants who are known to be vulnerable due to: Being aged under 18? Residing in institutional care (permanently or temporarily)? Having a learning disability? Having a mental health condition? Having physical or sensory impairments? Previous life experiences (e.g. victims of abuse)? Other (please specify)...		√
Will this research expose participants to any significant risk of physical or emotional harm?		√
Will this research involve any physically invasive procedures or the collection of bodily samples?		√
Will this research expose the researcher to any significant risk of physical or emotional harm?		√
Will this research involve deception of any kind?		√
Will this research involve access to personal information about identifiable individuals without their knowledge or consent?		√
I will inform immediately the School's Ethics Officer if I change the method(s) of data collection, the proposed sites of data collection, the means by which participants are accessed, or make any other significant changes to my research inquiry	√	

4.2 Before starting data collection

	Yes	No
I have read the *Research Code of Conduct* guidelines of the University of Nottingham, particularly section 4 on Data, and agree to abide by them: http://www.nottingham.ac.uk/ris/local/research-strategy-and-policy/Code_of_Conduct(Version_3_January_2010).pdf	√	
For those intending to work with children and/or vulnerable adults: I have read the University's *Guidance on arrangements for Protection of Children and Vulnerable Adults* http://www.nottingham.ac.uk/wideningparticipation/downloads/Guidance%20on%20the%20Protection%20of%20Children%20and%20Vunerable%20Adults.pdf	√	
My full identity will be revealed to all research participants	√	
All participants will be given accurate information about the nature of the research and the purposes to which the data will be put	√	
All participants will freely consent to take part, and this will be confirmed by use of a consent form. (An example of a consent form is available for you to amend and use.)	√	
One signed copy of the consent form will be held by the researcher and another will be retained by the participant	√	
It will be made clear that declining to participate will have no negative consequences for the	√	

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individual		
It will be made clear that participation is unlikely to be of direct personal benefit to the individual	√	
Participants will be asked for permission for quotations (from data) to be used in research outputs where this is intended	√	
Incentives (other than basic expenses) are offered to potential participants as an inducement to participate in the research. (Here any incentives include cash payments and non-cash items such as vouchers and book tokens.)	√	
For research conducted within, or concerning, organisations (e.g. universities, schools, hospitals, care homes, etc) I will gain authorisation in advance from an appropriate committee or individual. (This is in addition to any research ethics procedures required by those organisations, particularly health and social care agencies – see Section 2.)	√	

4.3 During the process of data collection

	Yes	No
I will provide participants with my University contact details, and those of my supervisor, so that they may make get in touch about any aspect of the research if they wish to do so	√	
Participants will be guaranteed anonymity only insofar as they do not disclose any illegal activities	√	
Anonymity will not be guaranteed where there is disclosure or evidence of significant harm, abuse, neglect or danger to participants or to others	√	
All participants will be free to withdraw from the study at any time, including withdrawing data following its collection	√	
Data collection will take place only in public and/or professional spaces (e.g. in a work setting). If fieldwork takes place in the respondent's home please outline in Section 6 what steps will be taken to ensure your safety. You may wish to consult the SRA researcher safety guidelines: http://www.the-sra.org.uk/guidelines.htm#safe	√	
Research participants will be informed when observations and/or recording is taking place	√	
Participants will be treated with dignity and respect at all times	√	

4.4 After collection of data

	Yes	No
Where anonymity has been agreed with the participant, data will be anonymised as soon as possible after collection	√	
All data collected will be stored in accordance with the requirements of the Data Protection Act 1998	√	
Data will only be used for the purposes outlined within the participant information sheet and consent form	√	
Details which could identify individual participants will not be disclosed to anyone other than the researcher, their supervisor and (if necessary) internal and/or external examiners without their explicit consent	√	
I will inform my supervisor and/or the School's research ethics officer and (if necessary) statutory services of any incidents of actual or suspected harm of children or vulnerable adults which are disclosed to me during the course of data collection	√	

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4.5 After completion of research

	Yes	No
Participants will be given the opportunity to know about the overall research findings	√	
Data must be submitted to the School office and will be retained (in a secure location) for 7 years from the date of any publication based upon them, after which time it will be destroyed.	√	
All hard copies of data collection tools and data which enable the identification of individual participants will be destroyed	√	

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SECTION 5: ETHICAL APPROVAL

Declaration of ethical research

1. **Undergraduate dissertation students who intend to conduct fieldwork** should include **one hard copies** of the checklist with their dissertations.
2. **By signing this form you are agreeing to work within the protocol which you have outlined and to abide by the University of Nottingham's Code of Research Ethics.** If you make changes to your protocol which in turn would change your answers to any of the above questions then you **must** complete a new form and submit a copy to your supervisor and the research ethics officer, Kate Millar.

Signed ..... Date 23/05/2017

3. ***If you ticked any of the shaded boxes in section 4 of this form, then you must complete SECTION 6 (overleaf). You must then discuss all ethical issues arising, record the outcome, including the supervisor's or REO's response, and have this form countersigned (see below)***

---

4. All forms should be countersigned by the REO.

Authorisation

This section **must** be completed in **all** cases – by type of investigator the form must be countersigned by the following personnel:

Undergraduate student → project / dissertation supervisor
Postgraduate taught student → project / dissertation supervisor

This form must then be sent to the internal SB e-mail address: SB-Biosciences-Ethics (SB-Biosciences-Ethics@nottingham.ac.uk) and Kate Millar (kate.millar@nottingham.ac.uk) by the supervisor (as the formal submission from a University e-mail address). If you make changes to your protocol which in turn would change your answers to any of the above questions then you **must** complete a new form and submit a copy to your tutor/supervisor and the ethics officer.

All forms should be countersigned (electronically confirmed) by the REO

Having reviewed the ethical issues arising from the proposed research:

- ☒ I am happy for the research to go ahead as planned.
- ☒ I have requested that changes be made to the research protocol. The principal researcher must complete and submit a revised form which integrates these changes
- ☒ This project must be referred for more detailed ethical scrutiny. A copy will be sent to the SSP REO for expert ethical review or this is to be referred to full SB REC Committee / School Management Group for consideration

Signed ..... Date 23/5/17

Designation ..... Project supervisor

School REO..... Date .....

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Note: **any** research protocols lodged with the School office may be subject to review by the School's Research Ethics Officer

SECTION 6: FURTHER INFORMATION & JUSTIFICATION OF METHODOLOGY

One box should be completed for **each** shaded box ticked in section 4 of this form.

Ethical issue: NA

Rationale for chosen methodology and/or how ethical issue is to be addressed:

NA

Supervisor/REO's response (including whether ethical issue has been satisfactorily addressed):

NA

Please continue on separate sheets if required

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Division of Food Sciences
School of Biosciences
University of Nottingham

Participant Consent Form

Project Title:
Effect of Sugar Reduction on Aroma Release in Carbonated Soft Drinks

In signing this consent form I confirm that:

	Yes	No
I have read the Participant Information Sheet and the nature and purpose of the research project has been explained to me.		
I have had the opportunity to ask questions.		
I understand the purpose of the research project and my involvement in it.		
I understand that my participation is voluntary and I may withdraw from the research project at any stage, without having to give any reason and withdrawing will not penalise or disadvantage me in any way.		
I understand that while information gained during the study may be published, any information I provide is confidential, and that no information that could lead to the identification of any individual will be disclosed in any reports on the project, or to any other party.		
I understand that data will be securely stored.		
I understand that I may contact the researcher (or supervisor) if I require further information about the research, and that I may contact the Research Ethics Officer of the School of Biosciences, University of Nottingham, if I wish to make a complaint relating to my involvement in the research.		
I agree to take part in the above research project.		

Participant's name (BLOCK CAPITAL)	Participant's signature	Date
Researcher's name (BLOCK CAPITAL)	Researcher's signature	Date

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University of Nottingham

Participant Information Sheet

Project Title:
Effect of Sugar Reduction on Aroma Release in Carbonated Soft Drinks

This in-vivo study is part of the research investigating the effect of sugar reduction on aroma release in carbonated soft drinks. The objective of this study is to collect breath data from human subjects during beverage consumption to understand aroma-matrix interactions.

What does the study involve?
The study involves the consumption of a range of beverages, each containing fructose, glucose or sucrose, citric acid and an apple style flavouring with or without carbonation.

During each session, 8 samples (10–20 mL each) will be consumed, with a rest period of 30 s in between during which you can cleanse your palate with water. 9 sessions will take place and each session will take ½ h at the maximum, unless a longer rest period is requested during the session. Thus, a total of 72 samples will be consumed.

Upon consumption of the beverage, a small, disposable plastic tubing will be inserted into one nostril, such that you could still breathe, drink and eat normally. Air will be sampled from the nostril into a 0.53 mm diameter deactivated fused silica capillary tube at a flow rate of 30–50 mL/min for a period of 30 s.

What are the benefits of participating in the study?
There will be a small payment fee of £15 upon completion of each block of 3 sessions (or 24 samples) for compensation of time and inconvenience.

What are the risks involved in the study?
There are little to no risks associated with consumption of the beverages, which are prepared in a food-grade environment. However, individuals with allergies to fructose, glucose, sucrose, citric acid, hexanal or ethyl butanoate and individuals with sugar restrictions in their diets (eg. individuals with diabetic conditions) are advised not to participate in the study. In addition, should you not feel well at any point during the study, please request for the study to be stopped immediately.

There are also minimal risks involved in sampling breath from human subjects as the atmospheric pressure chemical ionisation-mass spectrometer is a simple and non-invasive analytical method.

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What happens to the collected data?
While data collected during the study may be published, any information about participants which leaves the research unit will be anonymous and no identifiable personal data will be disclosed.

What are the conditions of participation?
Participation in the study is on a voluntary basis. Should you decide to take part, please keep the Participant Information Sheet for future reference and sign the Participant Consent Form before commencing the study. Should you no longer wish to take part at any point of the study, please request for a non-obligatory withdrawal.

Contact details
If you require further information, please contact the researcher, HuiQi Yeo, or the project supervisor, Dr Ian Fisk.

HuiQi Yeo: stxhqy@nottingham.ac.uk
Dr Ian Fisk: Ian.Fisk@nottingham.ac.uk

Complaint procedure
If you wish to discuss the way in which the research is being conducted or have any concerns about the research, then in the first instance please contact Dr Ian Fisk (Ian.Fisk@nottingham.ac.uk). If this does not resolve the matter to your satisfaction, then please contact the School's Research Ethics Officer, Dr Kate Millar (Kate.Millar@nottingham.ac.uk).

Thank you for taking part in this study.
Please keep this information sheet for your reference.

D. Panellist protocol for *in-vivo* aroma analysis

	Instructions
Breathe	1. Insert the plastic tubing connected to the APCI into the tip of your right nostril. 2. Inhale and exhale as per normal for 30 s. **<u>*Please keep your mouth closed at all times.*</u>
Drink	3. Remove your nose from the plastic tubing. 4. **Hold your breath.** 5. **Swirl** and open the bottle, **place your lips on the mouth of the bottle** and take in all the liquid. • Keep the liquid in your mouth. ***<u>Do not swallow</u>***. • Avoid any liquid and/or air movement in the mouth.
Swallow	6. **Hold your breath.** Insert the plastic tubing as previously done. 7. Consume all the liquid in ***<u>one swallow</u>***. Start breathing normally. **<u>*Please keep your mouth closed at all times.*</u>
Breathe	8. Inhale and exhale as per normal for 30 s. **<u>*Please keep your mouth closed at all times.*</u> 9. Remove your nose from the plastic tubing. 10. Take a 1 min rest. Have a cracker and a sip of water. Swirl the water in your mouth before swallowing to cleanse your palate.

E. ANOVA results

ANOVA results from analysis of predictive models for sugar concentration and type (Response surface linear model)

Monosaccharides (fructose, galactose and glucose)

Butanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	3.37E+12	3	1.12E+12	24.40	< 0.0001
Sugar conc	3.36E+12	1	3.36E+12	73.04	**< 0.0001**
Sugar type	7.12E+09	2	3.56E+09	0.08	0.9258
Residual	1.47E+12	32	4.60E+10		
Lack of Fit	1.95E+11	8	2.44E+10	0.46	0.8727
Pure Error	1.28E+12	24	5.32E+10		
Cor Total	4.84E+12	35			

Hexanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	1.30E+13	3	4.31984E+12	13.16	< 0.0001
Sugar conc	1.18E+13	1	1.1817E+13	35.99	**< 0.0001**
Sugar type	1.14E+12	2	5.71275E+11	1.74	0.1917
Residual	1.05E+13	32	3.28324E+11		
Lack of Fit	3.14E+12	8	3.92989E+11	1.28	0.2991
Pure Error	7.36E+12	24	3.06769E+11		
Cor Total	2.35E+13	35			

Octanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	1.77E+14	3	5.91E+13	15.28	< 0.0001
Sugar conc	1.74E+14	1	1.74E+14	45.05	**< 0.0001**
Sugar type	3.09E+12	2	1.54E+12	0.40	0.6742
Residual	1.24E+14	32	3.87E+12		
Lack of Fit	5.52E+13	8	6.90E+12	2.42	0.0452
Pure Error	6.85E+13	24	2.86E+12		
Cor Total	3.01E+14	35			

Decanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	1.31E+14	3	4.36547E+13	1.47	0.24
Sugar conc	1.16E+14	1	1.16162E+14	3.92	0.0563
Sugar type	1.48E+13	2	7.40111E+12	0.25	0.7803
Residual	9.47E+14	32	2.96028E+13		
Lack of Fit	2.44E+14	8	3.0456E+13	1.04	0.4356
Pure Error	7.04E+14	24	2.93183E+13		
Cor Total	1.08E+15	35			

Ethyl acetate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	6.50E+14	3	2.17E+14	245.51	< 0.0001
Sugar conc	6.43E+14	1	6.43E+14	729.02	**< 0.0001**
Sugar type	6.63E+12	2	3.31E+12	3.75	**0.0343**
Residual	2.82E+13	32	8.82E+11		
Lack of Fit	1.17E+13	8	1.47E+12	2.13	0.0722
Pure Error	1.65E+13	24	6.88E+11		
Cor Total	6.78E+14	35			

Ethyl butanoate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	3.29E+15	3	1.09659E+15	58.50	< 0.0001
Sugar conc	3.27E+15	1	3.26827E+15	174.35	**< 0.0001**
Sugar type	2.15E+13	2	1.075E+13	0.57	0.5692
Residual	6.00E+14	32	1.87449E+13		
Lack of Fit	3.16E+14	8	3.95439E+13	3.35	0.0102
Pure Error	2.83E+14	24	1.18119E+13		
Cor Total	3.89E+15	35			

Ethyl hexanoate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	4.04E+15	3	1.35E+15	67.73	< 0.0001
Sugar conc	3.96E+15	1	3.96E+15	198.95	**< 0.0001**
Sugar type	8.43E+13	2	4.21E+13	2.12	0.1369
Residual	6.37E+14	32	1.99E+13		
Lack of Fit	2.54E+14	8	3.18E+13	1.99	0.0919
Pure Error	3.83E+14	24	1.59E+13		
Cor Total	4.68E+15	35			

Ethyl octanoate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	1.18E+15	3	3.94543E+14	4.90	0.0065
Sugar conc	1.08E+15	1	1.07849E+15	13.40	**0.0009**
Sugar type	1.05E+14	2	5.25686E+13	0.65	0.5273
Residual	2.58E+15	32	8.05041E+13		
Lack of Fit	3.90E+14	8	4.87905E+13	0.54	0.818
Pure Error	2.19E+15	24	9.10753E+13		
Cor Total	3.76E+15	35			

Limonene					
Source	**Sum of squares**	**DF**	**Mean square**	**F value**	**Prob > F**
Model	1.18E+14	3	3.93E+13	1.12	0.3538
Sugar conc	7.21E+13	1	7.21E+13	2.06	0.1606
Sugar type	4.58E+13	2	2.29E+13	0.66	0.5262
Residual	1.12E+15	32	3.49E+13		
Lack of Fit	2.91E+14	8	3.64E+13	1.06	0.4245
Pure Error	8.27E+14	24	3.45E+13		
Cor Total	1.24E+15	35			

Disaccharides (lactose and sucrose)

Butanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	5.45E+12	2	2.72E+12	72.50	< 0.0001
Sugar conc	5.20E+12	1	5.20E+12	138.56	**< 0.0001**
Sugar type	2.42E+11	1	2.42E+11	6.44	**0.0191**
Residual	7.89E+11	21	3.76E+10		
Lack of Fit	4.19E+11	5	8.39E+10	3.63	0.0219
Pure Error	3.69E+11	16	2.31E+10		
Cor Total	6.23E+12	23			

Hexanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	6.09E+12	2	3.05E+12	32.55	< 0.0001
Sugar conc	4.56E+12	1	4.56E+12	48.76	**< 0.0001**
Sugar type	1.53E+12	1	1.53E+12	16.34	**0.0006**
Residual	1.97E+12	21	9.36E+10		
Lack of Fit	1.48E+12	5	2.95E+11	9.63	0.0002
Pure Error	4.90E+11	16	3.06E+10		
Cor Total	8.06E+12	23			

Octanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	9.00E+13	2	4.50E+13	12.99	0.0002
Sugar conc	4.10E+13	1	4.10E+13	11.82	**0.0025**
Sugar type	4.90E+13	1	4.90E+13	14.15	**0.0011**
Residual	7.27E+13	21	3.46E+12		
Lack of Fit	2.27E+13	5	4.55E+12	1.46	0.2586
Pure Error	5.00E+13	16	3.13E+12		
Cor Total	1.63E+14	23			

Decanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	7.07E+13	2	3.54E+13	2.59	0.0986
Sugar conc	3.22E+13	1	3.22E+13	2.36	0.1392
Sugar type	3.85E+13	1	3.85E+13	2.82	0.1078
Residual	2.87E+14	21	1.36E+13		
Lack of Fit	1.47E+14	5	2.94E+13	3.37	0.0288
Pure Error	1.40E+14	16	8.73E+12		
Cor Total	3.57E+14	23			

Ethyl acetate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	2.44E+14	2	1.22E+14	369.01	< 0.0001
Sugar conc	2.44E+14	1	2.44E+14	737.24	**< 0.0001**
Sugar type	2.60E+11	1	2.60E+11	0.79	0.3851
Residual	6.95E+12	21	3.31E+11		
Lack of Fit	1.10E+12	5	2.19E+11	0.60	0.7014
Pure Error	5.85E+12	16	3.66E+11		
Cor Total	2.51E+14	23			

Ethyl butanoate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	1.10E+15	2	5.50E+14	145.13	< 0.0001
A	1.08E+15	1	1.08E+15	284.60	**< 0.0001**
B	2.15E+13	1	2.15E+13	5.67	**0.0268**
Residual	7.96E+13	21	3.79E+12		
Lack of Fit	1.81E+13	5	3.62E+12	0.94	0.4802
Pure Error	6.15E+13	16	3.84E+12		
Cor Total	1.18E+15	23			

Ethyl hexanoate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	7.25E+14	2	3.62E+14	9.87	0.0009
Sugar conc	7.14E+14	1	7.14E+14	19.47	**0.0002**
Sugar type	1.04E+13	1	1.04E+13	0.28	0.6001
Residual	7.71E+14	21	3.67E+13		
Lack of Fit	3.53E+14	5	7.06E+13	2.71	0.0589
Pure Error	4.18E+14	16	2.61E+13		
Cor Total	1.50E+15	23			

Ethyl octanoate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	3.89E+14	2	1.94E+14	3.11	0.0659
A	3.35E+14	1	3.35E+14	5.35	**0.031**
B	5.40E+13	1	5.40E+13	0.86	0.3635
Residual	1.31E+15	21	6.26E+13		
Lack of Fit	1.92E+14	5	3.84E+13	0.55	0.738
Pure Error	1.12E+15	16	7.01E+13		
Cor Total	1.70E+15	23			

Limonene					
Source	**Sum of squares**	**DF**	**Mean square**	**F value**	**Prob > F**
Model	2.35E+13	2	1.17E+13	1.23	0.3118
Sugar conc	6.71E+12	1	6.71E+12	0.70	0.4106
Sugar type	1.68E+13	1	1.68E+13	1.76	0.1988
Residual	2.00E+14	21	9.52E+12		
Lack of Fit	2.47E+13	5	4.94E+12	0.45	0.8064
Pure Error	1.75E+14	16	1.10E+13		
Cor Total	2.23E+14	23			

ANOVA results from analysis of predictive models for sugar concentration and class (Response surface 2FI Model)

Butanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	0.65	3	0.22	47.11	< 0.0001
Sugar conc (A)	0.54	1	0.54	117.14	**< 0.0001**
Sugar class (B)	0.02	1	0.02	5.00	**0.0293**
A*B	0.02	1	0.02	4.53	**0.0378**
Residual	0.26	56	0.00		
Lack of Fit	0.02	4	0.00	1.09	0.3726
Pure Error	0.24	52	0.00		
Cor Total	0.91	59			

Hexanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	1.05	3	0.35	19.25	< 0.0001
Sugar conc (A)	0.58	1	0.58	32.08	**< 0.0001**
Sugar class (B)	0.24	1	0.24	13.29	**0.0006**
A*B	0.10	1	0.10	5.35	**0.0244**
Residual	1.01	56	0.02		
Lack of Fit	0.03	4	0.01	0.43	0.785
Pure Error	0.98	52	0.02		
Cor Total	2.06	59			

Octanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	0.60	3	0.20	12.51	< 0.0001
Sugar conc (A)	0.37	1	0.37	22.96	**< 0.0001**
Sugar class (B)	0.08	1	0.08	5.13	**0.0274**
A*B	0.07	1	0.07	4.21	**0.0448**
Residual	0.89	56	0.02		
Lack of Fit	0.03	4	0.01	0.43	0.7855
Pure Error	0.87	52	0.02		
Cor Total	1.49	59			

Decanal

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	0.33	3	0.11	4.33	0.0082
Sugar conc (A)	0.01	1	0.01	0.43	**0.5144**
Sugar class (B)	0.17	1	0.17	6.59	**0.013**
A*B	0.13	1	0.13	5.11	**0.0277**
Residual	1.42	56	0.03		
Lack of Fit	0.29	4	0.07	3.37	0.0159
Pure Error	1.12	52	0.02		
Cor Total	1.74	59			

Ethyl acetate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	3.54	3	1.18	400.38	< 0.0001
Sugar conc (A)	3.05	1	3.05	1035.58	**< 0.0001**
Sugar class (B)	0.09	1	0.09	31.93	**< 0.0001**
A*B	0.07	1	0.07	23.92	**< 0.0001**
Residual	0.17	56	0.00		
Lack of Fit	0.01	4	0.00	1.14	0.3471
Pure Error	0.15	52	0.00		
Cor Total	3.71	59			

Ethyl butanoate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	6.13	3	2.04	117.72	< 0.0001
Sugar conc (A)	4.36	1	4.36	251.47	**< 0.0001**
Sugar class (B)	0.45	1	0.45	25.93	**< 0.0001**
A*B	0.50	1	0.50	28.70	**< 0.0001**
Residual	0.97	56	0.02		
Lack of Fit	0.23	4	0.06	4.08	0.0059
Pure Error	0.74	52	0.01		
Cor Total	7.10	59			

Ethyl hexanoate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	2.71	3	0.90	66.97	< 0.0001
Sugar conc (A)	1.79	1	1.79	132.52	**< 0.0001**
Sugar class (B)	0.35	1	0.35	25.65	**< 0.0001**
A*B	0.23	1	0.23	16.83	**0.0001**
Residual	0.75	56	0.01		
Lack of Fit	0.09	4	0.02	1.73	0.1566
Pure Error	0.67	52	0.01		
Cor Total	3.46	59			

Ethyl octanoate

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	0.34	3	0.11	6.05	0.0012
Sugar conc (A)	0.27	1	0.27	14.27	**0.0004**
Sugar class (B)	0.02	1	0.02	1.05	0.3089
A*B	0.01	1	0.01	0.80	0.3737
Residual	1.04	56	0.02		
Lack of Fit	0.04	4	0.01	0.46	0.7642
Pure Error	1.01	52	0.02		
Cor Total	1.38	59			

Limonene					
Source	**Sum of squares**	**DF**	**Mean square**	**F value**	**Prob > F**
Model	0.10	3	0.03	1.10	0.3581
Sugar conc (A)	0.06	1	0.06	1.95	0.1682
Sugar class (B)	0.04	1	0.04	1.27	0.2645
A*B	0.00	1	0.00	0.00	0.9865
Residual	1.71	56	0.03		
Lack of Fit	0.06	4	0.02	0.51	0.7262
Pure Error	1.65	52	0.03		
Cor Total	1.81	59			

ANOVA results from analysis of predictive models for sucrose concentration and carbonation (Backward Elimination Regression Model with $\alpha_{exit} = 0.050$)

Ethyl butanoate

	I_{max}				
Source	**Sum of squares**	**DF**	**Mean square**	**F value**	**Prob > F**
Block	1.09E+15	4	2.72E+14		
Model	4.17E+14	4	1.04E+14	8.86	< 0.0001
Sucrose conc (A)	3.52E+12	1	3.52E+12	0.30	0.5857
Carbonation (B)	3.05E+14	1	3.05E+14	25.87	**< 0.0001**
A^2	6.17E+13	1	6.17E+13	5.24	**0.024**
A*B	4.74E+13	1	4.74E+13	4.02	**0.0473**
Residual	1.31E+15	111	1.18E+13		
Lack of Fit	4.25E+14	31	1.37E+13	1.24	0.2188
Pure Error	8.82E+14	80	1.10E+13		
Cor Total	2.81E+15	119			

	AUC				
Source	**Sum of squares**	**DF**	**Mean square**	**F value**	**Prob > F**
Block	7.12E+11	4	1.78E+11		
Model	1.58E+11	1	1.58E+11	35.26	< 0.0001
Carbonation	1.58E+11	1	1.58E+11	35.26	**< 0.0001**
Residual	5.11E+11	114	4.48E+09		
Lack of Fit	2.61E+11	34	7.68E+09	2.46	0.0005
Pure Error	2.50E+11	80	3.12E+09		
Cor Total	1.38E+12	119			

Non-significant terms (p > 0.05) were removed from the model.

Hexanal

I_{max}					
Source	Sum of squares	DF	Mean square	F value	Prob > F
Block	2.68E+14	4	6.69E+13		
Model	5.95E+13	1	5.95E+13	9.05	0.0032
Carbonation	5.95E+13	1	5.95E+13	9.05	**0.0032**
Residual	7.49E+14	114	6.57E+12		
Lack of Fit	2.24E+14	34	6.58E+12	1.00	0.4819
Pure Error	5.26E+14	80	6.57E+12		
Cor Total	1.08E+15	119			

Non-significant terms (p > 0.05) were removed from the model.

AUC					
Source	Sum of squares	DF	Mean square	F value	Prob > F
Block	2.07E+11	4	5.16E+10		
Model	3.02E+10	1	3.02E+10	17.96	< 0.0001
Carbonation	3.02E+10	1	3.02E+10	17.96	**< 0.0001**
Residual	1.92E+11	114	1.68E+09		
Lack of Fit	9.59E+10	34	2.82E+09	2.35	0.001
Pure Error	9.61E+10	80	1.20E+09		
Cor Total	4.29E+11	119			

Non-significant terms (p > 0.05) were removed from the model.