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Understanding aroma and flavour formation in baked confectionery products, as influenced by sugar and fat



Thesis presented by

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for the degree of

Doctor of Philosophy

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Declaration

"This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism."

Signature:

_____ **Date:** _____

Emer Garvey

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Abstract

This thesis directly addresses consumer demands for 'clean-label', sustainable and natural ingredients in baked confectionery products. Baked confectionery products are a prime matrix to explore alternative sucrose replacers due to the critical functionality of sucrose and fat in relation to desirable structural and organoleptic properties. The primary objective of this research was to elucidate the impact of modifying sugar and butter on the aromatic and sensory properties of selected baked confectionery products. Chapter 1 provides an updated review of reduced fat and sucrose research in baked confectionery products focussing on the association of aromatic volatiles and sensory perception. Chapter 2 outlines the development and optimisation of a headspace solid-phase microextraction gas-chromatography mass-spectrometry (HS-SPME-GC-MS) method for the extraction, separation and identification of volatile compounds from a baked confectionery matrix. Extraction parameters were optimised and the method was validated and applied throughout all subsequent volatile analysis in this thesis. Chapter 3 explored the influence of clean-label sucrose replacers on the sensory quality and volatile aroma of sponge cakes. Gas-chromatography-olfactometry (GC-O) was applied to assess the impact of sucrose replacers on aroma perception, in combination with consumer evaluation and ranking descriptive analysis (RDA) to better understand changes in sensory perception. The influence of sucrose particle size, and sucrose source (beet or cane sugar) in sponge cakes was explored in Chapter 4. Chapter 5 investigated if butter produced from pasture and non-pasture bovine diets, had different sensory characteristics, in a cross-cultural context. Consumer studies were conducted in Ireland, Germany and the USA, and, RDA was conducted in Ireland and Germany, with descriptive analysis (DA) carried out in the USA. The butters produced from these same bovine diets were incorporated into shortbread biscuits, and liking was evaluated using

consumers, in additional to temporal assessment, using temporal-check-all-that-applies (TCATA) to further understand the impact on the sensory properties of the shortbread biscuits (Chapter 6).

In summary this research demonstrated that the composition of sucrose replacers (particularly when containing reducing sugars), can accelerate Maillard (MR) and Caramelisation (CR) reactions, influencing sensory perception. 'Spicy/bready' furfural contributed most to the overall aroma of the sponge cake samples, and that 'fatty cake crust' heptanal and 'potato damp' methional, varied most between the control (100% sugar) and the 30% w/w reduced sugar sponge cakes with apple pomace powder and oligofructose, respectively. Sucrose source did not significantly affect ($P < 0.05$) the volatile profile of sponge cakes; however, reduced sugar crystal size positively influenced MR and CR compounds. No significant difference ($P < 0.05$) was identified in the overall liking, among USA, German and Irish consumers, of the experimental butters-although cross-cultural preferences were clearly evident. Sensory attribute differences based on cow diet were likely influenced by familiarity. The colour of shortbread biscuits formulated with pasture was perceived more favourable by consumers due to the golden colour, as a result of higher β-carotene content. The temporal profile of the shortbread biscuits, evaluated during the stages of oral processing; orthonasal, in-mouth and aftertaste, differentiated mainly due to variations in the fatty acid composition of the butter, which highlights that relatively minor changes in the fatty acid profile of butter can impact on the sensory characteristics of baked confectionery products where it is used as an ingredient

This PhD thesis has highlighted the potential of combining sensory techniques, volatile profiling and olfactometry to provide in-depth information to aid in understanding aroma development in baked confectionery products. This approach can evidently be used to

improve the sensory quality of baked confectionery products and especially products with modified sucrose and fat contents; however it is also very applicable to improving the quality of any food type and in new product development.

Publications

List of Publications

Peer-reviewed articles

1. Garvey, E. C., O'Sullivan, M. G., Kerry, J. P., & Kilcawley, K. N. (2020). Factors influencing the sensory perception of reformulated baked confectionery products. *Critical Reviews in Food Science and Nutrition*, *60*(7), 1160-1188.
2. Garvey, E. C., O'Sullivan, M. G., Kerry, J. P., & Kilcawley, K. N. (2020b). Optimisation of HS-SPME parameters for the analysis of volatile compounds in baked confectionery products. *Food Analytical Methods, 13*, 1314–1327.
3. Garvey, E. C., O'Sullivan, M. G., Kerry, J. P., Milner, L., Gallagher, E., & Kilcawley, K. N. (2020). Characterising the sensory quality and volatile aroma profile of clean-label sucrose reduced sponge cakes. *Food Chemistry* , 128124.
4. Garvey, E.C., Sander, T., O'Callaghan, T. F., Drake, M., Fox, S., O'Sullivan, M. G., & Kilcawley, K. N. (2020). A Cross-Cultural Evaluation of Liking and Perception of Salted Butter Produced from Different Feed Systems. *Foods*, *9*(12), 1767.

Conference

1. **Oral-** Application of Response Surface Methodology to Optimise Solid-Phase Microextraction for Volatile Analysis of Baked Confectionery Products, Institute of Food Science and Technology of Ireland, 47th Annual Food Science and Technology Conference, Dublin, Ireland, 6-7th December 2018.

2. **Poster-** Impact of 'clean label' sucrose replacers on the odour active compounds, aroma profile and sensory quality of sponge cakes, Pangborn Sensory Symposium 2019.

3. **Oral-** Piece of cake? Unravelling the contribution of sugar and fat to the desirable flavour of baked confectionery products. Walsh Fellow Seminar, 2019

Magazine

Garvey, E. C., O'Sullivan, M. G., Kerry, J. P., & Kilcawley, K. N. April 2020. 'Piece of Cake?' TResearch Magazine.

Awards

- Winner of 'Best Regional Presentation (Food Research Program)' at Teagasc Walsh Fellowship Seminar, 2019

- Winner of 'Best Oral Presentation' at the International Food Science and Technology Ireland Conference, 2018

List of abbreviations

AEDA = Aroma extraction dilution assay

ANOVA = Analysis of variance

APP = Reduced sucrose sponge cake formulated with 30% w/w apple pomace powder

CCD = Central Composite Design

CR = Caramelisation

DA = Descriptive analysis

FA = Fatty acid

FD = Factor dilution

FS-CLVR = Feed system including fresh pasture (perennial ryegrass/white clover)

FS-GRSS = Feed system including fresh pasture (perennial ryegrass)

FS-TMR = Feed system including total mixed ration

GC = Gas Chromatogrpahy

GC-O = Gas-chromatography-olfactometry

HS-SPME = Headspace solid-phase micro-extraction

JAR = Just about right

LO = Lipid oxidation

MR = Maillard Reaction

MS = Mass Spectrometry

OLIGO = Reduced sucrose sponge cake formulated with 30% w/w oligofructose

P&T = Purge and trap

PCA = Principal component analysis

PSD = Particle size distribution

QDA = Quantitative descriptive analysis

RDA = Ranking descriptive analysis

RSM = Response Surface Methodology

SAFE = Solvent assisted flavour evaporation

SB-C = Sponge cake formulated with sugarbeet control sugar

SB-GRSS = Shortbread biscuit formulated with butter produced from a fresh pasture diet

SB-LPS = Sponge cake formulated with sugarbeet large particle size sugar

SB-SPS = Sponge cake formulated with sugarbeet small particle size sugar

SB-TMR= Shortbread biscuit formulated with butter produced from a housed, indoor, concentrate diet

SC70 = Sucrose reduced (70%) sponge cake

SC-C = Sponge cake formulated with sugarcane control sugar

SC-LPS = Sponge cake formulated with sugarcane large particle size sugar

SC-SPS = Sponge cake formulated with sugarcane small particle size sugar

SDE = Simultaneous distillation extraction

SPS = Sugar particle size

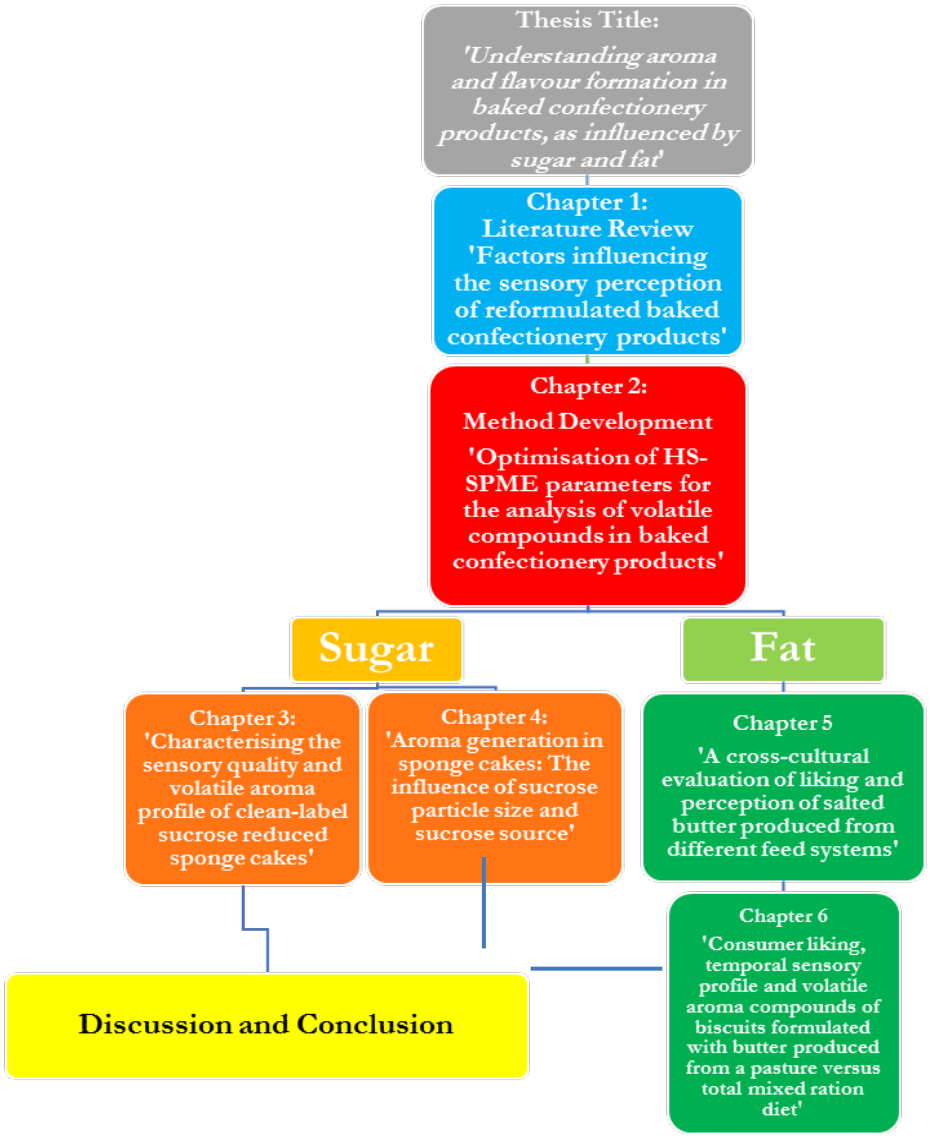
SS = Sucrose source

TCATA = Temporal check-all-that-applies

TD = Thermal desorption

TDS = Temporal Dominance of Sensations

WPP = Reduced sucrose sponge cake formulated with 30% w/w whey permeate powder


Thesis Title:
'Understanding aroma and flavour formation in baked confectionery products, as influenced by sugar and fat'
Chapter 1:
Literature Review
'Factors influencing the sensory perception of reformulated baked confectionery products'
Chapter 2:
Method Development
'Optimisation of HS-SPME parameters for the analysis of volatile compounds in baked confectionery products'
Sugar
Fat
Chapter 3:
'Characterising the sensory quality and volatile aroma profile of clean-label sucrose reduced sponge cakes'
Chapter 4:
'Aroma generation in sponge cakes: The influence of sucrose particle size and sucrose source'
Chapter 5
'A cross-cultural evaluation of liking and perception of salted butter produced from different feed systems'
Chapter 6
'Consumer liking, temporal sensory profile and volatile aroma compounds of biscuits formulated with butter produced from a pasture versus total mixed ration diet'
Discussion and Conclusion

Chapter 1. Literature Review: Factors influencing the sensory perception of reformulated baked confectionery products

This chapter has been published in *Critical Reviews in Food Science and Nutrition*

1.1 Introduction

Baked confectionery is an umbrella term used to categorize a variety of cakes, muffins, biscuits, cookies etc. (O'Sullivan, 2016). Globally, these products are highly appreciated by consumers across all populations. They are characterised by their aroma, flavour, texture, and aesthetic appeal, having the ability to induce a feeling of satisfaction and happiness when consumed (Poonnakasem et al. 2016). As cakes and other confectionery products are associated with celebrations, they are considered as a 'reward' or a 'treat' and are anticipated to be of high quality. These products are predominantly comprised of sugar, flour, water, fat, eggs, and a leavening agent. Combined in different ratios, these ingredients produce various products such as cakes, muffins, cookies etc. It is the individual contributions of these raw materials that deliver the desired organoleptic properties and therefore drive consumer liking. Fat and sugar have been identified as the most important contributors to the overall acceptability of sweet bakery products with both contributing to texture, mouthfeel, volume, colour, and flavour (Heenan et al. 2010; Manohar and Rao 1999; Zoulias, Oreopoulou, and Kounalaki 2002).

In 2016, 13% of the global adult population were reported obese with 39% of adults aged 18 years and over classified as overweight (WHO 2017). As a result, the food industry have become motivated to modify product formulations through sugar and fat reduction in order to aid consumer welfare, while simultaneously striving to retain the sensory appeal and maintain purchase intent. There is also a demand for 'clean label' products that are both nutritious and low in calories, yet consumers still expect a product that is not compromised in sensory quality. However, there is a vast quantity of literature exploring sugar (sucrose)/fat replacement or reduction, with the majority of results correlating sugar and fat reduction with a decrease in consumer acceptability (Cavalcante

and Silva 2015; Eslava-Zomeño, Quiles, and Hernando 2016; Giarnetti et al. 2015; Karp et al. 2016; Onacik-Gür et al. 2016; Serin and Sayar 2017; Zahn, Pepke, and Rohm 2010).

Taste and aroma are considered paramount to a consumer's acceptability of a food product. When a food is eaten, a complex mechanism occurs between the taste receptors in the mouth and aroma receptors in the nasal cavity that result in flavour perception (Naknean and Meenune 2010). Although non-volatile compounds and structural components contribute significantly to the recognition of taste, volatile aroma compounds are considered the major influencer in the overall liking and acceptability of food (Taylor and Linforth 1996). The process of baking induces many changes; structural enhancement, development of the desired texture, and improved digestibility, but the major effect is the transformation of the sensory attributes, specifically aroma formation (Mohsen et al. 2009). Baking promotes thermal reactions and other interactions within the matrix which are thought to be the main precursors of the 'characterising' volatile aroma compounds associated with baked goods (Pozo-Bayón, Guichard, and Cayot 2006a). Identification of the most significant compounds responsible for the desired flavour (taste and aroma) of baked confectionery products could be a stepping stone for innovative development of healthier confectionery that possess an integral appeal to the consumer.

The consumption of food is an elaborate process which includes mastication, salivation, tongue movement and swallowing (Piggott 2000), and therefore these events have an impact on the rate and intensity at which an aroma is perceived (Linforth, Baek, and Taylor 1999; Wilson and Brown 1997). In addition, the food matrix can possess a number of factors that influence aroma release; for example, viscosity (Hollowood, Linforth, and Taylor 2002), fat content (van Ruth, King, and Giannouli 2002), and the presence of hydrocolloids and emulsifiers (Koliandris et al. 2008). Different sensory methods can be employed to gain an insight into the consumer's experience during food

consumption and aftertaste. Combining instrumental data of volatile compounds with the application of an appropriate sensory methodology can yield important correlations between aroma and flavour perception and therefore, consumer acceptance (Heenan et al. 2009; Lee and Ahn 2009; Quílez, Ruiz, and Romero 2006). Gas chromatography coupled to mass spectrometry (GC-MS) is the separation technique usually applied for the identification and quantification of volatile aromatic compounds in foods (Kilcawley 2017). Although there may be a vast quantity of compounds present in a food product, only a fraction will impact on the flavour perception (Dunkel et al. 2014).

This review aims to provide information on the factors that impact the sensory acceptance of baked confectionery, especially in products where fat and/or sugar has been decreased or replaced.

1.2 Raw Materials

Although baked confectionaries share many similar ingredients, it is the proportion and ratio of the ingredients that generally defines them on an individual basis. Cakes and muffins are of a similar classification, as the finished products are characterised by a light aerated structure with a moisture content of 20-30% (Fiszman, Sanz, and Salvador 2013). Whereas, biscuits and cookies possess a much lower moisture content (1-4%) and aeration is not as critical as the desired texture of the end product is favourably described as 'crispy' or 'chewy' (O'Sullivan 2016). Before trying to decipher the complex mechanism of volatile production in baked confectionery products, it is noteworthy to consider the raw materials involved in the process, which act as precursors for the development of the desired aroma and flavour.

1.2.1 Flour

Wheat flour is a predominant ingredient in the bakery industry. Flour is mainly composed of starch and protein and is essentially the 'glue' that binds all ingredients of a bakery product together. The functional properties that flour provides are attributed to the quantity and quality of the proteins present. Gluten proteins make up 80-85% of total wheat protein and are responsible for its unique ability to form a viscoelastic dough. Gluten also plays a role in gas retention and determination of the overall quality of a baked product (Goesaert et al. 2005; Majzoobi et al. 2016). Although these properties are more important in bread manufacture, protein interactions are necessary for an adequate structure in sweet bakery products (Wilderjans et al. 2008).

In terms of its contribution to aroma and flavour production, compounds such as vanillin, 3-hydroxy-4.5-dimethyl-2(5H)-furanone, 4,5-epoxy-(E)-2-decenal and (E)-2-nonenal have been identified as the most odour active compounds in white wheat flour, with odour qualities ranging from vanilla-like to fatty (Czerny and Schieberle 2002). Widely utilised in baked confectionery, white wheat flour yields a soft, somewhat bland taste that allows the other ingredients to command flavour perception. Bakery products produced utilising grains and plants with nutritional benefits (high in fibre, antioxidant properties etc.) receive a lot more attention in literature due to the presence of coeliac disease in populations, and also the increasing demand for low glycaemic products fit for diabetic patients. As flour is usually the most abundant ingredient in a bakery product, replacement with a suitable alternative can be an opportunity to significantly enhance the nutritional profile.

Many flour replacement ingredients have been evaluated. Hedonic assessments by untrained panellists revealed increasing substitution of wheat flour for pea and broad bean derived flours lead to a decrease in organoleptic properties of sponge cakes (Belghith-Fendri et al. 2016). The aroma of 'cake like' doughnuts made with 20% and

30% cowpea meal was described as "slightly beany"; however, untrained panellists did not necessarily rate this as an adverse attribute (McWatters 1982). Similarly, cookies enriched with cowpea flour at 33% and 50% were described by untrained panellists as having a 'beany', 'nutty' or 'fishy' flavour (McWatters et al. 2003). Trained panellists have also described biscuits enriched with soya flour as 'beany' (Shrestha and Noomhorm 2002). Addition of resistant starch in muffins led to a significant decrease in the 'typical taste' and 'typical odour' by descriptive analysis (Baixauli et al. 2008). On replacement of ≥ 20% of wheat flour with β-glucan-rich hydrocolloids from oats, a descriptive sensory panel experienced an increase in 'cardboard flavour' and a decrease in 'sweetness' (Lee and Ahn 2009).

Chocolate chip cookies containing a mix of barley and wheat flour (30-70% replacement) were perceived by a semi-trained panel, using descriptive sensory analysis, as having an increase in 'baked barley' aroma but attributes such as 'chocolaty aroma', 'sweet flavour' and 'chocolaty flavour' were not impacted (Frost, Adhikari, and Lewis 2011). On replacement of 70% wheat flour with almond flour in Chinese moon cakes, quantitative descriptive analysis (QDA) yielded favourable results with trained panellists having appreciated the 'almond flavour' derived from methyl-butyraldehyde (Jia et al. 2008).

Although these substitutes demonstrate potential, it is apparent from the literature that none replicate the same sensory experience as traditional formulas made with white wheat flour.

1.2.2 Eggs

Eggs are widely utilised in baking due to their multifunctional composition. Egg white proteins are excellent foaming agents capable of forming a network of air bubbles which coagulate on heating to form a porous aerated stable structure desirable in cakes

and muffins (Arunepanlop et al. 1996). However, egg yolk also provides emulsifying capabilities, aids colour development, and contributes to the flavour and aroma of baked confectionery products (Yang and Baldwin 1995). Eggs are responsible for the Maillard compounds which produce 'roasty', 'sweet' and 'malty' aromas desirable in cakes and cake-like products. Literature regarding egg replacement in baked confectionery appears to be motivated by a number of factors; the cholesterol content of eggs and its association with cardiovascular disease, utilisation of cheaper plant based alternatives or the growing interest in vegetarian and vegan diets.

Shao, Lin, and Chen (2015) examined creating eggless cakes with the use of hydrocolloids. Sensory evaluation by trained panellists revealed a significant decrease in the intensity of 'egg taste' and 'egg smell' in eggless cakes compared to the control. Similarly, on evaluation of eggless cakes by QDA, trained panellists allocated a higher rating for 'egg flavour' in control cakes compared to the formula without egg (Kohrs et al. 2010). Angel cake and muffins reformulated with lentil protein as an egg/milk replacer were assessed by untrained panellists using a hedonic scale (Jarpa-Parra et al. 2017). The results demonstrated that the cocoa in the muffin formula appeared to mask the direct taste of the lentils (100% replacement of milk and egg), but a 'beany' taste was apparent. In the case of the angel cakes, panellists favourably described the flavour as 'nutty'.

The implementation of soy sources as an egg substitute in baked confectionery has been frequently reported. Muffins produced with soy flour as an egg replacement (Geera et al. 2011) resulted in untrained panellists rating the product as having the highest 'off-favour', lowest 'overall favour', and the most 'intense aftertaste', compared to that of other muffins formulated with egg substitutes. QDA of eggless cakes produced with soy protein isolate (SPI), assessed by trained panellists, yielded significantly different scores for the attributes 'beany taste', 'eggy taste' and 'overall aroma' compared to that of the control (Lin et al. 2017). Corresponding with these results, cakes reformulated with soy

alternatives, in place of egg, generally score significantly lower for overall acceptability on hedonic scales, compared to that of the control (Geera et al. 2011; Rahmati and Mazaheri Tehrani 2015).

On replacement of egg with baking powder in sponge cake, Pozo-Bayón et al. (2007) demonstrated that characterising 'malty', 'chocolate' (3-methylbutanal), 'roasty', 'nutty' (2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, trimethylpyrazine), 'caramel-like' (5-methylfurfural), and 'cherry', 'almond' (benzaldehyde) compounds were absent in the formulas made without egg. Similarly, Maire et al. (2013) identified that sponge cakes made without egg yolk were lacking methional ('musty'/'potato'). In addition, the authors noted less lipid oxidation (LO) compounds in the sponge cake made with egg, suggesting that egg phospholipids may as act an antioxidant (Haeyoung and Eunok 2008).

Sensory evaluation of sponge cake, by hedonic scales, found that replacement of egg white with 12.5% and 25% whey protein isolate (WPI) did not significantly impact the odour, flavour or appearance of the cake (Díaz-Ramírez et al. 2016). Although WPI may seem promising as an egg replacer, the incentive for egg replacement is also motivated by cost, which limits the application of WPI. It is evident that eggs contribute to overall flavour acceptability in addition to structural properties in baked confectionery products.

1.2.3 Fat

Fat has a major influence on the overall acceptability of baked confectionery products and is usually present in the form of hydrogenated shortenings or butterfat. In terms of functionality, fat plays a critical role in the incorporation of air bubbles; enabling an increase in volume and the development of a porous structure. Additionally, fat aids in the entrapment of moisture leading to a moist and tender crumb (Conforti 2006; Eslava-Zomeño et al. 2016). Through the interaction with starch in the baked product

matrix, fat forms lipid-amylose complexes, which hinder retrogradation; helping to maintain a desirable texture and hence extend shelf-life (Mert and Demirkesen 2016). However, due to the adverse health effects associated with saturated and trans fats, suitable alternatives are desirable.

Fat is a principle contributor to aroma and flavour perception. Fat has the ability to enhance palatability by imparting lubricity and a specific mouthfeel, whilst many aroma volatile compounds are fat soluble and bound within the lipid component of a product (O'Sullivan 2016; Zoulias, Oreopoulou, and Tzia 2002). Due to its unique fatty acid composition, butter is difficult to replace in recipes without having an adverse effect on the organoleptic qualities of the finished product. Compounds such as 2,3-butanedione, acetoin, δ-decalactone, δ-octalactone, and butyric acid are important contributors for the typical flavour/aroma of butter (Mallia, Escher, and Schlichtherle-Cerny 2008; Schieberle et al. 1993). Pastries produced with butter have been characterised by a 'sweet' and 'coconut' aroma originating from δ-decalactones (Gassenmeier and Schieberle 1994). Giarnetti et al. (2015) explored replacing butter in cookies with a combination of inulin and extra virgin olive oil at different percentages. Descriptive sensory analysis revealed that the reformulated cookies scored much lower in 'caramel odour', 'buttery odour', 'buttery flavour', and lacked a sweet perception, compared to the control. Similarly, 50% butter replacement with prune puree in cookies resulted in a decrease in 'butter flavour' intensity and a less desired product (Swanson and Perry 2007). It appears the amount of butter incorporated into a recipe strongly reflects the intensity of 'butter flavour' and 'butter aroma' perceived on consumption.

Margarine and shortening blends are more commonly used in bakery products due to their plasticity and lower cost compared to butter. The make-up of margarine is relatively simplistic, consisting of a water in oil emulsion, whereas shortening is comprised solely of an oil blend. Although the characterising compounds of butter are not as

abundant in margarines and shortenings, they are still capable of imparting positive attributes such as 'buttery', 'fruity' and 'sweet' derived from 2,3-butanedione, ethyl butanoate, and δ-decalactone, and δ-octalactone, respectively (Shiota et al. 2011). Shortening replaced with different fat replacers in cookies resulted in significantly lower intensity scores for 'vanilla' and 'sweet' on a descriptive scale compared to a control (Armbrister and Setser 1994), indicating that the source of these aromatic compounds was bound within the fat matrix. Similarly, biscuits formulated with vegetable shortening were identified by Free Choice Profiling to have stronger intensity in 'buttery', 'vanilla', 'coconut', and 'cinnamon' attributes than biscuits with the same percentage of dairy based shortening and liquid oils (Tarancón, et al. 2013). Hedonic scales usually reveal lower aroma and flavour acceptability when sensory panellists evaluate sweet bakery products where the fat has been removed or replaced (Psimouli and Oreopoulou 2013; Rodríguez-García, Salvador, and Hernando 2014; Singh and Kumar 2018). However, when hydroxypropylmethylcellulose was used as a fat replacer for margarine in biscuits, it did not appear to adversely affect the sensory properties of biscuits at a substitution rate of 15%, but at 30%, 'buttery' flavour was significantly reduced (Laguna et al. 2013).

Carbohydrate fat replacers have been extolled for their ability to replicate the texture of fat in the mouth as their globular structure can somewhat mimic the impression of creaminess (Meyer et al. 2011). However, maltodextrin and polydextrose were found unable to imitate the lubricity, taste, and flavour of fat in short dough biscuits (Sudha et al. 2007). Trained panellists associated an increase in 'floury' and a decrease in 'buttery' flavours with reduced fat biscuits formulated with N-DULGE® (a mixture of tapioca dextrin and tapioca starch) and resistant starch, by descriptive analysis (Laguna et al. 2012). Partial replacement of oil in chocolate muffins, with soluble cocoa-fibre, has been associated with an increase in "bitterness" by descriptive analysis (Martínez-Cervera et al. 2011). On the contrary, the addition of apricot kernel fibre to replace shortening in

cookies, did not adversely impact sensory perception (Seker et al. 2010). Fat reduction can also coincide with a decreased in sweetness perception, which has been reported in biscuits (Biguzzi, Schlich, and Lange 2014; Forker, Zahn, and Rohm 2012).

Butter replacement in cookies corresponded with a significant decrease in the levels of methyl ketones (2-butanone, 2-heptanone, 2-nonanone, and 2-undecanone) (Giarnetti et al. 2015), which are known to impact on 'buttery' and sweetness perception. As stated, the unique fatty acid profile of butter is comprised mostly of short and medium length fatty acids, having the capability to generate short chain methyl ketones via oxidation. These compounds contribute to the aroma of cookies and other sweet bakery products. On replacement of margarine with extra virgin olive oil in Madeira cakes, Matsakidou, Blekas, and Paraskevopoulou (2010) found that the alcohols ((Z)-2-pentenol, (Z)-3-hexenol, (E)-2-hexenol and (Z)-2-hexenol) were created from oxidation of the virgin olive oil. Although untrained panellists did not negatively rate the re-formulated sponge cake, the presence of these LO alcohols may have implications for product shelf-life as they can contribute to off-flavours over time.

Overall, there appears a lot more information is required to further understand the role of fat in consumer acceptability of confectionery products.

1.2.4 Sugar

Dominating a large proportion of the ingredient declaration for the majority of commercial cakes, muffins, biscuits etc., sugar or sucrose, is considered the most important raw material incorporated in baked confectionery products. Not only providing the characteristic sweetness, sugar also plays a vital role in creating and maintaining the structure, and texture of baked confectionery products. Sugar also restricts water activity, thus inhibiting microbial growth and contributing to the preservation of the product (Rodríguez, Magan, and Medina 2016). Sucrose is highly recognised in food

manufacturing for its ability to impart a clean, sweet taste. However, providing 4 kcals of energy per gram, and usually present in large quantities in baked confectionery, excess sucrose consumption is identified as a major contributor to the prevalence of obesity and type II diabetes worldwide (Hashem, He, and MacGregor 2016).

Sweeteners, both artificial and natural, are widely utilised for their ability to impart a conventional 'sweet flavour' with only a fraction of the calorific value to that of sucrose. Although these sweeteners influence the perception of sweetness, they cannot fully imitate the role sucrose plays in structural development, functionality, or colour formation (Struck et al. 2014). The sugar alcohol xylitol conjoined with bulking agents, such as oligofructose, has shown potential for reduced sugar cake formulation (Nourmohammadi and Peighambardoust 2016; Ronda et al. 2005), due to the synergistic effect of these substances. Xylitol imparts a high level of sweetness but is unable to partake in the Maillard reaction (MR), whereas bulking agents are less sweet by nature but are capable of aiding in structural and colour development, thus resulting in an acceptable product.

Steviol glycosides are widely used as a sucrose replacement with their popularity due to their 'clean label' status. Although these sweeteners deliver a high intensity of sweetness, 100-300 times sweeter than sucrose (Cardello, Da Silva, and Damasio 1999), they are unable to meet all the requirements of a sucrose substitute. Steviol glycosides have been shown to perform well with other bulking agents in confectionery systems (Periche et al. 2016; Shah, Jones, and Vasiljevic 2010). Sucrose reduction of 30% was achieved in muffins with the use of a steviol glycoside (*rebaudioside A*) in addition to inulin and polydextrose (Zahn et al. 2013). Flash sensory profiling revealed these formulas were associated with attributes such as 'buttery flavour', 'sweet', and 'aromatic'. However, on evaluation of muffins where sucrose was partially replaced with Stevia (25%), trained panellists identified the control (sucrose), on a hedonic scale, as having the highest

acceptability (Karp et al. 2016). Complete replacement of sucrose with stevia does not seem to be well received by consumers in baked confectioneries, but partial replacement shows potential (Wardy et al. 2018).

Although sucrose contributes hugely to the sweet flavour of baked confectionery, it can also play a role in the development of flavour and aroma that is not necessary related to sweetness. Reduced sucrose cookies have shown to have a significantly reduced perception of 'buttery' flavour (Laguna et al. 2013). Similarly, on replacement of sucrose with isomaltose, cakes were perceived as having a significantly less 'buttery' and 'caramel' flavour (Heenan et al. 2010). This may be explained by the interaction sugar has in thermal processes that occur during baking. When sucrose is removed from the equation, volatile compounds may be lost or suppressed due to the lack of monosaccharaides available to partake in the MR and caramelisation. Despite the desire for sugar to be reduced in food formulations, it is evident sucrose directly impacts on the appreciated flavour and aroma of baked confectionaries, as well as playing an important role in functional properties.

1.2.5 Other Ingredients

Introduction of non-conventional materials can also favour the production of desired aroma compounds in baked confectionery matrices and offers scope to improve the nutritional quality of a product. Wheat cookies supplemented with SPI at 10% scored significantly higher on a hedonic scale for 'aroma' and 'taste' compared to the control cookie (Mohsen et al. 2009). The addition of SPI, an additional source of amino acids, favoured the generation of 2-ethyl-5-methylpyrazine ('biscuit-like') and maltol ('cotton-candy') with concentrations of these compounds higher than that of the control. Cookies re-formulated with an emulsion gel containing inulin (Giarnetti et al. 2015), showed increased levels of 3-methylbutanal ('malty/chocolate'), methylpyrazine and trimethylpyrazine ('roasty/nutty'). The formation of these compounds can be explained

by the degradation of inulin that occurs during baking, producing mono- and di-saccharides that are then available to accelerate the MR. Similar results were found when inulin was added to wheat bread (Poinot et al. 2010). On replacement of whole meal flour with purple wheat flour in biscuits, Pasqualone et al. (2015) saw significantly higher amounts of potent aroma compounds 3-methylbutanal, 2-methylbutanal, benzaldehyde, and the furan compounds furfural, 5-methylfuran, and hydroxymethylfurfural (HMF).

Bi-products of wine fermentation, such as grape marc extract has been shown to increase the level of the benzaldehyde ('cherry'/'almond'), phenylacetaldehyde ('floral'/'honey'), and furans 2-methylfuran, 2-acetylfuran, 5-methylfurfural and 2-furanmethanol ('sweet'/'caramel') in biscuits, resulting in enhanced consumer acceptability and purchase intention (Pasqualone et al. 2014). Higher levels of furanic compounds were identified in the grape marc extract biscuits compared to the control. This can be explained by the acidic pH of this material, which is favourable for the formation of these compounds.

Varying yeast amounts have been shown to have an impact on compounds derived from the MR (Birch et al. 2013a; Birch et al. 2013b; Poinot et al. 2008; Zehentbauer and Grosch 1998b), which are associated with 'malty', 'sweet', and 'roasty' attributes, and hence important to the overall aroma of bakery products. The monosaccharide fructose, in the presence of high temperatures, has been shown to have a positive effect on the formation of HMF in cookies and biscuits (Ameur et al. 2007; Nguyen, Peters, and Van Boekel 2016; Zhang et al. 2012). HMF and furfural have also been shown to be influenced by salt (NaCl) content in cookies (Kocadağlı and Gökmen 2016; Van Der Fels-Klerx et al. 2014).

1.2.6 Matrix effect

It is well understood how the removal of key ingredients (fat and sugar) in product formulation can adversely impact on aroma and flavour of baked confectionery (Giarnetti et al. 2015; Struck et al. 2014; Sudha et al. 2007). The food matrix can also significantly influence how flavour and aroma are perceived. On consideration of manipulating the integral high sugar, high fat composition of a confectionery product, it is important to understand how aroma compounds can be retained or released from the matrix when concentrations of these ingredients are altered.

The main function of sucrose in the majority of formulas is to enhance palatability by imparting a sweet, clean taste. Sucrose has proven to have a significant impact on aroma release in sweetened beverages, with studies demonstrating that sugar increases aroma perception (Hansson, Andersson, and Leufvén, 2001; Nahon et al. 1998; Saint-Eve et al. 2009). This effect can be explained by the 'salting out' phenomenon, whereby sucrose saturates the solution and as free water is lost due to sugar hydration, aroma compounds are forced into the headspace (Nawar 1971). Headspace analysis of cereal bars showed increasing amounts of glucose solids had a pronounced effect on aroma release for some compounds (acetaldehyde, ethyl butyrate, ethyl methyl butyrate, and limonene) but not others (maltol and methyl cinnamate) (Heenan et al. 2012). As sugar has the ability to increase the aroma intensity of compounds, in theory, when sugar is removed, perception of aroma compounds can also decrease. Aroma addition has been suggested as a tool to compensate for the decline in sensory quality on sucrose reduction in food formulas (Hutchings, Low, and Keast 2018). However, this theory is drawn from liquid and semi-solid models. In order for this concept to apply to sugar reduction in baked confectioneries, more work on aroma-interactions in soft-solid matrices, as found in bakery products, is required (Poinot et al. 2013).

Sugar reduction is a difficult challenge as it is almost inevitable that sweetness perception decreases concurrently with sugar reduction (Biguzzi et al. 2014; Drewnowski, Nordensten, and Dwyer 1998; Martínez-Cervera et al. 2012), leading to diminished consumer acceptance. Fat and sugar are very much intertwined in the role of sensory perception in baked confectionery products. Fat contributes hugely to the texture and mouth-feel of food products. In addition, the perception of fat on consumption can be somewhat hard to define by consumers, with sweetness impression shown to decrease with a decrease of fat in biscuits (Biguzzi et al. 2014; Forker et al. 2012). Cognizance of the relationship between aroma and perception must be taken into account when sugar and fat are reduced so that consumer desirability is not adversely impacted.

Manipulation of components of the matrix can be an innovative way to enhance aroma perception and even improve the quality of reduced fat/sugar products. On variation of particle size distribution in chocolate, Afoakwa et al. (2009) demonstrated that with finer particle sizes, an increase in favourable compounds associated with 'cocoa-chocolate-praline' and 'caramel-sweet' notes were released into the headspace. Richardson et al. (2018) employed sugar particle size reduction in a chocolate brownie matrix. Replacing standard sugar crystals with a smaller particle size in the formula produced brownies that retained their conventional 'sweet' taste and were identified as significantly sweeter than the control. From these findings, the authors postulated that sucrose of smaller particle size can be used in product formulation to produce sugar reduced brownies of acceptable quality.

1.3 Precursors of Flavour- Volatile Formation

Aroma is considered a critical determinant to the overall quality of bakery products as it is one of the initial sensory attributes the consumer encounters. Even in

small quantities, low aroma threshold compounds can act as a determinant of product quality and consumer preference (Quílez et al. 2006). Aroma compounds can be produced as a result of enzyme activity, fermentation, or through thermal reactions (Pozo-Bayón et al. 2006a). Although the ingredients contribute immensely to the overall flavour perception of the product, it is the thermal reactions that occur during baking that significantly influence the aroma, and thus flavour. The following reactions are thought to generate the most characterising compounds associated with baked confectionery products.

1.3.1 The Maillard reaction

Maillard reactions are non-enzymatic reactions that occur on heating and have the ability to completely transform the flavour, aroma, and colour of food products. The MR is a complex cascade of chemical reactions and has been extensively studied (Hodge 1953; Nursten 1981). It is generally described as occurring in three main stages. The MR is instigated by a condensation reaction between a carbonyl group of a reducing sugar and a free amino group ($–NH^2$) originating from amino acids, peptides, or proteins, in a low moisture, high temperature environment, to produce amines, N-glycosylamine (aldose sugar) or fructosylamine (ketose sugar) (Parliament 1989). These products are colourless and not odour active. As the temperature increases internally in the food product and moisture is driven off, N-glycosylamine or fructosylamine rearrange to form an Amadori or a Heyns product, respectively. Amadori/Heyns products are inherently unstable and subsequently degrade, impacted by the pH of the matrix; this degradation by means of pH is known as dehydration. At pH ≤7, 1,2-enolization is promoted to form furfural and HMF, whereas in an alkaline environment (pH ≥7), 2,3 enolization occurs forming highly reactive reductones and dehydroreductones (Martins, Jongen, and Van Boekel 2000; Pozo-Bayón et al. 2006a). The temperature, nature of the reactants (amino acid, peptide

and sugar), and water activity also strongly influence the rate at which these reactions occur (Van Boekel 2006). Alternatively, Amadori and Heyns products can also undergo cyclization to produce nitrogen-containing heterocyclic compounds, such as pyrroles or pyridines (Jousse et al. 2002). Sugar fragmentation is another possible route of degradation for these products, a complex mechanism involving retro-aldol, hydrolytic, oxidative and amine-induced carbohydrate cleavages resulting in the production of α-dicarbonyl compounds which can recombine to yield HMF and other furans (Nursten 2007; Smuda and Glomb 2013; Taş and Gökmen 2017). The third potential pathway of Amadori/Heyns degradation is through means of Strecker degradation. In relation to the MR, Strecker degradation is brought about by α-dicarbonyls, and induces deamination and decarboxylation of free amino acids, resulting in the production of volatile aldehydes whose structure mimics that of their amino acid counterpart (Rizzi 2008; Yaylayan and Mandeville 1994). Compounds such as 3-methylbutanal, phenylacetaldehyde, and methional are well established as volatile compounds derived from Strecker degradation of leucine, phenylalanine, and methionine, respectively, and can be considered some of the most important products of the MR (Hofmann, Münch, and Schieberle 2000). In addition to aldehydes, aminoketones are also a result of α-dicarbonyl and amino acid reactions. These compounds have the ability to condense into heterocyclic compounds such as pyrazines, pyridines, thiazoles, pyrroles etc. (Shu 1998). As seen in Figure 1.1, each one of these pathways is capable of producing volatile intermediates that are important aroma compounds which influence the flavour of baked confectionaries. On further condensation, these compounds form polymers known as melanoidins (Zamora and Hidalgo 2005), yielding the characteristic golden brown colour of bakery products.

1.3.2 Caramelisation

Although the MR receives a lot of attention for the role it plays in the formation of volatile and non-volatile compounds during baking, caramelisation is also an important contributor to the development of the overall aroma and colour of baked products. Caramelisation is referred to as the decomposition of sugars and happens at temperatures >120°C, favoured by a pH of <3 or >9, and can be associated with a brown colour and 'caramel' odour in food (Lee and Lee 1997; Zhang et al. 2012). Isomerization of monosaccharides is generally the initial step in caramelisation, where sugar molecules experience enolization, and further degradation reactions lead to the formation of α-dicarbonyls (Kroh 1994). Sugar degradation produces compounds comparable to that of the early stages in the MR, but are produced at a slower rate due to the lack of a catalyst, the amino group (Van Boekel 2006). As the MR relies on the participation of reducing sugars, the extreme temperatures attained on the surface of the product during baking can induce starch and sucrose hydrolysis, thus leading reducing sugars to be available for both MR and caramelisation reactions simultaneously (Capuano et al. 2008). As the name suggests, caramelisation is associated with aroma compounds associated with a 'caramel' odour, which derive from furans, ketones, aldehydes, and lactones, aromatic compounds formed during thermal decomposition of sugars (Paravisini et al. 2015).

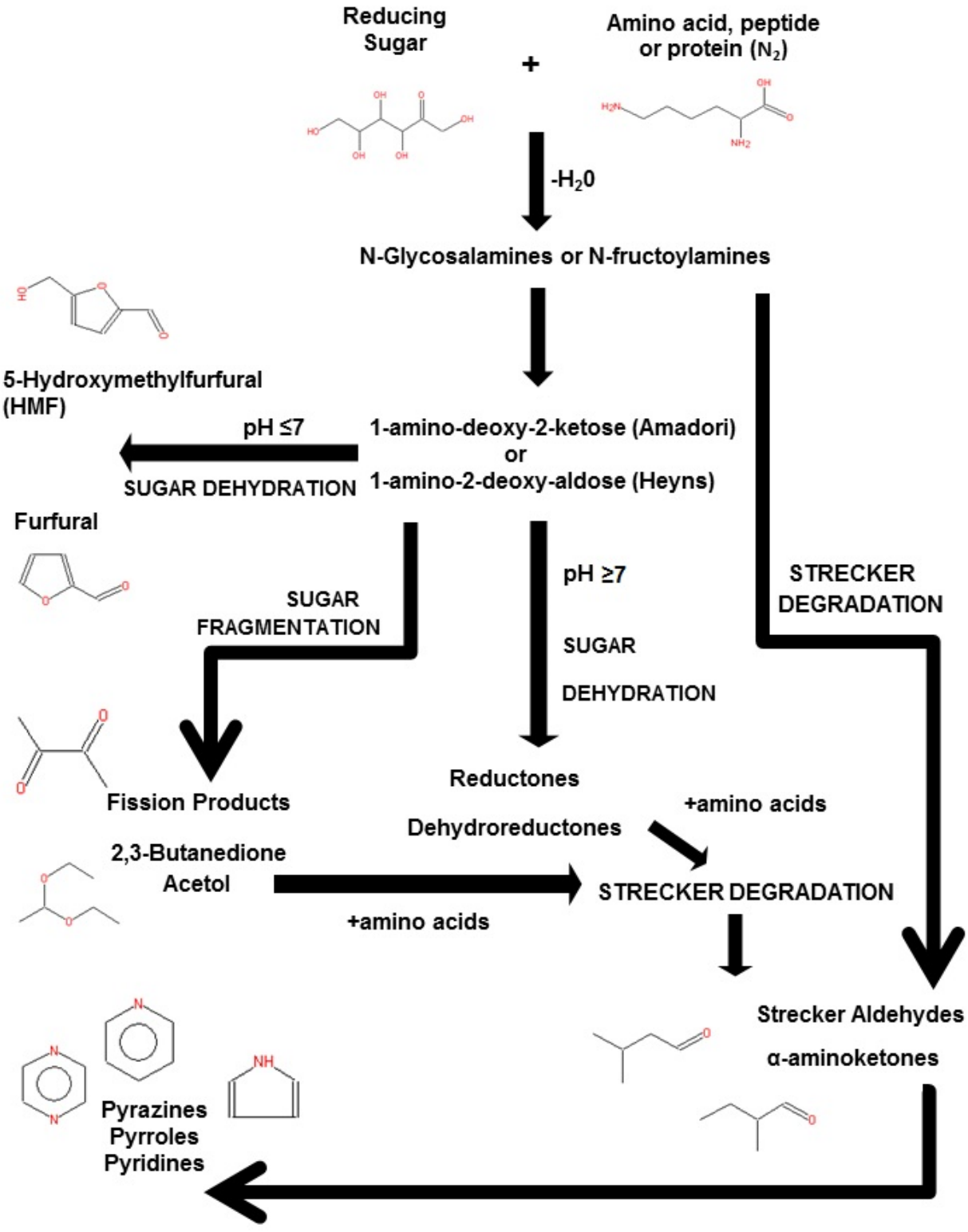


Figure 1.1 Flavour compound formation. The Maillard reaction (adapted from Pozo-Bay on Guichard, and Cayon 2006a)

1.3.3 Lipid oxidation

Unsaturated lipids are susceptible to LO, a problematic reaction leading to undesirable changes in flavour, nutritional quality, and shelf-life (Waraho, McClements, and Decker 2011). Auto-oxidation is the most common form of LO in bakery products (Maire et al. 2013) and can be described as a free radical chain reaction consisting of three stages; initiation, propagation and termination (Frankel 2014). Margarine and shortenings utilised in baking are an abundant source of oleic, linoleic, and linolenic acid and are thus prone to secondary oxidation. The formation of various aldehydes, ketones, and alcohols are indicators of LO in bakery products and these LO derived compounds can contribute up to a quarter of the volatile profile of bread (Jacobsen 1999; Pico, Bernal, and Gómez 2015). The main pathways of LO occur on ingredient preparation, in the presence of oxygen, at high temperatures of baking, and on storage, with hexanal being the primary marker of LO in sponge cake and other bakery products (Maire et al. 2013; Purcaro, Moret, and Conte 2008).

1.3.4 Processing factors

Processing factors have been shown to influence the formation of volatile compounds generated through thermal reactions in bakery products. Most work to date has focused on furanic compounds such as HMF and furfural, however, other volatile compounds are likely to be affected.

Compounds important to aroma and colour development in baked products are produced via thermal reactions, thus baking times and temperatures will have a pronounced effect on their formation and development. Rega et al. (2009) monitored volatile compounds produced during baking a sponge cake over a period of 0-25 minutes. Strecker aldehydes and pyrazines expressed linear behaviour and increased with baking time. However, HMF was formed mainly at the end of the baking processed. Longer

baking times coupled with higher baking temperatures were shown to have a positive effect on the formation of HMF in sponge cakes (Zhang et al. 2012). This may be reasoned by the longer period for caramelisation to occur, which brings about a pH shift in the matrix (slightly acidic), and therefore promotes the formation of this furanic compound. Varying mixing times, baking times, and baking temperatures have been shown to significantly impact the volatile composition of bread, and manipulation of these parameters can yield greater amounts of MR and caramelisation volatile compounds (Sabovics, Straumite, and Galoburda 2014).

1.4 Volatile Analysis of Baked Cereal Products

1.4.1 Gas chromatography

Sensory analysis acquires useful information on the perception and acceptance of foods but cannot provide information on the compounds responsible for a given flavour perception. Therefore, combining data from both flavour chemistry and sensory science can help identify the compounds responsible for a desired aroma or taste. Gas chromatography mass spectrometry (GC-MS) is a strategic technique used in food analysis to identify potent compounds with the ability to impact on aroma perception, and this information can be used to establish the impact of processes and raw materials on the overall flavour profile, as well as help predict product quality and market acceptance (Paraskevopoulou et al. 2014). The working principle of GC is separation of analytes based on volatility and affinity to a column phase. The analytes elute depending on characteristics such as volatility, molecular weight, vapour pressure, and polarity, and are detected by Mass selective and flame ionization detectors.

To maximize the efficiency and output of the GC instrument, there are a number of aspects that require optimisation depending upon the separation required. The type of column is one of the most important considerations. As seen from Table 1.1, a range of stationary phase columns of various polarities have been utilised in the analysis of baked cereal products. The criteria for the choice of column should suit the chemistry of the compounds extracted. Traditionally most analysis has been undertaken using one-dimensional chromatography, where a single column of selected polarity is used. However, in complex samples, volatiles may co-elute making identification and quantification difficult. The advent of two-dimensional or, comprehensive chromatography, improves separation using two columns of different polarity. In this case, all or part of the eluent of the first column is directed to a second column using modulation (thermal or flow) to create a three dimensional output. By employing this approach, Matsakidou, Blekas, and Paraskevopoulou (2010) were able to identify 92 compounds from the volatile fraction of Madeira cake.

Flame ionization detector is a popular detector as it has sensitivity for an extensive range of organic compounds, low noise level, excellent linear range, low cost, and excellent durability (Colón and Baird 2004). However, mass spectrometry (MS) has become the detector of choice due to its selectivity, sensitivity, and versatility (Milman 2015). MS operates as a detector through the mechanism of initial molecule ionization followed by resolution of the ionized molecule based on mass-to-charge (m/z) ratio (Croissant, Watson, and Drake 2011). As a result, a mass spectra is created for each compound and therefore enables the identification of compounds in the sample through comparison of library databases and retention indexes.

1.4.2 Chemistry of extraction

Prior to GC analysis, it is necessary to extract volatiles from the sample of interest. Currently no analytical technique can compare to the human nose in terms of sensitivity, therefore it is necessary to concentrate the volatiles during extraction to ensure an optimum representation of the sample is attained (Kilcawley 2017). In addition, compounds responsible for aroma and flavour perception in food range from a diverse mixture of chemical classes of different molecular weight, polarity, and volatility. Hence, the application of the most suitable extraction technique is crucial for creating an accurate depiction of the volatile profile of the product. Implementation of the appropriate extraction technique needs to take into account; type of analysis (trace, target, untargeted, profiling etc.), labour intensity, robustness, flexibility, cost, sample matrix, time, and sample preparation (Ebeler, Terrien, and Butzke 2000; Hyötyläinen and Riekkola 2008). All extraction techniques have advantages and disadvantages, but also an inherent degree of bias. Extraction techniques utilised to profile the aroma of baked confectionery products are as follows.

Table 1.1

Extraction techniques utilised in the volatile analysis of baked cereal matrices

Sample of Interest	Extraction Technique	Parameters Employed	NaCl Used in Extraction	GC COLUMN	Number of Volatiles Extracted	Reference
Cookies	**Simultaneous distillation extraction**	**Sample:** 10g mixed with 40mL distilled H20 **Solvent:** Dichloromethane **Concentrated 10 times under nitrogen**	N	HP5 ***Non-polar***	14	**Prost et al. 1993**
Cookies	**Thermal Desorption**	**Adsorbent Material:** Not stated **Purge Time:** 3 min **Desorption Time/ Temp:** 5 mins at 240°C **Gas/ Desorption Flow:** 200 mL Nitrogen min-1 **Temp of Cold Trap:** -20°C	N	DB-5 ***Non-polar***	5 (Compounds added and recovered)	**Heiderich and Reineccius 2001**
Sponge Cake	**SAFE**	**Sample:** 20g **Extraction Time:** 2 hours **Temp:** 30°C **Solvent:** Dichloromethane	N	DB-Wax ***Polar***	19 (Compounds added and recovered)	**Pozo-Bayón et al. 2006b**
Sponge Cake	**SAFE**	**Sample:** 70g mixed with 150mL distilled H20 **Time:** 2 hours **Temp:** 30°C **Solvent:** Dichloromethane	N	DB-Wax ***Polar***	77	**Pozo-Bayón et al. 2007**

Sponge Cake	**Purge and Trap**	**Adsorbent Material:** Tenax Ground cake **Temp**: 25°C **Purging Gas:** 25 mL/min with Nitrogen **Purge times:** 5, 15, 30 and 60 min and 14 hour	N	DB-Wax ***Polar***	90	**Pozo-Bayón et al. 2007**
Altamura Bread	**Purge and Trap**	**Adsorbent Material:** Tenax TA **Temp**: 40°C **Purging Gas:** 40 mL/min with helium **Purge time:** 15 mins	N	Supelcowax ***Polar***	89 in crust 74 in crumb	**Bianchi et al. 2008**
Wheat Bread	**SPME**	**Fibre:** 75µm DVB/ CAR/ PDMS **Extraction:** 30 mins at 35°C (shaken with magnetic bar) Bread sample crushed	N	DB-WAX ***Polar***	46	**Poinot et al. 2008**
Sponge Cake	**Purge and Trap**	**Adsorbent Material:** Tenax **Temp**: 25°C **Purging Gas:** 25 mL/min with Nitrogen **Purge times:** 5, 15, 30 60 min +14 h	N	DB-Wax ***Polar***	Amylose Interaction Study (looked at 19 compounds)	**Pozo-Bayón et al. 2008**
Cookies	**Simultaneous distillation extraction**	**Sample:** 100g +400mL distilled H20 **Solvent:** Diethyl ether-pentane	N	DB-5 ***Non-polar***	80	**Mohsen et al. 2009**

Sponge Cake	**SPME**	**Fibre:** 50/30µm DVB/ CAR/ PDMS and 75µm CAR/ PDMS and 100µm PDMS **Extraction:** 30 mins at 50°C	N	DB-Wax ***Polar***	49 (between 3 fibres)	**Rega et al. 2009**
Sponge Cake	**SPME**	**Fibre:** 50/30µm DVB/ CAR/ PDMS **Extraction:** 60 mins at 60°C (manual) Cake sample cryogenically ground	N	FFAP ***Polar*** and BP-5 ***Non-polar***	92	**Matsakidou, Blekas, and Paraskevopoulou 2010**
Oat Cake	**SPME**	**Fibre:** 85µm CAR/ PDMS **Extraction:** 15 mins (temperature not stated)	N	DB-1701 ***Low/ Mid Polar***	36	**Cognat et al. 2012**
Oat Cake	**Thermal Desorption**	**Adsorbent Material:** Tenax TA **Purge Time:** 1 min **Desorption Time/ Temp:** 5 mins at 240°C **Gas/ Desorption Flow:** 200 mL Nitrogen min-1 **Temp of Cold Trap:** -10°C	N	DB1701 ***Low/ Mid-polar***	46	**Cognat et al. 2012**
Pineapple Breads	**SPME**	**Fibre:** 75µm CAR/ PDMS **Extraction:** 10 mins at 40°C	Y	DB-5 ***Non-polar***	59	**Ying et al. 2012**

Sponge Cake	**SPME**	**Fibre:** 75µm DVB/ CAR/ PDMS **Extraction:** During Baking	N	DB-FFAP ***Polar***	72	**Maire et al. 2013**
Sponge Cake	**SPME**	**Fibre:** 75µm CAR/ PDMS **Extraction:** 37ºC for 40mins (agitated at 600 rpm)	Y	HP-5 ***Non-polar***	31	**Petisca et al. 2013**
Biscuits	**SPME**	**Fibre:** 75 µm CAR/PDMS **Extraction:** 40°C for 50 mins	N	HP-Innowax ***Polar***	60	**Pasqualone et al. 2014**
Triticale Bread	**SPME**	**Fibre:** 85µm CAR/PDMS **Incubation:** 15 mins at 40°C **Extraction:** 65 mins at 40°C	N	Elite-WAX ETR ***Polar***	26	**Sabovics, Straumite, and Galoburda, 2014**
Shortbread Cookies	**SPME**	**Fibre:** 50/30µm DVB/ CAR/ PDMS **Extraction:** 15 mins at 35°C	N	HP-Innowax ***Polar***	24	**Giarnetti et al. 2015**
Biscuits	**SPME**	**Fibre:** 75 µm CAR/PDMS **Extraction:** 40°C for 50 mins	N	HP-Innowax ***Polar***	56	**Pasqualone et al. 2015**

Crackers	**Thermal Desorption**	**Extraction time/ temp:** 20 mins at 30°C **Purge Time:** 2 min **Desorption Time/ Temp:** 5 mins and 150°C followed by 5 mins at 300°C Gas/ Desorption Flow: 50 mL Nitrogen min-1 **Temp of Cold Trap:** 30°C	N	DB-5 ***Non-polar***	49	**O'Shea, Kilcawley and Gallagher, 2017**
Sponge cake	**Headspace Trap**	**Thermostatting temperature:** 65 °C **Thermostatting time:** 15 min **No. of pressurization cycles:** 4 **Dry purge time:** 0.9 min **Water / sample amount ratio (dry basis):** 16 **Total amount (water + sample amount, dry basis)** 10 g	N	VF-WAXms ***Polar***	Targeted to Furan and Furfural recovery	**Cepeda-Vázquez, Blumenthal, Camel & Rega, 2017**

1.4.2.1 Simultaneous distillation extraction

Simultaneous distillation extraction (SDE) is one of the oldest, widely used methods of volatile extraction and is based on vapour differences over water (Veith and Kiwus 1977). This technique can recover significant amounts of volatiles of different chemical classes with good reproducibly (Chaintreau 2001). Using SDE, Prost et al. (1993) recovered 14 compounds representative of cookie odour, but the technique poorly recovered compounds such as vanillin, γ-butyrolactone, maltol, and 4-(4-hydroxyphenyl)-2-butanone, which are thought to be important constituents the characteristic cookie odour. Mohsen et al. (2009) applied the same technique and similar parameters in analysing wheat cookies. The authors were capable of identifying and quantifying γ-butyrolactone and maltol, as well as another 42 volatile aromatic compounds of diverse chemical classes. Although SDE has been widely used in food research, studies in baked matrices are limited. This is probably due to the elevated temperatures associated with distillation, leading to the formation of artefact compounds, particular those relating to the MR (Cai, Liu, and Su 2001; Engel, Bahr, and Schieberle 1999). In addition, solvents utilised in extraction discriminate against compounds of a similar polarity, and hence the recoveries may not provide a true representation of the sample.

1.4.2.2 Solvent-assisted flavour evaporation

Designed to overcome some of the short comings of SDE, solvent-assisted flavour evaporation (SAFE) is a well-established technique that is suitable for extraction of volatiles from a range of matrices (Drake, Miracle, and McMahon 2010; Mahajan, Goddik, and Qian 2004; Mayuoni-kirshinbaum et al. 2012; Xu, Fan, and Qian 2007). The practicality of the SAFE apparatus allows for reduced loss of highly volatile compounds as the extraction is contained within a single glassware unit and operates at lower temperatures than SDE, thus minimising the production of artefacts (Engel et al. 1999).

On correct application, this method has demonstrated higher sensitivity than other extraction techniques for compounds related to perceived aroma (Havemose et al. 2007; Majcher and Jeleń 2009; Murat et al. 2012). However, detailed knowledge of the product composition is beneficial to the successful operation of SAFE, as components such as fat and alcohols can interfere with the extraction process (Reineccius 2007).

Pozo-Bayón et al. (2006b) investigated SAFE as a mechanism for quantifying aroma compounds in sponge cake. Nineteen aroma compounds associated with a 'rich' and 'sweet' character were added to a sponge cake and SAFE recovered all compounds with quantification achieved for thirteen. Key volatiles such as 'acetoin', 'γ-decalactone', and 'vanillin' were quantified, highlighting the suitability of this technique for baked cereal matrices. In a similar study, Pozo-Bayón et al. (2007) employed SAFE to investigate the contribution of egg to the aroma of sponge cake. By combining the use of two extraction techniques, SAFE and Purge and Trap (P&T), the authors were capable of recovering an elaborate volatile profile of 100 compounds. Although it stated the two techniques were complimentary, SAFE had the advantage of isolating 1,2-dimethylbenzene, butan-1-ol, limonene, 2-methyl-dihydro-2(H)-furan-3-one, as well as 19 other compounds, which P&T was unable to recover. However, limitations of this technique include the tendency to favour the extraction of high molecular weight compounds (Thomsen et al. 2014). Solvent extraction techniques by nature retrieve most compounds in the sample, without accounting for the retention effect of the matrix; therefore the sample profile reflects heavier compounds that are bound in the matrix, which may not be truly representative (Kilcawley 2017). Other drawbacks include the copious amounts of solvents used during extraction, leading to the generation of hazardous waste, as well as the length of time the process requires, and the lack of automation.

1.4.2.3 Purge and Trap

P&T is a headspace technique that entails purging volatiles from a sample to a highly sorbent material (usually Tenax®) where they are concentrated prior to desorption to the GC (Lee et al. 2001). Some of the attractions to this technique include: a limited sample amount, large volume traps, and a solvent free technique (Pillonel, Bosset, and Tabacchi 2002). P&T has been mainly utilised for the analysis of pollutants in water and air, but has demonstrated successful recoveries in baked cereal matrices (Table 1.1). Pozo-Bayón et al. (2007) utilised P&T to evaluate the aroma profile of sponge cake, of which 90 compounds were isolated. P&T was capable of identifying 2,3-butanedione (diacetyl), acetoin, 2-ethyl-5-methyl-pyrazine, and δ-decalactone, not detected in SAFE extracts. The aroma of Altumura bread was also successfully characterised using P&T where 89 volatile compounds were identified in the crust, and 78 in the crumb (Bianchi et al. 2008). Purging time is an important parameter in the optimum operation of P&T. Studies in liquid matrices have shown that increasing purging times can actually decrease the rate of compound recovery (Campillo et al. 2004; Salemi et al. 2006). When equilibrium has been reached between sample, headspace, and sorbent material, the sorbent material reaches its full capacity and continuation of purging gas after this point can result in the loss of volatiles.

As seen in Table 1.1, Pozo-Bayón et al. (2007) utilised a range of different purging times and found 14 hours to be the most effective in extracting volatile compounds from a sponge cake. Similarly, long purging times were effective in studying the interaction of amylose with aroma compounds in a sponge cake (Pozo-Bayón, 2008). However, Bianchi et al. (2008) applied a purging time of 15 minutes and retrieved an ample profile of compounds from Altumura bread, comparable to that of Pozo-Bayón et al. (2007).

Complications with this technique can include (i) contamination of the sorbent material from samples (Schmidt 2003), (ii) moisture control, (iii) the catalytic activity

occurring on the adsorbent, which can lead to the generation of artefacts compounds (Pillonel et al. 2002), and similarly to SAFE, the length of time needed preform the technique.

1.4.2.4 Thermal desorption

Similar to the development of P&T, Thermal Desorption (TD) was designed for the analysis of air borne volatiles (Wauters et al. 1979). However, TD is now also widely used to extract aroma compounds from food. The sample is usually incubated and the volatiles are purged dynamically to pre-packed absorbent tubes (usually containing Tenax, or other absorbents such as charcoal or silica gel). The tubes are heated and the volatiles are directly injected into the GC, or further concentrated prior to transfer to the GC. Enhanced sensitivity and efficiency of reusable adsorbent tubes is a significant benefit, but the main appeal is the large adsorption capacity of the tubes (Madruga et al. 2009; Ramírez et al. 2010). This technique has been successful in extracting esters from cookies (Heiderich and Reineccius 2001), characterising crackers supplemented with barley (O'Shea, Kilcawley, and Gallagher, 2017), as well as differentiating fresh and rancid oat cakes by their volatile profile (Cognat et al. 2012). The main disadvantage associated with TD is moisture control (Pillonel et al. 2002), which may explain the lack of studies utilising this technique. However, it may be suitable for low moisture biscuit and cookie products, flours etc.

1.4.2.5 Headspace Solid-Phase Microextraction

Solid-phase microextraction (SPME) is widely utilised for the analysis of volatiles in foods (Cuevas-Glory et al. 2007; Frank, Owen, and Patterson 2004; Ruiz et al. 1998), mainly because it is highly automatable with good reproducibility. The working principle of SPME involves a fused silica fibre that is coated with a stationary phase. The phase

can be composed of multiple materials of different polarity to assist in extraction of a wide range of compounds or of single phases for targeted extraction of specific chemical classes, which is accomplished based on polarity, volatility, or molecular weight. The most common types of fibres utilised in literature are comprised of a multi-phase, consisting of a molecular sieve Carboxen (CAR), polar divinylbenzene (DVB), non-polar polydimethylsiloxane (PDMS), or a single phase polyacrylate (PA), which targets very polar analytes. The main application of SPME is in head-space (HS) analysis, where the fibre is exposed to the HS above the sample in a sealed container/vial. Consequently, the volatiles are adsorbed or absorbed onto the fibre through gentle agitation (Kataoka, Lord, and Pawliszyn 2000).

HS-SPME is the most popular technique for volatile extraction of foods, especially in baked cereal analysis (see Table 1.1). As well as being automatable, HS-SPME is an attractive extraction technique due to the simplicity of sample preparation, solvent free, relatively low cost, and can be targeted towards a wide range of chemical classes (Afoakwa et al. 2009). Rega et al. (2009) evaluated the efficacy of three fibres (50/30µ DVB/CAR/PDMS, 75µ CAR/PDMS and 100µ PDMS) to obtain a representative profile for sponge cake and found that the 50/30µ DVB/CAR/PDMS extracted the largest quantity of volatile compounds (See Table 1.2) and the 75µ CAR/PDMS was capable of isolating high boiling point compounds. It is essential that the appropriate parameters; extraction time, extraction temperature, suitable fibre for compounds of interest, and sample size, are taken into account to ensure optimum results are obtained in SPME analysis (Kataoka et al. 2000).

Table 1.2.

Comparison of different SPME fibres utilised in the volatile extraction of sponge cake (Rega *et al.* 2009)

Compound	CAR/ PDMS	PDMS	DVB/ CAR/ PDMS
2-Methylpropanal	x		
2-Methylbutanal	x		x
3-Methylbutanal	x	x	x
2-Pentanone	x		x
2,3-Pentanone	x	x	x
Hexanal	x		x
Heptanal	x		x
2-2-pentylfuran	x		x
Pentanol	x		x
2-Methylpyrazine	x		x
Octanal	x	x	x
1-Hydroxy-2-propanone	x		x
2,5-Dimethylpyrazine	x	x	x
2,6-Dimethylpyrazine			x
2,3-Dimethylpyrazine	x		x
Nonanal	x		x
Trimethylpyrazine	x	x	x
(E)-2-octenal	x		x
1-octen-3-ol	x		x
Acetic Acid	x	x	x
Furfural	x		x
Decanal	x		x
Benzaldehyde	x		x
(E)-2-nonenal			x
Octanol	x		x
Undecanal	x		x
Acetylpyrazine	x		x
Phenylacetaldehyde	x		x
Butyric Acid	x		x
Furfuryl alcohol	x		x
Nonanol	x		x
Dodecanal	x		x
2-Undecanal	x	x	x

(E,Z)-2,4-Decadienal	x		x
(E,E)-2,4-Decadienal	x		x
Hexanoic acid	x		x
Dimethylsulfone	x		x
2-Acetylpyrrole	x		x
Maltol			x
Pentadecane-2-one			x
Furaneol	x	x	x
Octanoic Acid	x		x
Tetradecanol		x	x
Nonanoic Acid	x		x
2,3-Dihydro-3,5-dihydroxy-6-methyl- 4(H)-pyran-4-one	x	x	x
5-Hydroxymethylfurfural	x	x	x

HS-SPME has been widely utilised for baked cereal products (Cognat et al. 2012; Giarnetti et al. 2015; Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Petisca et al. 2013; Poinot et al. 2007; Raffo et al. 2015; Rega et al. 2009; Sabovics 2014; Ying et al. 2012). Poinot et al. (2007) trialled 27 HS-SPME conditions, varying in extraction time, extraction temperature and SPME fibre, to optimize the extraction of volatile compounds most representative of bread odour. By permitting a panel of trained judges to compare the odour qualities of collected HS-SPME volatile extracts, the authors were able to conclude that an extraction time of 30 and 60 minutes at 35°C, using either 50/30 μm DVB/CAR/PDMS or a 75 μm CAR/PDMS fibre, can yield a volatile profile representative of bread odour. Raffo et al. (2015) found an extraction time of 60 minutes at 50°C (under agitation) with a DVB/CAR/PDMS fibre beneficial for providing a complete volatile profile of wheat bread. Through preliminary work, Matsakidou, Blekas, and Paraskevopoulou (2010) also identified a 60 minute extraction time at 60°C favourable for the recovery of volatiles representative of cake odour. It is likely that the extensive extraction time and relatively high extraction temperature contributed to the wide range of volatile compounds

identified (92 compounds). Shortbread cookies were examined with a 50/30μm DVB/CAR/PDMS fibre for 15 minutes at 35°C, enabling the recovery and identification of 24 volatile compounds (Giarnetti et al 2015). This result seems rather low compared to Mohsen et al. (2009) who were able to identify 42 compounds in cookies using the SDE technique. Pasqualone et al. (2014) utilised a 75μm CAR/ PDMS fibre for the extraction of compounds from biscuits (enriched with grape marc extract) at 40°C for 50 minutes, and yielded 60 compounds from a wide range of chemical classes; alcohols, aldehydes, ketones, esters, furans etc. The authors employed the same parameters to analyse biscuits enriched with purple wheat, yielding a similar result of 56 compounds (Pasqualone et al. 2015). However, the authors did consider that this fibre was more sensitive to compounds arising from LO, meaning, perhaps the profile depicted by these extraction conditions, was not a true representative of the sample.

On-line extraction of volatile compounds during the baking of sponge cake has been accomplished with SPME (Maire et al. 2013; Rega et al. 2009). By assembling a glass inlet hood from the oven to a refrigerated extraction chamber, volatile compounds generated during baking were captured at different stages throughout the baking process. Utilising this technique, Rega et al. (2009) monitored the development of compounds associated with LO, and the MR, at different time points. By employing the same technique, Maire et al. (2013) demonstrated how varying the flow rate of vapours from the chamber during baking impacted on the extraction of very volatile and semi-volatile compounds. A flow rate of 7.5 L min^{-1} at 40°C enabled the extraction of a higher volume of compounds and was particularly beneficial in extracting semi volatiles such as pyrans and furans, however, 1 L min^{-1} at 10°C yielded the extraction of very volatile compounds.

The major downside to SPME is the limited capacity of the fibre. This leads to competition on the fibre and results in the compounds with a higher affinity for the fibre phase displacing more volatile compounds. Fragility of the SPME fibre and the possible

carryover of compounds are also potential issues associated with SPME as an extraction technique (Prosen and Zupančič-Kralj 1999).

1.5 Potent Aroma Volatile Compounds in Baked Confectionery

As baked confectionery products exhibit similar formulations and baking procedures, their qualitative volatile profiles can be similar. However, the ratio of individual volatiles will vary significantly, thus impacting on consumer's perceptions (Table 1.3). The following covers the key volatile classes associated with baked confectionery products.

1.5.1 Aldehydes

On consumption of baked confectionery products, the perception of 'sweet' is un-doubtfully one of the initial attributes perceived during mastication, inherently due to the volume of non-volatile sucrose present in product formula. However, retronasal olfaction perception of 'sweet' can also result from specific aldehydes, such as benzaldehyde and phenylacetaldehyde, which are associated with 'almond', 'cherry', 'honey', and 'floral' notes in biscuit, cookies and cakes (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009). Egg yolk provides an abundance of amino acids and when subject to the high temperatures of baking, Strecker degradation occurs, resulting in aldehyde formation. Both benzaldehyde and phenylacetaldehyde are products of Strecker degradation of the amino acid phenylalanine (Chu and Yaylayan 2008). 2-Methylpropanal, 3-methylbutanal, and 2-methylbutanal are also Strecker aldehydes considered important to the aroma of baked goods and derive from valine, leucine, and isoleucine, respectively. 2-Methylpropanal has been identified as

imparting a 'sweet', 'mint', and 'floral' aroma in cakes (Maire et al. 2013), whereas 3-methylbutanal and 2-methylbutanal yield a more 'chocolate', 'malty' aroma in baked confectionery, with concentrations particularly high in the crust of cakes (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Pozo-Bayón et al. 2007). 'Fatty' and 'fruity' odours in cake and biscuits derive from aliphatic aldehydes such as octanal, nonanal and decanal (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009), whose presence is as of result of the auto-oxidation of linoleic or oleic acid (Fullana, Carbonell-Barrachina, and Sidhu, 2004; Whitfield and Mottram 1992). Similarly, hexanal, heptanal, and 2,4-decadienal, markers of auto-oxidation of linoleic acid (Fujisaki, Endo, and Fujimoto 2002), have been reported in bakery products as imparting a 'fruity', 'herbal', 'fresh cut grass' aroma (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009). Methional has been identified as a key contributor to the 'roasty' smell of baguettes (Zehentbauer and Grosch 1998a), and is generated from the Strecker degradation of the amino acid methionine (Escudero et al. 2000). Methional contributes a 'dusty', 'potato-like' odour and is perceived at very low levels in cake products (Maire et al. 2013; Pozo-Bayón et al. 2007; Rega et al. 2009).

1.5.2 Alcohols

Quite a number of alcohols have been identified in cake and biscuit/cookie products (Table 1.3). As mentioned, LO of the fat promotes the generation of alcohols through degradation of unsaturated fatty acids, particularly polyunsaturated fatty acids due to the presence of multiple double bonds. Depending on the fatty acid, and the point of cleavage, various alcohols of different odour qualities can be produced. Alcohols

positively associated with baked confectionery aroma include fatty 2-ethylhexanol, 1-octanol, 1-nonanol, and 1-decanol, identified as having odour qualities described as 'orange', 'rose', and 'sweet' (Maire et al. 2013; Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009). Other odour descriptions include 'cauliflower', 'cardboard', 'mushroom/fungal', and are associated with alcohols; 1-pentanol, 1-hexanol, and 1-octen-3-ol, respectively (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009). Linoleic acid is prone to oxidation and thus yields 1-hexanol and 1-octen-3-ol (Paraskevopoulou, Chrysanthou, and Koutidou 2012). Although these compounds may be perceived as unpleasant at high concentrations, in relatively low concentrations they add to the overall dynamic of baked and cereal products, with 1-octen-3-ol identified as a key compound in oat flakes (Klensporf and Jeleń 2008).

Flour is also identified as a contributor to the alcohol profile of baked confectionery (Maire et al. 2013). The process of milling induces the release of free fatty acids and propagates LO reactions, as well as microbial degradation to produce alcohols (Hansen and Hansen, 1994). Wheat flour starch has shown to have high levels of 2-ethylhexanol, a degradation product of LO (Sayaslan et al. 2000). This corresponds to Pozo-Bayón et al. (2007) and Maire et al. (2013) identifying this compound in the dough of sponge cakes, indicating this compound originates from the raw material, but formation is potentially promoted during baking preparation.

1.5.3 Ketones

Ketones are generally associated with favourable aromas. The MR and caramelisation can contribute some of the most characteristic volatile compounds associated with bakery products. The decomposition of sugar results in diketones such as

2,3-butanedione (diacetyl) and 2,3-pentadione, responsible for 'buttery', 'caramel', and 'butterscotch' notes in sweetened baked goods (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al, 2007). As previously mentioned the methyl ketones, 2-butanone, 2-heptanone, 2-nonanone, and 2-undecanone have been identified in cookies (Giarnetti et al. 2015) and are associated with 'buttery' and 'sweet' attributes. These compounds are generated from β-keto acids in milk fat when exposed to heating (Wong and Patton 1962), and contribute to the aroma of butter (Mallia et al. 2008).

Table 1.3.

Volatile compounds identified in baked confectionery products

Compound	Odour Description	Product	Reference
Alcohols			
Ethanol		Biscuit/ Cookie	**Pasqualone et al. 2014; Pasqualone et al. 2015;**
Propanol		Biscuit/ Cookie	**Pasqualone et al. 2014**
Butanol		Cake, Biscuit/ Cookie	**Pasqualone et al. 2014 Pozo-Bayón et al. 2007;**
1-Pentanol	Foot, cauliflower, pungent, fusel oil,	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
1-Hexanol	Cardboard, solvent, potatoes, fruity, sweet, green	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007**
Heptanol	Musty, leafy, violet, herbal, green, sweet, fresh, woody	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**

2-Ethylhexanol	Citrus, fresh, floral, oily	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010; Pozo-Bayón et al. 2007**
4-Hexen-1-ol		Biscuit/ Cookie	**Pasqualone et al. 2015**
2-Octanol		Cake	**Pozo-Bayón et al. 2007**
2-Butoxyethanol		Cake	**Pozo-Bayón et al. 2007**
1-Methoxy-2-propanol		Cake	**Pozo-Bayón et al. 2007**
1-Octen-3-ol	Mushroom, musty,fungal, earthy	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
2,6-Dimethyl-2,7-octadien-1,6-diol		Cake	**Matsakidou et al. 2010**
1-(2-Methoxypropoxy)-2-propanol		Cake	**Pasqualone et al. 2014; Pasqualone et al. 2015 Pozo-Bayón et al. 2007**
1-Octanol	Waxy, green, orange, aldehydic, fatty, rose	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
1-Nonanol	Fresh, clean, fatty, floral,	Cake, Biscuit/ Cookie	**Marie et al. 2013; Mohsen et al. 2009;**

	rose, orange, dusty, wet,		**Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
Decanol	Floral, fatty, orange, sweet, clean, watery	Cake	**Marie et al. 2013**
Dodecanol	Earthy, soapy, waxy, fatty, honey, coconut	Cake	**Marie et al. 2013**
Octadecanol		Cake	**Marie et al. 2013**
1-Penten-3-ol		Cake	**Matsakidou et al. 2010**
α-Terpineol		Cake	**Pozo-Bayón et al. 2007**
Borneol		Cake	**Pozo-Bayón et al. 2007**
1-(2-butoxyethoxy)ethanol		Cake	**Pozo-Bayón et al. 2007**
Benzyl alcohol		Cake, Biscuit/ Cookie	**Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007**
2-Phenylethanol		Cake, Biscuit/Cookie	**Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007**
Hexadecanol	Waxy, floral	Cake	**Marie et al. 2013; Pozo-Bayón et al. 2007**
Tetradecanol		Cake	**Rega et al. 2009**

2-Methylcyclopentyl alcohol		Biscuit/ Cookie	**Pasqualone et al. 2014; Pasqualone et al. 2015**

Aldehydes

Acetaldehyde	Pungent, fresh, aldehydic, refreshing, green	Cake	**Marie et al. 2013; Matsakidou et al. 2010**
2-Methylpropanal	Fresh, sweet, mint, floral	Cake, Biscuit/ Cookie	**Marie et al. 2013; Mohsen et al. 2009; Pozo-Bayón et al. 2007; Rega et al. 2009**
2-Methylbutanal	Musty, cocoa, coffee, nutty,malty	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010 Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007**
3-Methylbutanal	Chocolate, ethereal, aldehydic, peach, fatty, malty	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010 Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
Pentanal		Biscuit/ Cookie	**Pasqualone et al. 2014; Pasqualone et al. 2015**
2-Pentenal		Biscuit/ Cookie	**Mohsen et al. 2009**
Hexanal	Floral, fruity,		**Giarnetti et al. 2015**

	herbal, cut grass, green, sweaty	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010 Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
Methional	Musty, tomato, potato,earthy, vegetable, creamy	Cake	**Marie et al. 2013; Pozo-Bayón et al. 2007; Rega et al. 2009**
(*E*)-2-Hexenal		Biscuit/ Cookie	**Mohsen et al. 2009., Pasqualone et al. 2014, Pasqualone et al. 2015**
3-Hexenal		Biscuit/ Cookie	**Mohsen et al. 2009**
Heptanal	Fresh, green, sweet, herbal	Cake, Biscuit/ Cookie	**Marie et al, 2013; Matsakidou et al. 2010 Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
(E)-2-Heptenal		Cake, Biscuit/ Cookie	**Marie et al. 2013; Pasqualone et al. 2014; Pasqualone et al. 2015**
(Z)-4-Heptenal		Cake, Biscuit/ Cookie	**Matsakidou et al. 2010; Mohsen et al. 2009**
Octanal	Floral, citrus, fruit, orange peel	Cake, Biscuit/ Cookie	**Giarnetti et al. 2015 Marie et al. 2013; Matsakidou et al. 2010; Mohsen et al. 2009;**

			Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009
(*E*)-2-Octenal	Fried, Fatty, Unpleasant	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010 Pasqualone et al. 2014; Pozo-Bayón et al. 2007; Rega et al. 2009**
Benzaldehyde	Sweet, bitter, almond, sharp, cherry	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010 Mohsen et al. 2009; Pasqualone et al, 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
Phenylacetaldehyde	Rose, honey, floral, flowers, sweet, cocoa	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010 Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
Nonanal	Aldehydic, waxy, citrus, orange, green, peel	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010; Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007;**

			Rega et al. 2009
2-Nonenal	Vegetable, solvent, floral, musty, cucumber, green	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010 Pasqualone et al. 2015; Rega et al. 2009**
(*E*,*E*)-2,4-Heptadienal	Fatty, green, oily, aldehydic, cake, cinnamon	Cake , Biscuit/ Cookie	**Marie et al. 2013; Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015;**
Decanal	Floral, fruity, sweet, waxy, orange, peel, citrus	Cake	**Marie et al. 2013; Matsakidou et al. 2010 Pozo-Bayón et al. 2007; Rega et al. 2009**
(*E*)-2-Decenal	Waxy, fatty, earthy, coriander, green, mushroom	Biscuit/ Cookie	**Giarnetti et al. 2015 Marie et al. 2013; Pasqualone et al. 2015**
(*E*,*E*)-2,4-Decadienal	Rice, cooked, baked, fried potato, fatty, pumpkin nut, meat	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010; Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
(E,Z)-2,4-Decadienal	Fried oil, cooked, fatty, geranium, green	Cake	**Marie et al. 2013; Pozo-Bayón et al. 2007; Rega et al. 2009**
2,4-Nonadienal		Biscuit/ Cookie	**Pasqualone et al. 2015**

(*E,E)*-2,4-Nonadienal		Biscuit/ Cookie	**Mohsen et al. 2010; Pasqualone et al. 2014**
2-Dodecanal	Vegetable, floral, fatty, clean	Cake, Biscuit/ Cookie	**Marie et al. 2013; Pasqualone et al. 2014; Pasqualone et al. 2015; Rega et al. 2009**
2-Undecanal	Floral, bud, soapy, citrus, green, fatty, fresh laundry	Cake	**Marie et al. 2013; Rega et al. 2009**
Methylbenzaldehyde		Cake	**Pozo-Bayón et al. 2007**
Tridecanal	Fresh, clean, soapy, citrus, petal, waxy, grapefruit peep	Cake	**Marie et al. 2013**
Octadecanal	Oily	Cake	**Marie et al. 2013**
Vanillin	Sweet, vanilla, creamy, chocolate	Cake	**Marie et al. 2013**
Pyrazines			
Pyrazine		Cake, Biscuit/ Cookie	**Matsakidou et al. 2010 Mohsen et al. 2009**
Methylpyrazine		Cake, Biscuit/ Cookie	**Giarnetti et al. 2015; Matsakidou et al. 2010; Mohsen et al 2009; Pasqualone et al. 2014; Pasqualone et al. 2015;**

			Pozo-Bayón et al. 2007; **Rega et al. 2009**
2,5-Dimethylpyrazine	Solvent, hospital, perfumed rice, cake crust	Cake, Biscuit/ Cookie	**Giarnetti et al. 2015** **Matsakidou et al. 2010** **Pozo-Bayón et al. 2007;** **Rega et al. 2009**
2,6-Dimethylpyrazine	Cake, roasted, bread crust, rice, walnut, praline	Cake	**Matsakidou et al. 2010** **Pozo-Bayón et al. 2007;** **Rega et al. 2009**
Ethylpyrazine		Cake, Biscuit/ Cookie	**Matsakidou et al. 2010** **Pasqualone et al. 2014;** **Pozo-Bayón et al. 2007**
2,3-Dimethylpyrazine	Earthy, potatoes, green pea, perfumed rice, cake, crust, nutty, peanut butter, walnut, caramel, leather	Cake	**Marie et al. 2013** **Matsakidou et al. 2010** **Pozo-Bayón et al. 2007;** **Rega et al. 2009**
2-Ethyl-6-methylpyrazine	Roasted, burnt	Cake	**Matsakidou et al. 2010** **Pozo-Bayón et al. 2007;** **Rega et al. 2009**
2-Ethyl-5-methylpyrazine		Cake, Biscuit/ Cookie	**Matsakidou et al. 2010** **Rega et al. 2009**
Trimethylpyrazine	Herbal, earthy, potatoes, roasted, cake	Cake	**Matsakidou et al. 2010;** **Pozo-Bayón et al. 2007;** **Rega et al. 2009**
Vinylpyrazine		Cake	**Pozo-Bayón et al. 2007**
3-Ethyl-2,5-dimethylpyrazine		Cake	**Matsakidou et al. 2010;** **Pozo-Bayón et al. 2007;**

			Mohsen et al. 2009
2-Ethyl-3,5-dimethylpyrazine		Cake, Biscuit/ Cookie	**Matsakidou et al. 2010** **Pozo-Bayón et al. 2007**
2-Methyl-6-vinylpyrazine	Vegetables, potato	Cake	**Pozo-Bayón et al. 2007**
2-Methyl-5-vinylpyrazine		Cake	**Pozo-Bayón et al. 2007**
3,5-Diethyl-2-methylpyrazine		Cake	**Pozo-Bayón et al. 2007**
Dimethyl-2-vinylpyrazine (isomer)	Pungent, herbal, potatoes	Cake	**Pozo-Bayón et al. 2007**
Acetylpyrazine	Hazelnut, praline, cake	Cake	**Pozo-Bayón et al. 2007;** **Rega et al. 2009**
2-Methyl-5-(2-propenyl)-pyrazine		Cake	**Matsakidou et al. 2010**
2-Acetyl-5-methylpyrazine		Cake	**Pozo-Bayón et al. 2007**
2-Acetyl-6-methylpyrazine		Cake	**Pozo-Bayón et al. 2007**
Benzopyrazine		Cake	**Pozo-Bayón et al. 2007**
Ketones			
Acetone		Biscuit/ Cookie	**Giarnetti et al. 2015**
2,3-Butanedione (Diacetyl)	Butter, fruity, caramel, butterscotch	Cake, Biscuit/ Cookie	**Giarnetti et al. 2015** **Marie et al. 2013;** **Matsakidou et al. 2010;** **Pasqualone et al. 2014;** **Pozo-Bayón et al. 2007**
2-Butanone		Biscuit/ Cookie	**Giarnetti et al. 2015** **Mohsen et al. 2009;** **Pasqualone et al. 2015**

2-Pentanone		Cake, Biscuit/ Cookie	**Mohsen et al. 2009; Pasqualone et al. 2015; Rega et al. 2009**
2,3-Pentanedione	Pungent, sweet, butter, creamy, caramel, nutty	Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010 Pozo-Bayón et al. 2007; Rega et al. 2009**
Hydroxyacetone (1-Hydroxy-2-propanone)		Cake, Biscuit/ Cookie	**Marie et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
Acetoin (3-Hydroxy-2-butanone)		Cake, Biscit/Cookie	**Giarnetti et al. 2015; Pozo-Bayón et al. 2007**
2-Heptanone		Cake, Biscuit/ Cookie	**Giarnetti et al. 2015 Matsakidou et al. 2010 Mohsen et al. 2009; Pasqualone et al. 2015; Pozo-Bayón et al. 2007;**
1-Octen-3-one	Herbal, mushroom, earthy, musty	Cake	**Marie et al. 2013**
2-Octanone		Cake	**Matsakidou et al. 2010; Rega et al. 2009**
3-Octen-2-one		Cake	**Matsakidou et al. 2010**
2-Nonanone		Cake, Biscuit/ Cookie	**Giarnetti et al. 2015 Matsakidou et al. 2010; Pozo-Bayón et al. 2007;**

2-Decanone		Cake	**Marie et al. 2013; Matsakidou et al. 2010 Pozo-Bayón et al. 2007**
2,3-Methyloctanone		Cake	**Matsakidou et al. 2010 Rega et al, 2009**
2-Pentadecanone		Cake	**Rega et al. 2009**
2-Undecanone		Cake, Biscuit/ Cookie	**Giarnetti et al. 2015; Matsakidou et al. 2010**
2-Dodecanone		Cake	**Matsakidou et al. 2010**
6-Methyl-5-hepten-2-one		Cake	**Matsakidou et al. 2010; Pozo-Bayón et al. 2007**
(*E*,*E*)-3,5-Octadiene-2-one		Cake, Biscuit/ Cookie	**Pozo Bayon et al. 2007; Mohsen et al., 2009; Pasqualone et al., 2015**
Acetophenone		Cake	**Pozo-Bayón et al. 2007**
Acids			
Acetic acid	Unpleasant, earthy, sharp, pungent, sour, vinegar	Cake, Biscuit/ Cookie	**Giarnetti et al. 2015 Marie et al. 2013; Matsakidou et al. 2010 Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
Formic acid	Pungent, vinegar	Cake	**Marie et al. 2013**
Propanoic acid		Biscuit/ Cookie	**Pasqualone et al. 2015**

Butanoic acid	Sweat, fish, unpleasant	Cake, Biscuit/ Cookie	**Mohsen et al. 2009; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
Pentanoic acid		Cake	**Pozo-Bayón et al. 2007**
Hexanoic acid	Mild, sour, fatty, sweat, cheese, rancid	Cake, Biscuit/ Cookie	**Giarnetti et al. 2015; Marie et al. 2013; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
Heptanoic acid		Cake	**Pozo-Bayón et al. 2007**
Octanoic acid	Fatty, acid, sour	Cake, Biscuit/ Cookie	**Marie et al. 2013; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
2-Hexenoic acid		Biscuit/ Cookie	**Marie et al. 2013; Pasqualone et al. 2015;**
2,4-Hexadienoic acid		Biscuit/ Cookie	**Giarnetti et al. 2015; Pasqualone et al. 2014; Pasqualone et al. 2015**
Nonanoic acid	Waxy, dirty, cheese, cultured dairy	Cake, Biscuit/ Cookie	**Marie et al. 2013; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
Decanoic acid	Unpleasant, rancid, sour, fatty, citrus	Cake, Biscuit/ Cookie	**Marie et al. 2013; Mohsen et al. 2009; Pasqualone et al. 2015;**

			Pozo-Bayón et al. 2007;
Dodecanoic acid	Fatty, coconut, bay oil	Cake	**Marie et al. 2013;** **Pozo-Bayón et al. 2007**
Benzoic acid	Faint, balsm	Cake	**Marie et al. 2013;** **Pozo-Bayón et al. 2007**
Dodecanoic acid	Fatty, coconut, bay oil	Cake	**Marie et al. 2013;** **Pozo-Bayón et al. 2007**
Hexadecanoic acid	Slightly fatty, waxy	Cake	**Marie et al. 2013**
Furans			
2-Methylfuran	Sweet, pungent, caramel, burnt	Biscuit/ Cookie	**Pasqualone et al. 2014**
2-2-pentylfuran	Earthy, vegetable, beany, metallic	Cake, Biscuit/ Cookie	**Marie et al. 2013;** **Matsakidou et al. 2010** **Pasqualone et al. 2014;** **Pasqualone et al. 2015;** **Pozo-Bayón et al. 2007;** **Rega et al. 2009**
Dihydro-2-methyl-3(2H)-furanone	Roasted, biscuit, hazelnut, nutty	Cake, Biscuit/ Cookie	**Mohsen et al. 2009;** **Pozo-Bayón et al. 2007**
Furaneol (Strawberry Furanone)	Caramel-like, spice, cake, sweet, cotton candy, strawberry, sweet, fruity	Cake, Biscuit/ Cookie	**Marie et al. 2013;** **Matsakidou et al. 2010** **Mohsen et al. 2009;** **Rega et al. 2009**
2-Furanmethanol	Sweet caramel, burnt	Cake, Biscuit/ Cookie	**Matsakidou et al. 2010** **Pasqualone et al. 2014;** **Pasqualone et al. 2015;** **Pozo-Bayón et al. 2007;**

			Rega et al. 2009
Furfural	Earthy, potatoes, green pea, parfumed rice, cake, crust, sweet, woody, almond, fragrant, bready	Cake, Biscuit/ Cookie	**Giarnetti et al. 2015 Marie et al. 2013; Matsakidou et al. 2010 Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009**
2-Acetylfuran	Sweet, balsam, almond, cocoa, caramel, coffee	Cake, Biscuit/ Cookie	**Marie et al. 2013; Pasqualone et al. 2014; Pozo-Bayón et al. 2007**
5-Hydroxymethylfurfural (HMF)	Fatty, musty, waxy, caramel	Cake, Biscuit/ Cookie	**Marie et al. 2013; Pasqualone et al. 2015; Rega et al. 2009**
5-Methylfurfural	Biscuit, chocolate, roasted, cake, spice, caramel, maple	Cake, Biscuit/ Cookie	**Marie et al, 2013; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007**
2-Ethyl-5-methylfuran		Biscuit/ Cookie	**Mohsen et al. 2009**
5H-furan-2-one		Cake, Biscuit/ Cookie	**Giarnetti et al, 2015; Matsakidou et al. 2010; Pozo-Bayón et al. 2007**
Alkanes			
Octane	Gasoline	Cake	**Marie et al. 2013**

Decane		Cake	**Matsakidou et al. 2010**
Dodecane	Alkane	Cake	**Marie et al. 2013**
Hexadecane		Cake	**Marie et al. 2013**
Tridecane		Cake	**Matsakidou et al. 2010**
Tetracosane		Cake	**Marie et al. 2013**
Tetradecane	Mild Waxy	Cake	**Marie et al. 2013;** **Matsakidou et al. 2010**
Pentadecane	Waxy	Cake	**Marie et al. 2013**
Esters			
Ethyl Acetate		Cake, Biscuit/ Cookie	**Matsakidou et al. 2010;** **Pasqualone et al. 2014;** **Pasqualone et al. 2015**
Butyl Acetate		Cake	**Matsakidou et al. 2010**
Ethyl Butanoate		Cake	**Pozo-Bayón et al. 2007**
Ethyl Hexanoate	Vegetable, floral, fruity	Cake	**Pozo-Bayón et al. 2007**
2-Ethylhexanoic acid		Cake	**Pozo-Bayón et al. 2007**
Methyl Benzoate		Biscuit/ Cookie	**Pasqualone et al. 2015**
Ethyl Benzoate		Biscuit/ Cookie	**Pasqualone et al. 2015**
Methyl Decanoate		Biscuit/ Cookie	**Pasqualone et al. 2015**
Methyl Dodecanoate		Biscuit/ Cookie	**Mohsen et al. 2009**
Ethyl Decanoate		Biscuit/ Cookie	**Mohsen et al. 2009**

Isopropyl Tetradecanoate		Cake	**Pozo-Bayón et al. 2007**
Ethyl Octanoate	Fruity, wine, waxy, sweet, apricot, banana, brandy	Cake	**Marie et al. 2013**
Lactones			
γ-Butyrolactone		Cake, Biscuit/ Cookie	**Giarnetti et al. 2015; Mohsen et al. 2009; Pozo-Bayón et al. 2007**
γ-Hexalactone		Cake	**Pozo-Bayón et al. 2007**
γ-Octalactone		Cake	**Pozo-Bayón et al. 2007**
γ-Nonalactone		Cake	**Pozo-Bayón et al. 2007**
γ-Decalactone		Cake	**Pozo-Bayón et al. 2007**
δ-Decalactone		Cake, Biscuit/ Cookie	**Mohsen et al 2009.; Pozo-Bayón et al. 2007**
Sulfur Compounds			
Dimethyl Disulphide	Sulfurous, vegetable, cabbage, onion	Cake	**Marie et al. 2013; Pozo-Bayón et al. 2007;**
Dimethyl Trisulfide	Solvent, gas, wastewater, pungent	Cake	**Pozo-Bayón et al. 2007**
Dimethyl Sulfone		Cake	**Pozo-Bayón et al. 2007**
2-Acetyl-2-thiazoline		Cake	**Pozo-Bayón et al. 2007; Rega et al. 2009**

2-Acetylthiazole	Hazelnut, popcorn	Cake	**Matsakidou et al. 2010** **Pozo-Bayón et al. 2007**
Benzothiazole		Biscuit/ Cookie	**Pasqualone et al. 2014**
Aromatic Hydrocarbons			
Toulene		Cake	**Marie et al. 2013**
Pentylbenzene		Biscuit/ Cookie	**Pasqualone et al, 2014**
2-Methyl-propenylbenzene		Biscuit/ Cookie	**Pasqualone et al. 2014**
Hexylbenzene		Biscuit/ Cookie	**Pasqualone et al. 2014**
Octylbenzene		Biscuit/ Cookie	**Pasqualone et al. 2014**
Phenolic Compounds			
Phenol		Cake, Biscuit/ Cookie	**Pasqualone et al. 2014;** **Pasqualone et al. 2015;** **Pozo-Bayón et al. 2007**
2-Methoxyphenol (Guaiacol)		Cake	**Pasqualone et al. 2014;** **Pasqualone et al. 2015;** **Pozo-Bayón et al. 2007**
2-Methoxy-4-vinylphenol		Cake	**Pozo-Bayón et al. 2007**
Pyrroles			
1-H-Pyrrole		Cake, Biscuit/ Cookie	**Matsakidou et al. 2010;** **Mohsen et al 2009**

2-Acetylpyrrole		Cake	**Matsakidou et al. 2010** **Mohsen et al 2009;** **Pozo-Bayón et al. 2007;** **Rega et al. 2009**
2-Acetyl-1-pyrroline	Popcorn	Biscuit/ Cookie	**Mohsen et al. 2009**
Terpenes			
Verbenone		Cake	**Pozo-Bayón et al. 2007**
D-Limonene		Cake, Biscuit/ Cookie	**Giarnetti et al. 2015** **Matsakidou et al, 2010** **Pasqualone et al. 2014;** **Pozo-Bayón et al. 2007;**
Pyran			
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one		Cake, Biscuit/ Cookie	**Marie et al, 2013;** **Matsakidou et al. 2010** **Mohsen et al. 2009;** **Rega et al. 2009**
Maltol	Caramel, sweet, cotton candy, jam, fruity	Cake	**Marie et al. 2013;** **Matsakidou et al. 2010;** **Rega et al. 2009**
Pyridines			
N-acetyl-4(H)-pyridine	Walnut, popcorn	Cake	**Matsakidou et al. 2010**

1.5.4 Pyrazines

Similar to wheat bread, cake is composed of a crust and a crumb that are distinguishable by the quantitative differences of their volatile profile. The crust of cake is a concentrated source of thermal reactions, and therefore generates a greater quantity of heat derived compounds such as pyrazines; compounds responsible for the 'roasted', 'caramel', and 'nutty' odours in baked confectionery. Pyrazines are formed through the Strecker degradation of α-aminoketones during the high temperatures of baking, with formation being promoted in an alkaline pH (Jousse et al. 2002). A range of pyrazines have been identified in the crust and crumb of cakes (see Table 1.3), with 2,5-dimethylpyrazine, 2,6-dimethlypyrazine, 2,3-dimethylpyrazine, trimethylpyrazine, and 2-methyl-6-vinylpyrazine having high odour activity and noted to be the main contributors to the characteristic 'roasty' and 'perfumed rice' aroma of sponge cake (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Pozo-Bayón et al. 2007; Rega et al. 2009). Some pyrazines have high odor thresholds, thus requiring their concentration to be quite high before their 'roasty', 'nutty' aroma can be perceived in cereal products (Bredie et al. 1998). 'Biscuit like' 2-ethyl-5-methylpyrazine has been identified in cookies (Mohsen et al. 2009), as well as odour active 2,5-dimethylpyrazine and trimethylpyrazine (Giarnetti et al. 2015). It appears the abundance of pyrazine compounds is not as prominent in biscuits and cookies, compared to that of cake (Table 1.3). However, this could be a repercussion of the extraction technique and parameters taken to isolate these compounds (Pasqualone et al. 2015), thus more research is required to understand pyrazine contribution to biscuit/cookie aroma.

1.5.5 Furans

Furan and its derivatives are widespread in foods and beverages, with quantities present depending on heat exposure. These compounds generate interest due to their

ability to thrive in low moisture systems, with formation favoured in acidic environments (Kroh, 1994). The low moisture content of biscuit/cookie structures accelerates caramelisation and Maillard reactions, enhancing the concentration of furans (Ameur et al. 2007). Similar to pyrazines, the crusts of cakes reflect higher concentrations of furan compounds compared to the crumb (Matsakidou, Blekas, and Paraskevopoulou 2010; Pozo-Bayón et al. 2007). Furans have low odour thresholds and significantly contribute to the delicate aroma of bakery products. Fresh biscuits have been associated 'sweet', 'toasted', and 'caramel' attributes (Heenan et al. 2009), elucidated by the presence of furfural and HMF. Furanic compounds are described as the most potent compounds in biscuits and cookies, yielding a desirable 'bready', 'almond', 'pungent', and 'sweet' aroma (Giarnetti et al. 2015; Mohsen et al. 2009; Pasqualone et al. 2014; Pasqualone et al. 2015). Pyrolysis of hexose and pentose induce the formation of HMF and furfural, respectively (Petisca et al. 2014). Levels of furans have been shown to be significantly higher in fresh cookies compared to those after storage (Mohsen et al. 2009), demonstrating their importance in cookie aroma. Furaneol, 2-2-pentylfuran, and 2-furanmethanol have been identified in high amounts in the crust and crumb of sponge cakes (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Rega et al. 2009). Furaneol significantly contributes to sweet tasting fruits such as strawberries and pineapples, contributing a sweet taste and 'burnt sugar', 'caramel' aroma (Chen and Sidisky 2011; Elss et al. 2005; Sanz, Richardson, and Pérez 1995). Lipoxygenase-catalysed oxidation of linoleic acid can produce 2-2-pentylfuran which is associated with an 'earthy' 'beany' aroma (Vara-Ubol, Chambers, and Chambers 2004). Oxidation of flour lipids can also contribute to levels of 2-pentyl furan (Birch, Petersen, and Hansen 2013). 'Caramel-like' aroma derives from 2-furnamethanol, a compound associated with products exposed to high temperatures, with significant levels identified in coffee and chocolate (Afoakwa et al. 2009; Nebesny et al.

2007). It is apparent that furan and its derivatives are important to the perceived aroma of baked confectionery products.

1.5.6 Other compounds

Although the above chemical classes may dominate the profile of baked confectionery, many others can impact greatly on the perceived aroma of cakes, biscuits and cookies. Maltol, a pyran compound, is considered important to the aroma of cakes (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Rega et al. 2009), yielding a 'cotton candy' odour at low concentrations. This compound is a well-known product of the MR, with 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one acting as a precursor (Yaylayan and Mandeville 1994). N-Acetyl-4(H)-pyridine and 2-acetylthiazole have been identified in cake crust and associated with a 'walnut', 'hazelnut', 'popcorn' aroma (Matsakidou, Blekas, and Paraskevopoulou 2010), where 2-acetyl-1-pyrroline yields a 'popcorn aroma' and has been identified in cookies (Mohsen et al. 2009). This compound is known to give rice its characteristic aroma (Buttery et al. 1983). Ethyl esters of fatty acids, ethyl octanoate, and ethyl hexanoate, have also been identified in cake (Maire et al. 2013; Pozo-Bayón et al. 2007) and offer 'sweet', 'apricot', 'floral', and 'fruity' notes.

Baked confectionery in general are associated with having pleasant aroma, however, depending on ingredient preparation, or thermal processes, unfavourable compounds with low odour threshold can form. Although present in low quantities, carboxylic acids can be detected in baked confectionery ranging in a variety of unpleasant odours (Table 1.3). Hexanoic acid, octanoic acid, and nonanoic acid, auto-oxidation products of their corresponding aldehydes (Paradiso et al. 2008), have been identified in cakes, biscuits, and cookies (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Pasqualone, et al. 2014; Pasqualone et al. 2015; Pozo-Bayón

et al. 2007; Rega et al. 2009) and yield a 'fatty', 'rancid', 'cheese' aroma, risking deterioration to the sensory properties of these products. LO is the main precursor of off flavours and taints in many foods; therefore it is optimum to manage the cascade of reactions to retain the desirable aroma and flavour of bakery products (Maire et al. 2013).

1.6 Relating Volatile Compounds to Sensory Data

The aim of sensory analysis is to gain an insight into the way food is perceived by humans using visual, olfactometry, taste, touch, and auditory responses. It is beneficial for all those involved in product development to have knowledge and understanding of the types of sensory methodologies available. Application of the most suitable sensory method can aid evaluation of new and reformulated products, and yield insights into product acceptability. Although information on volatiles gives a comprehensive insight into the compounds that may affect aroma and flavour, it can only provide an estimate on how consumers may perceive a product; therefore it is of utmost benefit to use volatile information in conjunction with sensory analysis to obtain a better understanding of the relationship between aroma and sensory perception.

There are many sensory tests available to evaluate a food product, with the most suitable depending on the information required. Considerations such as complexity of the test, cost, resources, and training or commitment from panellists, must be all taken into account when choosing an appropriate sensory test (Lawless and Heymann 2010).

Sensory acceptance testing, though the use of hedonic scales, is a popular choice for consumer research as they are easily understood and assessors do not require in depth training. Hedonic scales normally assess the degree of liking or disliking of sensory attributes such as appearance, odour, taste, aroma, texture, and are popularly utilised to assess food and beverages (O'Sullivan 2016). Hedonic scales have been extensively

utilised in many studies to evaluate reformulated baked confectionery (Cavalcante and Silva 2015; Eslava-Zomeño et al. 2016; Giarnetti et al. 2015; Karp et al. 2016; Matsakidou, Blekas, and Paraskevopoulou 2010; Mohsen et al. 2009; Onacik-Gür et al. 2016; Serin and Sayar 2017; Wardy et al. 2018; Zahn et al. 2010). However, this type of sensory method can yield ambiguous information and can be difficult to correlate with volatile information.

Descriptive analysis is the most complete and informative tool for assessing the sensory attributes of food products (Lawless and Heymann 2010). Descriptive methodologies include; Flavour Profile Method (Caul 1957), Texture Profile Method (Brandt, Skinner, and Coleman 1963) and QDA (Stone et al. 2004). These are extensively utilised for their comprehensive evaluation of food and beverages (Murray, Delahunty, and Baxter 2001). In short, all descriptive analysis techniques involve the same principle steps. Initially, the generation of an agreed list of sensory attributes that best describe the product is developed. This is followed by panellist training; the selected attributes are defined using product references or standards, helping the assessors to distinguish clearly between attributes (O'Sullivan 2016). Subsequently, the panellists are permitted to assess the intensity of each attribute in respect to the product. Training and commitment of panellists is crucial for the success of this technique.

When trying to understand the intricate make-up of flavour, descriptive analysis used in conjunction with volatile analysis can elucidate relationships between aroma compounds and flavour perception. Utilising this strategy, Cognat et al. (2014) identified specific volatiles related to particular off-flavours perceived by panellists when monitoring oat biscuits over time, providing important information regarding product quality throughout shelf-life. Without complimenting volatile data with sensory analysis, it is impossible to know if the product continues to have approval on the market. The concentrations of volatile compounds that form the aroma fraction of bread are highly

susceptible to changes in processes and ingredients, however, combining sensory and chemical information have proven effective in characterising individual aroma profiles of similar breads (Heenan et al. 2009; Poinot et al. 2007). QDA has also been used to validate volatile information from reformulated biscuits and cookies (Pasqualone et al. 2014, Pasqualone 2015; Giarnetti et al. 2015).

In order to define a true relationship between volatile and sensory data, chemometric methods are often employed. Combining the principle concepts of multivariate statistical techniques, mathematics, and computer science, chemometrics enables important correlations to be realised between sensory attributes and volatile compounds through a simplistic, visual aid (Zielinski et al. 2014). Principal component analysis (PCA) is frequently used and attempts to identify the prominent factors (variables) that best explain the variance in a large data set (Kallithraka et al. 2001). PCA has been utilised to relate volatile compounds in different bread aroma extracts to sensory results (Poinot et al. 2007) as well as relating volatile compounds to colour data in biscuits supplemented with grape marc extract (Pasqualone et al. 2014). Partial least square (PLS) analysis another popular technique utilised to make connections between instrumental and sensory data. Depending on the information sought, PLS may be considered superior to PCA as this takes into consideration the correlation between the dependent variable and the independent variables.

Gas Chromatography-Olfactometry (GC-O) utilises the human nose as a detection device to aid in the identification of odour active fractions of a chromatograph (Wardencki, Chmiel, and Dymerski 2013). Although compounds may be present in large concentrations, it is dependent on their odour threshold whether they are relevant to the aroma quality of a product. GC-O is a pre-eminent technique for determining odour thresholds of key volatiles, but has limitations. Sensory perception is often a combination of multiple volatiles rather than individual compounds. Volatiles need to be

extracted/concentrated and therefore some compounds may be lost, underestimated or overestimated depending upon procedures used. Extraction methods, SAFE and SPME, have successfully been able to identify the odour active compounds which relate to the traditional aroma of a sponge cake (Matsakidou, Blekas, and Paraskevopoulou 2010; Pozo-Bayón et al. 2007; Rega et al. 2009). GC-O can be time consuming as human assessors require selection and training, with most approaches requiring multiple sessions (Delahunty, Eyres, and Dufour 2006; Zellner et al. 2008). However, on successful of application of this technique, the important volatiles responsible for the characteristic odour in a product can be established.

1.7 Conclusions and Future Work

Characterising the volatile aroma compounds in baked confectionery provides a basis for improving the quality of reduced fat/reduced sugar formulas. It is evident that the raw materials of baked confectionery have a major impact on flavour perception, and modification of these ingredients can have a significant impact on sensory quality. Although a small percentage of volatiles transfer directly from the raw materials, thermal degradation of components in the formula, through MR and CR reactions, generates the most potent and characterising compounds. Aldehydes, alcohols, pyrazines, ketones, and furans are by far the most prominent and potent compounds that appear to influence the sensory appeal of baked confectionery products. LO also appears to be an important contributor to the volatile profile of these products, and therefore reducing fat content or, changing lipid types, is likely to have implications for flavour perception and shelf-life. Further research is required in relation to how the sensory impact of the inclusion or exclusion of the fundamental raw materials influence the volatile profile and sensory character of baked confectionery. This challenge would be best achieved using a

chemometric approach to analyse sensory and flavour chemical data. In addition, the application of GC-O to determine the odour activity of key volatile compounds could also be useful in determining their direct impact on sensory perception and how they are influenced by production formulation changes.

1.8 References

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Chapter 2. Optimisation of HS-SPME parameters for the analysis of volatile compounds in baked confectionery products

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Abstract

Optimised extraction methods are required to better understand the impact of volatile compounds on the physical and organoleptic attributes of baked confectionery products (cakes, etc.). This is especially relevant with an increased focus on the reformulation of such products to aid in the reduction of diet-related chronic diseases. Headspace solid-phase microextraction (HS-SPME) has become one of the most widely used extraction techniques for volatile profiling of foods and beverages, mainly because it is very automatable, has a high sample throughput, is solvent-free and multiple fibre phases are available to target a wide range of volatile organic compounds. This study used response surface methodology to optimise HS-SPME parameters for the extraction of volatiles in baked confectionery products. After HS-SPME fibre selection, a central composite design was used to evaluate the effect of incubation time, extraction time and extraction temperature on 18 selected volatile compounds, representative of key volatiles in baked confectionery products, using a sponge cake crumb as the matrix. The most suitable fibre was the divinylbenzene/carboxen/polydimethylsiloxane. The results demonstrated that the final reduced models significantly ($p < 0.0001$) fitted the responses of 18 selected volatile compounds, with R2 values ranging from 0.8178 to 0.9871. The optimal conditions derived were an incubation time of 5 min, extraction time of 60 min and an extraction temperature of 60 °C. These were subsequently evaluated in three baked confectionery products, highlighting the effectiveness of this approach.

Keywords: Baked confectionery products, HS-SPME, Response surface methodology, GC-MS, Aroma

2.1 Introduction

Baked confectionery products (cakes, muffins, biscuits etc.) are consumed across all populations due to their desirable organoleptic properties. However, reformulation of these traditional 'high sugar', 'trans/saturated fat' food commodities has become a priority due to rising prevalence of chronic diseases, such as obesity and type II diabetes (Richardson et al. 2018; Silow et al. 2018). Reformulation is challenging as sugar and fat significantly contribute to the development of structure, texture, and shelf life, as well as playing a key role in creating the desired flavour and aroma. In order to comprehend how aroma is influenced by the raw materials, volatile compounds from the prominent reactions; Maillard reaction (MR), caramelisation (CR) and lipid oxidation (LO) are of interest as they are subject to modulate on reformulation of traditional recipes. Thus, having an optimised method to identify volatile compounds responsible for the desired aroma of baked confectionery products could provide useful in relation to the impact of process changes on product quality; and assist in the development of higher quality reformulated products (Chapter 1).

Volatile organic compounds responsible for aroma and flavour perception in baked confectionery products are derived from a range of chemical classes; alcohols, aldehydes, ketones, pyrazines, furans etc. with over 100 reported (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009). Volatile analysis of cake and cake-like products has been reported utilising different extraction techniques prior to gas chromatography mass spectrometry (GCMS) analysis, such as; simultaneous distillation extraction (Mohsen et al. 2009), solvent assisted flavour evaporation (SAFE) (Pozo-Bayón et al. 2007), purge and trap (Pozo-Bayón et al. 2007), and headspace solid-phase microextraction (HS-SPME) (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou et al.

2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Rega et al. 2009). HS-SPME is an attractive technique due to the simplicity of sample preparation, and the fact that it is solvent free, rapid, can be highly automated, and a range of single or multiple fibres phases are available, varying in polarity and molecular size to assist in efficient extraction of target analytes. The working principle of HS-SPME is based on obtaining equilibrium between the sample matrix and headspace, and between the headspace and fibre coating. Factors such as extraction time, extraction temperature, pH, and, sample concentration can influence the efficiency of the process (Prosen and Zupančič-Kralj, 1999). The influence of these factors will vary between sample types due to the changes in sample matrix, and therefore HS-SPME parameters need to be optimised to achieve the most comprehensive volatile profile possible for baked confectionery products.

In order to achieve precise optimisation, a copious amount of experimental runs may be required in order to assess the combined effects of a range of SPME parameters on volatile response. This can be reduced considerably by employing statistical and mathematical techniques to monitor the effect of these parameters (independent variables) on the volatile response (dependant variable). HS-SPME optimisation has been effectively achieved for various food matrices utilising response surface methodology (RSM) (Chmiel et al. 2017; Ma et al. 2013; Pérez-Palacios et al. 2012). RSM allows for the observation of the direct influence of a parameter on volatile response, but also the interaction effect of parameters on responses, thus reducing the number of experimental runs required.

Therefore the objective of this study was to develop an optimised HS-SPME extraction method for volatile analysis of baked confectionery products by GCMS. Initially the most appropriate SPME fibre was selected, an RSM approach was used to optimise HS-SPME extraction parameters, using sponge cake crumb as a test sample. The

effect of SPME fibre type, incubation time, extraction time, and extraction temperature on the extraction of 18 selected volatile compounds (Table 2.1), widely identified in baked confectionery products (Giarnetti et al. 2015; Matsakidou et al. 2010; Pasqualone et al. 2015; Pozo-Bayón et al. 2007), was explored. The optimised HS-SPME method was subsequently applied to three baked confectionery products (shortbread biscuit, sponge cake and chocolate brownie) to demonstrate its competency in comparison to published studies.

2.2 Materials and Methods

2.2.1 Sample preparation

The reference recipe of the sponge cake comprised of 400 g of plain cream flour (Odlums, Ireland), 220 g of caster sugar (Siucra, Nordzucker, Germany), 180 g of free range egg (local retailer), 180 g of cake margarine (Stork, UK), 140 g of water and 8 g of baking powder (Dr. Oetker, UK). Flour and baking powder were sifted into a bowl followed by the addition of sugar, margarine, eggs and water. The contents were mixed together using a household mixer (Kenwood Mixer, Model KMM710, UK) at minimum speed 1 for 30 seconds, and again at speed 2 for 2 min. The batter was poured into a round cake mould (12 inch) and baked at 180 °C for 40 min in a domestic convection oven (Zanussi, Bedfordshire, UK). This process was carried out in triplicate. The cakes were left to fully cool overnight at ambient temperature. The following morning, 1 cm of the outer crust of each cake was removed and the crumbs were broken down with a wooden spoon to form one homogenous bulk crumb. The bulk crumbed cake mixture was frozen at -20 °C until subsequent analysis.

For the method application part of this study, three different baked confectionery matrixes were chosen- a chocolate brownie, a shortbread biscuit and a sponge cake. The brownie product was prepared as per Richardson et al. (2018). Dark chocolate (85% cocoa, Aldi, Ireland) (175 g) and butter (Kerrygold, Ornua, Ireland) (175 g) were melted together and 250 g of caster sugar was added by and hand stirred for 1 min. Eggs (180 g) were beaten in a separate bowl and added to the mixture. Flour (115 g) was folded in gently and the mixture was stirred by hand until smooth (2 min). The batter was poured into baking trays (16.5 × 24 cm) and batches were baked for 30 min at 180 °C. The shortbread biscuits were prepared by mixing together 200 g of butter and 100 g of sugar until smooth, in a household mixer. Flour (300 g) was gently folded in until incorporated evenly to the sugar/butter mixture. The biscuit dough was compressed and rolled out to 1 cm thick, and shortbread biscuits were cut out using a cookie cutter (1 1/2 inch diameter). The shortbread biscuits were baked for 20 min at 160 °C. The sponge cake sample was produced as above. For the application part of the study, the end products were stored in an air tight container at room temperature until subsequent analysis, which took place within 24 hours after baking.

2.2.2 HS-SPME Method Development

2.2.2.1 Fibre selection

Fibre screening was carried out prior to HS-SPME optimisation. Four HS-SPME fibres; 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS), 75 μm CAR/PDMS, 85 μm Polyacrylate (PA), and 100 μm PDMS were compared for their efficacy of obtaining the most representative volatile profile of baked confectionery products. The HS-SPME fibres were exposed to 3 g of a cake crumb

(bulk batch produced as above) for 10 min incubation time, 50 min extraction time at 40 °C for each fibre, and analysed in triplicate. Fibres were conditioned according to manufacturer's instructions prior to use.

2.2.2.2 Optimisation of HS-SPME extraction parameters

RSM was employed to optimise the parameters involved in the HS-SPME method for the extraction of volatile compounds from baked confectionery products. Utilising a central composite rotatable design (CCRD, α= 1.68); the effect of incubation time (A), extraction time (B), and extraction temperature (C), on the extraction of volatile aroma compounds from a sponge cake matrix was investigated. The experimental design consisted of a 2^3-factorial design comprised of 20 experimental runs, which included 6 axial points (estimation of curvature) and 6 replicates of the centre point (estimating pure error) (Table 2.2). Data from individual responses (peak area value of compounds) were inputted into the statistical model and tested for lack of fit (ANOVA) and determination coefficient (R^2). Insignificant model terms were removed. The 'desirability function' in Design Expert allowed for the creation of one optimised method based on the maximum response of the 18 selected volatile compounds.

Table 2.1

Volatile compounds chosen for the optimisation of HS-SPME method

Chemical Class	Volatile Compound	Origin	Odour Description*
Aldehyde	Hexanal	LO	Floral, fruity, herbal grass,
	Heptanal	LO	Fresh, green, sweet, herbal
	Benzaldehyde	MR	Sweet, bitter, almond, cherry
	Phenylacetaldehyde	MR	Rose, honey, floral, sweet,
	Nonanal	LO	Aldehydic, waxy, citrus, orange
	(E,Z)-2,4-Decadienal	LO	Rice, baked, fried potato, fatty
Furan	Dihydro-2-methyl-3(2H)-furanone	MR/CR	Roasted, biscuit, hazelnut,
	2-Furanmethanol	MR/CR	Sweet caramel, burnt
	2-2-pentylfuran	MR/LO	Earthy, vegetable, beany
	Furfural	MR/CR	Sweet, woody, almond, bready
Ketone	2,3-Butanedione	MR/CR	Butter, caramel, butterscotch
	2-Pentanone	LO	Sweet, fruity, woody
Pyrazine	2,5-Dimethylpyrazine	MR	Coffee, peanut, cake crust
	2-Ethyl-5-methyl-pyrazine	MR	Herbal, earthy, potatoes,
Alcohol	1-Hexanol	LO	Cardboard, solvent, fruity
	1-Octen-3-ol	LO	Mushroom, musty, fungal,
Lactone	δ-Decalactone	RM	Coconut , fatty, buttery, milky
Terpene	d-Limonene	RM	Fresh, citrus

***Odour qualities taken from www.goodscentcompany.com

Origin of compounds from Chapter 1 & Maire et al. 2013

LO: lipid oxidation, MR: Maillard reaction, CR: carmelisation, RM: raw material

2.2.3 Volatile analysis by HS-SPME GCMS

Volatile analysis was carried out utilising a Gerstel MultiPurpose Sampler (GMPS) rail system (Anatune, Cambridge CB3 0NA, UK) connected to a Shimadzu GP2010 plus GC (Mason Technology Ltd, Dublin, Ireland). Cake crumb (3 g) was added to an amber 20 ml screw capped SPME vial (Apex Scientific Ltd, Co. Kildare, Ireland) and equilibrated for varying times (5-10 min), while exposed to heat with pulsed agitation for 5 seconds at 350 rpm using the GMPS agitator/heater. The SPME fibre was exposed to the headspace above the samples, at a depth of 21 mm, for varying incubation times (5-10 min), extraction times (20-60 min), at varying temperatures (40-60 °C), throughout the optimisation trial. The fibre was retracted, injected into the GC inlet and desorbed for 3 min at 250 °C using the GMPS fibre bakeout station. For each experimental run (Table 2.2), 3 g of cake crumb (bulk batch prepared as described earlier) was analysed in triplicate. An external standard stock solution (1-butanol, dimethyl disulphide, buty acetate, cyclohexanone) (Sigma-Aldrich,Arklow, Ireland) at 1000 ppm in methanol (Sigma-Aldrich, Ireland) was also analysed at the start and end of the sample set batch, and levels of each external standard were quantified and compared to expected values to ensure that both the SPME extraction and MS detection was performing within specification.

The GC analysis was performed on a Shimadzu 2010 Plus GC (Mason Technology Ltd, Dublin Ireland), equipped with a split/splitless injector, operating in splitless mode with a merlin microseal (Sigma-Aldrich, Wicklow, Ireland). The carrier gas was helium held at a pressure of 43.8 psi and a flow rate of 1.2 mL/min. The volatile compounds were separated on a DB-624 UI (60m x 0.32mm x 1.80μm) column (Agilent Technologies Ireland Ltd, Cork, Ireland). The temperature of the column oven was set at 40 °C, held for 5 min, increased at 5 °C/min to 230 °C then increased at 15 °C/min to

260 °C. The total GC run time was 65 min. Compound identification was carried out by a Mass Spectrometry detector-Shimadzu TQ8030 (Mason Technologies Ltd, Dublin, Ireland), ran in single quad mode. The ion source temperature was 220 °C and the interface temperature was set at 260 °C. The MS mode was electronic ionization (70eV) with the mass range scanned between m/z 35-250. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, the Shimadzu commercial library FFNSC version 2 and an in-house library created in GCMS Solutions software (Shimadzu, Japan) with target and qualifier ions and linear retention indices for each compound. Spectral de-convolution was also performed to confirm identification of compounds using AMDIS.

2.2.4 Model Validation

Validation of the model was performed by applying the optimised HS-SPME conditions to the bulk sponge cake matrix analysed in triplicate, and comparing the average response values obtained to the values predicted by the regression model. Subsequently, fifteen (replication of the centre point was removed) runs (Table 2.2) were chosen for repetition using freshly baked sponge cake.

Table 2.2

Experimental conditions applied for the optimisation of HS-SPME for baked confectionery matrices

Run	A: Incubation Time	B: Extraction Time	C: Extraction Temperature
1	11.7045	40	50
2	10	60	60
3	7.5	73.6359	50
4	5	60	60
5	7.5	40	50
6	7.5	40	50
7	7.5	40	33.1821
8	7.5	40	50
9	7.5	40	66.8179
10	10	20	60
11	5	20	40
12	7.5	40	50
13	10	60	40
14	5	20	60
15	7.5	40	50
16	7.5	40	50
17	7.5	6.36414	50
18	5	60	40
19	3.29552	40	50
20	10	20	40

2.2.5 Application of the Optimised HS-SPME Method

Once the HS-SPME GCMS method had been optimised, it was applied to three freshly baked confectionery matrices (sponge cake, shortbread biscuit and chocolate brownie), in triplicate. This was undertaken to prove the competency of the method in volatile recovery of typical baked confectionery products. The number of volatile compounds recovered were then compared to published studies on similar or related products to have an estimate of its effectiveness.

2.2.6 Statistical Analysis

The response surface methodology design and optimisation desirability function was accomplished with the aid of Design Expert Version 10 (Stat-Ease Inc. Minneapolis, MN). Statistical analysis for model validation was performed using ANOVA followed by a Tukey post hoc test to compare the difference in means. This was performed at a 0.05 alpha level, using SPSS Statistics Version 25 (SPSS, IBM, Chicago, IL, USA).

2.3 Results and discussion

2.3.1 SPME fibre screening

Of the four fibres tested, the DVB/CAR/PDMS fibre recovered the greatest abundance of volatile compounds (70) from the sponge cake matrix. Although the CAR/PDMS fibre was capable of recovering 52 compounds, and achieved good recovery of 2,3-butanedione, it was unable to recover high molecular weight aldehydes such as phenylacetaldehyde and (E,Z)-2,4-decadienal (), reported to be important to the aroma of baked confectionery products (Matsakidou et al. 2010; Pozo-Bayón et al. 2007). These results correspond with Rega et al. (2009) who also identified that the DVB/CAR/PDMS fibre was effective in extracting a greater quantity of compounds compared to the CAR/PDMS fibre, but found the CAR/PDMS fibre more suitable for extracting highly volatile compounds. The PA and PDMS fibre only recovered 27 and 20 compounds, respectively. The success obtained by the DVB/CAR/PDMS fibre is due to the chemical makeup of the various phases, a molecular sieve carboxen (CAR), polar divinylbenzene

(DVB) and non-polar polydimethylsiloxane (PDMS), and hence has the ability to target a wider range of compounds, thus this fibre was chosen for HS-SPME optimisation study.

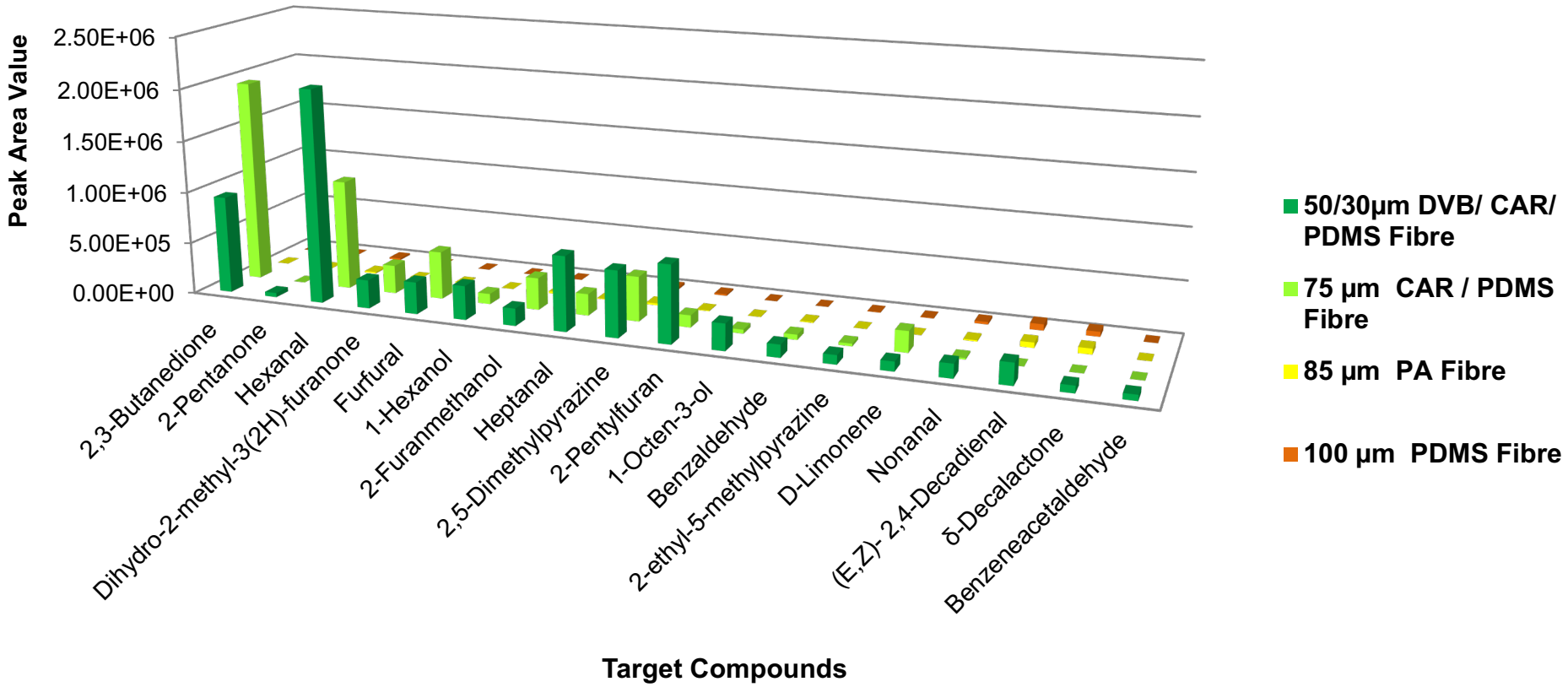


Figure 2.1

Efficacy of HS-SPME fibres on extraction of target compounds from sponge cake matrix

Average peak area values (n=3)

HS-SPME conditions: incubation time= 10 min, extraction time= 50 min, extraction temp= 40°C

2.3.2 Optimisation of HS-SPME procedure using response surface methodology

It is important that the most influential factors contributing to the successful extraction of volatile and semi-volatile compounds, from the matrix of interest, are established prior to optimisation of the HS-SPME method. This can be determined through a screening step of all factors that show potential influence on the extraction of volatiles by HS-SPME. In the case of a baked cereal matrix, there have been a number of studies involving HS-SPME GCMS analysis reported in literature (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Rega et al. 2009). Factors such as NaCl (salting out), pH, and sample amount have also been altered to recover volatile profiles in bread products (Raffo et al. 2015). However, as we were undertaking headspace analysis on solid samples in this study, we decided it was unnecessary to assess salting out or pH changes. The authors did not investigate sample amount as the volume of 3 g of crumbed sample in a 20 mL head-space vial was just below the depth of the SPME fibre after insertion and we felt that we need to ensure that we saturated the headspace with volatiles without any potential issues with fibre coming into contact with the sample. The parameters; incubation time, extraction time, and extraction temperature were evaluated for the extraction of volatile aroma compounds from the sponge cake matrix in this study.

Table 2.3
Significance of main effect, quadratic effect and interaction effect of extraction time (B) and extraction temperature (C), regression coefficient (R2) and lack of fit of final reduced models

Target Volatile Compound	Regression Equation	Main Effect		Quadratic Effect		Interaction Effect	Lack of Fit	R^2
		B	C	B2	C2	BC		
2,3-Butanedione	2102000 + 374135 * B + 607976 * C -262941 * B2 -189396 * C2	< 0.0001	< 0.0001	< 0.0001	0.0007		0.4826	0.9514
2-Pentanone	422551 + 85946.5 * B + 23507.4 * C -51628.5 * BC - 61329.3 * B2 - 36641.5 * C2	< 0.0001		0.0008	0.0233	0.0187	0.9111	0.8248
Hexanal	2561670+ 748832 * B + 892874 * C - 252297 * B2	< 0.0001	< 0.0001	0.004			0.7634	0.9362
Dihydro-2-methyl-3(2H)-furanone	235283 + 36331.8 * B + 6484.85 * C - 19190.3 * BC - 27963.3 * B2 - 30040.6 * C2	< 0.0001		0.0001	< 0.0001	0.0196	0.716	0.8812
1-Hexanol	345755 + 141744 * B + 162498 * C + 48046 * BC	< 0.0001	< 0.0001			0.0123	0.7664	0.9463
2-Furanmethanol	318121 + 129013 * B + 130818 * C + 81517.9 * BC	< 0.0001	< 0.0001			0.0002	0.4132	0.9304
Heptanal	1466000 + 376373 * B + 549193 * C + 160684 * BC - 98706.5 * B2	< 0.0001	< 0.0001	0.0047		0.0012	0.6048	0.9705
2,5-Dimethylpyrazine	831763 + 310830 * B + 405832 * C + 169432 * BC - 48533 * B2 + 44310.7 * C2	< 0.0001	< 0.0001	0.0082	0.0139	< 0.0001	0.2641	0.9871
2-2-pentylfuran	1198900 + 399745 * B + 571735 * C + 163799 * BC -100181 * B2	< 0.0001	< 0.0001	0.0111		0.0032	0.7127	0.9638
1-Octen-3-ol	428208 + 186721 * B + 259844 * C + 127385 * BC	< 0.0001	< 0.0001			< 0.0001	0.7377	0.9602
Furfural	225209 + 81422.3 * B + 92590 * C + 38905.4 * BC -15602.7 * B2	< 0.0001	< 0.0001	0.0155	0.167	0.0001	0.728	0.9741
Benzaldehyde	220312 + 85582.5 * B + 91273.2 * C + 49945.5 * BC	< 0.0001	< 0.0001			0.0004	0.228	0.9359
2-Ethyl-5-methyl-pyrazine	128134 + 48555.3 * B + 70261.7 * C + 26613.5 * BC -6906.43 * B2 + 8111.93 * C2	< 0.0001	< 0.0001	0.0299	0.0132	< 0.0001	0.5064	0.9847
d-Limonene	103261 + 61644.9 * B + 37586.3 * C + 44666.1 * BC	< 0.0001	0.0011			0.0023	0.6609	0.8178

Phenylacetaldehyde	81688.8 + 37055.3 * B + 69525.9 * C + 25867.5 * BC + 21380.6 * C2	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.4495	0.9859
Nonanal	278989 + 128152 * B + 220550 * C + 98340.6 * BC + 55618.4 * C2	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.5555	0.9773
(E,Z)-2,4-Decadienal	54448.9 + 32784.8 * B + 65852.3 * C + 28765.3 * BC + 28758.8 * C2	< 0.0001	< 0.0001	< 0.0001	0.0002	0.1505	0.9563
δ-Decalactone	29521.3 + 14626.2 * B + 35579 * C + 11001.8 * BC + 16134.5 * C2	< 0.0001	< 0.0001	< 0.0001	0.008	0.0846	0.9415

Main effect, quadratic effect and interaction effect data for incubation time (A) removed as identified as non-significant variable ($P > 0.05$)

Preliminary experiments (data not shown) were undertaken to optimise the HS-SPME method by evaluating extraction parameters individually; however, this approach does not take into account possible interactive effects, and therefore maximum volatile response may not be realised (Ma et al. 2013). The CCRD experimental results demonstrate the main, interaction and quadratic effect of the extraction parameters on volatile response (Table 2.3), generated using analysis of variance (ANOVA). The final reduced models were satisfactory ($p < 0.0001$) in explaining the variability of responses amongst the selected 18 compounds, with satisfactory determination coefficients (R^2) ranging from 0.8178-0.9871. The lack of fit was not significant ($p > 0.05$) for all compounds analysed, indicating the data fitted the regression model adequately. Response surface plots were capable of depicting the behaviour of compounds in relation to the varying extraction time and extraction temperature (Figure 2.2a &b).

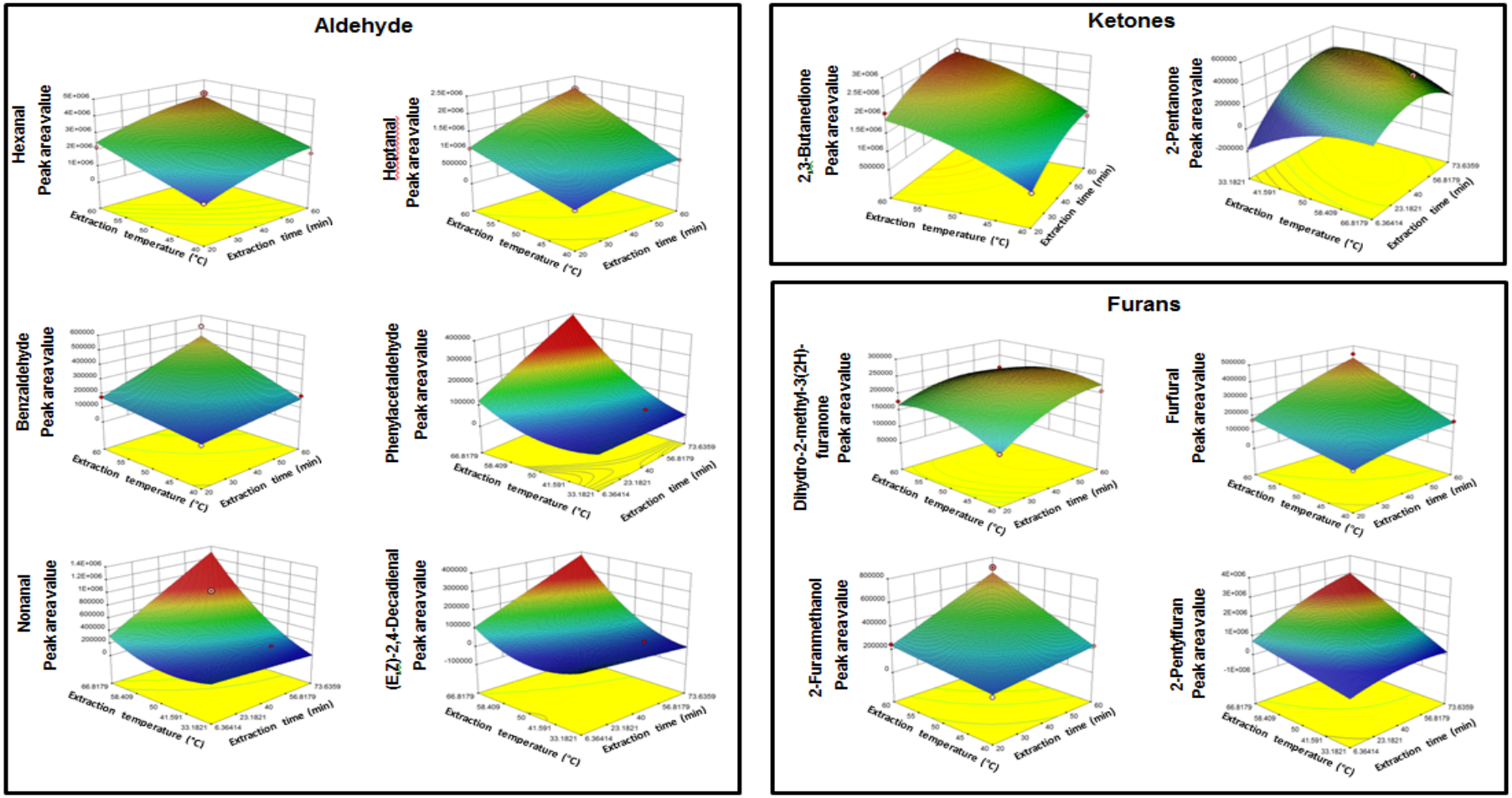


Figure 2.2a Response surface plots showing the influence of HS-SPME conditions (extraction time and extraction temperature; incubation time fixed at 5 min) on the response area value of target aldehydes, ketones and furans in the sponge cake matrix

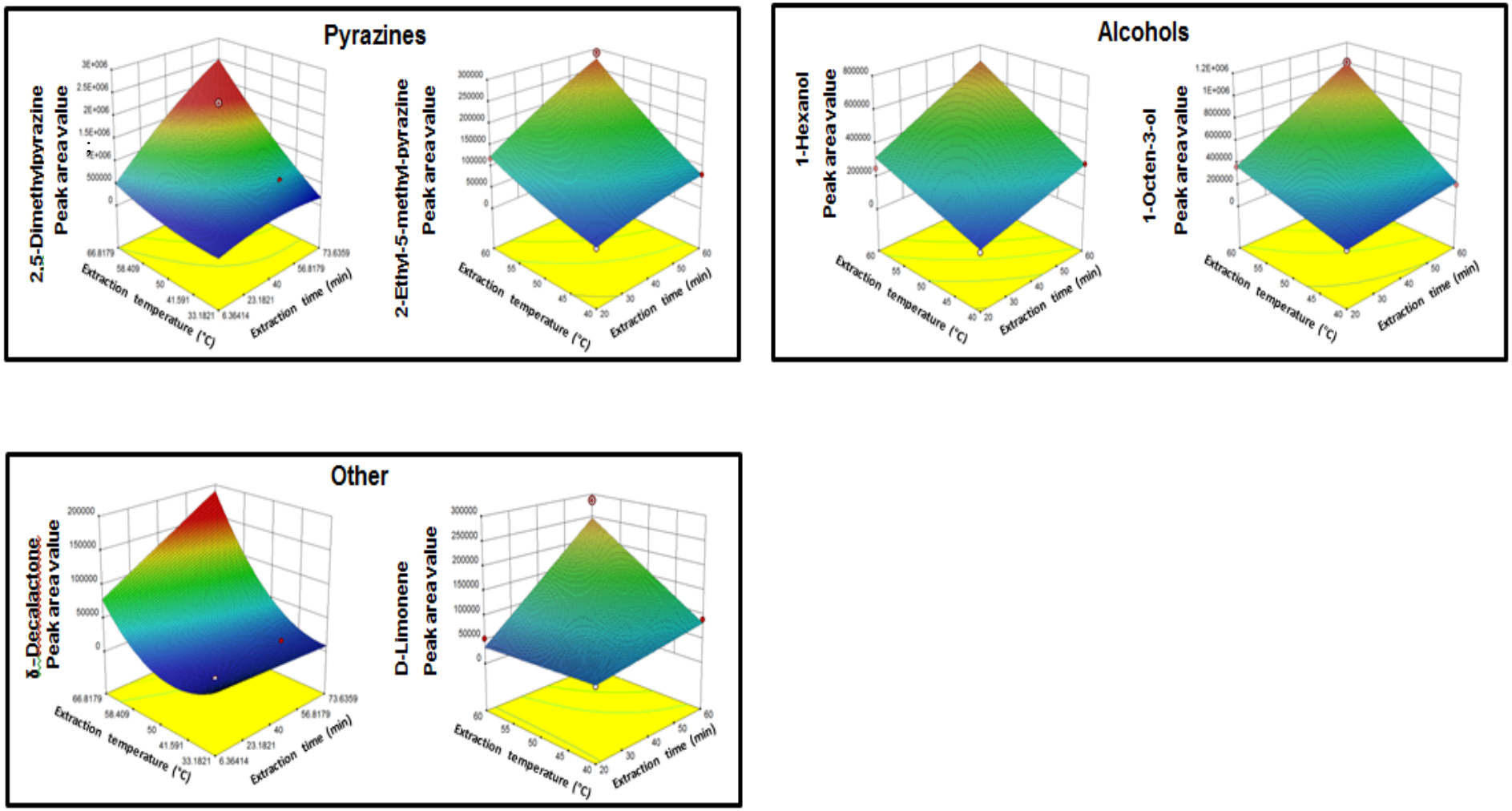


Fig 2.2b Response surface plots showing the influence of HS-SPME conditions (extraction time and extraction temperature; incubation time fixed at 5 min) on the response area value of target pyrazines, alcohols and other chemical class (lactone, terpene) in the sponge cake matrix

Incubation time is regarded as the length of time taken for volatile compounds to partition from the food matrix into the headspace. It is considered an important factor in saturating the headspace prior to compound adsorption/absorption onto the fibre (Mondello et al. 2005), and assists in compounds reaching equilibrium in the headspace, leading to a potentially greater recovery. However, incubation time (A) did not have a significant effect ($p > 0.05$) on the recovery of the 18 selected compounds from the sponge cake matrix in the time range studied, 5-10 min. In this study, the time range studied may have been insufficient to confer an effect. However, longer incubation times were applied in HS-SPME GCMS of honey, 7-23 min (da Costa et al. 2018), and beer 5-25 min (Moreira et al. 2013), without any significant effect. As this parameter demonstrated no significant effect, an incubation time of 5 min was selected. This incubation time has also been applied in a similar matrix (bread) without prior optimisation (Pico et al. 2018).

However, extraction time had the most pronounced effect on volatile extraction, with the response of all 18 selected compounds significantly ($p < 0.05$) impacted by the length of extraction time. Extraction time is important as it is the time taken for compounds to reach equilibrium on the fibre, including very volatile and semi-volatile compounds that may take longer to reach equilibrium (Prosen and Zupančič-Kralj, 1999). When the sample was exposed to varying extraction times throughout the study, the peak areas increased concurrently with extraction times (Figure 2.2a & b), for most of the 18 selected volatile compounds. Similar results were shown for volatiles in bread (Ruiz, Quilez, Mestres and Guasch, 2003).

Extraction temperature was shown to have a significant effect ($p < 0.05$) on the extraction of the majority of the 18 selected aroma compounds (Figure 2a&b). Depending on the matrix of the sample studied (i.e solid vs liquid), temperatures above ambient may

be required during HS-SPME GCMS analysis to assist the transition of compounds from the barrier of the sample matrix into the headspace, and subsequently onto the fibre (Prosen and Zupančič-Kralj, 1999). For most compounds, an extraction temperature of 60 °C or above demonstrated an increase in the response of the volatile compounds (Figure 2a &b). However, with any extraction technique, elevating temperatures increase the risk of artefact formation, particularly for furans and other compounds related to the Maillard reaction (Pérez-Palacios et al. 2012). However, previous HS-SPME studies of baked cereal products (Matsakidou et al. 2010; Raffo et al. 2015; Rega et al. 2009) have utilised temperatures of 50 °C and above, and have recovered a greater number of compounds compared to studies of utilising a temperature of 40°C and below (Giarnetti et al. 2015; Petisca et al. 2013). In addition, as sponge cakes, and other baked confectionery products, are already exposed to the extreme temperatures of baking, it is unlikely that furan formation would occur during HS-SPME extraction (Wang et al. 2017). Our study found that 2-pentanone and dihydro-2-methyl-3(2H)-furanone were not significantly influenced by extraction temperature and obtained the highest recovery at 40°C and 50°C, respectively.

The interaction effect of extraction time and extraction temperature is depicted in the experimental range by the response surface plots (Figure 2.2a &2.2b), with a fixed incubation time of 5 min. The interaction effect of these independent variables was significant ($p < 0.05$) on the volatile response of 16 of the selected 18 compounds, with no significant effect ($p > 0.05$) demonstrated on 2,3-butanedione or hexanal. This result is important as it highlights the benefit of employing RSM for optimisation. Single parameter optimisation (data not shown) was unable to achieve maximum volatile response as all experimental conditions (low temperature, long time and vice versa) were not trialled. The interaction effect of time and temperature demonstrates the efficiency

of RSM, as with just 20 experimental runs, all extraction conditions were evaluated on the ability to achieve maximum volatile response. Overall, the majority of compounds favoured a higher extraction temperature at longer extraction times. The optimised HS-SPME method was derived using the 'desirability function' on Design Expert version 10, whereby the optimum conditions for each response (volatile compound) are combined to identify a method that will achieve the highest desirability figure between 0-1, (ideally closer to 1). In this study, the optimum extraction conditions were identified as an incubation time of 5 min, an extraction time of 60 min and an extraction temperature of 60 °C, using a DVB/CAR/PDMS fibre. The optimisation desirability value of this proposed method was 0.872.

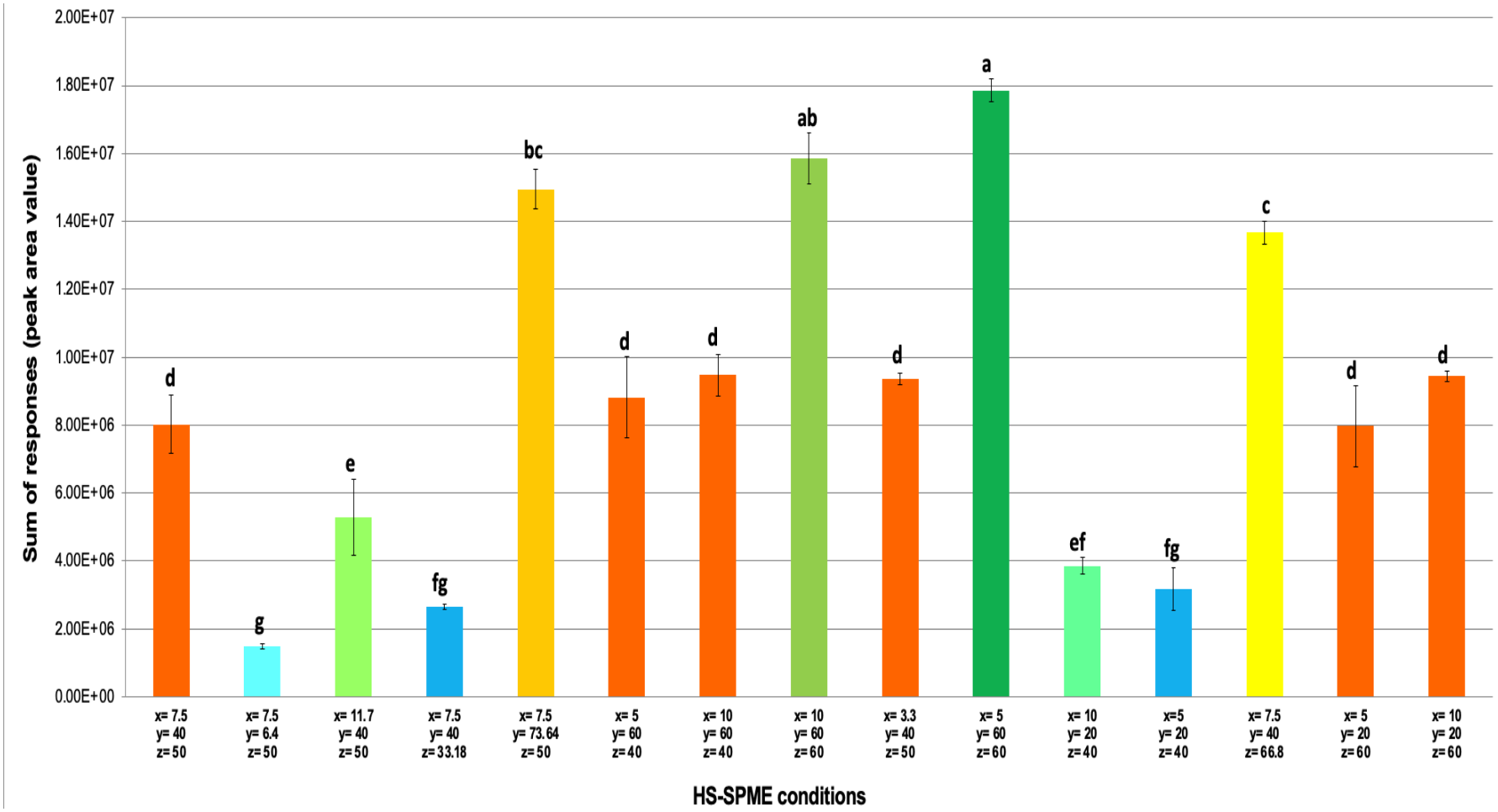


Figure 2.3 Combined peak area values of the 18 volatile compounds analysed, with the average (n= 3) of each compound peak area value used. Values with different lowercase letters are significantly different ($p < 0.05$)

x= incubation time, y= extraction time, z= extraction temperature

2.3.3 Model Validation

To validate the proposed HS-SPME model, the optimised extraction conditions (incubation time 5 min, extraction time 60 min and extraction temperature 60°C) were subsequently applied to the same bulk sponge cake sample, and analysed in triplicate. The results were compared to those predicted by the regression model and the values obtained for 2-pentanone, hexanal, furfural, hexanol, 2-furanmethanol, heptanal, 2,5-dimethylpyrazine, (E,Z)-2,4-decadienal, and δ-decalactone were within the range of predicted values (Table 2.4). However, values for 2,3-butanedione, dihydro-2-methyl-3(2H)-furanone, and, benzaldehyde were below the predicted values, whereas, 1-octen-3-ol, 2-ethyl-5-methyl-pyrazine, d-limonene, phenylacetaldehyde, and nonanal were above the predicted values (Table 2.4). Differences in real and predicated recoveries, for different chemical class compounds, are not unusual especially when dealing with headspace extractions from complex solid materials (Nongonierma et al. 2005). The efficiency of the adsorbent is impacted by the hydrophobicity, volatility and vapour pressure of the analytes and the sample/adsorbent partition coefficient, therefore responses are likely to vary more in non-homogenous solid materials, than in homogenous fluids (Spietelun et al. 2013). Another possible factor influencing recovery is the inherent instability of the product matrix. Previous work by Pico et al. 2017 demonstrated that cryogenically ground bread crumb stored at -21°C showed significant decreases in the levels of 2,3-butanedione, d-limonene, 1-octen-3-ol, benzaldehyde, and phenylacetaldehyde, over 4 weeks when analysed by solvent extraction. Jensen et al. (2011) also found that levels of nonanal and benzaldehyde increased in bread stored at ambient temperature over 3 weeks. Thus changes in volatiles may also be due to further reactions during frozen storage or thawing, such as lipid oxidation and strecker degradation reactions (Mohsen et al. 2009; Bueno et al. 2013).

Although 9 out of the 18 compounds studied achieved statistically similar levels to the predicted model, further experimental runs were required to fully validate the method. Of the 20 experimental runs devised by the RSM software, 15 (removing replication of centre point) were chosen for repetition using a freshly baked cake, to confirm the performance of the newly established HS-SPME method parameters. The results from experimental conditions are demonstrated in Figure 2.3, using the sum of the volatile peak areas, as seen in other HS-SPME optimisation studies (Moreira et al. 2013; Pérez-Palacios et al. 2012). On replication of all individual experimental conditions, the optimised parameters; incubation time of 5 min, extraction time of 60 min and extraction temperature of 60 °C, achieved a significantly ($p < 0.05$) higher volatile recovery compared to all other experimental conditions, therefore validating the defined optimal extraction conditions.

Table 2.4

Predicted values of the regression models for validation of the optimised HS-SPME method compared to actual values obtained

Compound	Predicted Peak Area Value	95% Prediction Interval low	Observed Mean (n=3)	95% Prediction Interval high
2,3-Butanedione	2631450	2366000	1887937*	2897000
2-Pentanone	382406	2860000	400405	4788000
Hexanal	3951080	3504000	3639652	4398000
Dihydro-2-methyl-3(2H)-furanone	200905	1648000	154399	2371000
Furfural	422524	3854000	459323	4593000
1-Hexanol	698044	6154000	738606	7807000
2-Furanmethanol	659470	5753000	583883	7436000
Heptanal	2134140	1937000	1985872	2332000
2,5-Dimethylpyrazine	1713640	1608000	1693876	1819000
2-pentylfuran	2234280	2005000	2056139	2464000
1-Octen-3-ol	1002160	8942000	1124044*	1110000
Benzaldehyde	447113	3929000	318358*	5014000
2-ethyl-5-methyl-pyrazine	274770	2556000	304142*	2939000
D-Limonene	247158	1874000	325845*	3069000
Phenylacetaldehyde	235518	2190000	284957*	2520000
Nonanal	778009	7214000	906908*	8346000
(E,Z)-2,4-decadienal	210610	1815000	196770	2397000
δ-Decalactone	382406	89223	94554	1245000

Average (n=3) compound values with an * did not meet regression model predictions

2.3.4 Application to baked confectionery matrices

The optimised HS-SPME GCMS method was applied to cake-like matrices to demonstrate its effectiveness. The method was applied to a freshly prepared shortbread biscuit, chocolate brownie and a sponge cake matrix in triplicate. In total 163 compounds were identified between the three matrices (Table 2.5). Among the main compounds identified were 25 aldehydes, 20 ketones, 19 esters, 18 furanic compounds and 12 pyrazines, as well as lactones, terpenes and phenols. For the sponge cake matrix, 70 compounds were identified, which is comparable to studies by Maire et al. (2013) who reported 70 volatile compounds in sponge cakes using a thicker HS-SPME fibre (75µm DVB/CAR/PDMS), and Pozo-Bayón et al. (2007) who recovered 77 compounds using SAFE which was previously shown to recover a larger quantity of compounds in comparison to HS-SPME in other studies (Murat et al. 2012; Majcher and Jeleń, 2009). Similarly, the number of volatiles identified in shortbread biscuits (99) demonstrated the efficacy of the method in comparison to previous studies which found 24 compounds in butter cookies (Giarnetti et al. 2015) and 60 and 56 in wheat biscuits, respectively (Pasqualone et al. 2014; Pasqualone et al. 2015). To our knowledge no studies have been published on the volatile profile of chocolate brownies to date. Although a direct comparison of the optimised HS-SPME extraction method to other extraction methods using the same samples was outside the scope of this study, the results are at worst comparable but typically better than published studies on similar baked confectionery matrices.

Table 2.5

Application of the HS-SPME-GC-MS method to baked confectionery matrices- Average (n=3) peak area values (x10^6) obtained for the volatile compounds identified in a chocolate brownie, biscuit and sponge cake matrix.

Compound	CAS Number	RI	REF RI	Chocolate Brownie Matrix	Shortbread Biscuit Matrix	Sponge Cake Matrix	Identification
Aldehydes							
Acetaldehyde	75-07-0	453	454.9	1.19	0.65	0.097	MS, RI
2-Propenal	107-02-8	522	524.9	0.052	0.079	n.d	MS, RI
Propanal	123-38-6	526	528.9	0.054	0.024	n.d	MS, RI
2-Methylpropanal	78-84-2	591	594.2	1.41	0.21	0.031	MS, RI
3-Methylbutanal	590-86-3	689	692.7	12.1	2.20	0.32	MS, RI
2-Methylbutanal	96-17-3	697	700.9	3.98	2.05	0.61	MS, RI
Pentanal	110-62-3	733	737.3	n.d	n.d	0.25	MS, RI
(E)-2-Pentenal	1576-87-0	803		n.d	n.d	0.10	MS
Hexanal	66-25-1	835	839.4	1.81	2.74	2.84	MS, RI
(E)-2-Hexenal	6728-26-3	901		n.d	0.80	n.d	MS
Heptanal	111-71-7	937	942.8	0.66	0.81	1.08	MS, RI
(E)-2-Nonenal	18829-56-6	967		0.44	n.d	n.d	MS
Benzaldehyde	100-52-7	1024	1030.3	7.66	1.41	0.17	MS, RI
Octanal	124-13-0	1040	1046.2	0.85	0.94	0.15	MS, RI
Phenylacetaldehyde	122-78-1	1112	1118.6	18.2	4.08	0.14	MS, RI
Nonanal	124-19-6	1142	1145.2	2.20	2.32	0.57	MS, RI
(E)-2-Nonenal	18829-56-6	1218		n.d	n.d	0.10	MS
Decanal	112-31-2	1245	1253.1	0.22	0.12	0.06	MS, RI
2,4-Nonadienal	6750-03-4	1284		n.d	n.d	0.017	MS
(2E)-2-Decenal	3913-81-3	1319		0.34	0.27	n.d	MS
2,4-Decadienal	2363-88-4	1363		n.d	n.d	0.17	MS
(E,Z)-2,4-Decadienal	25152-84-5	1386		0.34	0.35	0.16	MS

2-Undecenal	2463-77-6	1422		n.d	0.14	n.d	MS
Dodecanal	112-54-9	1449		n.d	0.05	n.d	MS
Vanillin	121-33-5	1543		n.d	0.03	n.d	MS
Alcohols							
Ethanol	64-17-5	506	508.4	0.20	0.16	0.026	MS, RI
(R)-2-Butanol	14898-79-4	644		0.04	n.d	0.02	MS
2-Methyl-3-buten-2-ol	115-18-4	655		0.02	n.d	n.d	MS
1-Butanol	71-36-3	710	715.4	n.d	0.08	n.d	MS, RI
1-Penten-3-ol	616-25-1	729		n.d	n.d	0.09	MS
(R)-(-)-2-Pentanol	31087-44-2	740		0.14	n.d	n.d	MS
3-Methyl-1-butanol	123-51-3	779		n.d	0.03	n.d	MS
2-Hexanol	626-93-7	785		1.47	n.d	n.d	MS
1-Pentanol	71-41-0	811	816.6	0.15	0.28	1.17	MS, RI
2,3-Butanediol	19132-06-0	863	862.5	9.64	n.d	n.d	MS, RI
3-Hexen-1-ol	544-12-7	905		n.d	n.d	0.09	MS
1-Hexanol	111-27-3	909	915.6	n.d	0.85	1.84	MS, RI
2-Propylheptanol	10042-59-8	1010		0.33	n.d	n.d	MS
1-Octen-3-ol	3391-86-4	1019		n.d	n.d	0.89	MS
2-Ethylhexanol	104-76-7	1070	1076.3	0.78	0.50	0.16	MS, RI
1-Undecanol	112-42-5	1288		n.d	0.14	n.d	MS
Ketones							
Acetone	67-64-1	531	534.3	2.19	0.94	0.12	MS, RI
2,3-Butanedione	431-03-8	628	631.7	2.95	1.30	0.74	MS, RI
2-Butanone	78-93-3	635	638.8	1.05	0.43	0.52	MS, RI
2-Pentanone	107-87-9	725	729.6	4.51	2.65	0.17	MS, RI
2,3-Pentanedione	600-14-6	733		n.d	n.d	0.23	MS
1-Hydroxy-2-propanone	116-09-6	731	735.3	11.6	46.6	0.23	MS, RI
Acetoin	513-86-0	774	778.1	6.23	0.84	0.46	MS, RI
2-Hexanone	591-78-6	827	832.8	n.d	0.64	0.23	MS, RI

1-Hydroxy-2-butanone	5077-67-8	832		0.42	0.30	n.d	MS
2-Hydroxy-3-pentanone	5704-20-1	869		0.75	n.d	n.d	MS
2-Heptanone	110-43-0	928	934.6	40.7	51.3	12.5	MS, RI
2,3-Hexanedione	3848-24-6	982		0.41	n.d	n.d	MS
4-Methyl-2-heptanone	6137-06-0	1032	1038.6	n.d	n.d	2.60	MS, RI
(3E,5E) -Octadien-2-one	30086-02-3	1127		n.d	n.d	0.01	MS
2-Nonanone	821-55-6	1132	1138.9	13.3	35.5	n.d	MS, RI
Acetophenone	98-86-2	1137	1144	0.66	n.d	n.d	MS, RI
3,5-Octadien-2-one	38284-27-4	1155		n.d	n.d	0.04	MS
2-Undecanone	112-12-9	1335	1344.2	3.17	12.5	n.d	MS, RI
2-Dodecanone	6175-49-1	1436		n.d	0.08	n.d	MS
2-Tridecanone	593-08-8	1553		0.67	2.71	0.04	MS
Furans							
Furan	110-00-9	517	519.7	0.02	n.d	n.d	MS, RI
2-Methylfuran	534-22-5	620	623.1	0.04	0.13	0.006	MS, RI
2-Ethylfuran	3208-16-0	715	720	0.03	0.02	0.02	MS, RI
2,5-Dimethylfuran	625-86-5	720		0.02	0.02	n.d	MS
2-Vinylfuran	1487-18-9	799		n.d	0.10	0.05	MS
Dihydro-2-methyl-3(2H)-furanone	3188-00-9	852		0.62	0.29	n.d	MS
Furfural	98-01-1	894	898.5	6.21	51.7	10.7	MS, RI
2-Butylfuran	4466-24-4	906	911.6	0.08	0.07	0.03	MS, RI
2-Furanmethanol	98-00-0	921		4.15	22.2	0.05	MS
Acetylfuran	1192-62-7	971		0.01	2.95	n.d	MS
2-2-pentylfuran	3777-69-3	1006	1012.4	1.96	1.81	1.98	MS, RI
5-Methyl-2-furfuryl alcohol	3857-25-8	1011		n.d	0.50	n.d	MS
Butyrolactone	96-48-0	1020	1026.3	5.40	n.d	n.d	MS, RI
2(5H)-Furanone	497-23-4	1023	1029.5	1.54	6.23	n.d	MS, RI

Furyl hydroxymethyl ketone	17678-19-2	1173		n.d	26.2	n.d	MS
4-Methyl-5H-furan-2-one	6124-79-4	1178		0.25	25.5	n.d	MS
4-Hydroxydihydro-2(3H)-furanone	5469-16-9	1356		n.d	0.64	n.d	MS
5-Hydroxymethylfurfural	67-47-0	1364		0.16	25.5	n.d	MS
Pyrazines							
Pyrazine	290-37-9	769	773.2	0.31	0.27	n.d	MS, RI
Methylpyrazine	109-08-0	858		5.38	n.d	0.20	
2,5-dimethylpyrazine	123-32-0	945	950.3	7.97	2.59	0.54	MS, RI
2,3-Dimethylpyrazine	5910-89-4	955	961.1	1.74	0.33	n.d	MS, RI
2-Ethyl-6-methylpyrazine	13925-03-6	1031		1.40	n.d	0.70	MS
Trimethylpyrazine	14667-55-1	1035	1041	7.72	0.50	n.d	MS, RI
2,5-Dimethyl-3-ethylpyrazine	13360-65-1	1108	114.5	2.18	n.d	n.d	MS, RI
Tetramethylpyrazine	1124-11-4	1116	1122.6	13.4	24.3	n.d	MS, RI
2-Ethyl-3,5-dimethylpyrazine	13925-07-0	1117		1.36	n.d	n.d	MS
2,5-Diethylpyrazine	13238-84-1	1122		0.85	n.d	n.d	MS
2-Ethyl-3,5,6-trimethylpyrazine	17398-16-2	1184	1192.1	1.43	n.d	n.d	MS, RI
2,5-Dimethyl-3-isoamylpyrazine	18433-98-2	1346		0.37	n.d	n.d	MS
Acids							
Acetic acid	64-19-7	686	690.4	75.5	26.4	0.04	MS, RI
Propanoic Acid	79-09-4	773	778.4	n.d	0.18	n.d	MS, RI
Butanoic acid	107-92-6	857	862.5	2.67	5.17	n.d	MS, RI
3-Methylbutanoic acid	503-74-2	910	916	19.3	n.d	n.d	MS, RI
2-Methylbutanoic acid	116-53-0	917		4.17	n.d	n.d	MS
Hexanoic acid	142-62-1	1043	1049.6	2.68	5.39	0.05	MS, RI
Octanoic acid	124-07-2	1234	1242.1	0.56	0.88	n.d	MS, RI

Esters							
Ethyl ether	60-29-7	515	517.3	0.07	0.17	0.007	MS, RI
Acetic acid, methyl ester	79-20-9	552		0.20	0.05	n.d	MS
Vinyl acetate	108-05-4	608		0.03	0.07	n.d	MS
Ethyl Acetate	141-78-6	639	642.6	0.07	0.01	n.d	MS, RI
Propanoic acid, 2-methyl-	79-31-2	829		4.36	n.d	n.d	MS
Allyl butanoate	2051-78-7	881		n.d	0.05	n.d	MS
Ethylbenzene	100-41-4	888	892.9	n.d	n.d	0.02	MS, RI
Isoamyl acetate	123-92-2	897	902.5	0.32	n.d	n.d	MS, RI
2-Methylbutyl acetate	624-41-9	901	906.1	0.07	n.d	n.d	MS, RI
Isovaleric acid	503-74-2	909	914.5	n.d	0.31	n.d	MS, RI
1-Methoxy-2-propyl acetate	108-65-6	991		4.05	n.d	n.d	MS
Isobutylacetic acid	646-07-1	1014		0.24	n.d	n.d	MS
Ethylhexanoic acid	149-57-5	1178		n.d	n.d	0.02	MS
Ethyl octanoate	106-32-1	1213	1222	0.23	n.d	n.d	MS, RI
Ethyl benzoate	93-89-0	1223	1231.8	0.01	n.d	n.d	MS, RI
Ethyl benzeneacetate	101-97-3	1296	1305.1	0.31	n.d	n.d	MS, RI
β-Phenethyl acetate	103-45-7	1311	1320.7	1.20	n.d	n.d	MS, RI
Ethyl dodecanoate	106-33-2	1659		5.60E+04	n.d	n.d	MS
Lactones							
α-Angelica lactone	591-12-8	935		n.d	0.61	n.d	MS
Butyrolactone	96-48-0	928		n.d	1.55	n.d	MS
δ-Caprolactone	823-22-3	1213		n.d	2.92	n.d	MS
δ-Hexalactone	66-25-1	1214		1.34	n.d	n.d	MS
γ-Heptalactone	105-21-5	1265	1273.7	n.d	0.19	n.d	MS, RI
Caprolactone	502-44-3	1269		n.d	0.05	n.d	MS
γ-Undecalactone (peach lactone)	104-67-6	1373		n.d	0.19	n.d	MS

γ-Octalactone	104-50-7	1374		0.23	n.d	n.d	MS
δ-Octalactone	124-13-0	1410		0.45	0.78	n.d	MS
γ-Nonalactone	104-61-0	1483	1492.9	0.25	0.18	n.d	MS, RI
δ-Nonalactone	3301-94-8	1527		n.d	0.02	n.d	MS
δ-Decalactone	705-86-2	1685	1620.9	8.83E+05	1.70	0.11	MS, RI
Terpenes							
o-Xylene*	95-47-6	886	891.4	n.d	0.18	n.d	MS, RI
p-Xylene*	106-42-3	923	928.1	0.20	0.28	n.d	MS, RI
Styrene	100-42-5	923	929.2	n.d	0.08	n.d	MS, RI
α-Pinene	80-56-8	950	955.6	1.17	1.07	0.18	MS, RI
β-Pinene*	127-91-3	998		0.18	n.d	n.d	MS
Sabinene	13466-78-9	999		0.17	n.d	n.d	MS
3-Carene	13466-78-9	1028	1034.4	0.87	n.d	n.d	MS, RI
d-Limonene	5989-27-5	1047	1053.9	1.24	1.90	0.06	MS, RI
o-Cymene*	527-84-4	1051	1057.1	0.37	0.74	0.28	MS, RI
Linalool	78-70-6	1138	1145.2	0.46	0.30	n.d	MS, RI
Phenols							
Phenol	13127-88-3	1088	1093.9	0.77	n.d	n.d	MS, RI
2-Methoxy-4-vinylphenol	7786-61-0	1400	1410.6	0.04	0.20	0.05	MS, RI
2,4-Di-tert-butylphenol	96-76-4	1624		0.51	0.60	n.d	MS
Other							
Carbon disulfide	75-15-0	545	548.3	0.97	11.3	0.53	MS, RI
Toluene	108-88-3	790	794.4	1.10	0.31	0.11	MS, RI
Methyl acetylacetate	105-45-3	936		n.d	n.d	8.45	MS
1-Octene	111-66-0	901		0.04	n.d	n.d	MS
2-Acetylthiazole	24295-03-2	1080		n.d	n.d	0.06	MS
Corylon (Maple Lactone)	80-71-7	1098	1105	0.36	n.d	n.d	MS, RI
Glycerin	56-81-5	1105		4.11	7.82	n.d	MS

2-Acetylpyrrole	1072-83-9	1148		2.76	n.d	n.d	MS
2-Pyrrolidinone (γ-Butyrolactam)	616-45-5	1185	1193.4	1.71	n.d	n.d	MS, RI
2-Phenylethanol	60-12-8	1193	1201.2	6.12	2.38	n.d	MS, RI
Maltol	118-71-8	1196		0.91	4.90	n.d	MS
3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	28564-83-2	1236		0.71	12.7	n.d	MS
2-Phenyl-2-butenal	4411-89-6	1358		0.27	n.d	n.d	MS
2,5-Dihydrothiophene	1708-32-3	1381		0.07	n.d	n.d	MS
5,6-Dihydro-2H-pyran-2-one	3393-45-1	1393		0.66	n.d	n.d	MS
Glycerine diacetate	102-62-5	1405		n.d	0.03	n.d	MS
6-Pentyl-5,6-dihydro-2H-pyran-2-one	54814-64-1	1662		0.23	n.d	n.d	MS

2.4. Conclusion

Application of RSM enabled the optimisation of an HS-SPME method to extract a range of aromatic volatiles from baked confectionery products. Fibre type, extraction time, and extraction temperature were shown to have the most pronounced effect on the extraction of 18 selected volatile compounds from a sponge cake matrix. The optimal and validated conditions derived for HS-SPME analysis of baked confectionery were an incubation time of 5 min, an extraction time of 60 min at an extraction temperature of 60°C. This method is suitable to study the volatile profile of a wide range of baked confectionery products.

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Chapter 3. Characterising the sensory quality and volatile aroma profile of clean-label sucrose reduced sponge cakes

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Abstract

The sensory and aroma quality of 30% (w/w) sucrose reduced sponge cakes incorporating clean-label replacers were investigated. The sensory quality of the reformulated sponge cakes varied, with those containing apple pomace powder (APP) showing the greatest difference to the control (SC100). Volatile profiles mainly differed in relation to compounds derived from the Maillard reaction, caramelisation and lipid oxidation. Thirty six aroma active volatile compounds were identified in the SC100, APP and oligofructose (OLIGO) sponge cakes by olfactometry. Furfural 'spicy bready' contributed most to the overall aroma of all samples, with factor dilution values differing the most for heptanal 'fatty cake crust', methional 'potato damp', and 2,5-dimethylpyrazine 'cake crust, nutty'. This study provides an in-depth insight into the impact of sugar reduction reformulation on the sensory perception of sponge cakes and demonstrates how this approach can be used to improve the sensory perception of reduced sucrose sponge cakes.

Keywords:

Reformulation, Odour active, Olfactometry, by-products, volatiles

3.1 Introduction

With increasing awareness of dietary sugar intake and associated chronic diseases (obesity and type II diabetes), there is a need to reformulate foods to reduce the refined sugar content. Baked confectionery products are a prime matrix to explore alternative sucrose replacers due to the critical functionality of sucrose to the desirable structure and organoleptic properties. Sucrose replacement in these products has been extensively reviewed (Chapter 1; Struck, Jaros, Brennan, & Rohm, 2014). Trends of sucrose reduction/replacement in the past mainly consisted of the incorporation of artificial sweeteners and/ or sugar alcohols (polyols), due to their ability to mimic sucrose in terms of functionality and sweetness (Martínez-Cervera, Salvador, & Sanz, 2014; Ronda, Gómez, Blanco, & Caballero, 2005; Zoulias, Piknis, & Oreopoulou, 2000). However, current trends are shifting towards a more 'clean' mechanism of sucrose reduction, as manufacturers include statements such as 'free from artificial additives' on their products to evoke consumer interest and purchase intent (Asioli et al., 2017).

Although no concrete definition for clean-label ingredients/ products is established, foods of natural origin, organic, or free from additives/ preservatives are generally deemed superior by consumers. Similarly, by-products which contribute specific functional properties (e.g. antioxidant, added fibre) are also highly regarded by consumers due to the attraction of sustainable production practises and potential health benefits (Pasqualone et al., 2014). There has been a wide spectrum of studies involving the use of clean-label ingredients; OptiSol™, a natural ingredient derived from flaxseed, was used to replace 30% fat and was found to be capable of producing a clean-label sponge cake with similar physiochemical and sensory properties to that of a control sponge cake (Eslava-Zomeño, Quiles, & Hernando, 2016). Steviol glycosides, from Stevia rebaudioside, were combined with inulin or polydextrose to replace 30% sucrose in a

muffin with the resulting products providing similar sensory attributes to a control without sucrose reduction (Zahn, Forker, Krügel, & Rohm, 2013). Fructans such as inulin and oligofructose are also widely used as functional and clean-label ingredients due to their prebiotic classification and are found naturally in the chicory plant. Fructans, due to their level of sweetness, also have potential as sucrose replacers in products. Volpini-Rapina, Sokei, and Conti-Silva (2012) added an Orafti® Synergy1 ingredient, an inulin/ oligofructose mixture, to orange cakes and found it impacted on the crust colour, dough colour and cake hardness, but did not affect the 'orange aroma', 'orange flavour', or 'sweet taste'. Consumers also preferred the reformulated cake over a commercial cake. Following the addition of the same ingredient to gluten-free chocolate cookies, da Silva and Conti-Silva (2018) reported an increase in 'caramel aroma', 'chocolate aroma', 'sweet taste' and 'caramel flavour' with increasing replacement of rice flour on a w/w basis. This indicates the potential of fructans as a clean-label sucrose replacement ingredient to positively influence the aroma and flavour of baked confectionery products. As stated, food industry by-products are an attractive option as sucrose replacers due to the sustainability of utilising and reducing food waste, the potential of contributing added nutritional value, and the fact they are clean-label. Fruit pomaces are by-products of juice/ alcoholic beverage processing and are comprised mainly of peel and seeds, contributing additional advantages of added fibre and possible antioxidant activity (Ktenioudaki & Gallagher, 2012). The application of apple pomace powder as an ingredient in bakery products has been successfully achieved for biscuits (Alongi, Melchior, & Anese, 2019) and sponge cakes (Sudha, Baskaran, & Leelavathi, 2007).

Sucrose imparts a clean, sweet taste appreciated by consumers and previous studies exploring 100% sucrose replacement in baked confectionery frequently report a decline in sensory quality (Ronda et al., 2005). Identification of odour active aroma

compounds in reduced sucrose products, in comparison to a standard sucrose control product, is likely to provide important information on factors impacting sensory quality. For example, Pasqualone et al. (2014) identified higher levels of 'favourable' biscuit compounds; benzaldehyde (cherry/almond), phenylacetaldehyde (floral/honey) and furans; 2-methylfuran, 2-acetylfuran, 5-methylfurfural and 2-furanmethanol (sweet/caramel), after the addition of grape marc (a by-product of wine fermentation). Correlating sensory and volatile data aids in establishing how raw materials influence aroma and thus the flavour of baked confectionery products. This approach in theory, can aid in the development of optimal reduced sugar products from a consumer perspective by potentially manipulating ingredients to evoke a desired sensory response or by eliminating or reducing mal-odours. This is particularly relevant for products of the Maillard reaction (MR) and caramelisation (CR), as they are important to the overall aroma of baked confectionery products (Chapter 1). Gas chromatography-olfactometry (GC-O) is a technique utilised to aid in the identification of aroma compounds that are actually contributing to sensory perception, and thus can provide very useful additional information in comparative studies. Although the volatile profiles of different baked confectionery products have been explored, to the best of our knowledge, no studies on how sucrose reduction/replacement impacts the volatile aroma profile of sponge cakes have been published.

Therefore, the objective of this study was to explore the impact of clean-label sucrose alternatives (apple pomace, whey permeate, and oligofrucrose) on the sensory properties and volatile profile of 30% w/w reduced sucrose sponge cakes. To achieve this we used an optimised headspace solid-phase microextraction gas-chromatography mass pectrometry (HS-SPME-GC–MS) method to determine their volatile profile

(Chapter 2), with GC-O and ranking descriptive analysis (RDA) to determine key volatile changes that influence sensory perception.

3.1 Materials and methods

3.2.1 Analytical Standards

Olfactometry training standards were of analytical grade; ethyl butyrate, octanal, p-cresol, and dimethyl disulphide and heptanal of ≥99% and ≥95% purity respectively (Merck Ireland, Arklow, Co. Wicklow, Ireland), were prepared at 0.3% (w/v) in methanol and stored at −18 °C until required. For each GC-O training session, a stock solution was diluted to 0.03% (w/v) in distilled water to allow the odours to be of adequate potency.

External standards used to confirm the identity of odour active compounds were also of analytical grade; linalool, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol), 2-nonanone, and 2-methylpyrazine (Merck Ireland). For linalool, 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 2-nonanone, a 0.3% (w/v) solution was prepared in methanol, with a 0.5% (w/v) solution in methanol prepared for 2-methylpyrazine. As above, for adequate potency, these compounds were analysed at 0.03% (w/v) and 0.05% (w/v) in distilled water, respectively.

3.2.2. Sponge cake preparation

Five sponge cake formulations and baking conditions described by Milner, Kerry, O'Sullivan, and Gallagher (2020) were employed. A number of preliminary baking trials, involving incremental reduction of sucrose levels, with the aim of maximum sucrose reduction without adverse changes to sponge cake structure were undertaken. Physiochemical analysis was carried out on the resulting products and the ideal sucrose replacement level was established at 30%, which enables these products to be classified as reduced sugar. A sponge cake formula with 30% reduced sugar was referred to as sucrose reduction 70% (SR70). This formed the base formulation for the clean label reduced sucrose sponge cakes (replaced by 5% replacer on a flour weight basis). The sucrose replacers incorporated into this base sponge cake formula were apple pomace powder (APP), whey permeate powder (WPP), and oligofrucrose (OLIGO). The control sponge cake (100% sucrose), was referred to as SC100. Plain flour (200 g) (Odlums, Ireland) and baking powder (4 g) (Dr. Oekter, UK) were sifted into a bowl followed by the addition of caster sugar (110 g for control, 77 g for sucrose reduced) (Súcra, Nordzucker, Germany), sucrose replacer (10 g), cake margarine (90 g) (Stork, UK), free range eggs (90 g) (local retailer), and water (70 g). The contents were mixed using a household mixer (Kenwood Mixer, Model KMM710, UK) at speed 1 for 30 s, scraped and mixed again for a further 2 min at speed 2. Miniature loaf tins (80 mm × 60 mm × 40 mm) were filled with 80 g of cake batter and baked at 180 °C for 45 min in a domestic convection oven (Zanussi, Bedfordshire, UK). The cakes were left to cool and placed in sealed storage bags until subsequent volatile, GC-O or sensory analysis (which took place within 24 h after baking).

3.2.3. Sensory evaluation

Hedonic sensory evaluation and ranking descriptive analysis were carried out with 30 consumers (female = 63%, male= 37%, age range = 22- 50) recruited from Teagasc Food Research Centre (Moorepark, Ireland). Panellists were chosen based on their frequency to consume sponge cake and familiarity with sensory evaluation, but did not receive formal training. Evaluation took place in accordance to international standards (ISO 11136, 2014), where panellists were presented with the five sponge cake formulations simultaneously. Samples were presented on white paper plates with randomised three digit codes assigned to each sample, alongside water and a saltine cracker for palate cleansing. Panellists were asked to rate their liking of each sample based on the colour, odour, flavour, texture and overall acceptability on a nine-point hedonic scale, which ranged from "9 = extremely like" to "1 = extremely dislike". Once the hedonic portion of the evaluation was completed for a sample, panellists were prompted to evaluate the same sample cake on its sensory attributes, using a ranking descriptive analysis (RDA) method. Attributes were generated by a focus group consisting of 7 people, comprised of members from the Food Quality and Sensory Science department at the Teagasc Food Research Centre. The established list of attributes was chosen based on their relevance to sponge cakes, whilst having descriptors targeted at profiling the formulated samples. Panellists were briefly coached on the explanation of each attribute in relevance to sponge cake, and asked to evaluate the intensity of each on a 9 cm continuous scale. Sensory analysis was conducted in duplicate over two separate occasions.

3.2.4. Volatile analysis

Volatile analysis was carried out as described Chapter 2. Each sponge cake was sliced vertically and 1 cm of the outer crust was removed. Cake crumb (3 g) was added to an amber 20 mL screw capped headspace vial (Apex Scientific Ltd, Co.Kildare, Ireland) and equilibrated for 5 min, at 60 °C with pulsed agitation for 5 s at 350 rpm, using the Gerstal MultiPurpose Sampler (GMPS) agitator/ heater. Volatile analysis was carried out utilising a GMPS rail system (Anatune, Cambridge CB3 0NA, UK) connected to a Shimadzu GP2010 plus gas chromatograph (GC) (Mason Technology Ltd, Dublin, Ireland) using headspace solid-phase microextraction (HS-SPME). The SPME fibre; 30/50 μm DVB/CAR/PDMS (Supelco), was exposed to the headspace above the samples, at a depth of 21 mm, for 60 min at 60 °C. The fibre was retracted, injected into the GC inlet and desorbed for 3 min at 250 °C using the GMPS fibre bakeout station. Each sponge cake formula was analysed in triplicate.

3.2.5. Identification of odour active compounds by gas chromatography-olfactometry

HS-SPME-GC-O analysis was only undertaken on APP and OLIGO sponge cakes, and compared to the control (SC100). APP was chosen as it demonstrated obvious contrasts in sensory results, whereas OLIGO showed similarities to both APP and SC100 sponge cakes. Sponge cake samples were sliced and blitzed in a food processor (NutriBullet 600, Australia) to combine cake crust and crumb uniformly to represent a masticated sample. Three sniffer assessors were chosen for the GC-O analysis based on their performance in an olfactometry assessment using three different Sniffin' Sticks tests (identification, discrimination, and threshold) (Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997). Prior to sample analysis, the panellists were exposed to a standard stock solution

(as described in 3.2.1), designed for GC-O training, comprised of 5 compounds; dimethyl disulphide ('sulphur','decomposing'), ethyl butyrate ('fruity', 'pineapple'), heptanal ('fatty', 'green'), octanal ('orange', 'fruity') and ϱ-cresol ('barnyard'). This step allowed panellists to familiarise themselves with the GC-O process and software, as well as the range of odours they could potentially encounter during the GC-O analysis of sponge cake samples. Multiple batches (a minimum of three) of each freshly baked sponge cake formula were produced as required. Volatile extraction was carried out by the HS-SPME procedure (as previously described) using 3 g of the crumb and crust mixture. GC-O analyses was performed on an Agilent 7890 GC with a flame ionization detector, 5973N mass detector (Agilent Technologies, Ltd, Cork, Ireland), and an Gerstel ODP-3 olfactometry detector port (Anatune Ltd, Cambridge, UK). The volatile compounds were separated on DB-624 UI (20 m × 1.8 mm × 1 μm) (Agilent Technologies Ltd, Ireland) column. Eluting compounds were split 1:1:1 into the MS detector, flame ionisation detector and the sniffing port simultaneously by means of a column flow splitter. The carrier gas was helium, held at a pressure of 9.8 psi and a flow rate of 1.209 mL min−1. GC conditions consisted of an initial oven temperature of 80 °C, held for 2 min and increased at 10 °C/min to 220 °C. The GC run time was shortened to 21 min to reduce the risk of assessors experiencing fatigue during a sniffing session (however it still encompassed the volatile range of interest). In addition, the transfer line to the sniffing port was conditioned with humidified air to reduce olfactometry fatigue and prevent the occurrence of condensation droplets collecting in the nasal port. Panellists conducted GC-O analysis of each sponge cake in duplicate. The ion source temperature was 220 °C and the interface temperature was set at 260 °C. The MS mode was electronic ionization (70 eV) with the mass range scanned between m/z 35–250. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, comparison of LRI to the mid polar column from the previous analysis and to standards where possible.

Spectral de-convolution was also performed to confirm identification of compounds using AMDIS. If an aroma was detected by at least 3 out of 6 assessments, it was established as odour active (Koutidou, Grauwet, Van Loey, & Acharya, 2017). To determine the threshold at which each odour active compound could be perceived, Aroma Extraction Dilution Analysis (AEDA) was carried out by manipulation of the GC injection split ratio (Feng et al., 2015). The operating mode of GC analysis was changed from splitless to split injection and the split ratio was adjusted to 1:1, 1:2, 1:5, 1:10, 1:20, 1:50, 1:100 and 1:150, allowing for adequate dilution to determine the most odour active compounds in the sample. Undertaking AEDA using the splitless approach to dilute removes any potential matrix effects that can occur if the sample itself was diluted. The assessor who demonstrated the highest olfactometry perception in the previous analysis was chosen for the AEDA study. The last split ratio at which a compound could be detected was referred to as the factor dilution (FD) for that compound.

3.2.6. Statistical analysis

Data analysis was handled accordingly based on the normality of the data. Hedonic scale data was analysed using Welch test with post hoc Games-Howell. Analysis of variance (ANOVA) with post hoc Tukey significant test was applied to RDA data; both analyses were conducted working at an alpha level of 0.05. Volatile data was treated with ANOVA or Welch test, based on the result of Levenes test (specified in Table 3.2), with difference in means identified with Tukey or Games Howell post hoc test, respectively, both working at an alpha level of 0.05. Statistical analysis was performed using IBM SPSS Statistics 24 for windows (SPSS Inc., IBM Corporation, NY, USA). Principle component analysis (PCA) was constructed using "FactoMinoR" and visualised

using "factoextra" packages in R (v 3.4.1, R Foundation for Statistical Computing, Vienna, Austria).

3.3 Results and discussion

3.3.1. Sensory quality of sponge cakes

The average results of the sensory evaluation of reformulated sponge cakes are presented in Table 3.1. Overall, the sucrose reduced formulas (SR70, WPP, and OLIGO) were not perceived significantly different in terms of liking of colour, odour, flavour, texture and overall liking, compared to SC100. However, the APP sponge cake scored significantly ($P < 0.05$) lower for all attributes. The texture and flavour of the SR70 sponge cake was not significantly different ($P > 0.05$) from the APP sponge cake, highlighting reduced consumer acceptance of the SR70 sponge cake. RDA was undertaken to further interpret the difference in each formulated sponge cake. Panellists were asked to assess the attributes relative to colour (crumb colour & crust colour), odour (vanilla, fresh cake, nutty & roasty), flavour (sweet, toasty, off-flavour & aftertaste) and texture on chewing. For crust colour and crumb colour, the APP sponge cake was ranked significantly ($P < 0.05$) darker (0 = very light, 9 = very dark), compared to all other samples (Figure 3.1). However, both the OLIGO and WPP sponge cakes were also perceived significantly ($P < 0.05$) darker to SC100 and SR70 sponge cakes for crust colour and crumb colour.

Pomace refers to a combination of apple peel, pulp and seed, resulting in a reddish/brown dried powder raw material, thus contributing to the overall darker colour of the APP cake. However, the individual sugars present in apple pomace are comprised mainly of fructose (Milner et al., 2020). This reducing sugar is likely accelerating MR and CR reactions during baking, and contributing to the darker crust and crumb of the APP

sponge cake. The darker colour of the APP sponge cake may also explain the low hedonic score achieved for cake colour. Thus, in this study a 5% w/w addition of apple pomace on a flour weight basis was perceived negatively by panellists.

Table 3.1

Average (n=2) results and standard deviation of Hedonic and Ranking Descriptive Analysis evaluation of control sponge cake and sucrose reduced formulas.

Values with a change in letter indicate significant difference ($P < 0.05$)

	SC100	SR70	APP	WPP	OLIGO
Hedonic					
Colour	7.77 ± 0.85 [a]	7.47 ± 0.96 [a]	5.05 ± 1.90 [b]	7.40 ± 0.79 [a]	7.72 ± 0.75 [a]
Odour	7.20 ± 0.86 [a]	6.87 ± 1.01 [a]	5.35 ± 1.41 [b]	7.10 ± 1.00 [a]	7.13 ± 1.05 [a]
Flavour	7.23 ± 0.86 [a]	6.52 ± 1.20 [ab]	5.35 ± 1.94 [b]	6.92 ± 1.04 [a]	7.07 ± 1.14 [a]
Texture	6.73 ± 1.04 [a]	6.17 ± 1.31 [ab]	5.40 ± 1.78 [b]	6.83 ± 1.17 [a]	6.77 ± 1.48 [a]
Overall Liking	7.18 ± 0.80 [a]	6.55 ± 1.24 [a]	5.15 ± 1.83 [b]	7.05 ± 1.01 [a]	7.13 ± 1.21 [a]
Ranking Descriptive Analysis					
Colour					
Crust Colour (0=very light, 9=very dark)	4.43 ± 1.06[d]	4.33 ± 0.98 [d]	7.53 ± 1.09 [a]	6.12 ± 1.18 [b]	5.56 ± 1.38 [b]
Crumb Colour (0=very light, 9=very dark)	3.27 ± 1.25 [c]	3.16 ± 1.34 [c]	7.23 ± .82 [a]	3.56 ± 1.04 [c]	4.42 ± 1.23 [b]
Odour					
Vanilla Odour	3.09 ± 2.04 [a]	3.17 ± 1.79 [a]	2.27 ± 1.61 [a]	3.10 ±1.87 [a]	2.97 ± 1.75 [a]

Fresh Cake Odour	5.28 ± 1.77 [a]	4.88 ± 1.90 [a]	3.57 ± 1.72 [b]	4.88 ± 1.70 [a]	4.48 ± 2.05 [ab]
Nutty Odour	2.54 ± 1.52 [b]	2.73 ± 1.80 [b]	4.21 ± 1.83 [a]	3.07 ± 1.89 [ab]	3.11 ± 1.77 [ab]
Roasty Odour	2.74 ± 1.57 [b]	2.78 ± 1.54 [b]	4.81 ± 1.95 [a]	3.38 ± 1.95 [b]	3.50 ± 1.73 [b]
Flavour					
Sweet Flavour	5.27 ± 1.46 [a]	4.45 ± 1.62 [ab]	3.7± 1.68 [b]	4.70 ± 1.58 [ab]	4.72 ± 1.70 [ab]
Toasty Flavour	2.56 ± 1.47 [b]	2.84 ± 1.72 [b]	4.30 ± 1.89 [a]	3.4 ± 1.98 [ab]	3.28 ± 1.88 [ab]
Aftertaste	2.65 ± 1.63 [a]	2.81 ± 1.62 [a]	3.68 ± 1.96 [a]	2.87 ± 1.77 [a]	2.98 ± 1.79 [a]
Off-Flavour	1.34 ± 0.71 [b]	1.46 ± 0.81 [ab]	2.32 ± 1.51 [a]	1.45 ± 0.72 [ab]	1.56 ± 1.10 [ab]
Texture					
Texture on Chewing (0=very dry, 9= very moist)	4.51 ± 1.39 [ab]	4.3 ± 1.70 [ab]	3.62 ± 1.44 [b]	4.68 ± 1.33 [a]	4.68 ± 1.45 [a]

The addition of 20% apple pomace powder to sponge cakes (Sudha et al., 2007) and 15% to cookies (Toledo, Nunes, Silva, Spoto, & CanniattiBrazaca, 2017) did not negatively impact consumer's perception of colour/appearance liking. However, this is likely also related to differences in the apple pomace powders used. Similarly, oligofructose contains approximately 5–6% free sugars consisting of glucose, fructose and sucrose (Milner et al., 2020), and as lactose is the primary carbohydrate component of whey permeate, it can be reasoned that the reducing sugars present in these formulas are likely responsible for the significant ($P < 0.05$) difference in colour perception, in comparison to the SC100 and SR70 sponge cakes. Similar outcomes were evident when oligofructose was added to orange cakes (Volpini-Rapina et al., 2012) and chocolate cookies (da Silva & ContiSilva, 2018).

'Fresh cake' odour was rated the highest for the SC100 sponge cake, however, only the APP sponge cake was rated significantly ($P < 0.05$) lower. Again, the low association of 'fresh cake' odour with the APP sponge cake may correspond to the low liking of APP odour in the hedonic scale evaluation. The APP sponge cake scored significantly ($P < 0.05$) higher for 'nutty' and 'roasty' odour compared to SC100 and SR70, which may indicate that these odour qualities may dominate over the 'fresh cake' odour, in this sample. Torbica, Škrobot, Janić Hajnal, Belović, and Zhang (2019) also reported that addition of 10% apple pomace powder to wholegrain wheat bread resulted in trained panellists perceiving the formulated bread to have a lower association with 'cereal aroma'. It is interesting to note that the clean-label reduced sucrose sponge cakes, WPP, and OLIGO were not perceived significantly different to APP for 'nutty' odour. The darker appearance of the formulated cakes APP, WPP and OLIGO, may be also influencing odour perception (Maric & Jacquot, 2013).

The reduced sucrose sponge cakes; SR70, WPP, and OLIGO, were not perceived to be significantly ($P > 0.05$) different to SC100 in terms of 'sweet' flavour. However, the

APP sponge cake was perceived significantly ($P < 0.05$) less sweet than the SC100 sponge cake, despite all sucrose replacers containing equal levels of sucrose. Alongi et al. (2019) incorporated APP, 20% w/w on a wheat flour basis, into a shortbread biscuit, without a decline in the perception of sweetness. 'Toasty' flavour was perceived significantly ($P < 0.05$) stronger in the APP sponge cake compared to the SC100 and SR70 sponge cakes. 'Toasty' flavour of the WPP and OLIGO sponge cakes were not statistically ($P > 0.05$) different to the APP sponge cake. It is plausible that the higher scores for 'nutty' odour and 'toasty' flavour are also linked to MR and CR reactions due to the presence of reducing sugars in the formulations.

The APP sponge cake scored significantly ($P < 0.05$) higher for 'off-flavour' compared to SC100, which may be due to a 'fruit flavour' often associated with apple pomace (Alongi et al., 2019) (however we did not capture additional details from the panellists as to the nature of the perceived off-flavour). The aim of including the term 'off-flavour' was to identify any unconventional tastes not usually associated with a traditional sponge cake. No significant differences ($P > 0.05$) were identified for 'aftertaste' or 'vanilla' odour between all the sponge cakes.

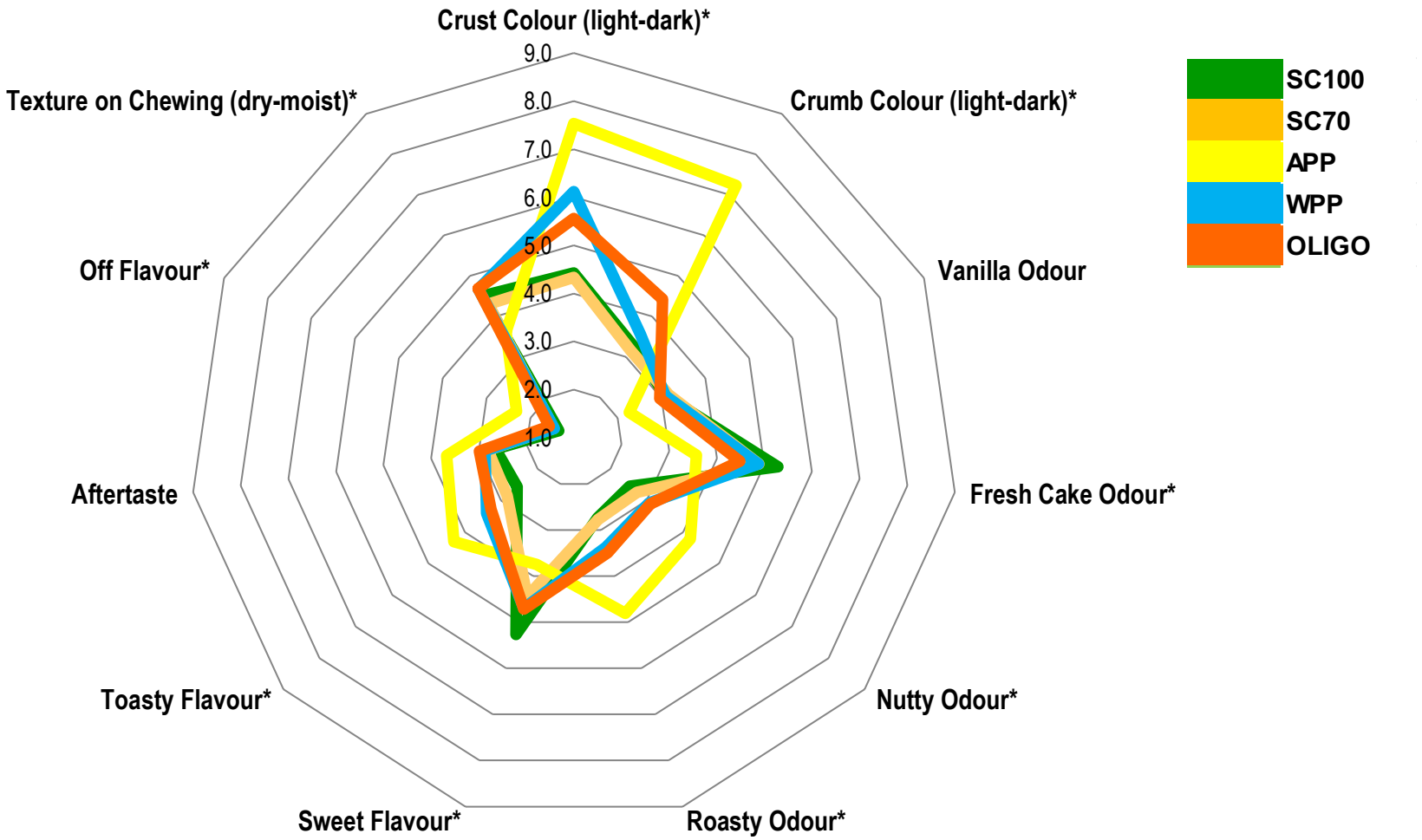


Figure 3.1. Average results (n=2) from ranking descriptive analysis of control sponge cake and sucrose reduced cakes formulated with clean-label sucrose replacers.

Attributes annotated with * indicate significant difference ($P < 0.05$) within samples.

In terms of 'texture on chewing' the APP sponge cake was perceived as the driest (closest to "very dry" on scale) compared to the SC100, WPP and OLIGO sponge cakes, however there was no significant difference between the APP and SR70 sponge cakes. As none of the sponge cakes scored higher than 5 on the RDA scale, panellists did not perceive them to be overly moist. Instrumental measurements for moisture content on identical sponge cake preparations (Milner et al., 2020) found that there was no significant difference between the moisture contents of these formulations. The desired soft texture of a sponge cake is partially due to the typical moisture content of 20–30%. As sucrose is known to play an important role in moisture retention (Struck et al., 2014), sucrose replacement can therefore adversely impact texture (Martínez-Cervera et al., 2014; Ronda et al., 2005), leading to a decrease in palatability (Martínez-Cervera et al., 2014). Although there was no significant ($P > 0.05$) difference in the instrumental moisture measurement of the cake formulas, the APP sponge cake was perceived significantly drier during mastication compared to other formulas, which also likely contributes to the low hedonic score for texture (Table 3.1). Apple pomace powder is often incorporated into bakery products to enhance the nutritional value through added fibre and polyphenols (Sudha et al., 2007). As APP had the highest fibre content out of the five formulated sponge cakes (Milner et al., 2020), it also likely contributes to the increased perception of dryness during mastication (Figure 3.1). SR70 was possibly perceived slightly drier than those sponge cakes with the added sucrose replacers (OLIGO and WPP) due to both the 30% reduction in sucrose and the lack of additional hygroscopic ingredients to enhance the perception of moisture.

3.3.2. Volatile aroma profile of sponge cakes

HS-SPME-GC–MS analysis of the sponge cakes identified a total of 77 volatile compounds across all sponge cakes (Table S3), with the compounds influenced the most by sucrose replacement outlined only in Table 3.2. Ketones, aldehydes, furans, pyrazine, terpenes and alcohols were the main chemical classes contributing to the volatile profile of the SC100 sponge cake and the reformulated reduced sucrose sponge cakes. To gain an initial insight into the differences, a PCA was undertaken with the strongest associations depicted in Figure 3.2. The first two components of the PCA explain ~53% of the total variance among the samples. To gain further information, a difference in means using ANOVA or Welch test was applied. Some distinct trends were observed in relation to the volatiles responsible for the differences between these samples.

Pyrazine compounds, (pyrazine, methylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, trimethylpyrazine) were identified in high levels in the SC100 sponge cake (Table 3.2). Both the SC100 and OLIGO sponge cakes contained significantly ($P < 0.05$) higher levels of methylpyrazine and 2,3-dimethylpyrazine compared to all other samples. SC100, OLIGO and APP sponge cakes had the highest abundance of 2,5-dimethlypyrazine and trimethlypyrazine. Pyrazines are favourable in baked confectionery products for imparting 'roasty', 'nutty', 'cake crust' aromas (Matsakidou, Blekas, & Paraskevopoulou, 2010; Pozo-Bayón, Ruíz-Rodríguez, Pernin, & Cayot, 2007), and are formed by aminoketone degradation (Martins, Jongen, & Van Boekel, 2000), as a result of α-dicarbonyl and amino acid reactions in the early stages of the MR. Furan derived compounds are formed primarily through sugar dehydration/ sugar fragmentation during the MR (Martins et al., 2000) or CR through direct decomposition of sugar moieties (Zhang et al., 2012). Their aroma impressions have been described as 'earthy', 'caramel-

like', and 'biscuit' in sponge cakes (Matsakidou et al., 2010; Pozo-Bayón et al., 2007). The levels of furan compounds differed immensely amongst the samples (Table 3.

2). The WPP sponge cake had significantly ($P < 0.05$) higher levels of 2-methylfuran, furfural and 2(5H)-furanone compared to all other samples (APP was similar ($P > 0.05$) for furfural). The high level of furans in this sample may be explained by the amount of lactose present in whey permeate, which has been shown to greatly influence furan formation when heated in the presence of protein. For example, when lactose was used to replace 60% sucrose in sponge cakes, 5-hydroxymethylfurfural generation was accelerated (Zhang et al., 2012).

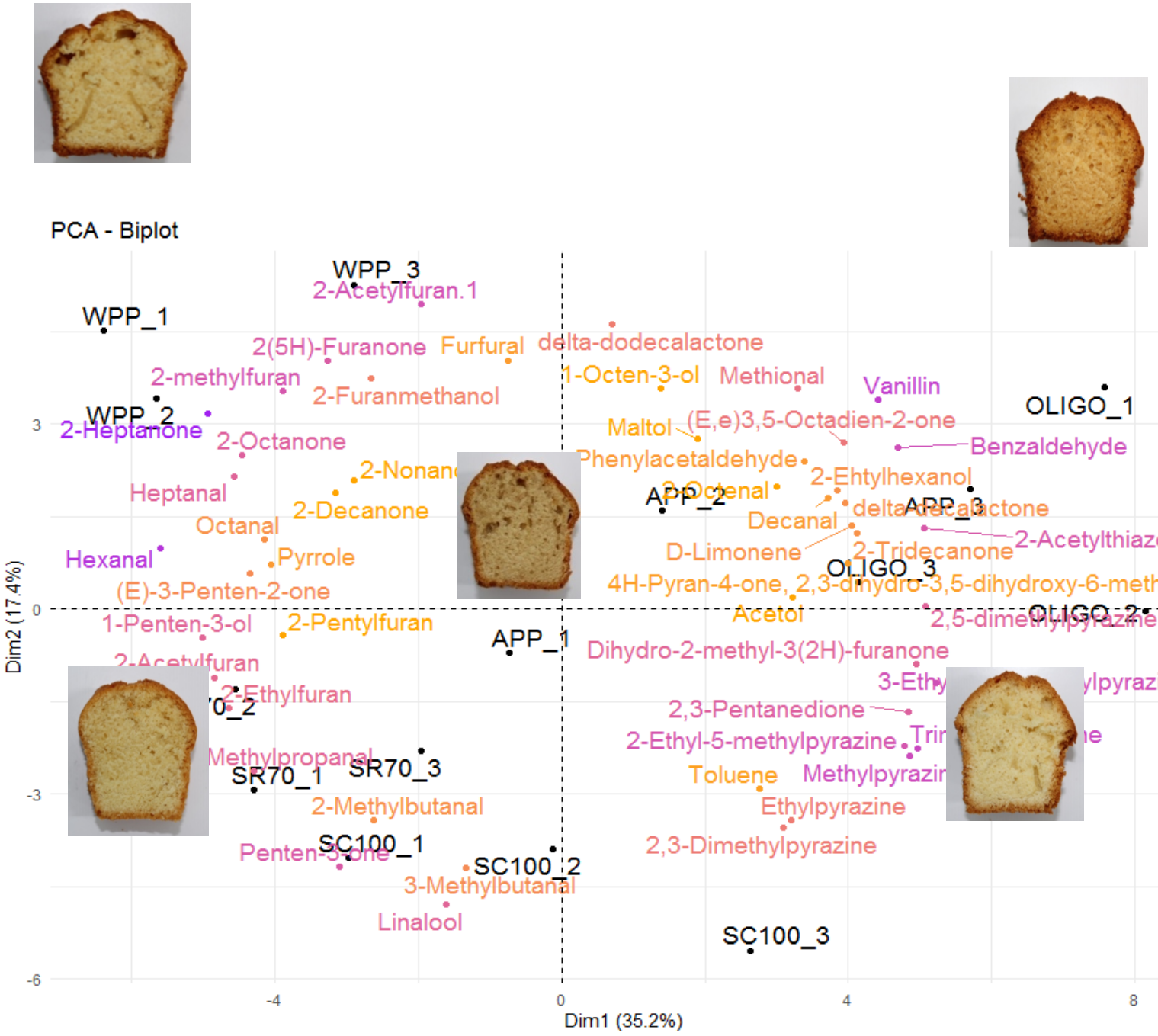

PCA - Biplot
WPP_3
2-Acetylfuran.1
WPP_1
2(5H)-Furanone
Furfural
delta-dodecalactone
2-methylfuran
1-Octen-3-ol
Methional
Vanillin
2-Furanmethanol
OLIGO_1
WPP_2
2-Heptanone
Maltol
(E,e)3,5-Octadien-2-one
2-Octanone
Benzaldehyde
Phenylacetaldehyde
2-Nonanone
2-Ehtylhexanol
Heptanal
APP_2
APP_3
2-Decanone
delta-decalactone
Octanal
Decanal
2-Acetylthiazole
Hexanal
Pyrrole
D-Limonene
2-Tridecanone
OLIGO_3
(E)-3-Penten-2-one
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
Acetol
OLIGO_2
1-Penten-3-ol
2-Pentylfuran
2,5-dimethylpyrazine
APP_1
2-Acetylfuran
Dihydro-2-methyl-3(2H)-furanone
2-Ethylfuran
2,3-Pentanedione
2-Ethyl-5-methylpyrazine
Methylpropanal
SR70_1
SR70_3
Toluene
2-Methylbutanal
Ethylpyrazine
SC100_1
2,3-Dimethylpyrazine
SC100_2
3-Methylbutanal
Linalool
SC100_3
Dim2 (17.4%)
Dim1 (35.2%)
3
0
-3
-6
-4
0
4
8
contrib
2.5
2.0
1.5
1.0

Figure. 3.2 Principle component biplot of the volatile compounds associated with control (SC100) and reformulated SR70 (30% reduced sucrose), SR-APP (30% reduced sucrose with apple pomace powder), SR-WPP (30% reduced sucrose with whey protein permeate), SR-OLIGO (30% reduced sucrose with oligofructose) sponge cakes analysed by headspace solid-phase microextraction gas chromatography mass spectrometry. Score plot represents sample replicates (n=3).

Lactose can directly be degraded by MR or CR, whereas sucrose has to be exposed to heating for much longer to be hydrolysed to its monosaccharides; glucose and fructose. Furfural was significantly higher in the APP sponge cake compared to all other samples (SC100, SR70 and OLIGO), most likely due to the higher level of fructose and glucose in the apple pomace (Milner et al., 2020). As depicted in Figure 2., dihydro-2-methyl-3(2H)-furanone (coffee furan) was in highest abundance in the SC100, APP and OLIGO sponge cakes and has been previously been identified as 'caramel-like' in the crust of sponge cakes (Matsakidou et al., 2010). Its formation is likely via the degradation of glucose during CR reactions (Umano, Hagi, Nakahara, Shyoji, & Shibamoto, 1995).

Ketone compounds, 2,3-butanedione (diacetyl) and 2,3-pentanedione, are identified as important contributors to the desirable aroma of baked confectionery, with both yielding 'butterscotch', 'caramel' impressions (Chapter 1). The abundance of these α-diketones are at risk of being suppressed on sucrose reduction as they are products of sucrose decomposition during CR or sugar fragmentation during MR, and hence cake aroma may be adversely affected. In this study, the level of 2,3-butandione did not significantly ($P > 0.05$) differ across the five sponge cake formulations, however, 2,3-pentanedione was significantly ($P < 0.05$) lower in the WPP sponge cakes compared to SR100 and OLIGO sponge cakes. Poisson, Auzanneau, Mestdagh, Blank, and Davidek (2018) proposed that 2,3-pentanedione can be generated directly from sucrose, whereas 2,3-butanedione can be formed from sucrose fragments, and monosaccharides contribute to the formation of both, explaining the consistent level of 2,3-butanedione across all samples and the higher abundance of 2,3-pentanedione in the OLIGO and SC100 sponge cakes. Acetol (1-hydroxy-2-propanone) has been previously identified in sponge cakes (Maire, Rega, Cuvelier, Soto, & Giampaoli, 2013; Matsakidou et al., 2010; Pozo-Bayón et al., 2007; Rega, Guerard, Delarue, Maire, & Giampaoli, 2009) and is generated from the

decomposition of sugars during CR. The OLIGO sponge cake contained higher levels of acetol compared to all other formulas, with the APP sponge cake containing the least ($P < 0.05$). The abundance of acetol in the OLIGO sponge cake sample indicates CR was accelerated during baking, which may be explained by the susceptibility of fructooligosaccharides to degrade when exposed to higher temperatures of baking. 2-Heptanone was significantly ($P < 0.05$) higher (nearly double) in the WPP sponge cake compared to the SC100 sponge cake. Although 2-heptanone has not been reported as odour active in sponge cakes, this compound likely originates from the whey permeate.

Comparing the abundance of aldehyde compounds between the sponge cake samples, the negative component of PC1 (SC100, APP and OLIGO) is more associated with the Strecker aldehydes; phenylacetaldehyde and methional, which are derived from amino acids phenylalanine and methionine, respectively. Phenylacetaldehyde is appreciated for a 'sweet', 'rose', 'honey' aroma and has been shown to contribute to sponge cake odour (Matsakidou et al., 2010; Pozo-Bayón et al., 2007), whereas methional has been identified as having a 'potato' like odour (Pozo-Bayón et al., 2007). Both of these Strecker aldehydes had the highest abundance in the APP sponge cake, with phenylacetaldehyde significantly ($P < 0.05$) higher than the SC100 sponge cake. Both phenylalanine and methionine amino acids have been identified in the flesh of honey crisp apples (Zhang, Li, & Cheng, 2010), which may indicate that the apple pomace is contributing to the higher amounts of these Strecker aldehydes. Benzaldehyde has been characterised as a 'cherry', 'almond' odour in sponge cakes (Maire et al., 2013; Matsakidou et al., 2010; Pozo-Bayón et al., 2007; Rega et al., 2009). Benzaldehyde can also be formed from phenylalanine and there was no significant ($P > 0.05$) difference between the SC100 sponge cake and the reformulated sponge cakes with sucrose replacers, but levels of benzaldehyde in SR70 were significantly ($P < 0.05$) lower compared to the OLIGO

sponge cake. The SR70 sponge cake formula had the least simple sugars (Milner et al., 2020), and thus Strecker degradation may not have been as abundant, possibly evident by the fact that it had a significantly ($P < 0.05$) lower abundance of phenylacetaldehyde. Considering Figure 3.2., the aldehydes associated with the sponge cake samples SR70 and WPP are primarily lipid derived (hexanal, heptanal & octanal), and were most abundant in the WPP sponge cake sample (Table 3.2), with heptanal significantly ($P < 0.05$) higher in the WPP and SR70 sponge cakes. This is difficult to interpret, but it may be due the fact that the other samples had more volatiles deriving from MR and CR, which have been shown to exhibit antioxidant activity (Benjakul, Visessanguan, Phongkanpai, & Tanaka, 2005). SC100, APP and OLIGO sponge cakes had significantly ($P < 0.05$) lower amounts of heptanal compared to SR70 and APP, which may be due to the high levels of fructose in these formulas, which has been identified as a powerful oxygen scavenger (Benjakul et al., 2005).

The APP and OLIGO sponge cakes were also found to have the highest levels of 2-acetylthiazole, a compound identified as having a 'hazelnut', 'popcorn' aroma in sponge cakes (Matsakidou et al., 2010; Pozo-Bayón et al., 2007). This compound is a product of the MR and is shown to be accelerated by the presence of the amino acid cysteine (Pripis-Nicolau, De Revel, Bertrand, & Maujean, 2000). The WPP sponge cake also contained significantly ($P < 0.05$) higher amounts of maltol, an odour active compound recognised for its 'sweet', 'cotton candy' odour in sponge cakes (Matsakidou et al., 2010). Although maltol can be created directly from sucrose, in the presence of glycine, lactose is capable of being converted to maltol on heating (Patton, 1950), which may explain its higher abundance in the WPP sponge cake.

Table 3.2

Average (n=3) peak area values (x10^6) of selected volatile compounds identified in control (SC100), and reduced sucrose reduced (SR70, APP, WPP, OLIGO) sponge cakes.

Compound	CAS Number	RI	SC100	SR70	APP	WPP	OLIGO
Ketones							
2,3-Butanedione	431-03-8	632	2.98 ± 0.785	2.46 ± 0.189	2.23 ± 0.295	2.28 ± 0.260	3.13 ±0.316
1-Penten-3-one[1]	1629-58-9	727	0.035 ± 0.005^{ab}	0.039 ± 0.008^{a}	0.017 ±0.002^{b}	0.021 ± 0.004^{b}	0.016 ±0.002^{b}
1-Hydroxy-2-propanone (Acetol)	116-09-6	737	5.19 ±1.796	2.38 ± 1.194	0.823 ± 0.110	4.47 ±2.981	14.9 ±9.989
2,3-Pentanedione[1]	600-14-6	736	1.11 ± 0.557^{ab}	0.561 ±0.212^{b}	0.813 ±0.110^{b}	0.306 ±0.063^{b}	1.82 ± 0.302^{a}
2-Heptanone[1]	591-78-6	936	7.97 ±2.226^{b}	10.5 ±1.542^{ab}	9.15 ±2.165^{b}	14.1 ±1.353^{a}	8.11 ±0.814^{b}
2-Nonanone	821-55-6	1141	1.77 ±0.425	1.87 ±0.298	1.81 ±0.336	2.04 ±0.116	1.86 ±0.288
3,5-Octadien-2-one	30086-02-3	1163	0.751±0.007	0.0682±0.007	0.0733±0.007	0.0878±0.006	0.0740±0.009
Aldehydes							
2-Methylpropanal	78-84-2	594	0.14 ±0.045	0.116 ±0.026	0.0888 ±0.032	0.112 ±0.036	0.0582 ±0.010
3-Methylbutanal	590-86-3	693	1.09 ±0.279	0.774 ±0.128	0.713 ±0.193	0.638 ±0.135	0.671 ±0.159
2-Methylbutanal	96-17-3	701	0.866 ±0.215	0.624 ±0.101	0.594 ±0.151	0.596 ±0.108	0.486 ±0.072
(E)-2-Pentenal[1]	1576-87-0	807	0.146 ±0.019^{ab}	0.149 ±0.006^{ab}	0.161 ±0.018^{a}	0.135 ±0.010^{ab}	0.121 ±0.009^{b}
Hexanal[1]	66-25-1	840	3.39 ±0.765^{abc}	4.30 ±0.418^{ab}	3.11 ±0.631^{bc}	4.76 ±0.724^{a}	2.72 ±0.215^{c}

Heptanal[1]	111-71-7	944	10.0 ±2.874[b]	21.3 ±3.657[a]	9.77 ±2.603[b]	22.3 ±2.503[a]	12.9 ±1.135 [b]
Methional[1]	3268-49-3	974	0.0178 ±0.002 [c]	0.0270 ±0.017 [bc]	0.0902 ±0.017 [a]	0.0551 ±0.002[abc]	0.0711±0.028[ab]
Benzaldehyde[1]	100-52-7	1032	2.27 ±0.296[ab]	1.56 ±0.268[b]	2.60 ±0.561[ab]	2.22 ±0.408[ab]	3.25 ±0.592[a]
Octanal[1]	124-13-0	1048	0.346 ±0.077 [a]	0.398 ±0.023 [a]	0.277 ±0.034 [b]	0.418 ±0.054 [a]	0.347 ±0.039 [ab]
(E)-2-Octenal[2]	13019-16-4	1121	0.479±0.035 [bc]	0.378 ±0.022 [c]	0.831 ±0.018 [ab]	0.498 ±0.025 [bc]	0.571 ±0.119 [b]
Phenylacetaldehyde[2]	122-78-1	1122	0.479±0.035 [b]	0.290 ±0.033 [c]	0.688 ±0.031 [a]	0.498 ±0.025 [b]	0.571 ±0.119 [ab]
(E)-2-Nonenal[2]	18829-56-6	1226	0.109 ±0.016 [b]	0.143 ±0.025 [b]	0.394 ±0.052 [a]	0.136±0.004 [b]	0.153 ±0.055 [b]
Vanillin	121-33-5	1544	0.022 ±0.003	0.0229 ±0.004	0.0341 ±0.010	0.028 ±0.004	0.0361 ±0.005
Furans							
2-Methylfuran[2]	534-22-5	623	0.017 ± 0.003[b]	0.021 ±0.001 [b]	0.014 ±0.004 [b]	0.072 ±0.023[a]	0.013 ±0.002 [b]
2-Ethyl-5-methylfuran[1]	1703-52-2	817	0.054 ±0.010 [a]	0.059 ±0.008 [a]	0.017 ±0.006 [c]	0.061 ± 0.012[a]	0.030 ±0.002 [b]
Dihydro-2-methyl-3(2H)-furanone[2]	3188-00-9	857	0.382 ±0.029[a]	0.137 ±0.015[b]	0.294 ±0.067[ab]	0.170 ±0.042[b]	0.583 ±0.181[ab]
Furfural[2]	98-01-1	899	1.38 ±0.406[b]	0.513 ±0.086[b]	3.33 ±0.210[a]	3.86 ±0.716[a]	1.40 ±0.607[b]
2-Furanmethanol	98-00-0	929	1.23 ±0.764	1.04 ±0.804	1.14 ±0.779	22.7 ±13.269	2.87 ±2.602
2-Acetylfuran	1192-62-7	978	0.179 ±0.038 [c]	0.135 ±0.016 [c]	0.591 ±0.031 [b]	1.24 ±0.178 [a]	0.355 ±0.066 [c]
2-2-pentylfuran	3777-69-3	1014	7.22 ±2.287	6.04 ±0.615	6.81 ±2.027	6.98 ±0.381	4.62 ±0.892
2(5H)-Furanone[2]	497-23-4	1031	0.717 ± 0.183[b]	0.694 ±0.124[bc]	0.430 ±0.066 [b]	5.07 ±0.377 [a]	0.780 ±0.061 [b]
Pyrazines							

Pyrazine[2]	290-37-9	773	0.519 ± 0.020[a]	0.267 ±0.002[bc]	0.215 ±0.024[b]	0.543 ±0.079[ac]	0.246 ±0.045[b]
Methylpyrazine[1]	109-08-0	864	3.07 ±0.637 [a]	1.10 ±0.069[b]	1.78 ±0.317[b]	0.913 ±0.146[b]	3.37 ±0.659 [a]
2,5-Dimethylpyrazine[2]	123-32-0	951	5.94 ±2.416[ab]	2.48 ±0.573[ab]	4.77 ±0.974[a]	0.481 ±0.052[b]	25.0 ±6.466 [ab]
2,3-Dimethylpyrazine[1]	5910-89-4	962	0.253 ±0.099[a]	0.0941 ±0.023[b]	0.0968 ±0.030 [b]	0.0646 ±0.019 [b]	0.195 ±0.053[ab]
Trimethylpyrazine[1]	14667-55-1	1043	0.646 ±0.281 [ab]	0.234 ±0.068 [bc]	0.266 ±0.051[bc]	0.0392 ±0.006[c]	0.906 ±.232 [a]
3-Ethyl-2,5-dimethylpyrazine[1]	13360-65-1	1117	0.078 ±0.035 [ab]	0.021 ±0.015 [bc]	0.0307 ±0.027[bc]	0.0102± 0.006[c]	0.0133±0.021 [a]
Terpenes							
d-Limonene	5989-27-5	1056	0.717±0.096	0.515±0.036	0.705±0.186	0.587±0.083	0.834±0.249
o-cymene*[1]	527-84-4	1059	0.218 ±0.035[ab]	0.163 ±0.015[b]	0.226 ±0.033[ab]	0.197 ±0.024[ab]	0.284 ±0.056[a]
p-cymene*[1]	99-87-6	1077	0.0267 ±0.004 [ab]	0.0168 ±0.003 [b]	0.0278 ±0.009 [ab]	0.0223 ±0.004 [ab]	0.0405 ±0.010 [a]
Linalool[1]	78-70-6	1147	0.528 ±0.148[a]	0.436 ±0.087[ab]	0.121 ±0.019[c]	0.239 ±0.041[bc]	0.177 ±0.035[c]
Alcohols							
1-Penten-3-ol[1]	616-25-1	732	0.047 ±0.004[ab]	0.049 ±0.005[ab]	0.036 ±0.004[bc]	0.055 ±0.005 [a]	0.024 ±0.007[c]
1-Hexanol[2]	111-27-3	919	0.31±0.071[b]	0.452±0.126[ab]	0.459 ±0.486[ab]	0.673 ±0.025[a]	0.501 ±0.205[ab]
1-Octen-3-ol	3391-86-4	1025	2.18 ±0.385	2.00 ±0.345	2.18 ±0.295	2.47 ±0.310	2.61 ±0.369
2-Ethylhexanol	104-76-7	1079	0.025 ±0.005	0.018 ±0.010	0.031 ±0.020	0.022 ±0.009	0.037 ±0.022
Lactones							

γ-dodecalactone[1]	706-14-9	1496	0.0342±0.005^{b}	0.0377±0.003^{b}	0.0376±0.004^{b}	0.0562 ±0.005^{a}	0.0532±0.007^{a}
δ-decalactone[1]	705-86-2	1638	0.122±0.016^{b}	0.108±0.013^{b}	0.223±0.036^{a}	0.118±0.029^{b}	0.168±0.019^{ab}
Other							
Toluene[2]	108-88-3	785	0.266±0.066^{abc}	0.195±0.011^{b}	0.276±0.023^{a}	0.140±0.013^{c}	0.236±0.019^{ab}
Pyrrole[1]	109-97-7	835	0.0748±0.012 ^{ab}	0.0523 ±0.007 ^{bv}	0.0441±0.009 ^{c}	0.0892±0.016 ^{a}	0.0482±0.002 ^{bc}
2-Acetylthiazole[2]	24295-03-2	1086	0.266±0.066^{abc}	0.195±0.011^{b}	0.276±0.023^{a}	0.140±0.013^{c}	0.236±0.019^{ab}
2-Acetylpyrrole	1072-83-9	1158	0.0748±0.012 ^{ab}	0.0523 ±0.007 ^{bv}	0.0441±0.009 ^{c}	0.0892±0.016 ^{a}	0.0482±0.002 ^{bc}
Dodecane	112-40-3	1203	0.266±0.066^{abc}	0.195±0.011^{b}	0.276±0.023^{a}	0.140±0.013^{c}	0.236±0.019^{ab}
Maltol[2]	118-71-8	1211	0.101 ±0.146^{b}	0.0563±0.082^{b}	0.104 ±0.099^{b}	0.853 ±0.629^{a}	0.995 ±1.231^{b}
2,3-Dihydro-3,5-dihydroxy-6-methyl 4(H)-pyran-4-one	28564-83-2	1249	0.0126±0.0146	0.00241±0.000	0.0232±0.012	0.00843±0.0021	0.343±0.318

[1]Values for compound with a change in letter indicate significant difference identified using **ANOVA and Tukey post hoc test**

[2]Values for compound with a change in letter indicate significant difference identified using **Welch test and Games Howell post hoc test**

Full list of identified compounds can be found in Supplementary Materials- Table S3.

Compounds marked with an * indicate tentative identification due to isomer.

3.3.3 Odour active compounds in the SC100, APP and OLIGO sponge cakes

Thirty six odour active compounds (Table 3.3) were detected in the SC100, APP & OLIGO sponge cake samples, with the identity of 33 confirmed through comparison of molecular ion matching, RI indices (using the procedure described in Section 2.4) and analytical standards. Co-elution of aroma compounds is common, and in this study, benzaldehyde co-eluted with 1-octen-3-ol, 2-ethyl-5-methylpyrazine with trimethylpyrazine, phenylacetaldehyde with 3-ethyl-2,5-dimethylpyrazine and furaneol with 2-nonanone. The aroma descriptions of the 3 unknown compounds were also included in Table 3.3. The FD values in Table 3.3 highlights the intensity of the aroma, thus values of 0 indicate that the aroma could not be perceived by the trained assessor from a splitless injection, and a value of 1 indicates perception operating at splitless, 2 indicates the maximum perception by the trained assessor was from a 2:1 split injection, 10 indicates a 10:1 split injection etc. Thus, the higher the FD value the greater the contribution of that compound to the overall aroma and flavour of the sample.

In total, ten aldehydes were found to be odour active in these samples. Heptanal had the largest contribution to the aroma of SC100 sponge cake due to the large FD value (1 5 0) and was described as having a 'fatty, sweet, cake crust' aroma (Table 3.3). Heptanal had a lower contribution to the aroma of both APP and OLIGO sponge cake samples (FD 50), however, the abundance of heptanal was not significantly different amongst the SC100, APP and OLIGO sponge cakes on volatile analysis of cake crumb (Table 3.2). Although the difference may be due to other factors influencing perception, the GC-O analysis was undertaken on a combination of crust and crumb sample. Factors such as the matrix effect are also likely contributing (Frank, Eyres, Piyasiri, & Delahunty, 2012), where compositional differences impact on the release of volatiles in food. Previous HS-SPME/Solvent Assistant Flavour Evaporation (SAFE)-GC-O analysis of sponge cakes

did not identify heptanal as odour active (Matsakidou et al., 2010; Pozo-Bayón et al., 2007). However, levels are likely to differ considerably in sponge cake formulas dependent upon lipid content and other factors impacting on oxidation. Strecker aldehydes; 2-methylpropanal, 3-methylbutanal and 2-methylbutanal arise from branch chain amino acids and their contribution to odour activity was similar in the SC100 and OLIGO sponge cakes, but different in the APP sponge cakes. Different levels of these aldehydes have been previously identified in the crust of sponge cakes (Maire et al., 2013; Matsakidou et al., 2010; Pozo-Bayón et al., 2007), which explains the difference in FD values. 3-Methylbutanal and 2-methylbutanal, were perceived as 'sweet', 'caramel' and 'spicy, sweet', respectively, whereas 2-methylpropanal was perceived as 'creamy', 'spicy'. Methional is also a Strecker derived volatile and has a distinct 'potato' odour quality and was previously reported as having a high detection frequency in both the crust and crumb of sponge cakes (PozoBayón et al., 2007). In this study, methional had FD values of 50, 150 and 100 in the SC100, APP and OLIGO sponge cakes, respectively, and significantly contributed to the aroma of all three sponge cakes. The abundance of methional across all samples was low (Table 3.2), but as it has an extremely low odour threshold, reported as 0.09 ppm in water (Giri, Osako, & Ohshima, 2010), highlighting its potential importance. A 'potato-like' odour may not be considered appealing in bakery products, but methional has been identified as having the highest FD in wheat bread (Rega et al., 2009), therefore contributing to the dynamic roasty odour of wheat bread. Methional may be responsible for the significantly lower score for 'odour liking' of the APP sponge cake (Table 3.1), and also the significantly higher score for the 'roasty' attribute (Figure 3.2). Strecker aldehydes benzaldehdye and phenylacetaldehyde, were found to co-elute with other volatiles, making it difficult to discern their true contribution to the odour and flavour perception of these samples. Phenylacetaldehyde appeared to have the biggest impact in the OLIGO sponge cake and the impact of benzaldehdye was similar in both

the APP and OLIGO sponge cakes, but absent in the SC100 sponge cake. Nonanal and (E)-2-octenal, are also products of lipid oxidation. The FD values for nonanal were 20, 10 and 50 for the SC100, APP and OLIGO sponge cakes, respectively. Thus, it had the greatest contribution to the OLIGO sponge cake sample. The FD values followed a similar trend for (E)-2-octenal, with a FD 50 value for the OLIGO sponge cake and an FD 20 for both the SC100 and APP sponge cakes.

As mentioned, furans are important volatiles from MR and CR reactions in baked confectionery products. Furfural was described as having a 'spicy', 'sweet', 'caramel', 'bready' aroma (Table 3.3), and was the most odour active compound in all three sponge cakes samples, with the highest DF (1 5 0) in the APP sponge cake and an FD of 100 in SC100 and OLIGO sponge cakes. The abundance of furfural was significantly ($P < 0.05$) higher in APP sponge cake compared to SC100 and OLIGO sponge cakes (Table 3.2), which explains its perceived odour intensity. Pozo-Bayón et al., 2007 identified furfural in the SAFE extract of sponge cakes but described it as 'earthy', 'potato', although it was coeluting with 2-ethyl-3,5-dimethylpyrazine, which likely accounts for the different aroma descriptors. Matsakidou et al. (2010) used identical HS-SPME extraction parameters as this study (60 min at 60 °C) and identified furfural in only the crust of sponge cakes and did not identify it as odour active. Three other furanone compounds also contributed to the aroma in these sponge cakes; 2-furanmethanol (furfuryl alcohol), dihydro-2-methyl 3(2H)-furanone (coffee furanone), and 2-acetylfuran. Of these three furans, furfuryl alcohol had the greatest impact with FD values of 20, 100 and 50 for SC100, APP and OLIGO sponge cakes, respectively. Thus, in this case the sucrose replacers appear to be enhancing the contribution of this compound to the overall aroma and flavour. Table 3.2 depicts no significant difference between the samples for furfuryl alcohol in the crumb, however, MR and CR reaction compounds, such as furans, are always likely to be higher

in the crust (Chapter 1). 2-Acetylfuran had FD values of 20, and 10 in SC100, and OLIGO sponge cakes, respectively and was not detected in the APP sponge cake. The reduction in sucrose, or change in composition, appears to have decreased the contribution of 2-acetylfuran to the overall aroma and thus flavour. The impact of coffee furanone was much less with a FD value of 1 achieved for each sample. The contribution of furaneol was also difficult to elucidate as it was not identified in the crumb. The odour of the analysed analytical standard of furaneol corresponded to the intense "sweet" aroma identified by panellists in the sponge cakes (Table 3), therefore potentially demonstrating its presence in sponge cake crust. It has been previously been identified in sponge cake (Pozo-Bayón et al., 2007).

Pyrazine compounds were strongly associated with all of the sponge cake samples. 2,5-Dimethylpyrazine was described as 'cake crust', 'sweet', 'nutty' and was perceived up to a FD of 100 in both SC100 and OLIGO sponge cakes, and at FD of 50 in the APP sponge cake. 2,3- Dimethylpyrazine, described as 'bready', was more odour active in the SC100 and APP sponge cakes (FD 100), compared to the OLIGO (FD 50) sponge cake. Although Table 3.2 shows similarities in the abundance levels of these compounds, it also appears that differences in composition due to the matrix effect may have also influenced their perception. The prevalence of pyrazines in the crust of sponge cakes (Matsakidou et al., 2010; Pozo-Bayón et al., 2007) likely contributed to the difference. Pozo-Bayón et al. (2007) identified 2,5-dimethylpyrazine in the crust and crumb of sponge cakes with panellists describing the odour as 'solvent', 'hospital'. Similarly, Pozo-Bayón et al. (2007) and Matsakidou et al. (2010) both identified 2,3-dimethylpyrazine in sponge cakes, however, neither were reported as odour active. Methylpyrazine and ethylpyrazine had less of an odour impact, methylpyrazine had GC-O values of 10, 2 and 5, and ethylpyrazine had FD values of 5, 2 and 2, in the SC100, APP and OLIGO sponge cakes,

respectively. The odour of methyl- and ethyl-pyrazine was described as 'fruity', 'sweet' and 'bready' (Table 3.3). 2-Ethyl-5-methyl-pyrazine and trimethylpyrazine co-eluted, but had FD values of 2, 10 and 20 in the SC100, APP and OLIGO sponge cakes, respectively.

Six ketones were identified as odour active in these sponge cakes; 2,3-butanedione, 2,3-pentanedione, 2-nonanone, acetol, and (3,5)-octadien-2-one. The potential sources of these ketones are heat derived MR + CR reactions and lipid oxidation. 2,3-Butanedione was characterised by 'butterscotch', 'sweet', 'toffee' with FD values of 10, 5 and 20 for the SC100, APP and OLIGO sponge cakes, respectively. The higher FD for 2,3-butanedione in the OLIGO sponge cake was likely due to additional CR reactions due to the increased presence of monosaccharides. Although no significant difference in the abundance of 2,3- butanedione was evident between these samples (Table 3.2), levels were higher in the OLIGO sponge cake (this may be due to differences related to sampling between volatile assessment and GCO as previously stated). 2,3-Pentanedione was described as having 'spicy, woody' character and had lower FD values, at 10, 2 and 1, for the SC100, APP and OLIGO sponge cakes, respectively. Again, the higher perception in the SC100 sponge cake may be explained by slightly higher abundance (Table 3.2). Another odour active ketone identified was acetophenone, which in this study was described as 'sweet', 'cake crust', and was most odour active in the OLIGO (FD 50) sponge cake, but not perceived in the APP sponge cake, which corresponds with values in Table 3.2. Acetophenone is found in chicory (Baek & Cadwallader, 1998), which may explain the higher FD value for the OLIGO sponge cake sample. It had a FD of 20 in the SC100 sponge cake. This compound has been identified in star apple fruit as having a 'cherry' aroma (Lasekan, Khatib, Juhari, Patiram, & Lasekan, 2013).

2-Acetylpyrrole was present in all samples, and had the highest perception in the APP sponge cake (FD 100). The FD values for the SC100 and OLIGO sponge cakes were 10 and 50, respectively. 2- Acetylpyrrole is also a MR product, with higher concentrations depending on the amino acid and reducing sugar source. The odour descriptors for 2-acetylpyrrolye, for the three sponge cakes were 'cotton candy', 'sweet' and 'fruity'. Previously 2-acetylpyrrole has been described as having a 'chocolate-like' aroma in soy sauce (Feng et al., 2015). This may be related to the product, or that the odour quality of this compound may differ depending on its concentration. In this study it was relatively low across all the sponge cakes (Table 3.2). Pozo-Bayón et al. (2007) identified 2-acetylpyrrole in the volatile fraction of sponge cakes, yet did not identify it as being odour active. 2-Acetylthiazole was also present in each sample, the odour activity varied from FD 50 in the OLIGO sponge cake, to 10 in the APP sponge cake, and to 2 in the SC100 sponge cake. Thus the odour activity was much higher in the two clean-label reduced sucrose samples. 2-Acethylthiazole is also a MR product and thus levels appear to be influenced by the inclusion of the apple pomace powder and oligofructose. Maltol and 2,3-dihydro-3,5- dihydroxy-6-methyl4(H)-pyran-4-one were perceived as 'sweet', 'fruity', 'spicy' and 'bready' in all three sponge cakes, with highest odour activity in the OLIGO (FD 50) sponge cake. Maltol is a product of MR and CR, and Matsakidou et al. (2010) identified maltol as 'sweet, caramel' in sponge cakes, highlighting its contribution to the desirable aroma of baked goods. 2,3-Dihydro-3,5-dihydroxy-6-methyl4(H)- pyran-4-one is a precursor of maltol and is also derived from MR/ CR reactions (Rega et al., 2009).

Table 3.3

Odour active compounds with corresponding RI value, odour impression and factor dilution value identified in S100, APP and OLIGO sponge cakes.

Order of identification	Retention Index		Volatile compound	Odour Impression	FD Values			Identification*
	DB-624 UI (volatile analysis)	DB-624 UI (GC-O analysis)			SC100	APP	OLIGO	
1	594	606	2-Methylpropanal	spicy, cake crust	2	5	2	MS, RI, Odour
2	632	632	2,3-Butanedione	Butterscotch, sweet, toffee	10	5	20	MS, RI, Odour
3	693	701	3-Methylbutanal	Sweet, toffee, pineapple	50	10	50	MS, RI, Odour
4	701	707	2-Methylbutanal	Spicy, sweet	50	10	50	MS, RI, Odour
5	736	738	2,3-Pentanedione	Woody, spicy	10	2	1	MS, RI, Odour
6	737	755	Acetol	Sweet, fruity, cotton candy	0	1	50	MS, RI, Odour
7	-	805	Unknown 1	Toasted, bready, cake crust	20	0	0	MS, RI, Odour
8	857	857	Dihydro-2-methyl-3(2H)-furanone	Woody, bready	1	1	1	MS, RI, Odour
9	864	864	Methylpyrazine	Butterscotch, sweet, cake crust	10	2	5	MS, RI, Odour, Std
10	899	901	Furfural	Spicy, bready	100	150	100	MS, RI, Odour

11	928	930	2-Furanmethanol	Biscuit, cake crust, caramelized	20	100	50	MS, RI, Odour
12	944	943	Heptanal	Fatty, oily, cake crust	150	50	50	MS, RI, Odour
13	951	950	2,5-Dimethylpyrazine	cake crust, nutty, bready	100	50	100	MS, RI, Odour
14	958	955	Ethylpyrazine	Roasty, bready	5	2	2	MS, RI, Odour
15	962	966	2,3-Dimethylpyrazine	Bready, caramel	100	100	50	MS, RI, Odour
16	974	973	Methional	potato damp	50	150	100	MS, RI, Odour
17	978	977	2-Acetylfuran	Bready, caramel	20	0	10	MS, RI, Odour
18	-	1021	Unknown 2	Sweet, fruity, caramel	20	5	10	MS, RI, Odour
19	1025/1038	1026	Benzaldehyde / 1-Octen-3-ol	Mushroom, mouldy, sweet, almond, fruity	0	10	10	MS, RI, Odour
20	1042/1043	1039	2-ethyl-5-methyl-pyrazine/ Trimethylpyrazine	Musty, mouldy, cake crust	2	10	20	MS, RI, Odour
21	1086	1080	2-Acetylthiazole	Bready, biscuit, cake crust	2	10	50	MS, RI, Odour
22	1117/1122	1113/ 1115	Phenylacetaldehyde/ 3-ethyl-2,5-dimethylpyrazine	Fatty, fruity, cake crust, bready	20	20	50	MS, RI, Odour
23	1121	1119	(E)-2-Octenal*	Damp, earthy, mouldy	20	20	50	MS, RI, Odour
24	1141	1140	Furaneol/ 2-Nonanone	Roasty, cake crust, sweet	50	20	50	MS, RI, Odour, Std
25	1147	1146	Acetophenone	Sweet, cake crust, burnt	20	0	50	MS, RI, Odour
26	1152	1149	Nonanal	Bready, cake crust	20	10	50	MS, RI, Odour
27	1159	1157	2-Acetylpyrrole	cotton candy, fruity, sweet	10	100	50	MS, RI, Odour

28	1163	1161	(3E,5Z)-Octadien-2-one*	Caramel, butterscotch	10	20	50	MS, RI, Odour
29		1192	Unknown 3	Cake crust, sweet, butterscotch	20	50	50	MS, RI, Odour
30	1203	1200	Dodecane	Musty, damp,earthy	5	20	5	MS, RI, Odour
31	1207	1204	Maltol	Sweet, spicy, fruity	20	20	50	MS, RI, Odour
32	1249	1241	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Spicy, bready, roasty	20	10	50	MS, RI, Odour

* Identification by comparison with MS spectra, LRI matching from internal library and volatile analysis, odour comparison to literature and retention time of analytical standard

Dodecane is an alkane, and had a pronounced 'earthy', 'damp', 'musty' aroma and is produced by lipid oxidation (Maire et al., 2013). Dodecane was most odour active (FD 20) in the APP sponge cake. 1- Octen-3-ol, a product of lipid oxidation, co-eluted with benzaldehyde but was likely responsible for the mushroom aroma found in both the APP and OLIGO (FD 10) sponge cakes, it was absent in the SC100 sponge cake.

Figure 3.3 is a pie-chart of the differences in aroma perception of the ten most odour active volatile compounds in the APP and OLIGO sponge cake samples in comparison to the control (SC100). This clearly highlights the significant impact of formulation changes on the odour activity of individual volatile components in the resultant sponge cake.

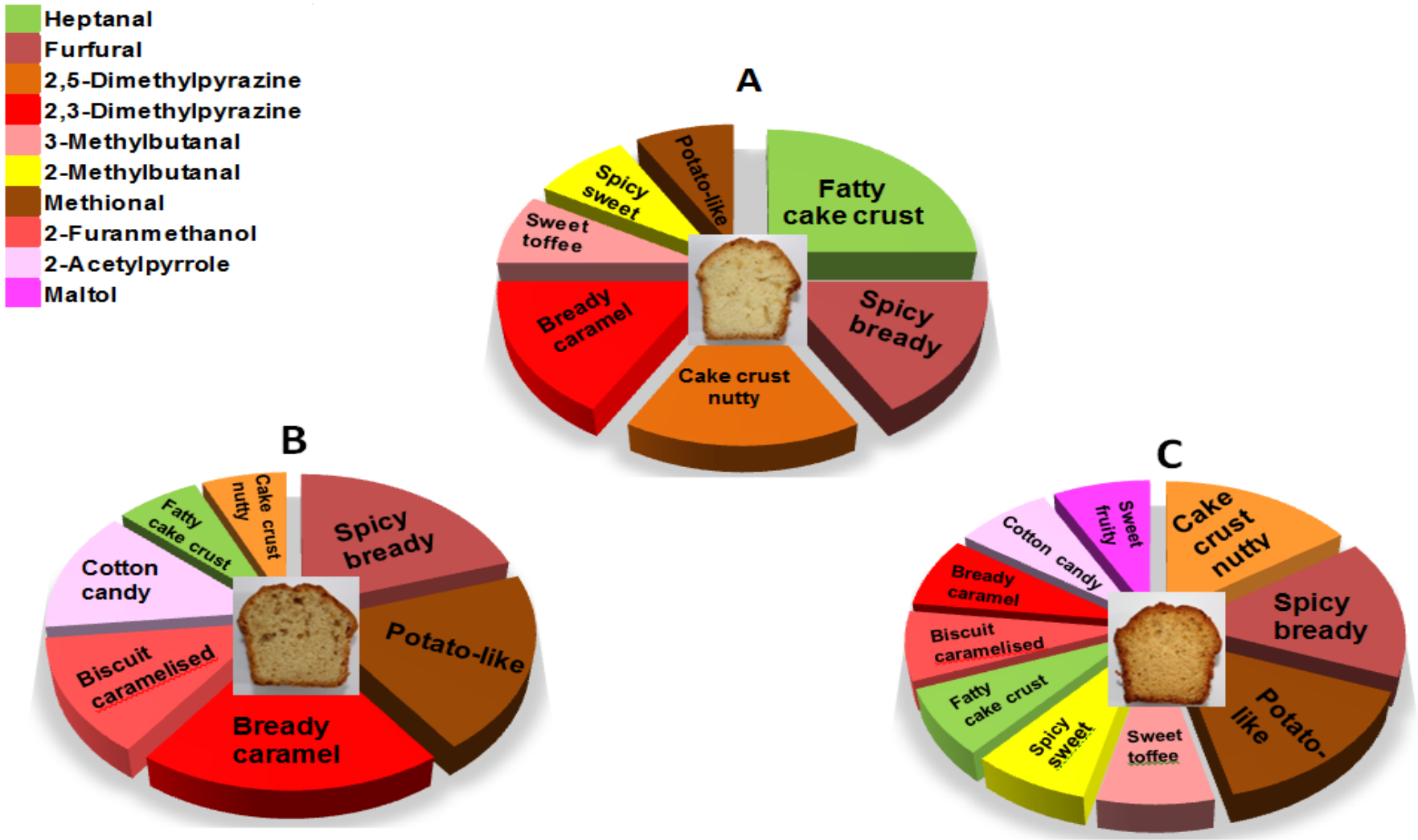

Heptanal
Furfural
2,5-Dimethylpyrazine
2,3-Dimethylpyrazine
3-Methylbutanal
2-Methylbutanal
Methional
2-Furanmethanol
2-Acetylpyrrole
Maltol
A
Fatty cake crust
Spicy bready
Cake crust nutty
Bready caramel
Sweet toffee
Spicy sweet
Potato-like
B
Spicy bready
Potato-like
Bready caramel
Biscuit caramelised
Cotton candy
Fatty cake crust
Cake crust nutty
C
Cake crust nutty
Spicy bready
Potato-like
Sweet toffee
Spicy sweet
Fatty cake crust
Biscuit caramelised
Bready caramel
Cotton candy
Sweet fruity

Figure 3.3 Graphical representation of the most odour active compounds in the **A** SC100 (control), **B** APP (30% reduced sucrose with apple pomace powder) and **C** OLIGO (30% reduced sucrose with oligofructose) sponge cake formulas. Pie chart segments represent the dilution factor values of the main odour active compounds with larger segments indicating compounds perceived at higher dilutions (Table 3.3). Colour chart reflects the volatile compounds present in the pie charts. Pie charts contain compounds from ≥FD 50 only.

3.4 Conclusion

The impact of reducing sucrose with the inclusion of clean-label ingredients on the sensory quality and aroma of sponge cakes was explored. The hedonic liking assessment found no significant differences between the control and reformulated sponge cakes, apart from significantly lower scores for the APP sponge cake, for all attributes, and a reduction for texture and flavour for the SR70 sponge cake. RDA highlighted many significant differences in the perception of crust and crumb colour in the reformulated sponge cakes (APP, OLIGO and WPP), in comparison to the SC100 and SR70 sponge cakes. 'Roasty odour', 'toasty flavour' and 'off-flavour' were significantly higher in the APP sponge cake. 'Nutty odour' was perceived higher in reformulated sponge cakes in comparison to the SC100 sponge cake. 'Fresh cake odour' and 'sweet flavour' were also significantly reduced in the APP sponge cake, in comparison to the SC100 sponge cake. Significant differences in volatile profiles between all the samples were evident, especially in those derived from MD and CR reactions, and lipid oxidation. Aroma active studies carried out on the SC100, APP and OLIGO sponge cakes, provided insightful information regarding differences in aroma activity between the reformulated samples and the SC100 control sponge cake. Thirty six aroma active compounds were identified, with furfural, methional, heptanal, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-furanmethanol having the greatest impact on sensory perception. Differences in the perception of other branched chain aldehydes, ketones, furans, pyrazine, pyrroles and terpene alcohols were also evident between SC100, APP and the OLIGO sponge cakes, but these had lower aroma intensities. This study has clearly demonstrated a significant deviation in the abundance of aroma active compounds influencing sensory perception in reduced sucrose sponge cakes with added clean-label ingredients in comparison to the control. However, it also highlights that considerable scope exists to manipulate added

clean-label ingredients to make the aromatic profile more similar to the control or enhance desired aromas and thus flavour profiles.

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Chapter 4. Aroma generation in sponge cakes: The influence of sucrose particle size and sucrose source

Abstract

The influence of sucrose source, sugarbeet and sugarcane, and sucrose crystal size, on volatile formation in sponge cakes was investigated. Sugarbeet and sugarcane were sourced from local retailers. Six sponge cake formulas were created; sugarbeet control (SB-C) and sugarcane control (SC-C) which had crystal size range of the commercial package, small crystal size (< 250 μm) beet (SB-SPS), cane (SC-SPS) and large crystal size (> 710 μm) beet (SB-LPS), cane (SC-LPS). Volatile analysis and gas-chromatography-olfactometry were performed on all sponge cake formulas. Overall, sucrose source did not have a significant influence on volatile formation. Sponge cakes formulated with SPS sucrose, particularly sugarbeet-SPS, yielded higher levels of Maillard reaction and caramelisation compounds such as furans and pyrazines, presumably due to the lower melting point of smaller crystals. However, Strecker aldehydes and pyrazine compounds were significantly influenced by the interaction effect between sucrose source and crystal size. Thirty-one aroma active volatile compounds were identified in the sponge cakes by olfactometry. 'Potato-like' methional, 'spicy/bready' furfural, 'cake crust' 2,3-dimethlypyrazine, 'fatty/oily' heptanal and 'earthy/damp' (E)-2-octenal contributed to odour active profile of all sponge cakes. Pronounced levels of 'spicy/bready' furfural were identified in SPS sponge cakes, a key contributor to the desirable aroma of sponge cakes, whereas LPS appeared to supress the generation of many aromatic sponge cake volatiles. Application of smaller sucrose crystals in baked confectionery formulations appears to be a method that can aid in the development of sucrose reduced products but retain even enhance their desired aroma.

Keywords: Bakery, volatile compounds, reformulation, odour active, gas-chromatography-olfactometry

4.1 Introduction

The growing demand for food products formulated with minimal ingredients, and the expectations of food manufactures to refrain from the use of artificial additives and preservatives, challenges the conventional approach to new product development, especially in relation to sugar and fat reduction. Sucrose reduction is a primary objective for food manufacturers globally in an attempt to tackle the burden of health-related chronic disease, such as obesity and type II diabetes. Polyols and artificial sweeteners were once considered solutions to sucrose reduction in sugar laden food and beverage matrices (Ghosh & Sudha, 2012; Hendriksen, Tijhuis, Fransen, Verhagen, & Hoekstra, 2011; Struck, Jaros, Brennan, & Rohm, 2014), however, in recent years substantial interest has occurred in the area of clean-label formulation (Asioli, Aschemann-Witzel, Caputo, Vecchio, Annunziata, Næs, et al., 2017).

Baked confectionery products are of continuous interest to food scientists in relation to reformulation, particularly sucrose reduction (Chapter 1; Luo, Arcot, Gill, Louie, & Rangan, 2019; Sahin, Zannini, Coffey, & Arendt, 2019). Clean-label sucrose reduction has been employed in sponge cakes utilising apple pomace powder, whey permeate powder and oligofructose ingredients (Chapter 3; Milner, Kerry, O'Sullivan, & Gallagher, 2020). Sponge cakes formulated with apple pomace powder and oligofructose had higher 'off-flavour' (apple pomace powder) and were more pronounced in 'toasty', 'nutty' attributes, than the control. In a recent study by Maruyama, Streletskaya, and Lim (2021), consumers identified sugarcane as the most 'natural' ingredient across a range of different raw materials, with fructose classed the least 'natural' in the sugar category. This suggests consumers are likely more receptive to some natural sugars over others possibly due to familiarity and tradition.

Sucrose, otherwise known as sugar (or table sugar), is traditionally derived from sugarbeet (*Beta vulgaris*) and sugarcane (*Saccharum officinarum*), available as a refined, granulated sugar with a sucrose content of ≥ 99%. Although the sugars are considered as virtually identical in terms of chemical composition, differences in their sensory characteristics have been recognized. Urbanus, Cox, Eklund, Ickes, Schmidt, and Lee (2014) found by descriptive analysis, using a trained panel, that sugarbeet was characterised by 'off-dairy', 'oxidized', 'earthy', and 'barnyard' aromas, whereas sugarcane was profiled as 'fruity' in mouth aroma, with a sweet aftertaste. Similarly, Urbanus, Schmidt, and Lee (2014) found the inclusion of sugarbeet with sugarcane created an 'off-aroma' in sugar syrup and pavlova, where the volatile fraction of raw sugarbeet juice has been shown to contain 2-methoxy-3-(1-methylpropyl)pyrazine, dimethyl disulfide, and geosmin (Pihlsgård, Larsson, Leufvén, & Lingnert, 2000)), with geosmin postulated to be the biggest contributor to 'off' aromas (Freidig & Goldman, 2014). Another study has characterised sugarcane containing primarily alcohols (1-pentanol, 2-butanol, 2-octanol) (Wang, Wang, Deng, Cai, & Chen, 2019). Differences in thermal behaviour of sugarbeet and sugarcane have also been identified (Lu, Thomas, & Schmidt, 2017), an important consideration for the influence these sugars could impart to the sensory quality of baked confectionery products.

Richardson, Tyuftin, Kilcawley, Gallagher, O'Sullivan, and Kerry (2018) investigated manipulating sugar crystal size in chocolate brownies, experimenting with small, medium and large particle fractions. The authors identified smaller sugar crystals yielded a chocolate brownie with statistically higher 'sweet taste' using consumers, compared to that of the control, and postulated that small sugar particles could be employed to reduce overall sugar content in baked confectionery products, whilst retaining the fundamental sensory appeal.

Although manipulation of ingredient particle size has shown to result in baked confectionery products with similar sensory properties as traditional formulas, there is potential for the aroma volatile profile to be altered. The particle size of sucrose and cocoa liquor mixture, for chocolate manufacture, has been shown to influence the release of 'characteristic chocolate' aroma compounds; 3-methylbutanal, 2-phenylethanol, furfuryl alcohol, acetic acid, methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine and trimethylpyrazine, when analysed by gas chromatography-olfactometry (Afoakwa, Paterson, Fowler, & Ryan, 2009).

Gas-chromatography-olfactometry was successfully employed to investigate the influence of sucrose replacers on the odour active compounds of clean-label sponge cakes (Chapter 3) and the objective of this study was to use a similar approach to investigate the influence of sucrose source (SS); (*sugarcane* and *sugarbeet)*, and particle size (PS) on the volatile aroma profile of sponge cakes. Volatile analysis was conducted using an optimised headspace solid-phase micro-extraction gas-chromatography mass-spectrometry (HS-SPME-GS-MS) method (Chapter 2) and gas-chromatography-olfactometry (GC-O).

4.2 Materials and methods

4.2.1 Analytical standards

Olfactometry training standards of analytical grade; ethyl butyrate, octanal, p-cresol, and dimethyl disulphide and heptanal of ≥99% and ≥95% purity respectively (Merck Ireland, Arklow, Co. Wicklow, Ireland), were prepared at 0.3% (w/v) in methanol and stored at −18 °C until required. For each GC-O training session, a stock solution was

diluted to 0.03% (w/v) in distilled water to allow the odours to be of adequate potency, as described in Chapter 3.

4.2.2 Sucrose samples

Sugarbeet samples (sucrose 99.7%, crystal size= 0.45 – 0.65 mm) were purchased in a local supermarket in the form of granulated sugar, under the brand name Siucra (Nordzucker, Germany). Sugarcane (sucrose content not declared, crystal size= 0.3 – 0.9 mm) was sourced from Equal Exchange (Sunderland, UK). Both producers supplied product specifications. Sugar was stored in airtight plastic containers at ambient temperatures of 20 °C prior to sieving. For effective sieving, sugar was dried at 70 °C for 1 hr in an oven (Binder, ED 115, Germany) to reduce moisture content.

4.2.3 Sugar sieving

The dried sugars were sieved using a laboratory test sieve shaker (Octagon 200 test sieve shaker, Endecotts Ltd, London, UK) using a range of sieve sizes (125, 250, 500, 710 and 1800 μm). To increase the amount of small particle sizes retained in a given sieve, and to reduce the labour of manually grinding the sugar crystals, a portion of the sugars were blitz in a blender (NutriBullet 600, Australia). All sugars were sieved in batches of 200 g at an 8-mm amplitude for 5 min. On evaluation of the sieved sugar, particle sizes ≤250μm, referred to as small particle sizes (SPS), and ≥710μm; large particle sizes (LPS), were selected to be incorporated into sponge cake matrices, and compared to the control sugars (sugar taken directly from the commercial packages). Each fraction was stored in airtight plastic containers until utilised for baking.

4.2.4 Particle size distribution

The particle size distributions (PSD) of each powder fraction were analysed in triplicate, measured by laser light scattering using a Mastersizer 3000 (Malvern Instruments Ltd., UK), equipped with an Aero S dry powder dispersion unit. Particle size measurements were expressed as smallest particle size (D10), mean particle size (D50) and largest particle size (D100), corresponding to the maximum diameters of 10%, 50%, and 90% of the particles, respectively (in % of total volume).

4.2.5 Sponge cake preparation

The fractionated sugar crystals were assigned to six sponge cake formulations; sugarbeet control (SB-C), sugarcane control (SC-C), sugarbeet small particle size (SB-SPS), sugarcane small particle size (SC-SPS), sugarbeet large particle size (SB-LPS) and sugarcane large particle size (SC-LPS). All sponge cake samples consisted of identical formulas and independent baking trials were carried out in duplicate.

Plain flour (200 g) (Odlums, Ireland) and baking powder (4 g) (Dr. Oekter, UK) were sifted into a bowl followed by the addition of the trial sugar (Súcra, Nordzucker, Germany; Equal Exchange, UK), cake margarine (90 g) (Stork, UK), free range eggs (90 g) (local retailer), and water (70 g). The contents were mixed using a household mixer (Kenwood Mixer, Model KMM710, UK) at speed 1 for 30 seconds, scraped and mixed again for a further 2 min at speed 2. Miniature loaf tins (80 mm × 60 mm × 40 mm) were filled with 80 g of cake batter and baked at 180°C for 45 min in a domestic convection oven (Zanussi, Bedfordshire, UK). The cakes were left to cool and placed in sealed

storage bags until subsequent volatile or GC-O analysis (which took place within 24 hours after baking).

4.2.6 Volatile analysis

Volatile analysis was carried out as described Chapter 2. Each sponge cake was sliced vertically and 1cm of the outer crust was removed. Cake crumb (3g) was added to an amber 20 ml screw capped headspace vial (Apex Scientific Ltd, Co.Kildare, Ireland) and equilibrated for 5 min, at 60°C with pulsed agitation for 5 seconds at 350 rpm, using the Gerstal MultiPurpose Sampler (GMPS) agitator/heater. Volatile analysis was carried out utilising a GMPS rail system (Anatune, Cambridge CB3 0NA, UK) connected to a Shimadzu GP2010 plus gas chromatograph (GC) (Mason Technology Ltd, Dublin, Ireland) using headspace solid-phase microextraction (HS-SPME). The SPME fibre; 30/50µm DVB/CAR/PDMS (Agilent Technologies Ltd., Ireland), was exposed to the headspace above the samples, at a depth of 21 mm, for 60 min at 60°C. The fibre was retracted, injected into the GC inlet and desorbed for 3 min at 250 °C, followed by 3 min at 270 °C in GMPS fibre bake-out station, to minimise the carryover of compounds. Each sponge cake formula was analysed in triplicate.

4.2.7 Identification of odour active compounds by gas chromatography-olfactometry

HS-SPME-GC-O analysis was undertaken on all experimental samples. Sponge cake samples were sliced and blitzed in a food processor (NutriBullet 600, Australia) to combine cake crust and crumb uniformly to represent a masticated sample. Ten sniffer assessors were chosen for the GC-O analysis based on their performance in an

olfactometry assessment using three different Sniffin' Sticks tests (identification, discrimination, and threshold) (Hummel, Sekinger, Wolf, Pauli and Kobal, 1997). Prior to sample analysis, the panellists were exposed to a standard stock solution (as described in 2.1), designed for GC-O training, comprised of 5 compounds; dimethyl disulphide ('*sulphur*','*decomposing*'), ethyl butyrate ('*fruity*', '*pineapple*'), heptanal ('*fatty*', '*green*'), octanal ('*orange*', '*fruity*') and ϱ-cresol ('*barnyard*'). This step allowed panellists to familiarise themselves with the GC-O process and software, as well as the range of odours they could potentially encounter during the GC-O analysis of sponge cake samples. Samples were evaluated in a randomized incomplete block design, allowing for each sample to be evaluated 5 times. If an aroma was detected by at least 3 out of 5 evaluations, it was established as odour active (Koutidou, Grauwet, Van Loey, & Acharya, 2017).

Volatile extraction was carried out by the HS-SPME procedure (as previously described). GC-O analyses was performed on an Agilent 7890 GC with a flame ionization detector, 5973 N mass detector (Agilent Technologies, Ltd, Cork, Ireland), and an Gerstel ODP-3 olfactometry detector port (Anatune Ltd, Cambridge, UK). The volatile compounds were separated on DB-624 UI (20m x 1.8mm x 1 μm) (Agilent Technologies Ltd, Ireland) column. Eluting compounds were split 1:1:1 into the MS detector, flame ionisation detector and the sniffing port simultaneously by means of a column flow splitter. The carrier gas was helium, held at a pressure of 9.8 psi and a flow rate of 1.209 mL min^{-1}. GC conditions consisted of an initial oven temperature of 80 °C, held for 2 min and increased at 10 °C/min to 220°C. The GC run time was shortened to 21 min to reduce the risk of assessors experiencing fatigue during a sniffing session (however it still encompassed the volatile range of interest). In addition, the transfer line to the sniffing port was conditioned with humidified air to reduce olfactometry fatigue and prevent the occurrence of condensation droplets collecting in the nasal port. The ion source

temperature was 220 °C and the interface temperature was set at 260 °C. The MS mode was electronic ionization (70eV) with the mass range scanned between m/z 35-250. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, comparison of LRI to the mid polar column from the previous analysis and to standards where possible. Spectral de-convolution was also performed to confirm identification of compounds using AMDIS.

To determine the threshold at which each odour active compound could be perceived, Aroma Extraction Dilution Analysis (AEDA) was carried out by manipulation of the GC injection split ratio (Feng, Cai, Sun-Waterhouse, Cui, Su, Lin, et al., 2015). Samples were analysed at a splitless (1:1 ratio), and for subsequent analysis, the operating mode of GC analysis was changed from splitless to split injection and the split ratio was adjusted to 1:10, 1:20, 1:50, 1:100, 1:150, 1:200, and 1:250, allowing for adequate dilution to determine the most odour active compounds in the samples. For this task, three assessors were chosen for the AEDA portion of the study, with each split ratio analysed in duplicate. The last split ratio at which a compound could be detected was referred to as the factor dilution (FD) for that compound.

4.4.8 Statistical Analysis

Data analysis was handled accordingly based on the normality of the data. To identify differences in area values within the volatile compound data, Welch test was applied, with difference in means identified by Games Howell post hoc test, working at an alpha level of 0.05. A two-way analysis of variance (ANOVA) was applied to volatile compound data to identify the main effects of independent variables sucrose source (SS) and sugar particle size (SPS) on the area peak value of volatile compounds (dependent

variable). The two-way ANOVA also analysed the interaction effect amongst the independent variables. Statistical analysis was performed using IBM SPSS Statistics 24 for windows (SPSS Inc., IBM Corporation, NY, USA). Principle component analysis (PCA) was constructed using the "factoextra" and "Facto- MinoR" packages in R (v 3.4.1, R Foundation for Statistical Computing, Vienna, Austria).

4.3 Results and discussion

4.3.1 Particle size distribution

PSD of sugarbeet, and sugarcane are shown in Figure 4.1. Particle size diameter means of the control sugars; SB-C and SC-C were 601 μm and 657 μm, respectively and demonstrate similar distribution range (SB-C; 52-1630 μm, SC-C; 46- 1850) (Figure 4.1). The SPS crystals, which were subject to sieving and grinding also had a similar distribution range (SB-SPS; 3.5-586 μm, SC-SPS; 1.1-666), with SB-SPS and SC-SPS having a diameter mean of 198 μm and 178 μm, respectively. The largest disparity was identified in the LPS sucrose, obtained from using a 710 μm sieve. As demonstrated in Figure 4.1., SC-LPS spans from 500 - 3000 μm. Diameter means for SB-LPS and SC-LPS were 867 μm and 1100 μm, respectively.

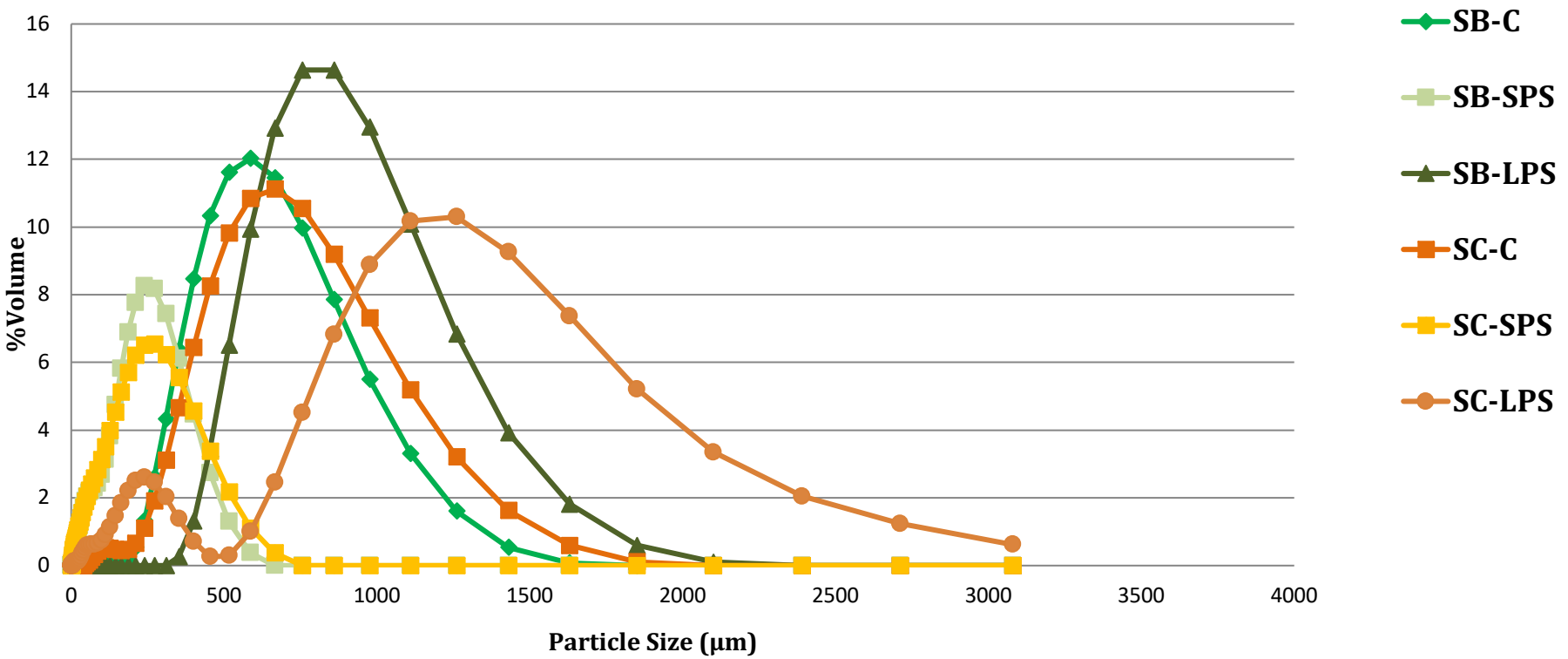


Figure 4.1. Particle size distribution of experimental sugars.

4.3.2 Volatile profile of sugarcane and sugarbeet sponge cakes

4.3.2.1 Influence of sugar source on volatile formation

Volatile analysis identified a total of 61 volatile compounds across all samples (Table 4.1), with ketones, aldehydes, furans, pyrazine, alcohols and acids as the main chemical classes. Principle component analysis (PCA) was conducted to identify any associations between individual volatiles and the different sponge cake samples (Figure 4.2). Figure 4.2 depicts the PCA plot of each sponge cake formulation and the volatiles and accounted for 77% of the total variance amongst the samples. Based on results of a two-way ANOVA, overall as an independent variable, SS did not confer an effect on volatile formation in this study, with the exception of toluene ($P = 0.001$), which was significantly ($P < 0.05$) higher in SB-C, compared to SC-C. Although raw sugarcane contains a small quantity of molasses (< 2%), as a result of the production process, it did not influence volatile compound generation.

4.3.2.2 Influence of crystal size on volatile formation

The inclusion of small sugar crystals in sponge cakes had a significant ($P < 0.05$) influence on some volatile compounds, particularly furans and pyrazines. A noticeable increase in furanic compounds across sponge cakes samples formulated with SPS sucrose was identified (Table 4.1). SB-SPS and SC-SPS had higher levels of furfural and 2-furanmethanol compared to SB-LPS and SC-LPL (SB-SPS significantly ($P < 0.05$) higher compared to LPS formulas). Although sucrose will not accelerate furan formation as strongly as reducing sugars such as glucose, fructose and lactose etc. in baked goods (Cepeda-Vázquez, Rega, Descharles, & Camel, 2018; Zhang, Song, Hu, Liao, Ni, & Li,

2012), the inclusion of smaller crystals sizes induces a lower melting point (Richardson, Tyuftin, Kilcawley, Gallagher, O'Sullivan, & Kerry, 2018), which may accelerate the hydrolysis of sucrose, resulting in more CR and MR, and thus yield higher levels of furans. The presence of higher levels of 2-acetylfuran in SB-SPS and SC-SPS sponge cakes may also indicate greater hydrolysis as it has also been identified to derive from glucose (Wang, Juliani, Simon, & Ho, 2009).

Pyrazines were also significantly impacted by particle size, with both SB-C and SC-C, and SB-SPS and SC-SPS formulas having greater abundances (Figure 4.2). Afoakwa et al. (2009), also found that the inclusion of large particle sizes (sucrose and cocoa liquor mix) in chocolate formulation decreased a range of pyrazines, including methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine. As α-dicarbonyl compounds are produced through sugar fragmentation/ dehydration during CR and MR, and play a role in pyrazine formation (Kocadağlı, Methven, Kant, & Parker, 2020), it is possible that the increased, surface area, due to the lower crystal size, may accelerate the generation of α-dicarbonyl compounds, leading to increased pyrazine levels. SB-SPS was particularly high in 2,5-dimethylpyrazine, trimethylpyrazine and 2-ethyl-5-methylpyrazine, which again may also be linked to Strecker degradation (Table 4.1).

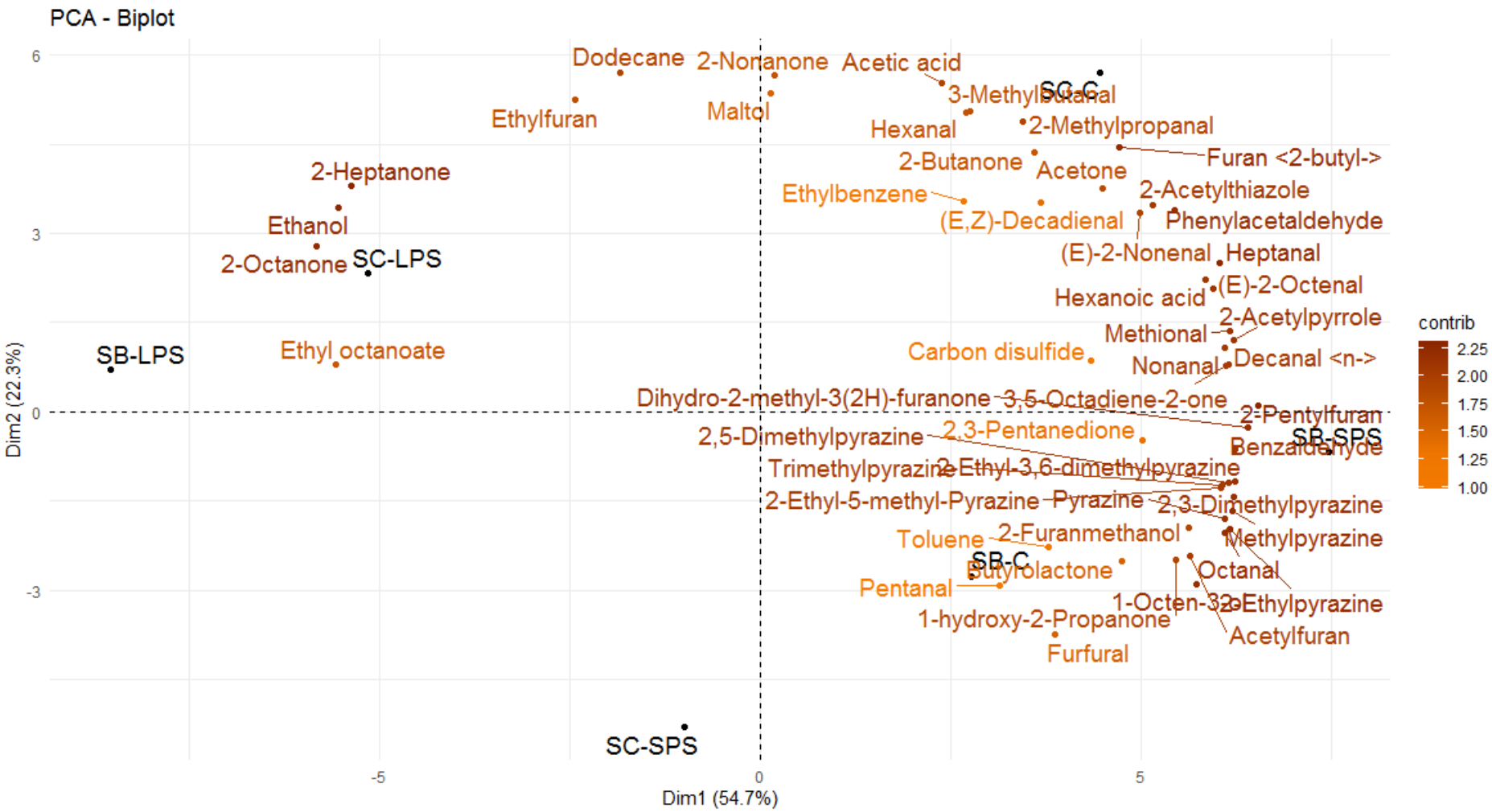


Figure 4.2. Principle component analysis of the volatile compounds associated with sugarbeet control (SB-C), sugarcane control (SC-C), sugarbeet small particle size (SB-SPS), sugarcane small particle size (SC-SPS, sugarbeet large particle size (SB-LPS) and sugarcane large particle size (SC-LPS) sponge cakes analysed by headspace solid-phase microextraction gas chromatography mass spectrometry.

As depicted in Figure 4.2, the inclusion of LPS crystals had the least influence on aroma volatile formation in the sponge cakes, with the exception of some methyl ketones, and ethanol (Table 4.1). Methyl ketones, 2-heptanone, 2-octanone, and 2-nonanone, were present in higher levels in LPS formulas, particularly in SB-LPS. On heating, methyl ketones can be formed by the decarboxylation of β-keto fatty acids, or the β-oxidation and decarboxylation of free saturated fatty acids (Deeth & Lewis, 2017), and therefore the larger particle size appears to be positively influencing this reaction. As stated, ethanol was present in greater amounts in the LPS formulas, and may be as a result of heighted microbiological activity (Pasqualone, Bianco, Paradiso, Summo, Gambacorta, & Caponio, 2014). However, the high volatility of ethanol makes it difficult to analyse by HS techniques.

4.3.2.3 Influence of the interaction effect of sucrose source and crystal size on volatile formation

A significant ($P < 0.001$) interaction effect was identified, between SS and PS, for a number of compounds. Strecker aldehydes; 2-methylpropanal and 3-methylbutanal, were significantly ($P < 0.001$) influenced by SS and PS, with SB-SPS and SC-C having significantly ($P < 0.05$) higher levels of 2-methylpropanal and 3-methylbutanal compared to the other samples (except SC-LPS, $P > 0.05$) (Table 4.1). 2-Methylpropanal and 3-methylbutanal derive from Strecker degradation reactions of amino acids, valine and leucine, respectively, and are identified as important outputs of MR, with sugar form (mono-, di- saccharide) an influential factor in their generation (Martin & Ames, 2001). It is difficult to discern how SS and PS interacted in the formation of these compounds, as no distinct trend is evident (Table 4.1). However, the presence of molasses in raw cane sugar may be a contributory factor as cane molasses has previously been reported to contain higher levels of the monosaccharides; glucose and fructose, compared to

sugarbeet molasses (Palmonari, Cavallini, Sniffen, Fernandes, Holder, Fagioli, et al., 2020). Although present in minor amounts, these simple sugars from molasses retained in raw cane sugar, may potentially accelerate MR reactions and thus contribute to the greater abundance of these Strecker aldehydes in SC-C. How these Strecker aldehydes were supressed in SC-SPS and SC-LPS is difficult to discern.

Levels of the aliphatic aldehyde heptanal were also influenced by the interaction between SS and PS, with SC-C having significantly ($P < 0.05$) higher levels compared to SB-C and SB-LPS. Levels of lipid oxidation aldehydes heptanal, (E)-octenal and (E)-nonanal were identified in higher levels in SC-C sponge cakes (Table 4.1). Again an occurrence that is difficult to interpret as the levels decrease when sugar size is manipulated. However, the green rind of sugarcane appeared to be high in lipid oxidation reaction aldehydes and alcohols (Wang et al. 2019), which may indicate these volatiles are inherently present in the molasses, or are heightened on the molasses removal process.

2-Butanone and ethylfuran levels were influenced by the interaction effect of SS and PS in the sponge cakes. SB-SPS and SC-C had the highest abundance of 2-butanone, with this compound identified as having 'butterscotch', 'artificial', 'vanilla' aroma in liquid sugarbeet (Pihlsgård, Larsson, Leufvén, & Lingnert, 2000). SC-C yielded significantly ($P < 0.05$) higher levels of 2-butanone compared to SB-C, as this compound has been suggested to be driven from MR and CR reactions in baked confectionery products (Lee, Bousquières, Descharles, Roux, Michon, Rega, et al., 2020; Pasqualone, Bianco, Paradiso, Summo, Gambacorta, Caponio, et al., 2015), it is also likely driven by the presence of molasses in SB-C. A similar behaviour was identified with the formation of 2-ethylfuran, with SB-C significantly ($P < 0.05$) lower than SC-C (Valli, Gómez-Caravaca, Di Nunzio, Danesi, Caboni, & Bordoni, 2012).

Table 4.1.

Average (n=6) peak area values (x10^6) of volatile compounds identified in sugarbeet control (SB-C), sugarcane control (SC-C), sugarbeet small particle size (SB-SPS), sugarcane small particle size (SC-SPS), sugarbeet large particle size (SB-LPS) and sugarcane large particle size (SC-LPS) sponge cakes analysed by headspace solid-phase microextraction gas chromatography mass spectrometry.

Volatile Compound	Sugarbeet Cakes			Sugarcane Cakes			Two-way ANOVA (α=0.001)	
	SB-C	SB-SPS	SB-LPS	SC-C	SC-SPS	SC-LPS	Interaction Effect (SS x SPS)	Particle Size
Ketones								
Acetone	0.44 ± 0.2 ^{ab}	0.92 ± 0.3 ^{ab}	0.39 ± 0.1 ^{b}	0.85 ± 0.1^{a}	0.42 ±0.1 ^{b}	0.65 ± 0.3 ^{ab}	**<0.001**	**n.s**
2,3-Butanedione	8.34 ± 1.6 ^{ab}	9.49 ± 1.0 ^{a}	8.56 ± 0.3 ^{ab}	8.81 ± 1.0 ^{ab}	7.67 ± 0.6 ^{b}	9.67 ± 1.7 ^{ab}	**n.s**	n.s
2-Butanone	0.18 ± 0.04 ^{b}	0.50 ± 0.3 ^{ab}	0.24 ± 0.021 ^{ab}	0.49 ± 0.2 ^{a}	0.22 ± 0.02 ^{ab}	0.35 ± 0.1 ^{ab}	**<0.001**	**n.s**
2-Pentanone	0.20 ± 0.1	0.24 ± 0.1	0.30 ± 0.1	0.21 ± 0.1	0.13 ±0.1	0.16 ± 0.1	**n.s**	n.s
2,3-Pentanedione	8.58 ±8.6 ^{ab}	7.3 ± 0.7 ^{a}	5.80 ± 0.4 ^{b}	8.43 ± 1.47 ^{a}	7.64 ± 4.94 ^{ab}	6.65 ± 1.54 ^{ab}	**n.s**	n.s
1-hydroxy-2-Propanone	34.06 ± 17.7	65.63 ± 31.2	12.18 ± 3.8	26.94 ± 14.1	37.60 ± 28.4	14.41 ± 11.2	**n.s**	n.s
2-Heptanone	37.36 ± 9.2 ^{bc}	34.18 ± 5.1^{c}	59.03 ± 4.4 ^{a}	48.67 ± 3.6 ^{ab}	39.24 ± 11.1^{bc}	58.93 ± 13.3^{ab}	**n.s**	**<0.001**
2,5-Hexanedione	5.91 ± 1.3	6.29 ± 1.5	1.27 ± 1.9	2.80 ± 2.6	3.72 ± 2.8	3.06 ± 2.3	**n.s**	**n.s**
2-Octanone	42.94 ± 10.5 ^{ab}	38.74 ± 5.0 ^{b}	58.32 ± 1.6 ^{a}	45.81 ± 3.8 ^{b}	41.56 ± 11.4 ^{ab}	53.45 ± 11.9 ^{ab}	**n.s**	**n.s**

2-Nonanone	34.71 ± 7.0 [ab]	34.69 ± 4.3 [b]	42.16 ± 1.1 [a]	54.71 ± 25.6 [ab]	33.94 ± 3.2 [b]	38.42 ± 5.0 [ab]	**n.s**	n.s
Acetophenone	0.57 ± 0.1 [a]	0.91 ± 0.4 [a]	0.18 ± 0.01 [b]	0.59 ± 0.2 [a]	0.34 ± 0.1 [ab]	0.26 ± 0.2 [ab]	**n.s**	n.s
3,5-Octadiene-2-one	0.97 ± 0.2 [ab]	1.21 ± 0.3 [a]	0.61 ± 0.01 [b]	1.28 ± 0.8 [abc]	0.91 ± 0.4 [abc]	0.55 ± 0.04 [c]	**n.s**	n.s
Undecan-2-one	7.22 ± 1.4	7.55 ± 1.1	7.29 ± 0.4	7.34 ± 1.2	6.24 ± 0.4	6.77 ± 0.4	**n.s**	n.s
Aldehydes								
2-Methylpropanal	0.39 ± 0.1 [b]	0.75 ± 0.2 [a]	0.38 ± 0.21 [b]	0.77 ± 0.2 [a]	0.32 ± 0.02 [b]	0.64 ± 0.1 [ab]	**<0.001**	n.s
3-Methylbutanal	3.43 ± 1 [ab]	6.34 ± 1.3 [a]	4.08 ± 0.3 [a]	6.29 ± 0.5 [ab]	3.13 ± 0.6 [b]	5.56 ± 1.7 [ab]	**<0.001**	n.s
2-Methylbutanal	4.26 ± 1.0	5.43 ± 1.0 [a]	3.92 ± 0.2 [a]	4.31 ± 1.9 [b]	3.00 ± 0.3 [b]	5.35 ± 1.7 [a]	n.s	n.s
(E)-2-Pentenal	1.56 ± 0.2	1.53 ± 0.3	0.61 ± 0.5	1.32 ± 0.2	0.85 ± 0.5	0.96 ± 0.5	**n.s**	**n.s**
Hexanal	22.08 ± 4.6	20.93 ± 4.2	19.45 ± 1.4	23.88 ± 2.5	17.76 ± 5.9	21.94 ± 4.8	**n.s**	n.s
Heptanal	19.52 ± 2.4[b]	24.74 ± 5.9[ab]	12.84 ± 0.5[c]	25.88 ± 1.9[a]	14.77 ± 7.0[abc]	14.36 ± 3.8[bc]	**<0.001**	**0.001**
Methional	0.38 ± 0.1 [a]	0.42 ± 0.2 [ab]	0.19 ± 0.05 [b]	0.39 ± 0.1 [ab]	0.24 ± 0.1 [ab]	0.28 ± 0.1 [ab]	**n.s**	**n.s**
Benzaldehyde	5.32 ± 1.5 [a]	6.72 ± 3.6 [ab]	2.11 ± 0.04[b]	4.52 ± 0.7 [a]	3.38 ± 0.9 [ab]	2.91 ± 0.8 [b]	**n.s**	**n.s**
Octanal	3.95 ± 1.2 [ab]	3.93 ± 1.0 [a]	1.86 ± 0.1 [b]	3.28 ± 0.6 [a]	3.14 ± 1.9 [ab]	2.04 ± 0.2 [b]	**n.s**	**n.s**
(E)-2-Octenal	4.46 ± 0.3 [a]	4.74 ± 1.0 [a]	1.33 ± 0.1 [b]	4.96 ± 0.7 [a]	2.08 ± 1.1 [b]	2.82 ± 1.8 [ab]	**<0.001**	**<0.001**
Phenylacetaldehyde	4.46 ± 0.4 [a]	4.68 ± 1.0 [ab]	3.11 ± 0.9 [ab]	5.48 ± 1.2 [a]	3.35 ± 0.6 [b]	3.74 ± 1.0 [ab]	**n.s**	**n.s**
Nonanal	6.14 ± 1.7 [ab]	6.14 ± 09 [a]	4.10 ± 0.6 [ab]	6.64 ± 1.9 [ab]	4.86 ± 1.0 [ab]	4.14 ± 0.4 [b]	**n.s**	**n.s**
(E)-2-Nonenal	0.96 ± 0.1 [ab]	0.90 ± 0.1[b]	0.55 ± 0.1 [c]	1.31 ± 0.2 [a]	0.57 ± 0.2 [c]	0.60 ± 0.3 [bc]	**0.001**	**<0.001**
Decanal	1.52 ± 0.6[ab]	1.44 ± 0.3[a]	0.51 ± 0.1[c]	1.52 ± 0.6[abc]	0.84 ± 0.3[b]	0.70 ± 0.2 [b]	**n.s**	**<0.001**
(E,Z)-Decadienal	2.67 ± 0.7 [a]	1.86 ± 0.6 [ab]	1.56 ± 0.7 [ab]	3.45 ± 1.3 [ab]	1.48 ± 0.3 [b]	1.64 ± 0.7 [ab]	**n.s**	**<0.001**
Furan								
Ethylfuran	0.21 ± 0.1 [b]	0.37 ± 0.1 [ab]	0.44 ± 0.02[a]	0.42 ± 0.1 [a]	0.25 ± 0.1 [b]	0.46 ± 0.03 [a]	**<0.001**	**0.001**

Dihydro-2-methyl-3(2H)-furanone	1.76 ± 0.4 [a]	2.87 ± 1.2 [ab]	0.71 ± 0.1[c]	2.22 ± 0.9 [ab]	1.72 ± 0.3 [ab]	0.90 ± 0.4 [bc]	**n.s**	**n.s**
Furfural	34.59 ± 5.5[ac]	46.66 ± 20.3[ab]	19.74 ± 4.5[b]	37.65 ± 9.4[a]	60.43 ± 23.7[a]	19.81 ± 8.5[bc]	**n.s**	**<0.001**
2-Butylfuran	0.51 ± 0.1 [ab]	0.61 ± 0.1 [a]	0.46 ± 0.03 [b]	0.67 ± 0.1 [a]	0.47 ± 0.2 [ab]	0.54 ± 0.1 [ab]	**n.s**	**n.s**
2-Furanmethanol	18.69 ± 6.9 [ab]	34.98 ± 15.2 [a]	7.69 ± 3.0 [b]	24.15 ± 10.2 [ab]	28.45 ± 22.0 [ab]	8.82 ± 7.1 [b]	**n.s**	**n.s**
Acetylfuran	1.79 ± 0.3 [a]	2.47 ± 0.8 [ab]	1.01 ± 0.2 [bc]	1.94 ± 0.5 [a]	2.23 ± 0.8 [abc]	0.99 ± 0.3 [c]	**n.s**	**<0.001**
2-2-pentylfuran	84.08 ± 16.8 [a]	110.27 ± 13.7 [a]	40.22 ± 1.3 [b]	93.72 ± 23.7 [ac]	73.19 ± 45.4 [abc]	59.63 ± 23.3 [bc]	**n.s**	**n.s**
Butyrolactone	0.56 ± 0.1	0.69 ± 0.3	0.34 ± 0.2	0.61 ± 0.2	0.75 ± 0.3	0.50 ± 0.1	**n.s**	**n.s**
Pyrazines								
Pyrazine	4.18 ± 1.0 [a]	6.07 ± 1.9 [a]	2.11 ± 0.3 [b]	4.13 ± 0.5 [a]	4.32 ± 0.5 [a]	2.63 ± 0.8 [b]	**n.s**	**<0.001**
Methylpyrazine	35.16 ± 8.1 [a]	50.01 ± 17.6 [a]	16.37 ± 2.4 [b]	33.36 ± 4.4 [a]	32.79 ± 6.5 [a]	18.14 ± 7.0 [b]	**n.s**	**<0.001**
2,5-Dimethylpyrazine	52.05 ± 12.6 [a]	81.82 ± 31.1 [a]	23.81 ± 5.2 [b]	50.87 ± 8.0 [a]	45.94 ± 14.0 [ab]	26.65 ± 12.0 [b]	**n.s**	**<0.001**
2-Ethylpyrazine	6.71 ± 2.1 [a]	9.26 ± 3.1 [a]	2.77 ± 0.5 [b]	6.11 ± 1.2 [a]	6.26 ± 1.5 [a]	3.08 ± 1.3 [b]	**n.s**	**<0.001**
2,3-Dimethylpyrazine	3.13 ± 1.0 [a]	4.64 ± 2.0 [ab]	1.35 ± 0.3 [b]	2.96 ± 0.5 [a]	2.76 ± 0.9 [ab]	1.49 ± 0.7 [b]	**n.s**	**<0.001**
2-Ethyl-5-methyl-Pyrazine	7.28 ± 2.3 [a]	12.77 ± 5.6 [a]	2.70 ± 0.8 [b]	6.99 ± 1.7 [a]	6.39 ± 2.7 [ab]	3.05 ± 1.7 [b]	**n.s**	**<0.001**
Trimethylpyrazine	9.68 ± 2.7 [a]	16.28 ± 7.3 [ab]	3.78 ± 1.1 [b]	9.07 ± 2.2 [a]	8.10 ± 3.4 [ab]	4.15 ± 2.4 [b]	**n.s**	**<0.001**
2-Ethyl-3,6-dimethylpyrazine	0.63 ± 0.2 [a]	1.00 ± 0.5 [ab]	0.18 ± 0.2 [ab]	0.58 ± 0.2 [ab]	0.49 ± 0.2 [ab]	0.25 ± 0.2 [b]	**n.s**	**0.001**
Alcohol								
Ethanol	38.88 ± 42.6 [b]	41.37 ± 46.9 [b]	316.84 ± 34.5 [a]	163.56 ± 179.0 [ab]	86.07 ± 7.4 [b]	296.64 ± 10.8 [a]	**n.s**	**<0.001**
1- Hexanol	8.26 ± 2.5	5.47 ± 0.4	5.57 ± 1.0	4.75 ± 0.5	5.85 ± 3.7	6.34 ± 1.5	**n.s**	**n.s**
1-Octen-3-ol	15.06 ± 2.7 [a]	14.57 ± 2.2 [a]	7.56 ± 0.9 [b]	11.86 ± 2.8 [ab]	12.91 ± 8.6 [ab]	9.35 ± 2.0 [b]	**n.s**	**n.s**

2-Ethyl-hexanol	0.70 ± 1.0	1.44 ± 1.3	1.01 ± 0.04	1.91 ± 0.8	1.57 ± 1.1	1.10 ± 0.1	**n.s**	**n.s**
Acid								
Acetic acid	2.88 ± 3.1	2.73 ± 2.1	2.75 ± 0.6	4.47 ± 2.0	1.70 ± 0.8	2.57 ± 1.9	**n.s**	**n.s**
Hexanoic acid	2.55 ± 0.7 [a]	2.85 ± 0.9 [a]	0.98 ± 1.1 [ab]	3.05 ± 2.2 [ab]	0.96 ± 1.1 [ab]	1.07 ± 0.4 [b]	**n.s**	**n.s**
Pyrrole								
2-Pyrrolidinone	0.09 ± 0.1 [b]	0.13 ± 0.1 [ab]	0.16 ± 0.1 [ab]	0.35 ± 0.1 [a]	0.21 ± 0.03 [ab]	0.17 ±0.1 [ab]	**n.s**	**n.s**
2-Acetylpyrrole	0.46 ± 0.2	0.72 ± 0.6	0.22 ± 0.2	0.73 ± 0.5	0.46 ± 0.4	0.26 ± 0.3	**n.s**	**n.s**
Lactone								
γ-Nonalactone	52.76 ± 7.4 [ab]	54.04 ± 8.6 [ab]	60.08 ± 2.2 [a]	54.93 ± 9.0 [ab]	46.82 ± 4.3 [b]	51.86 ± 2.5 [b]	**n.s**	**n.s**
Hexanoic acid, anhydride	0.57 ± 0.1 [ab]	0.59 ± 0.1 [ab]	0.62 ± 0.02 [a]	0.59 ± 0.1 [ab]	0.50 ± 0.05 [b]	0.54 ± 0.03 [b]	**n.s**	**n.s**
Esters								
Ethyl Acetate	0.68 ± 0.7 [ab]	0.67 ± 0.7 [ab]	0.20 ± 0.005 [ab]	0.17 ± 0.2 [ab]	0.09 ± 0.03 [ab]	0.25 ± 0.1 [ab]	**n.s**	**n.s**
Ethyl octanoate	0.05 ± 0.1 [b]	0.11 ± 0.1 [ab]	0.22 ± 0.05 [a]	0.10 ± 0.1 [ab]	0.15 ± 0.1 [ab]	0.19 ± 0.1 [ab]	**n.s**	**n.s**
Other								
Carbon disulfide	111.22 ± 69.3 [ab]	59.17 ± 23.4 [a]	13.17 ± 1.9 [b]	96.14 ± 19.4 [a]	50.30 ± 16.6 [a]	61.75 ± 34.3 [ab]	n.s	**0.001**
Toluene	1.46 ± 0.6 [ab]	1.27 ± 0.2 [a]	0.64 ± 0.1 [b]	0.68 ± 0.3 [b]	0.70 ± 0.1[b]	0.83 ± 0.2 [ab]	**n.s**	n.s
D-Limonene	5.38 ± 2.1 [ab]	4.83 ± 0.7 [a]	2.47 ± 0.1 [b]	4.50 ± 2.6 [ab]	5.70 ± 3.0 [ab]	2.56 ± 0.8 [b]	**n.s**	**n.s**
2-Acetylthiazole	1.49 ± 0.3 [ac]	1.58 ± 0.7 [ab]	0.79 ± 0.05 [b]	2.04 ± 0.2 [a]	1.10 ± 0.2 [bc]	1.36 ± 0.3 [ab]	**n.s**	0.001
Dodecane	1.25 ± 0.4	1.19 ± 0.1	1.31 ± 0.05	1.41 ± 0.1	1.21 ± 0.1	1.35 ± 0.1	**n.s**	**n.s**
Maltol	1.45 ± 1.0	1.75 ± 1.3	1.90 ± 0.7	1.94 ± 1.2	1.27 ± 1.4	1.57 ± 0.9	**n.s**	**n.s**

4.3.3 Influence of sugar particle size on the odour active volatile profile of sponge cakes

Thirty one odour active compounds (Table 4.2) were identified in all sponge cakes, with the identity of 25 confirmed through comparison of molecular ion matching and RI values. Co-elution of aroma compounds is common, and in this study, benzaldehyde co-eluted with 1-octen-3-ol, 2-ethyl-5-methylpyrazine with trimethylpyrazine, phenylacetaldehyde with 3-ethyl-2,5-dimethylpyrazine and furaneol with 2-nonanone, as previously identified in Chapter 3. The aroma descriptions of 6 unknown compounds were also included in Table 4.2.

Seven aldehydes were found to be odour active in the sponge cake samples. The Strecker aldehyde methional was identified as odour active across all sponge cake samples, yielding a 'potato-like' odour, with FD values ranging from 50-200. Methional contributes largely to the aroma profile of SB-C and SC-C, reflecting a similar trend to levels of methional identified in the crumb (Table 4.1). Methional is derived from the Strecker degradation of amino acid methionine, and has also been identified with a similar 'potato' odour in sponge cakes (Chapter 3; Pozo-Bayón, Ruíz-Rodríguez, Pernin, & Cayot, 2007) and has been identified as an important contributor to wheat bread crust (Boeswetter, Scherf, Schieberle, & Koehler, 2019; Pu, Zhang, Zhang, Sun, Ren, & Chen, 2019). 'Oily/fatty' heptanal was identified as odour active in all sponge cake samples, however, it was identified as having the largest contribution to the aroma of SB-C, SB-SPS and SC-C formulas, reflecting the abundance levels identified in the crumb (Table 4.1). As stated, heptanal is a product of LO, deriving from the auto-oxidation of oleic acid (Whitfield & Mottram, 1992), and it is worth noting that the influence of PS appears to not only be specific to MR and CR reactions (Zamora & Hidalgo, 2005). Chapter 3 previously identified heptanal as strongly influencing the aroma of sponge cakes formulated with

caster sugar. Similarly, another LO aldehyde formed from the auto-oxidation of linoleic acid (Whitfield & Mottram, 1992), (E)-2-octenal, perceived as 'earthy/damp' in sponge cakes, was also found to influence the aroma of all formulas, with an FD value ranging from 50 (SB-SPS) to 150 (SB-C), with the rest of the formulas identified at FD 100. The differences in FD values in relation to area values for the crumb (Table 4.1), is relative to the inconsistent thermal and degradation reactions happening in the crust. (E)-2-Octenal has also been previously shown to influence the aroma of sponge cakes formulated with clean-label ingredients (Chapter 3). 'Spicy/cake' 2-methylpropanal was perceived at an FD 20 in SC-C and SC-LPS, an FD of 10 in SB-LPS and was not detected in the other sponge cake formulas. 'Toffee/sweet/butterscotch' 3-methylbutanal was detected at an FD of 20 across all formulas. These Strecker aldehydes are formed predominately in the crust of the cake (Pozo-Bayón, Ruíz-Rodríguez, Pernin, & Cayot, 2007), which may explain differences in their values. The true contribution of 'sweet' benzaldehyde and 'honey/floral' phenylacetaldehyde was difficult to discern due to co-elution, however, both were identified as an FD 50 in SB-C and SB-SPS, which is likely due to increased MR and CR reactions in these formulas. Nonanal, which was identified as having a 'sweet/cake crust' aroma, was also perceived in all sponge cake formulations, with similar FD values (10-20).

Table 4.2.

Odour active compounds with corresponding odour impression and factor dilution (FD) values identified in sugarbeet control (SB-C), sugarcane control (SC-C), sugarbeet small particle size (SB-SPS), sugarcane small particle size (SC-SPS), sugarbeet large particle size (SB-LPS) and sugarcane large particle size (SC-LPS).

Volatile compound	Descriptor	Sugarbeet			Sugarcane		
		SB-C	SB-SPS	SB-LPS	SC-C	SC-SPS	SC-LPS
2-methylpropanal	Spicy, cake	n.d.	n.d.	10	20	n.d.	20
2,3-Butanedione	Butterscotch, caramel	20	20	20	20	1	20
3-Methylbutanal	Toffee, sweet, butterscotch	20	20	20	20	20	20
Acetol	Fruity, sweet	n.d.	n.d.	20	n.d.	n.d.	20
Unknown 1 [4.3]	Toasty, floral	n.d.	n.d.	10	n.d.	n.d.	20
Furfural	Spicy, bready	100	150	100	100	100	50
2-Furanmethanol	Bisuit, cake crust	50	50	n.d.	50	n.d.	n.d.
Heptanal	Oily, fatty	100	100	50	100	10	10
2,5-Dimethylpyrazine	Nutty, bready	20	20	10	50	100	1
2,3-Dimetylpyrazine	Cake crust	150	50	200	50	150	10
Methional	Potato-like	200	150	150	200	50	100
2-Acetylfuran	Bready, sweet	50	n.d	10	50	n.d.	n.d.
2-2-pentylfuran *	Bready	1	n.d	10	10	n.d.	n.d.
1-octen-3-ol /Benzaldehyde	Mushroom / sweet	50	10/ 50	10/50	20	20	10/20
2-ethyl-5-methyl-pyrazine/ Trimethylpyrazine	Sweet, cake crust, mouldy, woody, soil	20	1	10	1	10	20

2-Acetylthiazole	Sweet, bready, cake crust	20	10	20	20	1	20
Phenylacetaldehyde	Honey, floral	50	50	10	20	n.d	10
E- 2 - octenal	Earthy, damp	150	100	100	100	50	100
Furaneol 2-Nonanone	Cake crust, bready, sweet, cherry	100	50	100	50	50	50
Acetophenone	Cake crust. Toasty	50	n.d	20	10	20	50
Nonanal	Sweet, cake crust	20	20	10	20	10	20
2-Acetylpyrrole	Sweet, cotton candy	50	1	100	20	20	20
(3,5) Octadien2one	Caramel, cake crust, sweet	**n.d.**	20	10	1	10	20
Unknown 2 [9.7]	Cake crust, bready, butterscotch	50	1	10	1	20	20
Dodecane	Damp, musty	50	20	20	50	20	50
Unknown 3 [10]	Fresh, woody, citrus	1	1	50	50	1	1
Unknown 4 [10.40]	Biscuit, bready, floral	1	n.d	n.d.	n.d.		
2,3-Dihydro-3,5-dihydroxy-6-methyl 4(H)-pyran-4-one	Cake crust, spicy, roasty	50	50	20	20	100	20
Unknown 5 [10.60]	Nutty, roasty	n.d.	1	50	1	n.d.	n.d.
Unknown 6 [11.3]	Tea, citrus	n.d.	n.d.	1	20	n.d.	n.d.

*

Identification by comparison with MS spectra, LRI matching from internal library and volatile analysis, odour comparison to literature and retention time of analytical standard. n.d= not detected.

As discussed, furanic compounds were greatly influenced by the manipulation of PS in this study. As depicted in Figure 4.3, 'spicy/bready' furfural appears to play an important role to the desired aroma of all sponge cakes, with the highest FD of 150 identified in SB-SPS. The high area values of furfural, and its prominent aroma, is due to the favoured generation pathway of 1,2-enolization that takes place during sponge cake baking (Cepeda-Vázquez, Rega, Descharles, & Camel, 2018). 2-Furanmethanol was detected in SB-C, SB-SPS and SC-C formulas, at an FD 50, with a perceived odour of 'biscuit/cake crust'. Our results correspond to Afoakwa et al. (2009) who identified that 2-furanmethanol yielded a 'caramel-like/ sweet' odour in chocolate, and on application of large crystal sizes in chocolate production, aroma release of 2-furanmethanol decreased similar to this study, as 2-furanmethanol was not detected by GC-O in LPS sponge cakes. 'Bready/sweet' 2-acetylfuran and 'bready' 2-pentylfuran were also detected in sponge cakes, with 2-acetylfuran perceived the most in SB-C and SC-C at an FD 50, with no consistent trend identified with the perception of 2-2-pentylfuran, having highest FD values (10) in SB-LPS and SC-C. As shown in Fig 4.1., LPS formulas appear to have increased LO reactions, which may be more pronounced in the crust. As SC-C is comprised of many LPS crystals, this may be postulated as to why aroma perception of 2-pentryfuran is higher in SC-C. 2-2-pentylfuran has been perceived in sponge cakes as 'earthy/vegetable', using GC-O (Matsakidou, Blekas, & Paraskevopoulou, 2010).

Similarly, to furanic compounds, pyrazine formation was positively influenced by inclusion of SPS sugar crystals in sponge cake formulas. 2,3-Dimethylpyrazine was perceived as 'cake crust' in all sponge cake formulas, with highest FD values in SB-C (150), SB-LPS (200) and SC-SPS (150), contributing vastly to the overall aroma of these formulas (Figure 4.3). The GC-O results do not mimic the levels identified in the sponge cake crumb (Table 4.1), however, it is well established that MR and CR reactions are

accelerated in the crust, hence leading to the higher prevalence of pyrazines. Paraskevopoulou, Chrysanthou & Koutidou, (2012) identified 2,3-dimethylpyrazine to have higher detection frequency in the crust of wheat bread, and wheat bread formulated with lupin protein isolate, compared to the levels detected in the crumb. Levels of 'nutty/bready' 2,5-dimethylpyrazine were quite low in comparison to 2,3-dimethylpyrazine, with the highest FD identified in SC-C (50) and SC-SPS (100). Co-eluting compounds, 2-ethyl-5-methyl-pyrazine and trimethylpyrazine were perceived as 'sweet/ cake crust' and 'woody/soil', with levels varying across the formulas (Table 4.3).

Five ketones were identified as odour active in the sponge cake formulas; 2,3-butanedione, acetol, 2-nonanone and (3,5)-octadien-2-one. 2,3-Butanedione was characterised by 'butterscotch/caramel' aroma and perceived up to an FD value of 20 in all sponge cake formulas, except for SC-SPS. 2,3-Butanedione is formed during CR and/or MR reactions during sucrose fragmentation, with multiple routes of formation (Poisson, Auzanneau, Mestdagh, Blank, & Davidek, 2016); it may suggest that it's generation is not easily suppressed in sponge cakes. Acetol was only perceived in sponge cakes formulated with LPS sugar crystals (Table 4.1), and is characterised as having a 'fruity/sweet' odour and was perceived up to FD 20. This does not correspond to values identified in crumb, however, the levels of acetol in SB-LPS and SC-LPS had the lowest standard deviation in their area values (Table 4.1). 2-Nonanone was perceived as having a 'cake crust/bready' odour but was identified as co-eluting with furaneol by the distinct 'cherry/sweet' aroma, also identified in Chapter 3. 'Cake crust/ toasty' acetophenone was identified across all sponge cake samples, with the highest FD value of 50 identified in SB-C.

Compounds from other chemical classes also contributed to the aroma profile of the formulated sponge cakes. 2-acetylpyrrole, described as having a 'sweet/cotton candy'

aroma, was identified in all formulas, with highest perception (FD 100) in SB-LPS. 2-Acetylpyrrole is proposed to be generated through intact sugar fragments during MR reactions (Totlani & Peterson, 2007), indicating its irregular generation in cake crust, as also identified in Chapter 3. 'Spicy/roasty' 2,3-dihydro-3,5-dihydroxy-6-methyl 4(H) pyran-4-one contributed greatly to the aroma profile of SC-SPS, however, this compound was not identified in the crumb of any sponge cake formula (Table 5.1). 'Sweet/bready/cake crust' 2-acetylthiazole and 'damp/musty' dodecane also contributed to odour active profile of the sponge cake formulas.

Figure. 4.3. illustrates compounds perceived at a split ratio of 1:50 and above, thus these aroma compounds are the most influential contributors to the overall perceived aroma of the sponge cake formulas. It is interesting to note that only methional ('potato-like') and furfural ('spicy, bready') are significantly contributing the aroma of all the formulations. (E)-2-Octenal ('earthy, damp') considerably contributed to the aroma of all samples, except SC-SPS. Heptanal ('fatty, oily') influenced the aroma of SB-C, SB-SPS and SC-C, while 2,3-dimethyl pyrazine significantly contributed to the aroma of SB-C, SB-LPS and SC-SPS. 2-Nonanone/furaneol ('bready, cherry') only significantly contributed to the aroma of SB-C and SB-LPS, where 2-acetylpyrrole ('sweet, cotton candy') only significantly contributed to the aroma of SB-LPS. 2-5-Dimethylpyrazine ('nutty, bready') only majorly contributed to the aroma of SC-SPS, while 2-3-Dihydro-3,5-dihydroxy-6-methyl-4-(H)-pyran-4-one ('spicy, roasty') only significantly contributed to the aroma of SC-LPS, and was not actually detected in the initially HS-SPME analysis (Table 4.1), indicating its prominence in the crust.

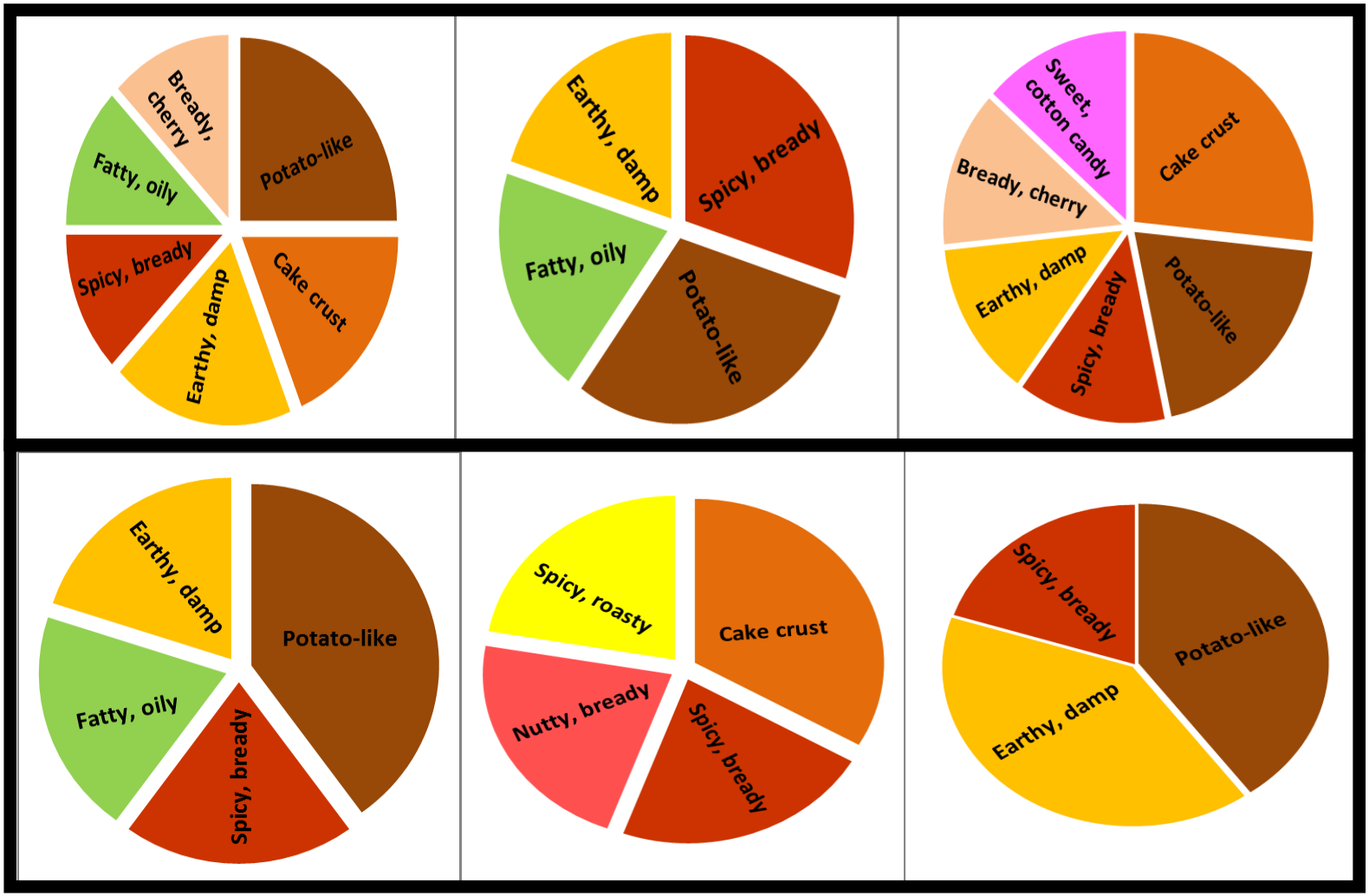


Figure 4.3 Odour active profiles of Sugarbeet; SB-C (A), SB-SPS (B) and SB-LPS (C), and Sugarcane; SC-C (A), SC-SPS (B) and SC-LPS (C) sponge cakes. Pie chart segments represent the dilution factor values of the main odour active compounds with larger segments indicating compounds perceived at higher dilutions (Table 4.2), with compounds from ≥FD 50 only. Colour chart reflects the volatile compounds present in the pie charts.

4.4 Conclusion

The interaction between sucrose source and particle size demonstrated to have a significant effect on the generation of several aroma compounds, hypothesised to be as a result of the molasses present in the raw sugarcane sucrose. When smaller particle size crystals were incorporated into the sponge cake formulas, particularly from sugarbeet, MR and CR reactions were clearly accelerated, presumably due to the lower melting point of smaller sugar crystals, leading to higher levels of prominent heat derived volatile compounds, such as furans and pyrazines. Larger particle size crystals, for both types of sucrose, appeared to increase levels of methyl ketones and ethanol, indicating how generation pathways are directly affected by particle size. Gas-chromatography-olfactometry identified 31 odour active compounds in these sponge cake samples, with methional, furfural, heptanal, 2,3-dimethylpyrazine and (E)-2-octenal having the greatest contribution to the overall aroma. The FD value of compounds differed as a result of formulation, however, the difference in volatile compounds, and volatile formation in the crust also contributed. To the best of our knowledge, no study has been conducted to explore the influence of sugar particle size on the volatile aroma compounds of sponge cakes. This data demonstrates that small particle sucrose crystals produce significantly higher levels of 'bready/spicy' furans, 'cake crust' pyrazines and 'fatty/oily' heptanal in sponge cakes. The findings in this study can be considered during reformulation of sucrose reduced sponge cakes, and other baked confectionery products, as application of a reduced quantity of small particle sizes proves potentially better at reproducing the same level of desirable aroma compounds as traditional formulas.

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Chapter 5. A cross-cultural evaluation of liking and perception of salted butter produced from different feed systems

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Abstract

Perception and liking among Irish, German and USA consumers of salted butter produced from different feed systems: outdoor grass (FS-GRSS), grass/clover (FS-CLVR), and indoor concentrate (FS-TMR), was investigated. A consumer study was conducted in all three countries. Irish and German assessors participated in ranking descriptive analysis (RDA), whereas descriptive analysis (DA) was carried out by a trained panel in the USA. Volatile analysis was conducted to identify differences in aroma compounds related to cow diet. Overall, there was no significant difference in overall liking of the butters, amongst USA, German and Irish consumers, although cross cultural preferences were evident, which did not impact on overall liking. Sensory attribute differences based on cow diet were evident across the three countries as identified by German and Irish assessors and trained USA panellists, which are likely influenced by familiarity. The abundance of specific volatile aromatic compounds, especially some aldehydes and ketones were significantly impacted by the feed system and may also contribute to some of the perceived sensory attribute differences in these butters.

Keywords: dairy, cow diet, preference, descriptive analysis, consumer study, volatiles

5.1 Introduction

Globally, consumers are increasingly aware of their food choices, with respect to country of origin, production practices, sustainability, and potential health promoting properties, prior to purchase (Aschemann-Witzel, Varela & Peschel, 2019). Satisfaction of these extrinsic aspects can influence overall liking, and thus purchase intent and even willingness to pay a premium, particularly for meat and dairy products (Bir, Widmar, Thompson, Townsend, Wolf, 2020; Furnols et al. 2011; García-Torres, López-Gajardo & Mesías, 2016; Napolitano, Braghieri, Piasentier, Favotto, Naspetti, Zanoli, 2010; Scozzafava, Gerini, Boncinelli, Contini, Marone & Casini, 2020). There has been substantial interest in exploring consumer's perception towards meat and dairy products produced from a pasture/grazing diet. A recent review by Stampa, Schipmann-Schwarze et al. (2020), mainly focusing on studies undertaken in Europe and the United States of America (USA), outlined that attitudes towards environmental practices and health benefits of consuming pasture-raised livestock products were the main drivers for consumption and willingness to pay premium prices for these types of products.

Farming practices in Ireland consist of fresh pasture for the majority of lactation, allowing for utilisation of a low-cost, readily available feed source to produce high quality milk products (O'Brien et al. 1999; Whelan et al. 2017). Since the abolishment of milk quotas in Europe in 2015, milk production in Ireland has increased substantially, with a 13% increase in milk intake from 2015 to 2017 (CSO, 2015; CSO, 2017; McKay, Lynch, Mulligan, Rajauria, Miller & Pierce, 2019). In 2019, Ireland's dairy sector grew in value by 11%, with butter being the largest export category (Bord Bia, 2019) and further opportunities exists to expand current markets and develop new markets.

The most apparent differences in dairy products produced from cows on a pasture rich diet, versus concentrates, are changes to the fatty acid (FA) profile and colour. Inclusion of fresh pasture significantly increases levels of unsaturated FAs in milk (Croissant, Washburn, Dean & Drake, 2007; O'Callaghan, Hennessy, McAuliffe, Kilcawley, O'Donovan, Dillon, Ross & Stanton, 2016; White, Bertrand, Wade, Washburn, Green & Jenkins, 2001) and β-carotene levels, enhancing a yellow colour, particularly obvious in butter as β-carotene is fat soluble (Martin, Verdier-Metz, Buchin, Hurtaud & Coulon, 2005). Although milk and dairy products are not regarded as a dietary source of omega-3 FAs, higher amounts of α-linoleic acid and conjugated linoleic acid (CLA) are present in bovine milk from pasture based diets (Croissant et al. 2007; Khanal, Dhiman, Ure, Brennand, Boman, & McMahon, 2005; Liu, Pustjens, Erasmus, Yang, Hettinga, & van Ruth, 2020; O'Callaghan et al. 2016; Van Valenberg, Hettinga, Dijkstra, Bovenhuis & Feskens, 2013). β-carotene derived from fresh pasture gives a yellow hue to butter, with the intensity of yellow positively correlating to the amount of fresh pasture grazed (Agabriel, Cornu, Journal, Sibra, Grolier & Martin, 2007; O'Callaghan, Faulkner, McAuliffe, O'Sullivan, Hennessy, Dillon, Kilcawley, Stanton & Ross, 2016). β-carotene is also a precursor for fat-soluble vitamin A, and a powerful antioxidant, and therefore also beneficial in the diet (Jayedi, Rashidy-Pour, Parohan, Zargar, & Shab-Bidar, 2019). Butter hardness and spreadability are also dictated by the FA profile and thus impacted by the cow's diet, with higher numbers of unsaturated FAs lowering the melting point (Walstra, 1995).

Butter is coveted for its rich sensory attributes, with butter flavour being a significant driver for liking (Krause, Lopetcharat & Drake, 2007). Although flavour is mainly dictated by the milk fat itself and added salt, aroma volatile compounds also play an important role in the sensory perception of butter. Previous studies have identified a

range of potentially important volatiles including lactones, ketones, acids, esters, aldehydes, pyrroles and sulphur compounds, thought to influence the sensory perception of butter or sweet cream butter (Li, Wang, Yuan, Li & Zhang, 2020; Lozano, Miracle, Krause, Drake & Cadwallader, 2007; Mallia, Escher, Dubois, Schieberle & Schlichtherle-Cerny, 2009), and further work is required to determine factors that may influence the generation of these volatiles, such as cow diet. Although the influence of diet on the sensory properties of butter has been previously investigated (Hurtaud, Faucon, Couvreur & Peyraud, 2010; O'Callaghan et al. 2016b), studies are limited and no studies have been published exploring the cross-cultural liking of salted butter.

Therefore, an objective of this study was to investigate the liking and perception of salted butters, produced from cows outdoors on two pasture-based diets; perennial ryegrass, or perennial ryegrass/white clover, and cows indoors on a concentrate diet (total mixed rations) by consumers (Ireland, Germany and the USA), untrained assessors (Ireland and Germany) and trained panelists (USA). In addition, volatile analysis was performed to elucidate potential differences in sensory perception. The information generated should result in an improved understanding of the cross-cultural perception of Irish dairy products beneficial for the export markets.

5.2 Materials and Methods

5.2.1. Experimental diets and milk production

Fifty four spring calving Friesian cows were selected from the general herd at the Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork. The cows were randomised based on milk yield, milk solids yield, calving date and lactation number, and allocated to one of three experimental feed systems (FS) (n=18) (18 cows in each FS); outdoor pasture grazing on perennial ryegrass (*Lolium perenne* L.) (FS-GRSS), outdoor pasture grazing on perennial ryegrass supplemented with white clover (*Trifolium repens* L.) (FS-CLVR) or housed indoor provided with a diet of TMR (FS-TMR). In depth details of the three diets was outlined by O'Callaghan et al. (2016b). The TMR diet comprised of 8.3 kg of concentrates, 7.15 kg of grass silage and 7.15 kg of maize silage, on a dry matter basis. Morning and evening milks from each of the experimental herds (FS-GRSS, FS-CLVR and FS-TMR) were collected and assigned as per feed system to 5000L refrigerated tanks. Combined milk was kept at 4°C prior to sample collection, which took place within 24 hours after milking.

2.2. Butter manufacture

Butter production took place on 3 separate occasions over a 3 week period. Butter produced from each experimental feed system was produced in triplicate (producing 3 batches per feed system). The procedure was identical to that as outlined by O'Callaghan, et al. (2016b).

The butter was packed into 200 ± 20 g sticks using an extruder and wrapped in grease-proof paper followed by an outer wrapping of aluminium foil. Butter was vacuum

packed and stored at -20°C until subsequent sensory and volatile analysis. Butter was defrosted in a refrigerator 24 hr before the relevant analysis. Prior to each sensory study, the butter was tempered at room temperature for 1 hr.

5.2.3. Consumer study

5.2.3.1. Consumer selection

Three consumer sensory panels from three countries, Ireland, Germany and USA, were created for the purpose of this study. Panels comprised of 108 German (70% female, 30 male, age= 18-68), 103 USA (79% female, 21% male, age= 21-64) and 96 Irish consumers (68% female, 32% male= 18-60), who regularly consumed butter. The German consumers consisted of a mixture of faculty and students, recruited in the University of Applied Sciences, Muenster, Germany. The USA consumers were recruited by the Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, North Carolina, USA. The Irish consumers consisted of students and staff from Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland and St. Angela's Food Technology Centre, Sligo, Ireland.

5.2.3.2. Product evaluation by hedonic, intensity and just-about-right scales

Butter evaluation took place in accordance with international standards (ISO 2014, ISO 11136), in each country. The ballots were identical in all three countries, except that salt liking was not included in the USA ballot. The consumer study evaluation was designed to collect information on attribute liking, intensity perception and optimum levels of each attribute by applying a just-about-right scale (JAR). Liking was assessed for overall appearance, colour, flavour, and texture attributes, intensity for colour, flavour,

saltiness, freshness, and firmness, and JAR scale evaluation was used to assess colour, flavour, saltiness and texture. Each attribute was evaluated for liking on a 9-point hedonic scale; 9= ”extremely like” and 1=”extremely dislike”, and intensity; 9=”high intensity” and 1=”low intensity”. Five-point JAR scales were employed with extremes; 1=”much too little”, 5=”much too much”. Since 9 butters were produced in total from the 3 experimental diets (3 butters produced in triplicate), the samples were presented to panelists in an incomplete block design, randomly allocated and single blinded using three-digit codes; allowing for even distribution of each treatment and butter trial. Therefore order of presentation was balanced not randomized and the ballot order for each product was identical as per standard consumer tests. Panelists were presented with three individual 5 ± 0.02 g servings of butter alongside water crackers and water. Crackers were provided for palatability purposes, the butter was presented on the crackers, but consumers were clearly instructed that the sensory evaluation was to be carried out solely on the sensory attributes of the butter. Panelists were encouraged to rinse their palate thoroughly after each sample and a 1 min rest between each sample was enforced. Consumers from the University of Applied Sciences, Muenster and Teagasc Food Research Centre completed the ballot questionnaire on paper. Consumers at North Carolina State University and St. Angela’s College undertook the questionaire *via* Compusense Cloud (Guelph, Canada) and FIZZ (Biosystems, Couternon, France), respectively.

5.2.4. Ranking Descriptive Analysis

Ranking descriptive analysis (RDA) (Richter, de Almeida, Prudencio, & de Toledo Benassi, 2010), a modification of flash profiling, was performed by untrained assessors at the University of Applied Sciences, Muenster, Germany, and at the Teagasc Food Research Centre, Ireland. As stated, assessors were recruited on their frequency to consumer butter, and had previous sensory analysis experience. Each panel was comprised of 20 German and 20 Irish assessors, respectively. Attributes were generated by a focus group consisting of 7 people, comprised of members from the Food Quality and Sensory Science Department at the Teagasc Food Research Centre, Ireland. The established list of attributes was chosen on their ability to best describe the different butter samples produced by each treatment. Irish and German assessors were asked to assess the attributes relative to colour (yellow colour), aroma (buttery, milky, grassy & rancid), flavour (salty, sweet, creamy, sour, stale) and texture (melt in the mouth). Similar to the consumer study, each butter trial sample was presented to assessors on a water cracker for evaluation. The presentation for the RDA was a complete block design. Assessors were briefly coached on the explanation of each attribute in relevance to butter and asked to evaluate the intensity of each on a 9 cm continuous scale. Sensory analysis was conducted in duplicate over two separate occasions.

5.2.5. Descriptive analysis evaluation

Butter flavour was evaluated by a trained descriptive sensory panel using an established flavour language for butter (Krause et al. 2007) (Table 3). All sensory testing was conducted in accordance with the North Carolina State University Institutional Review Board for Human Subjects guidelines. Panellists (n = 6) had more than 100 h of previous experience with the sensory analysis of dairy products. Prior to this study, panellists participated in 20 h of additional training on the three butters, FS-GRSS, FS-

CLVR and FS-TMR to calibrate and confirm sensory attributes. Samples were prepared 24 h in advance and refrigerated at 4°C. Prior to evaluation, butters were tempered to 15°C. A cube of butter (~20 g) was placed in 3-digit-coded, 60-mL lidded cups (Sweetheart Cup Company, Owings Mills, MD). Samples were evaluated on a 15-point intensity scale, in duplicate, on paper ballots by each panellist in a randomized balanced block design.

5.2.6. Volatile Analysis by HS-SPME-GC-MS

Volatile analysis was carried out by headspace solid phase micro-extraction gas chromatography mass spectrometry (HS-SPME-GC-MS) utilising a Gerstel MultiPurpose Sampler (GMPS) rail system (Anatune, Cambridge CB3 0NA, UK) connected to a Shimadzu GP2010 plus GC (Mason Technology Ltd., Dublin, Ireland). A 50/30 µm divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre was employed for analysis (Agilent Technologies Ireland Ltd, Cork, Ireland). The chosen HS-SPME parameters were as described by O'Callaghan et al. (2016b) with modifications. More sample was used and a longer extraction time was applied in an attempt to recover a higher number compounds. Butter was thawed overnight at room temperature and 3 g was added to an La-Pha-Pack amber 20 ml screw-capped SPME vial with magnetic caps and silicone/polytetrafluoroethylene 1.3mm 45° shore A septa (Apex Scientific Ltd., Co. Kildare, Ireland) and equilibrated for 10 min while exposed to a temperature of 40°C, with pulsed agitation for 5 s at 350 rpm using the GMPS agitator/heater. The SPME fibre was exposed to the headspace above the samples, at a depth of 21 mm, for an extraction time of 60 min at 40°C. The fibre was retracted, injected into the GC inlet with a merlin microseal (Merck, Arklow, Ireland) and desorbed for 3 min at 250 °C, followed by 3 min at 270°C in the GMPS fibre bakeout station, to

minimise carryover of compounds. Each butter trial was analysed in triplicate. An external standard solution (1-butanol, dimethyl disulphide, butyl acetate, cyclohexanone) (Merck, Arklow, Ireland) at 1000 ppm in methanol (Merck, Arklow, Ireland) was also analysed at the start and end of each batch, and levels of each external standard were quantified and compared to reference values to ensure that both the SPME extraction and MS detection were performing within specification.

The GC analysis was performed on a Shimadzu 2010 Plus GC (Mason Technology Ltd., Dublin, Ireland), equipped with a split/splitless injector, operating in splitless mode. The carrier gas was helium held at a pressure of 43.8 psi and a flow rate of 1.2 mL/min. The volatile compounds were separated on a DB-624 UI (60 m × 0.32 mm × 1.80 μm) column (Agilent Technologies Ireland Ltd., Cork, Ireland). The temperature of the column oven was set at 40 °C, held for 5 min, increased at 5 °C/min to 230 °C then increased at 15 °C/min to 260 °C. The total GC run time was 65 min. Compound identification was carried out by a Shimadzu TQ8030 mass spectrometry detector (Mason Technologies Ltd., Dublin, Ireland) ran in single quad mode. The ion source temperature was 220 °C and the interface temperature was set at 260 °C. The MS mode was electronic ionization (70 eV) with the mass range scanned between m/z 35–250 amu. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, the Shimadzu commercial library FFNSC (Flavour and Fragrance Natural and Synthetic Compounds library) version 2 and an in-house library created in GCMS Solutions software (Shimadzu, Japan) created with standards (where possible), and with target and qualifier ions and linear retention indices for each compound Van (Den Dool, Kratz, 1963). Spectral deconvolution was also performed to confirm identification of compounds using AMDIS (Automated Mass Spectral Deconvolution and Identification System, www.amdis.net).

5.2.7. Statistical analysis

Data analysis was handled accordingly based on the normality of the data. Hedonic data from the sensory evaluation was analysed using non-parametric Kruskal Wallis test (α=0.05), with post hoc Mann-Whitney to identify the significant differences between samples. Bonferroni adjustment was applied to account for type 1 error, therefore working at an alpha level of 0.017. Analysis of variance (ANOVA) with post hoc Tukey significant test was applied to RDA and descriptive analysis data, working at an alpha level of 0.05. Just about right (JAR) data was assessed using chi-square statistic. A combination of parametric and non-parametric tests was used to evaluate the volatile data (specified in Table 4). All parametric and non-parametric tests were performed using SPSS IBM SPSS Statistics 24 for windows (SPSS Inc., IBM Corporation, NY, USA).

5.3. Results and Discussion

5.3.1. Consumer evaluation

The average results of the sensory evaluation of consumers liking towards FS-GRSS, FS-CLVR and FS-TMR butters are presented in Table 5.1. Overall, there were no significant ($P < 0.05$) differences in overall liking of all three butters within each consumer cultural group; however, cross-cultural differences were evident.

5.3.1.1. Irish consumers

There was no significant ($P > 0.05$) difference amongst Irish consumers liking of the sensory attributes (overall appearance, colour, flavour, saltiness, texture) or overall liking

of the three butters (Hedonics Table 5.1). These results contradict a previous study, by O'Callaghan et al. (2016b) who found that Irish consumers preferred the appearance and flavour of butters produced from grass and clover diets, compared to butter produced from TMR. However, in this study Irish consumers did perceive the colour intensity of FS-GRSS and FS-CLVR butters significantly ($P < 0.017$) higher than FS-TMR butter (Intensity Scale Evaluation Table 5.1), presumably due to higher β-carotene levels, with 41.6% of panellists grouping the FS-TMR butter as 'not yellow enough', (JAR Evaluation Table 5.1). However, this did not negatively influence their liking of the FS-TMR butter.

5.3.1.2. German consumers

Similar to Irish consumers, there was no significant ($P > 0.05$) difference in liking by German consumers for all three butters for sensory attributes, or for overall liking (Hedonics Table 5.1). In agreement with Irish consumers, German consumers rated the colour of both FS-GRSS and FS-CLVR butter significantly ($P < 0.017$) more intense than FS-TMR butter (Intensity Scale Evaluation Table 5.1) and showed a significant ($P < 0.05$) higher score for 'not yellow enough' for FS-TMR butter (JAR Evaluation Table 5.1). German consumers also found the salt intensity of FS-GRSS and FS-CLVR butters, significantly ($P < 0.017$) higher than FS-TMR butter, even though identical levels of salt were added to each batch. Additionally, there was a significant ($P < 0.017$) difference in the perception of firmness intensity with FS-TMR butter perceived as firmer than FS-GRSS and FS-CLVR butters. Although FA analysis was not undertaken in this study, butters from an identical experimental trial O'Callaghan et al. (2016b), and other studies (Couvreur, Hurtaud, Lopez, Delaby & Peyraud, 2006; Silva, Silva, Prates, Bessa, Rosa & Rego, 2019) have characterised butter produced from pasture diets higher in unsaturated FAs, therefore the pasture derived butter is likely to be softer, due to a lower melting

point. The higher salty intensity perception of FS-GRSS and FS-CLVR butter as perceived by the German consumers may also relate by the softer texture of these butters and their behaviour in the mouth; more rapid melting compared to FS-TMR butter (Figure 5.1). This would also appear to be confirmed by the JAR Evaluation of texture for FS TMR butter which was deemed significantly ($P < 0.05$) higher for 'much too firm'. Dadalı and Elmacı (2019) found that margarine with the lowest score for hardness was perceived as the saltiest, despite having identical salt contents.

5.3.1.3. USA consumers

For overall appearance and colour liking, USA consumers scored FS-CLVR and FS-TMR butters significantly ($P < 0.017$) higher than FS-GRSS butter (Hedonics Table 5.1). When attempting to elucidate the drivers for liking of butter, Krause et al. (2007) identified from a focus group that USA consumers found a light yellow colour desirable in butter, which likely explains why USA consumers in this study rated their liking of appearance and colour of FS-TMR butter the highest (Hedonics Table 5.1), promoting the theory of familiarity dictating preference (Kim, Jombart, Valentin, & Kim, 2015; Pagès, Bertrand, Ali, Husson, & Lê, 2007). However, it is difficult to interpret why consumers liked the FS-CLVR butter similarly to FS-TMR butter for appearance and colour, yet rated the FS-GRSS butter significantly lower for these same attributes. In the JAR Evaluation, USA consumers also scored FS-CLVR and FS-TMR butters significantly ($P < 0.05$) higher for 'just about right' for yellow colour. However, these same consumers also scored FS-TMR butter significantly ($P < 0.05$) higher for 'not enough yellow', compared to FS-GRSS and FS-CLVR butter. Interestingly, FS-GRSS butter flavour was perceived as the most favourable by USA consumers, however, it was not significantly ($P > 0.017$) different from FS-TMR butter. This may reflect the trend in the USA for value

added grass fed milk and other dairy products (Harwood & Drake, 2018). In terms of texture liking, USA consumers liked the FS-CLVR butter significantly ($P < 0.017$) more than FS-TMR butter. Krause et al. (2007) also identified a cluster of USA consumers, referred to as 'margarine lovers', who were also butter users, but preferred the sensory attributes of margarines i.e. the soft texture. This result corresponds to that of the JAR Evaluation, where consumers ranked FS-CLVR and FS-GRSS butters higher for 'just about right' texture, with FS-CLVR butter significantly ($P < 0.05$) higher compared to FS-TMR butter. In addition, USA consumers ranked FS-TMR butter significantly ($P < 0.05$) higher for 'much too firm'.

Table 5.1.

Cross-cultural comparison of liking, intensity rating and just-about-right scale evaluation by Irish, German and United States of America consumers, of butters produced by different diets; FS-GRSS, FS-CLVR and FS-TMR.

	Irish Consumers			German Consumers			USA Consumers		
	FS-CLVR	**FS-GRSS**	**FS-TMR**	**FS-CLVR**	**FS-GRSS**	**FS-TMR**	**FS-CLVR**	**FS-GRSS**	**FS-TMR**
Hedonics									
Overall appearance	6.51 ± 1.56 ^{x}	6.46 ± 1.65 ^{x}	5.99 ± 1.76 ^{y}	5.62 ± 1.59 ^{y}	5.69 ± 1.65 ^{y}	5.44 ± 1.69 ^{y}	6.46 ± 1.63 ^{abx}	6.33 ± 1.57 ^{bx}	6.98 ± 1.42 ^{ax}
Colour	6.44 ± 1.61 ^{x}	6.19 ± 1.80	5.80 ± 1.80 ^{y}	5.66 ± 1.50 ^{y}	5.88 ± 1.56	5.56 ± 1.38^{y}	6.43 ± 1.64 ^{abx}	6.18 ± 1.72 ^{b}	6.91 ± 1.57 ^{ax}
Flavour	6.46 ± 1.74	6.44 ± 1.67 ^{y}	6.28 ± 1.68 ^{x}	6.19 ± 1.65	6.38 ± 1.66 ^{y}	5.89 ± 1.69 ^{y}	6.52 ± 1.75^{b}	7.10 ± 1.55 ^{ax}	6.76 ± 1.79 ^{abx}
Salt	6.00 ± 1.63	5.73 ± 1.79	5.78 ± 1.68	5.83 ± 0.75	5.87 ± 0.73	5.52 ± 0.72			
Texture (firmness for USA)	6.54 ± 1.58	6.35 ± 1.72 ^{x}	6.05 ± 1.75 ^{x}	6.40 ± 1.84	6.52 ± 1.51^{x}	6.17 ± 1.84^{x}	6.26 ± 1.67 ^{a}	5.95 ± 1.69 ^{aby}	5.50 ± 1.81 ^{by}
Overall liking	6.46 ± 1.67 ^{x}	6.36 ± 1.76 ^{y}	6.27 ± 1.66 ^{y}	6.04 ± 1.74^{y}	6.23 ± 1.61^{y}	5.89 ± 1.90^{z}	6.59 ± 1.65 ^{x}	7.13 ± 1.47 ^{xy}	6.85 ± 1.67^{x}
Intensity Scale Evaluation									
Colour	6.14 ± 1.89 ^{a}	5.68 ± 2.41^{ab}	5.22 ± 2.40 ^{bx}	6.31±0.80 ^{a}	6.02 ± 0.76 ^{a}	3.27 ± 0.81 ^{ay}			
Flavour	6.34 ± 1.78	5.73 ± 2.01	6.21 ± 1.60 ^{x}	6.38±1.79 ^{a}	6.14 ± 1.87 ^{a}	5.29 ± 2.23 ^{by}			
Salt	5.31 ± 2.00 ^{y}	5.15 ± 2.15 ^{y}	5.06 ± 1.96	6.17±1.88 ^{ax}	5.95 ± 1.81^{ax}	5.28 ± 1.89 ^{b}			
Freshness	6.34 ± 1.73 ^{x}	6.10 ± 1.93	5.95 ± 1.82	5.56±0.89 ^{y}	5.78 ± 0.82	5.80 ± 0.97			
Firmness	5.46 ± 2.05 ^{x}	5.34 ± 2.15 ^{x}	5.61 ± 1.94 ^{x}	3.62±2.08 ^{by}	3.42 ± 2.06 ^{by}	4.53 ± 1.98^{ay}			

JAR Evaluation										
Colour	**Not Yellow Enough**	23.96%	31.25%	41.67%	7.41% [b]	9.26% [b]	51.85% [a]	2.9%[b]	4.9% [b]	26.2% [a]
	Just About Right	62.50%	53.125%	42.71%	37.96%	38.89%	42.59%	57.3%[ab]	49.5% [b]	72.8% [a]
	Too Yellow	13.54%	15.625%	15.63%	54.63%	51.85%	5.56%	39.8%	45.6%	1.0%
Flavour	**Not Enough Flavour**	18.75%	26.04%	17.71%	18.52%	24.07%	42.59%	26.2%	21.4%	33.0%
	Just About Right	62.50%	60.42%	56.25%	51.85%	53.70%	41.67%	58.3%	70.9%	63.1%
	Too Much Flavour	18.75%	13.54%	26.04%	29.63%	22.22%	15.74%	15.5%[a]	7.8% [ab]	3.9% [b]
Salt	**Not Enough Salt**	18.75%	27.08%	16.67%	17.59%	21.30%	30.56%			
	Just About Right	62.50%	51.04%	59.38%	38.89%	41.67%	37.04%			
	Too Much Salt	18.75%	21.88%	23.96%	43.52%	34.26%	32.41%			
Texture	**Not Firm Enough**	2.08%	10.42%	10.42%	42.59%	38.89%	19.44%	0.0%	0.0%	1.0%
	Just About Right	76.04	63.54	52.08	55.56%	57.41%	64.81%	58.3%[a]	48.5%[ab]	35.9%[b]
	Much too Firm	21.88	26.04	37.50	1.85%[b]	3.70% [b]	15.74%[a]	41.7%[b]	51.5% [ab]	63.1%[a]

Within each consumer Cultural group: values in the same row not sharing the same superscript (a, b) indicate significant difference in perception (identified using Kruskall Wallis and Mann-Whitney test for multiple comparison, $\alpha = 0.017$).

Cross cultural comparison: values in the same row, under the identical diet headings, not sharing the same superscript (x, y, z) indicate significant difference in perecption (identified using Kruskall Wallis and Mann-Whitney test for multiple comparison, $\alpha = 0.017$).

No superscript indicates no significant difference identified.

5.3.1.4. Cross-cultural perceptions of butters

Irish and USA consumers scored the overall appearance liking of FS-GRSS and FS-CLVR butters significantly ($P < 0.017$) higher compared to German consumers (Hedonics Table 5.1). However, Irish and German consumers both scored FS-TMR butter statistically ($P < 0.017$) lower for liking of appearance. Cross-cultural liking of colour corresponds to liking of appearance, with Irish and USA consumers liking FS-CLVR butter significantly ($P < 0.017$) more than German consumers. Irish consumers are accustomed to yellow butter and previously showed highest liking for butter produced from grass and clover, compared to TMR (O'Callaghan et. al. 2016b). Referring to the study by Krause et al. (2007), the same cluster of USA consumers who liked softer 'margarine like' butters also preferred those butters and spreads which were darker in colour, in agreement with this study. Similarly, USA consumers scored the FS-GRSS butter significantly ($P < 0.017$) higher for liking of flavour than Irish and German consumers. Both Irish and German consumers had similar liking for the texture of FS-GRSS butter, which was significantly ($P < 0.017$) higher compared to USA consumers. Overall acceptability of FS-GRSS butter was rated significantly ($P < 0.017$) higher by USA consumers compared to Irish and German consumers, which may be driven by their high liking for flavour. Both USA and Irish consumers considered FS-CLVR butter to be significantly ($P < 0.017$) higher for overall acceptability compared to German consumers. Salt intensity was perceived significantly ($P < 0.017$) higher by German consumers compared to Irish consumers for both FS-GRSS and FS-CLVR butter (Intensity Scale Evaluation Table 5.1). Irish consumers are familiar with soft butter; and did not perceive a significant difference in salt taste in agreement with a previous study (O'Callaghan et al. 2016b). Butter sold in Germany however is typically unsalted which may explain the higher perceived salt intensity in the pasture butters, although texture as discussed earlier

is also likely a contributory factor. Perception of freshness intensity was significantly lower ($P < 0.017$) by German consumers compared to Irish consumers for FS-CLVR butters. Irish consumers found FS-GRSS butter to be significantly ($P < 0.017$) firmer compared to German consumers, which again, is likely to be related to familiarity (O'Callaghan et al. 2016b).

Significant ($P < 0.017$) differences were identified for liking of FS-TMR butter, with USA consumers rating the overall appearance, colour and overall liking significantly ($P < 0.017$) higher, compared to Irish and German consumers, with a similar trend identified by Krause et al. (2007). There was no significant ($P > 0.017$) difference in liking of flavour of FS-TMR butter between Irish and USA consumers, which differed to O' Callaghan et al. (2016b), where Irish consumers rated liking of flavour of TMR butter significantly lower than butter produced from milk from grass and clover diets. There was significant ($P < 0.017$) difference in the liking of texture of FS-TMR butter by Irish and German consumers compared to USA consumers. For intensity ratings, Irish consumers ranked colour, flavour and firmness of FS-TMR butter significantly ($P < 0.05$) higher than German consumers. No significant differences were evident in relation to JAR Evaluation for colour, flavour, salt (USA consumers did not assess salt) or texture between the cultural groups. Overall, the cross-cultural perception of butter attributes by the 3 consumer groups was within a similar range on the hedonic scale, signifying a liking of butter amongst all three consumer groups.

5.3.2. Ranking descriptive analysis

The average results of Irish and German consumer's perception of butters are portrayed in Figures. 5.1a & 5.1b, respectively, and in Table 5.2. Corresponding to the results for yellow intensity (Table 5.1), both Irish and German assessors rated FS-TMR butter significantly ($P < 0.05$) lower for yellow colour. Both German and Irish assessors rated FS-CLVR butter as having a more intense yellow colour (Figure 5.1a & 5.1b), compared to FS-GRSS butter, although not significant, this may suggest that FS-CLVR butter contained higher amounts of β-carotene (β-carotene levels vary with intake of grass and clover outdoors). O'Callaghan et al. (2016) identified grass butters as having the highest levels of β-carotene, however Panthi, Sundekilde et al. (2019) identified Massdam cheeses produced from cows supplemented with white clover as having higher amounts of β-carotene compared to cheese produced from only grass. As mentioned, β-carotene content in milk will vary due to the levels of grass and clover ingested by cows due to differences in availability within the pasture. German assessors perceived FS-TMR butter as significantly ($P < 0.05$) darker than Irish assessors; however, this does not match results from the intensity scale portion of the consumer study (Table 5.1). This result may be due to the fact that in Ireland only butter derived from pasture is commercially available, while in Germany, butters from both pasture or concentrate are widely available, and therefore Irish consumers may have scored TMR butter lower for colour intensity due to a lack of familiarity.

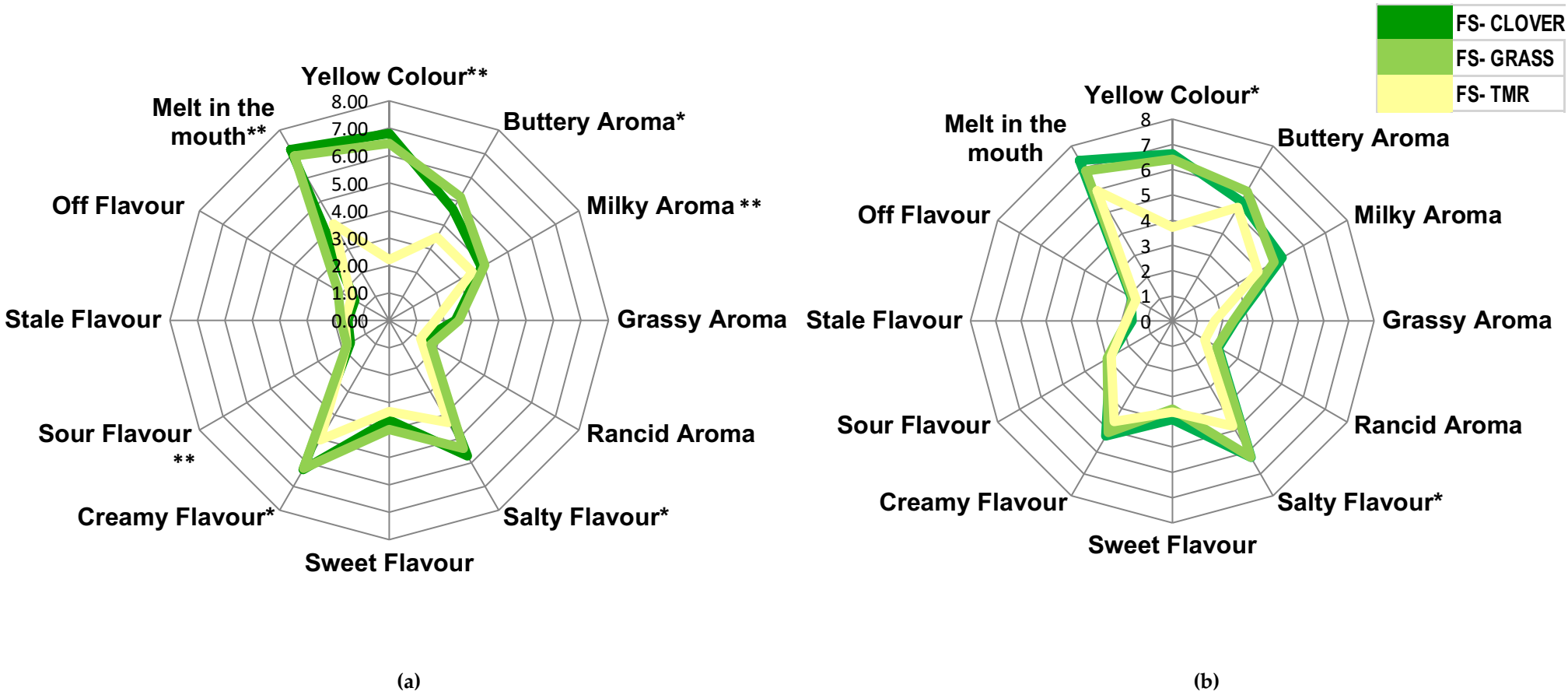


Figure 5.1. Average results (n=20) from ranking descriptive analysis evaluation of FS-GRSS, FS-CLVR and FS-TMR butters by Irish (a) and German (b) assessors.

* Indicates a significant difference identified in the perception of butters, within the respective cultural group

** Indicates a significant difference in the perception of the respective attribute, between Irish and German assessors

Irish assessors perceived the aroma of FS-GRSS and FS-CLVR butters as significantly ($P < 0.05$) more buttery, compared to FS-TMR butter, similar to O'Callaghan et al. (2016), where consumers rated butter produced from grass significantly higher for diacetyl aroma compared to TMR butter. There was no significant difference ($P < 0.05$) detected amongst the German assessors for buttery aroma. German assessors perceived FS-GRSS butters to be significantly more milky compared to Irish assessors. Grassy and rancid aroma were not identified as significantly ($P > 0.05$) different by either German or Irish assessors. Both Irish and German assessors perceived significant ($P < 0.05$) differences in saltiness perception, with values for FS-GRSS and FS-CLVR butters significantly higher, compared to FS-TMR butter. This is likely linked to the texture; melt in the mouth attribute, where Irish assessors perceived FS-GRSS and FS-CLVR butters to melt much more rapidly ($P < 0.05$) in the mouth. As previously mentioned, this is likely due to changes in fatty acid profile, which influence spreadability and melting properties of butter (Hurtaud & Peyraud, 2007). Similarly, Irish assessors perceived the flavour attribute creamy as significantly ($P < 0.05$) higher in both FS-GRSS and FS-CLVR butters, than in FS-TMR butter, which is also likely directly related to the texture, and in turn to the unsaturated fatty acid profile of these butters. Irish assessors have previously perceived pasture butters as significantly more creamy (O'Callaghan et al. 2016b). Irish assessors in this study also found that FS-GRSS and FS-CLVR butters were significantly higher ($P < 0.05$) for melt in the mouth than FS-TMR butter. German assessors scored melt in the mouth statistically higher ($P < 0.05$) for TS-TMR butter than Irish assessors. German assessors also found FS-CLVR butter significantly ($P < 0.05$) more sour compared to Irish assessors, and this may relate to the lower rating of freshness as perceived in the consumer study (Intensity Scale Evaluation Table 5.1)

Table 5.2.

Cross-cultural comparison of RDA evaluation by Irish and German assessors of butters produced by different diets; FS-GRSS, FS-CLVR and FS-TMR. Table presents average values and standard deviation.

	Irish assessors			German assessors		
	FS-GRSS	**FS-CLVR**	**FS-TMR**	**FS-GRSS**	**FS-CLVR**	**FS-TMR**
Colour						
Yellow Colour	6.81±0.9^{a}	6.33±1.37^{a}	2.41±0.85^{by}	6.63±1.12^{a}	6.41±1.46^{a}	3.72±1.37^{bx}
Aroma						
Buttery	4.66±1.53^{a}	5.13±1.51^{a}	3.48±1.22^{b}	5.48±1.86	5.93±1.99	5.20±1.93
Milky	3.89±1.45^{y}	4.05±1.29	3.40±1.24	5.03±2.56^{x}	4.66±2.25	3.93±2.08
Grassy	2.41±1.17	2.54±1.34	1.71±0.72	2.50±1.92	2.42±1.97	1.69±1.11
Rancid	1.39±0.69	1.76±1.40	1.31±0.73	2.11±1.76	2.05±1.64	1.49±1.08
Flavour						
Salty	5.71±1.13^{a}	5.33±1.66^{a}	4.16±1.77^{b}	6.24±2.00^{a}	6.23±1.47^{a}	4.81±1.77^{b}
Sweet	3.64±1.50	3.99±1.45	3.34±1.41	3.92±1.95	3.50±1.76	3.61±1.84
Creamy	6.29±1.25^{a}	6.24±1.37^{a}	4.91±1.46^{b}	5.27±1.90	5.10±1.90	4.60±2.03
Sour	1.64±0.57^{y}	1.76±1.15	1.83±0.79	2.85±1.67^{x}	2.97±2.20	2.79±1.82
Stale	1.51±0.81	1.76±1.07	1.61±0.74	1.59±0.78	1.80±1.49	1.83±1.44
Off Flavour	1.44±0.63	2.16±1.84	1.56±1.01	1.90±1.33	1.81±1.25	1.66±1.33
Texture						
Melt in the mouth	7.19±0.85^{a}	6.79±1.26^{a}	4.19±1.73^{by}	7.36±1.20	6.86±1.46	5.96±2.21^{x}

Within consumer group: values in the same row not sharing the same superscript (a, b) indicate significant difference (Confidence level 5%, identified using ANOVA and Tukey post)
Cross cultural comparison: values in the same row not sharing the same superscript (x, y) indicate significant difference (confidence level 5%, identified using student t-test).
Values provided after ± are standard deviations.

5.3.3. Descriptive analysis evaluation of FS-GRSS, FS-CLVR and FS-TMR butters by trained USA panellists

The results of the descriptive analysis (DA) undertaken by trained USA panellists are listed in Table 3. There was no significant ($P > 0.05$) difference perceived for cooked/nutty, milkfat, and salty taste, between all three butters. Grassy was rated significantly ($P < 0.05$) higher in FS-GRSS butter, followed by FS-CLVR butter, however, it was not detected in FS-TMR butter, corresponding to results from Cheng et al. (2020), who reported significant difference in grassy/hay perception of bovine skim milk powder, produced from pasture versus indoor TMR diets, as assessed by a descriptive panel. Villeneuve et al. (2013) identified that grassy intensity was higher from milk produced by pasture and correlated to the higher levels of the aldehyde pentanal. The attribute stale was not perceived in any of the butters, and the levels of saltiness perception were similar (Table 5.3). Panelists found a significant ($P < 0.05$) difference in the colour intensity of the three butters, in the following order FS-CLVR > FS-GRSS > FS-TMR. Mothball flavour was noted in FS-CLVR butter but not in FS-GRSS or FS-TMR butters. This is a feed specific flavour that has been documented in previous studies (butter, cheese, dried ingredients) manufactured from pasture feeding, and appears to be specific to certain types of pastures (Drake et al. 2005; Krause et al. 2007).

Table 5.3.

Sensory attribute means from trained USA panel evaluation of FS-GRSS, FS-CLVR and FS-TMR butters. Table presents DA averages and standard deviations.

	Feed System		
Sensory attribute	**FS-CLVR**	**FS-GRSS**	**FS-TMR**
Cooked/ Nutty	3.1 ± 0.1	3.06 ± 0.2	3.3 ± 0.1
Milkfat	3.1 ± 0.1	3.1 ± 0.2	3.2 ± 0.1
Grassy	1.2 ± 0.1^{b}	1.4 ± 0.1^{a}	ND ^{c}
Mothball	1.3 ± 0.1^{a}	ND ^{b}	ND ^{b}
Stale	ND	ND	ND
Salty Taste	11.1 ± 0.1	10.9 ± 0.7	11.2 ± 0.1
Colour Intensity	4.2 ± 0.1^{a}	3.4 ± 0.3^{b}	1.8 ± 0.1^{c}

Means represent duplicate evaluations from three experimental replications by 6 highly trained panellists. Attributes were scored using a 0 to 15 point universal intensity scale consistent with the Spectrum descriptive analysis method. ND – not detected. Means in a row followed by different superscript letters are different ($P < 0.05$).
Values provided after ± are standard deviations.

5.3.4. Volatile compounds

HS-SPME-GC-MS analysis of the butters identified a total of 30 volatile compounds across the three feeding systems (Table 5.4). Aldehydes, ketones, acids, terpenes and lactones were the main chemical classes contributing to the volatile profile of all three butters. We have only discussed those compounds where the abundances are significantly different with respect to the feed systems.

The aldehyde compounds; pentanal, hexanal, heptanal and decanal were most influenced by the different feed systems. All of these compounds are associated with lipid oxidation (Clarke, O'Sullivan, Kerry & Kilcawley, 2020). Levels of pentanal were significantly ($P < 0.05$) more abundant in FS-CLVR butter compared to FS-GRSS and FS-TMR butters in agreement with previous studies on butter (O'Callaghan et al. 2016b) and pasteurised bovine milk (Kilcawley, Faulkner, Clarke, O'Sullivan, Kerry, 2018). Pentanal is derived from the fatty acids arachidonic and linoleic acid has the potential to adversely impact sensory perception by yielding a paint-like, cardboard aroma (Kilcawley et al. 2018). Hexanal, derived from linoleic acid (Fujisaki, Endo & Fujimoto, 2002) can confer a grassy off-flavour in butter (Panseri, Soncin, Chiesa, & Biondi, 2011), and was significantly more abundant in FS-CLVR and FS-TMR butter, compared to FS-GRSS butter (Table 4.4). Heptanal was significantly ($P < 0.017$) more abundant in FS-CLVR and FS-GRSS butters in comparison to FS-TMR butter, and is also a product of linoleic acid, which has a green sweet aroma in dairy products (Friedrich & Acree, 1998). Decanal, a compound of oleic acid degradation, was significantly ($P < 0.05$) more abundant in FS-CLVR butter compared to FS-GRSS butter and has been identified as having a green, fatty aroma in sweet cream butter (Lozano et al. 2007). Faulkner et al. (2018) found a similar trend in decanal levels from raw milk produced from grass, grass/clover and TMR

diets. Although the relative abundance of precursor unsaturated fatty acids is important, other factors such as the presence of natural pro- and anti-oxidants are also important.

Six ketones were identified in the three butter samples; 2,3-butanedione (diacetly), a very odour active compound with a characteristic buttery aroma (Mallia, Escher & Schlichtherle-Cerny, 2008; Schieberle, Gassenmeier, Guth, Sen, & Grosch, 1993), was significantly ($P < 0.05$) more abundant in FS-CLVR and FS-GRSS butters compared to FS-TMR butter (Table 5.4). Diacetyl was not detected in any of the butters by O'Callaghan et al. (2016b), but grass derived butter was rated higher for diacetyl aroma. It is difficult to discern why more diacetyl would be present in the butters produced from milk derived from pasture, but it could be that precursors of diacetyl are higher in those milks, due to different microbial activities in the rumen. Diacetyl is derived from pyruvate where α-acetolactate synthase converts it to α-acetolactate, which is subsequently converted to diacetyl by non-enzymatic oxidative decarboxylation (Liu, Chen, Dorau, Lillevang, Jensen & Solem, 2020). FS-CLVR butter also had significantly ($P < 0.05$) more 2-butanone compared to FS-GRSS butter, with similar results seen in previous studies on butter (O'Callaghan et al. 2016b) and milk (Faulkner et al. 2018). This methyl ketone also derives from pyruvate metabolism (Valero, Villamiel, Miralles, Sanz, & Martınez-Castro, 2001), similarly to diacetyl. Acetone was significantly ($P < 0.05$) higher in FS-GRSS and FS-CLVR butters than in FS-TMR butter, and has also been identified as a product from concentrate feed (Contarini, Povolo, Leardi, & Toppino, 1997). Previous studies on milk and skim milk powder produced from pasture and TMR diets did not find differences in the abundance of acetone based on diet (Cheng et al. 2020; Faulkner et al. 2018).

Table 5.4. Average (n=9) peak area values (x106) of volatile compounds identified in FS-GRSS, FS-CLVR and FS-TMR butters.

Volatile Compound	CAS NUMBER	Odour descriptor¥	RI	REF RI	Feeding System		
					FS-GRSS	FS-CLVR	FS-TMR
Aldehyde							
Pentanal[2]	110-62-3	Cardboard like, off flavour	731	733	0.054 ± 0.027 [b]	0.489 ± 0.394 [a]	0.049 ± 0.018 [b]
Hexanal[3]	66-25-1	Cardboard like, off flavourGreen, fatty	836	839	0.032 ± 0.013 [b]	0.060 ± 0.035 [a]	0.049 ± 0.018 [a]
Heptanal[3]	111-71-7	Fatty, green	937	943	0.020 ± 0.008 [a]	0.035± 0.027 [a]	0.010 ± 0.004 [b]
Benzaldehyde	100-52-7	Almond, sweet cherry	1026	1028.9	0.020 ± 0.012	0.019 ± 0.011	0.028 ± 0.011
Nonanal	124-19-6	[a]flora	1143	1150	0.039 ± 0.040	0.035 ± 0.028	0.024 ± 0.017
Decanal[3]	112-31-2	Sweet, waxy, citrus	1246	-	0.001 ± 0.001 [b]	0.003± 0.001 [a]	0.002 ± 0.002 [ab]
Ketone							
Acetone[2]	67-64-1	Earthy, strong fruity, wood pulp, hay	529	533	0.224 ± 0.125 [ab]	0.216 ± 0.052 [a]	0.124 ± 0.027 [b]
Diacetyl (2,3-Butanedione) [2]	431-03-8	Buttery, caramel	628	-	0.044 ± 0.015 [a]	0.079 ± 0.057 [ab]	0.022 ± 0.009 [b]

Butanone[2]	78-93-3	Buttery, sour milk	635	639	0.106 ± 0.019 [b]	0.286 ± 0.156 [a]	0.170 ± 0.082 [ab]
2-Heptanone	113-43-0	Blue cheese, spicy, roquefort	929	936	0.062 ± 0.011	0.077 ± 0.034	0.060 ± 0.007
2-Nonanone	821-55-6	Malty, fruity, hot milk, smoked cheese	1133	1140	0.017 ± 0.003	0.037 ± 0.033	0.013 ± 0.005
2-Pentanone	107-87-9	Orange peel, sweet, fruity	725	-	0.029 ±0.012	0.032 ± 0.010	0.031 ± 0.014
Acid							
Butanoic acid[1]	107-92-6	Sweaty, butter, cheese, strong	860	864	0.015 ± 0.007 [b]	0.024 ± 0.004 [a]	0.017 ± 0.008 [ab]
Hexanoic acid[1]	142-62-1	Acidic, sweaty, cheesy, sharp, goaty	1045	1052	0.023 ± 0.009	0.041 ± 0.02	0.026 ± 0.010
Nonanoic acid[2]	112-05-0	Waxy, cheese, cultured dairy	22.7	-	0.014 ± 0.007 [b]	0.026 ± 0.005 [a]	0.014 ± 0.008[b]
Hydrocarbons							
Toluene[2]	108-88-3	Nutty, bitter, almond, plastic	789	794	0.794 ± 0.26 [b]	1.793 ± 0.708 [a]	0.139 ± 0.031 [c]
***o-Xylene**	108-38-3	Geranium	895	-	0.371 ± 0.304	0.303 ± 0.386	0.572 ± 0.449
***p-Xylene**	106-42-3		923	-	0.177 ± 0.187	0.087 ± 0.143	0.222 ± 0.136
Lactone							
δ-Hexalactone	823-22-3	Creamy fruity coconut	1215	-	0.129 ± 0.033	0.114 ± 0.026	0.117± 0.022
δ-Octalactone	698-76-0	Coconut, dairy, sweet	1413	-	0.026 ± 0.006	0.024 ± 0.007	0.022 ± 0.005
δ-Decalactone	705-86-2	Coconut, peachy, creamy, dairy	1691	1620.9	0.020 ± 0.007	0.021 ± 0.010	0.019 ± 0.008
Sulphide							
Dimethyl sulphide	75-18-3	Sulfury onion sweet	534	538	0.011 ± 0.010	0.018 ± 0.016	0.009 ± 0.002
Carbon disulphide	75-15-0		542	548	0.057 ± 0.018	0.132 ± 0.115	0.088 ± 0.088

Ester							
Ethyl Acetate[2]	141-78-6	Solvent, pineapple, fruity	639	-	0.013 ± 0.010 [ab]	0.033 ± 0.019 [a]	0.010 ± 0.009 [b]
Ethy benzene	113-18-8	Heavy, floral	887	-	0.072 ± 0.046	0.103 ± 0.160	0.206 ± 0.213
Ethyl ether	60-29-7	Ethereal	512	-	0.020 ± 0.022	0.024 ± 0.025	0.024 ± 0.026
Other							
Ethanol	64-17-5	Alcohol, dry	503	506	0.038 ± 0.020	0.032 ± 0.018	0.028 ± 0.021
1-Pentene	109-67-1		565	-	0.061 ± 0.024	0.047 ± 0.024	0.076 ± 0.077
α-Pinene	80-56-8	Pine, green	950	951	0.038 ± 0.032	0.014 ± 0.010	0.036 ± 0.026
Dodecane	112-40-3	Musty, damp	1193	-	0.006 ± 0.004	0.007 ± 0.002	0.005 ± 0.002

RI: Retention index. REF RI: Reference retention index. # CAS: Chemical Abstracts Service Number. Values in the same row not sharing the same superscript (a, b) specify significant difference in peak area value average. Volatile compound annotated with a 1 indicate statistical analysis carried out by ANOVA and Tukey post hoc test, $\alpha = 0.05$. Volatile compounds annotated with a 2 indicate statistical analysis carried out by Welch test and Games-Howell post hoc test, $\alpha = 0.05$. Volatile compounds annotated with a 3 indicate statistical analysis carried out by Kruskal–Wallis and Mann–Whitney, $\alpha = 0.017$. * Tentative identification due to isomers. Values provided after ± are standard deviations. ** Odor descriptors sourced from http://www.thegoodscentscompany.com/.- No REF RI available.

Both butanoic and nonanoic acid were significantly ($P < 0.05$) more abundant in FS-CLVR butter in comparison to FS-TMR and FS-GRSS butter. The presence and concentration of these acids are potentially important in flavour perception, with butanoic acid described as dirty sock in butter, and quite odour active (Lozano et al. 2007). Bovolenta et al. (2014) found that when cows were exposed to high levels of pasture, there was a significant influence on nonanoic acid in Montasio cheese, with this compound not detected in cheese produced from milk derived from a low pasture diet.

Although not recognised to play such a significant role in odour and sensory quality of butter, toluene was significantly more abundant ($P < 0.05$) in both FS-CLVR and FS-GRSS butter than in FS-TMR butter. Toluene is a by-product of β-carotene and isoflavones metabolism (Clarke, Griffin, Rai, O'Callaghan, O'Sullivan, Kerry, Kilcawley, 2019), demonstrating its potential as a biomarker for pasture derived dairy products (Clarke et al. 2020). As FS-CLVR butter was rated most yellow in this study, this may also suggest higher levels of β-carotene and hence the abundance of toluene.

The greater abundance of ethyl acetate in FS-CLVR butter is difficult to discern except that it is a product of ethanol and acetic acid. The sources of both precursors may be influenced by cow diet (Kilcawley et al. 2018).

5.4. Conclusion

This research assessed the cross-cultural perception and liking of butters produced by three different feed systems. Overall, Irish, German, and USA consumers did not discriminate their overall liking of butters based on feed system; although some clear cultural differences were evident. Familiarity was postulated to contribute to differences for appearance liking and colour liking by USA consumers, where the indoor TMR feed system scored highest. However, this did not impact overall liking of butter produced from pasture (FS-GRSS and FS-CLVR) by USA consumers. Both Irish and German consumers rated the colour intensity of the pasture butters (FS-GRSS and FS-CLVR) higher than FS-TMR butter. German consumers also found that pasture (FS-GRSS and FS-CLVR) butter was more salty and that FS-TMR butter was firmer. It seems plausible that the texture and salty differences noted by German consumers are likely related to differences in their FA profile, which directly impacts on texture, but also indirectly on salty perception as the greater unsaturated FAs lower the melting point in the pasture butters. RDA by Irish and German assessors also identified important cross-cultural differences; German assessors perceived FS-GRSS butter as significantly more sour and milky, and scored FS-TMR butter higher for melt in the mouth, in comparison to Irish assessors. Both Irish and German assessors found that FS-TMR butter had less yellow colour in agreement with consumers, and German assessors found it darker than Irish assessors. Both Irish and German assessors found that FS-CLVR butter was the most intense yellow. USA panelists also found that colour intensity ranged from FS-CLVR>FS-GRSS>FS-TMR. These results correlate with previous studies highlighting the impact of carotenoids, specifically β-carotene on the yellow colour of pasture derived dairy products. The trained USA panelists also found that a grassy flavour was highest in FS-GRSS butter but absent in FS-TMR butter, and that FS-CLV butter had a mothball attribute absent in FS-GRSS and FS-TMR butter. Volatile analysis identified a number of

compounds that were statistically different based on diet; aldehydes, ketones, acids, toluene and ethyl acetate. The aldehydes, ketones and acids in the butter from the pasture diets, may be influencing the grassy, milky and sour flavours as perceived by USA and German consumers. Diacetyl may also be influencing enhanced buttery aroma as perceived by Irish assessors in pasture (FS-GRSS and FS-CLVR) butter.

This study demonstrates that different feed systems affect cross cultural perception of butter and that familiarity of products from specific feeds systems is a factor, but that it does not adversely impact butter acceptance in terms of overall liking within these cultural groups.

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Chapter 6. Consumer liking, temporal sensory profile and volatile aroma compounds of biscuits formulated with butter produced from a pasture versus total mixed ration diet

Abstract

This study was conducted to explore the use of butter produced from two diets; outdoor pasture (GRSS) and indoor, total mixed ration (TMR), to identify if the differences in sensory quality of the butters have potential to translate to a baked biscuit product. Shortbread biscuits were chosen due to their high fat ratio. Two biscuits; shortbread with GRSS butter (SB-GRSS) and shortbread with TMR butter (SB-TMR) were subjected to sensory evaluation and aroma volatile analysis. Consumers (n=133) evaluated the biscuits for colour, flavour, texture and overall liking. Temporal check-all-that-applies technique

(TCATA) was employed to understand oral processing and aroma and flavour release during consumption of the biscuits. Volatile analysis was carried out to better understand differences in aroma and flavour perception. Results showed SB-GRSS scored significantly higher for colour liking, which is due to the translation of β-carotene from pasture to butter, yielding a desirable golden colour biscuit. TCATA analysis showed that sensory quality of shortbread biscuits can be profiled from cow diet, with stages of oral processing; orthonasal, in-mouth and aftertaste, differentiating mainly due to the difference in fatty acid profile. Particularly, SB-TMR was highlighted as having significantly higher 'artificial butter' aroma and flavour throughout TCATA evaluation, which is deriving from specific aroma volatile compounds. Volatile analysis elucidated information on aroma formation in biscuits, particularly Strecker aldehydes and furanic compounds, with butter biscuits differentiating in these compounds due to the fatty acid profiles of SB-TMR and SB-GRSS.

Keywords: Temporal Check-All-That-Applies, Biscuit, Pasture, Volatile analysis, Oral processing

6.1 Introduction

There is a shift in consumer preference towards clean-label products, with low tolerance for foods formulated with unknown and artificial ingredients, with an increased demand for a natural, sustainable approach to reformulation (Aschemann-Witzel, Varela, & Peschel, 2019; Asioli, Aschemann-Witzel, Caputo, Vecchio, Annunziata, Næs, et al., 2017). Baked confectionery products are commonly formulated with vegetable fats, such as margarine, or specially formulated shortenings (Tarancón, Fiszman, Salvador, & Tárrega, 2013), whereas dairy butter is primarily

utilised in premium product ranges, due to the higher cost per kg. Fat is an important contributor to the desirable organoleptic properties of biscuits, from producing optimum structure and crumb (Baltsavias, Jurgens, & van Vliet, 1999), to imparting favourable 'buttery', 'melt in mouth', 'crumbly' sensory attributes (Arepally, Reddy, Goswami, & Datta, 2020; Chapter 1). Reformulation on margarine/shortening replacement has been widely studied (Laguna, Primo-Martín, Varela, Salvador, & Sanz, 2014; Rodríguez-García, Laguna, Puig, Salvador, & Hernando, 2013; Sudha, Srivastava, Vetrimani, & Leelavathi, 2007; Tarancón, Fiszman, Salvador, & Tárrega, 2013), however, the impact of butter replacement in biscuits is limited and has shown a definitive decrease in consumer acceptability when butter is substituted for alternative fat replacers (Curti, Federici, Diantom, Carini, Pizzigalli, Wu Symon, et al., 2018; Giarnetti, Paradiso, Caponio, Summo, & Pasqualone, 2015).

Butter is appreciated for its rich sensory attributes, with sensory quality strongly influenced by feed choice (Chapter 5; Hurtaud, Faucon, Couvreur, & Peyraud, 2010). O' Callaghan et al. (2016) reported significant changes to physiochemical properties; texture, thermal and colour, of butter when cows were farmed out-door on fresh pasture (perennial ryegrass; *Lolium perenne* L.), a widely practiced diet in Ireland, in contrast to a total mixed ration (TMR) diet, housed indoors as extensively practiced in North America and mainland Europe. In addition, nutritional value of butter was improved from pasture feeding by altering the fatty acid (FA) profile, with increased levels of n-3 FA's and especially conjugated linoleic acid (CLA), as well as lowering the thrombogenicity index. Vitamin A precursor; β-carotene, and vitamin E form; α-tocopherol levels were also increased in butter as a result of extensive pasture feeding (Silva, Silva, Prates, Bessa, Rosa, & Rego, 2019). Consumption of butter produced from a pasture diet offers scope to include these

health promoting compounds/micronutrients in the diet, with CLA recognised for anti-carcinogenic, anti-inflammatory properties (Fuke & Nornberg, 2017; Kim, Kim, Kim, & Park, 2016), as well as free-radical scavenging, and inclusion of potent antioxidants β-carotene and α-tocopherol (Jayedi, Rashidy-Pour, Parohan, Zargar, & Shab-Bidar, 2019). Pasture-reared dairy and meat products are viewed by consumers as more natural in terms of sustainability, animal welfare and nutritional aspects (Jayedi, Rashidy-Pour, Parohan, Zargar, & Shab-Bidar, 2019), hence incorporation of butter produced from a pasture diet, when consumed in moderation, could potentially be formulated to improve the nutritional profile of butter biscuits, whilst maintaing the integral sensory appeal, and refraining from use of artificial ingredients.

Hedonic scales are commonly implemented to evaluate consumer's perception of fat reformulated biscuits (Forker, Zahn, & Rohm, 2012; Laguna, Primo-Martín, Varela, Salvador, & Sanz, 2014; Tarancón, Salvador, Sanz, Fiszman, & Tárrega, 2015), due to the simplicity of the test, and the importance of product acceptability for new product development. However, if a deviation of liking and acceptability is identified between the traditional (control) and the reformulated formula, it is beneficial to perform further sensory analysis to help elucidate information on the drivers impacting liking. On fat replacement in biscuits, it is of interest to consider the implications to texture perception during consumption, as well as flavour and aroma attributes. Temporal sensory methodologies are advantageous in understanding the dynamics of oral processing and the transformation of attributes over time, throughout consumption (Cadena, Vidal, Ares, & Varela, 2014; Di Monaco, Su, Masi, & Cavella, 2014), with Temporal Dominance of Sensations (TDS) and Temporal check-all-that-applies (TCATA) emerging as innovative techniques to assist in understanding consumer perception. TDS involves a list of pre-defined attributes presented to panellists, with the task of

selecting the attributes that present as 'dominant' during consumption (Pineau, Schlich, Cordelle, Mathonnière, Issanchou, Imbert, et al., 2009). TDS has been employed to study the impact of fat replacement/reduction in biscuits (Laguna, Varela, Salvador, & Fiszman, 2013; Le Calvé, Saint-Léger, Gaudreau, & Cayeux, 2019), as well as highlighting the ability to elucidate differences in consumption of commercial biscuits with subtle formulation differences (Rizo, Jimenez-Pérez, Camacho-García, Fiszman, Pérez-Soriano, & Tárrega, 2019). Alternatively, TCATA is a relatively new technique utilised for temporal sensory evaluation, being described as an extension of the check-all-that-applies methodology (Castura, Antúnez, Giménez, & Ares, 2016). In contrast to TDS, panellists are required to describe every attribute being perceived during consumption, versus just limiting to the dominant attributes (Ares et al. 2015), thus providing a full sensory profile characterisation of the product, during each moment of evaluation. This method can be of particular benefit to gain an insight into dynamic sensory properties such as texture during consumption and aftertaste (Makame, Cronje, Emmambux, & De Kock, 2019; Tan, Wee, Tomic, & Forde, 2019). To the best of our knowledge, TCATA has not been previously employed to study oral processing of biscuits.

Determination of volatile aroma compounds in biscuits can reveal important information in relation to flavour formation reactions taking place as a result of ingredient manipulation. Biscuit aroma is comprised of volatile compounds deriving predominately from heat-induced Maillard (MR) and caramelisation (CR) reactions (Ameur, Rega, Giampaoli, Trystram, & Birlouez-Aragon, 2008; Chapter 1), however, the lipid component can also heavily influence the compounds derived from lipid oxidation (LO) reactions. Butter replaced with an olive oil/inulin gel, in shortbread cookies, was observed to produce a higher abundance of LO derived hexanal, due to the unsaturated profile of olive oil (Giarnetti et al. 2015). In addition, methyl ketones

2-heptanone, 2-nonanone and 2-undecanone were suppressed on 100% butter replacement in the cookies. Identification of volatile compounds generated or suppressed by ingredient reformulation in biscuits can complement sensory data and aid in understanding aroma and flavour perception of reformulated products.

As reformulating and replacing butter in high fat biscuits proves challenging from a sensory perspective, this study investigates the influence of butter source as an ingredient of having been produced from different cow diets (grass and TMR) on the sensory and volatile characteristics of shortbread biscuits. From a previous study, which examined the cross-cultural liking and perception of butter produced from pasture and TMR diets (Chapter 5), differences in sensory perception of the butters was identified. As a diet of concentrate/TMR is commonly practised worldwide, it is of interest to compare the two butters in the biscuit matrix, to understand if feed type in the initial production process can in fact translate into a baked biscuit product.

Therefore, the objectives of this study were to explore the impact of butter produced from an outdoor, fresh pasture (GRASS) diet, versus indoor concentrate (TMR), on the sensory properties and volatile profile of shortbread butter biscuits. TCATA was implemented to aid in comprehending the differences in oral processing of the two formulated biscuits. Volatile analysis was undertaken to determine if the volatile profile was impacted by dietary source of the butter.

6.2 Materials and methods

6.2.1. Butter production

6.2.1.1. Experimental feed systems

Friesian cows were selected from the general herd at the Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork. The cows were randomised and allocated to one of the experimental feed systems; outdoor pasture grazing on perennial ryegrass (*Lolium perenne L.*) (FS-GRASS) or housed indoor provided with a diet of TMR (FS-TMR). In depth detail of the diets is outlined by O'Callaghan et al. (2016).

6.2.1.2. Butter manufacture

Butter produced from each experimental feeding system was produced in triplicate, thus producing 3 batches per feed system group. The following procedure is per O'Callaghan et al. (2016). The butter manufacturing took place in Moorepark Technology Ltd. (MTL, Moorepark, Fermoy, Co. Cork, Ireland). For each butter manufacturing trial, milk was pasteurised (Unison pasteurizer, Unison Engineering Ltd., Limerick, Ireland) at 72°C for 15 secs and skimmed at 50°C using a cream separator (Westfalia separator d-4740, GEA, Naas, Ireland). The cream was subsequently standardised, yielding a cream with 38-40% fat content. Cream was stored in sealed containers for 72 hrs at 5°C to promote milk fat crystallization.

Butter manufacture was carried out using a butter churn with a cream capacity of 50 L (A.S.T.A eismann GmbH, Neubeckum, Germany). Butter was produced by churning cream at 7°C, at 150 rpm until the fat globules were adequately broken down and butter grains were clearly observed. Buttermilk was drained from the churner and the butter grains were washed with cold (10°C) osmosis-treated water to the point that all residual buttermilk was removed. The butter was then weighed and 1.8% salt (Saxa table

salt, Premier Foods, UK) slurry was prepared using osmosis-treated water (60% w/w solution). The salt slurry was poured onto the mass of butter and kneaded by the churner at 60 rpm to ensure uniform distribution of salt crystals. The butter was distributed into 200 ±20 g sticks using an extruder and wrapped in grease-proof paper followed by an outer wrapping of aluminium foil. Butter was vacuum packed and stored at -20°C until subsequent sensory (temporal check all that applies) and volatile analysis, whereas butter for the consumer evaluation was stored at 5°C and used within a week from the production date.

6.2.2. Shortbread biscuit preparation

The shortbread biscuits were produced using a conventional formula consisting with plain flour (300 g) (Odlums, Ireland), caster sugar (Siucra, Nordzucker, Germany) (100 g) and the experimental FS-GRASS or FS-TMR butters (200 g). Sugar and butter were creamed together using a household mixer (Kenwood Mixer, Model KMM710, UK) at speed 1 until mixture was combined. The sides were scraped and mixed again for a further 2 min at speed 2 while the flour was added in gradually. The biscuit dough was compressed and rolled out to 1-cm thickness, and shortbread biscuits were cut out using a cookie cutter (3.8 cm diameter). The shortbread biscuits were baked for 18 min at 160 °C in a domestic convection oven (Zanussi, Bedfordshire, UK). The biscuits were left to cool and placed in sealed storage bags until subsequent volatile or sensory analysis, which took place within 24 hours after baking. Shortbread biscuits containing grass and TMR

butters were referred to as SB-GRSS and SB-TMR, respectively. Baking trials were performed in at least duplicate or more, depending on the analysis.

6.2.3. Consumer evaluation of shortbread biscuits

To access a large consumer group, sensory evaluation was carried out with 133 naïve consumers recruited at the 'Moorepark 2019- Teagasc National Dairy Open Day'; an event held at Teagasc Moorepark (Fermoy, Ireland), directed at the general public, dairy farmers and dairy industry stakeholders. Consumers consisted of a wide demographic and recruited based on their frequency to consume butter and shortbread biscuits. Temporary sensory booths were installed to ensure minimal distraction amongst consumers and sensory evaluation was carried out on iPad tablets (Apple, California), using consumer evaluation software (RedJade Sensory Solutions, LLC, California). Consumers were presented the biscuit samples, one at a time, on white paper plates with unique randomised three digit codes assigned to each sample, alongside water for palate cleansing between biscuits. Panellists were asked to rate their liking of each sample based on the colour, flavour, texture and overall acceptability on a 9-point hedonic scale, which ranged from "9=extremely like" to "1=extremely dislike".

6.2.4. Sensory evaluation- Temporal check-all-that-applies (TCATA)

6.2.4.1. Panel training

Temporal sensory evaluation of SB-GRSS and SB-TMR biscuits was conducted in Teagasc Ashtown (Dublin, Ireland), using an 8 member (4 females, 4 males, age= 45-71) trained, external panel. Panel members had a minimum of 2 years' experience working

as part of a descriptive panel, on a weekly basis. Panellists were screened for their familiarity of the product, perception of product attributes, availability, and taste/smell defects. Overall, panellist received 6 hrs of training prior to the temporal check-all-that-applies (TCATA) analysis. Training initially began with two 1 hr sessions of attribute generation, where a broad list of attributes was produced. As it is recommended to keep the number of attributes between 8-10 for temporal methodologies (Pineau et al. 2012), a following 1 hr training session consisted of the panellists consuming the biscuits and completing a check-all-that-applies ballot to further reduce attributes. A final list of 10 attributes was established, through consensus of researchers and panellists. Two 1 hr sessions were allocated to familiarise the panellists with the TCATA procedure and the method interface. During these sessions, panellists participated in several trial evaluations to establish an appropriate evaluation duration, evaluation with/without Fade feature (Ares et al. 2016), and calibration of panellists to ensure uniform time of consumption, swallowing etc. To interpret how the presence of aroma volatiles in the biscuits influenced aroma perception, orthonasal evaluation was implemented into the TCATA protocol, similar to the study conducted by Harwood, Parker, and Drake (2020). Once method timings were established and the panellists felt comfortable with navigating the software, the TCATA evaluation period was set at 80 s. The protocol involved three stages: aroma/orthonasal evaluation (0-20 s), in-mouth evaluation (20-50 s) and aftertaste evaluation (50-80 s). Panellists were instructed to de-select attributes perceived in aroma/orthonasal evaluation at 20 s to clearly illustrate the aroma evaluation segment of the analysis had ceased. In addition, further direction was given to the panellists that if an attribute left a persistent aftertaste, to leave the relevant attribute checked at 70 s, to indicate it was still lingering in the palate after consumption.

6.2.4.2. Biscuit Evaluation

TCATA evaluation of SB-GRSS and SB-TMR was performed according to Meyners and Castura (2018), in triplicate, over three sessions. Biscuit samples (whole biscuit) were presented in polystyrene cups labelled with a unique three-digit code, and presented in a randomised order for each panellist. Prior to initiating analysis (pressing "start" on timer), panellists were advised to take a couple of seconds to familiarise themselves with the positioning of the attributes on the screen. Panellists initiated the onscreen timer and broke the biscuit in half to aid with orthonasal evaluation, and began to select and deselect attributes as they perceived them. At 20 s, panellists deselected the attributes relevant to aroma and were prompted by a message on screen to consume the biscuit. As discussed in the training, panellists felt 30 s was adequate time for mastication evaluation. At 50 s, panellists were prompted to swallow the biscuit and evaluate the aftertaste of the biscuit. Evaluation ceased at 80 s. Panellists were prohibited from stopping the timer but understood to deselect attributes if they felt the aftertaste had subsided. Panellists were forced to take a 60 s break between samples and encouraged to cleanse their palate with water and a water cracker. Sensory data was collected using the TCATA function on Compusense Cloud (Compusense Inc, Guelph, Canada) and evaluation took place in sensory booths under red hue lighting.

6.2.5. Volatile analysis

Volatile analysis was carried out as described in Chapter 2. Biscuits (3g) were blitzed in a food processor (NutriBullet 600, Australia) to produce a uniform crumb and added to an 20 ml amber La-Pha-Pack screw capped headspace vial (Apex Scientific Ltd, Co. Kildare, Ireland) with magnetic caps and silicone/polytetrafluoroethylene septa (1.3

mm 45° shore A) and equilibrated for 5 min, at 60°C with pulsed agitation for 5 seconds at 350 rpm, using the Gerstal MultiPurpose Sampler (GMPS) agitator/heater. Volatile analysis was carried out utilising a GMPS rail system (Anatune, Cambridge CB3 0NA, UK) connected to a Shimadzu GP2010 plus gas chromatograph (GC) (Mason Technology Ltd, Dublin, Ireland) using headspace solid phase microextraction (HS-SPME). The SPME fibre; 30/50µm DVB/CAR/PDMS (Agilent Technologies Ltd., Ireland) was exposed to the headspace above the samples, at a depth of 21 mm, for 60 min at 60°C. The fibre was retracted, injected into the GC inlet and desorbed for 3 min at 250 °C, followed by 3 min at 270 °C in GMPS fibre bake-out station, to minimise the carryover of compounds. Biscuits were produced in duplicate for volatile analysis and each baking trial was analysed in triplicate. Details of the GC parameters are as described in Chapter 2.

6.2.6. Statistical analysis

All statistical analysis was performed at an α-level of 0.05.

6.2.6.1. Consumer evaluation

Hedonic data was assessed using non-parametric Mann-Whitney U test, conducted using Statistical Package for the Social Sciences (SPSS) software, Version 24 (IBM Statistics Inc., Armonk, NY, USA).

6.2.6.2 Volatile analysis

Independent-sample t-test was applied to volatile data, performed on SPSS.

6.2.6.3 TCATA data analysis

Data analysis of TCATA evaluation was conducted as described by Meyners and Castura (2018). TCATA data is yielded as a series of 1's and 0's, defining an attribute as perceived (checked=1), or not perceived (not checked=0), for every second of evaluation. TCATA curves are generated using proportion of citations; the % of panellists citing an attribute at a given time. This is calculated by averaging panellists replicates for each attribute at each time point, i.e. if 'buttery' is perceived in 3 sessions at 10 s; (1+1+1)/3 = 1, 1 is annotated as the average for 'buttery' at 10 s. All panellist citation proportions were combined and averaged, and TCATA curves were produced using the tempR package (Castura, 2018) using RStudio (ver. 1.1.383, Boston, MA, USA) and R software (ver. 3.4.3, R Foundation for Statistical Computing, Vienna, Austria). To ensure panellist session replicates were consistent, and that all panellists were in agreement with each other for the perception of each attribute (Meyners, 2011; Meyners & Castura, 2018), Gwet's AC1 coefficient and its 95% confidence bands, along with raw agreement and mean citation rate, were conducted through the functions 'similarity.tcata.repeatability' and 'similarity.tcata.replication' on tempR, respectively. Significant differences in citation proportions for each attribute, at each second, were determined using a two-sided Fishers Exact Test, in SPSS. A bold line overlay is used to identify the region of significance, between the two biscuit samples, on the TCATA curves. The average proportion of citations was also calculated (McMahon, Culver, Castura, & Ross, 2017), and this represents the number of seconds that an attribute had been perceived (checked) over the duration of evaluation of analysis (80 s), also referred to as the 'area under the curve' (AUC). AUC values were aggregated for each evaluation stage; ortho-nasal, in-mouth,

and aftertaste. Mann-Whitney U tests were performed to identify significant differences in AUC values, using SPSS.

6.3 Results and discussion

6.3.1 Consumer liking of shortbread biscuits produced from grass and TMR butter

Results from the consumer evaluation of SB-GRSS and SB-TMR are illustrated in Fig. 6.1. Overall, the only significant difference identified was for biscuit colour, with SB-GRSS rated significantly ($P < 0.05$) higher for liking colour. The difference in colour liking of the biscuits is a direct result of the stark colour contrasts of FS-GRSS and FS-TMR butter. FS-GRSS butter has been shown to yield a more intense yellow hue compared to FS-TMR (Chapter 5; O'Callaghan et al. 2016), due to the presence of ß-carotene in fresh pasture. As a result, this yellow hue translates to more 'golden' tone shortbread biscuit, which has been found to be perceived favourably amongst consumers (Mieszkowska & Marzec, 2016).

a a a a

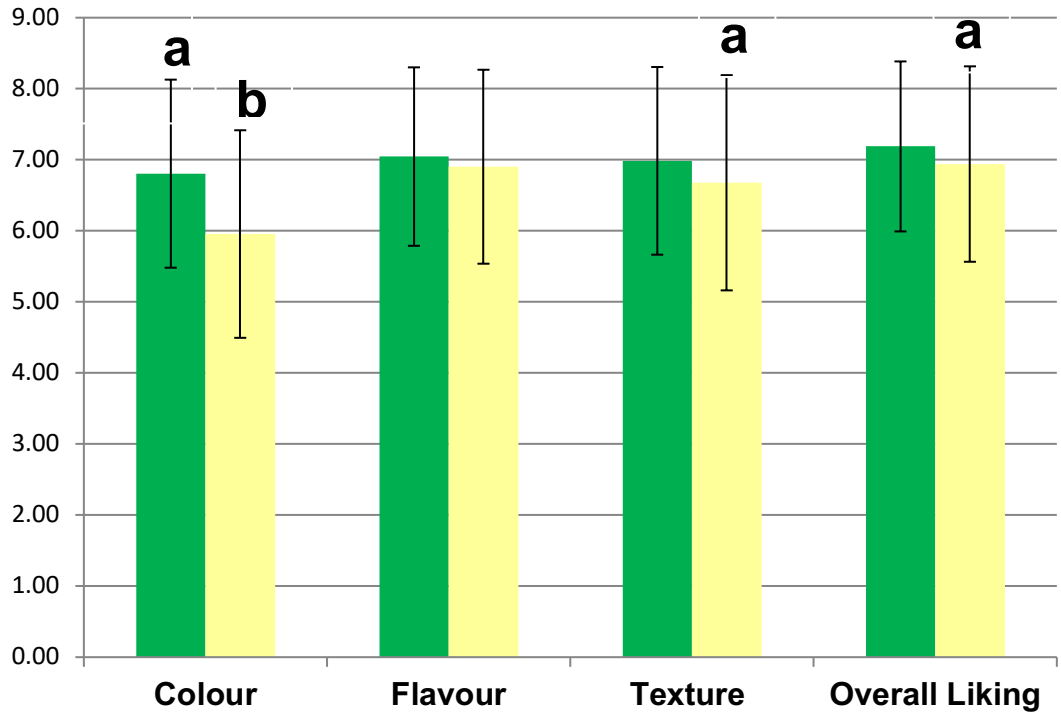


Figure 6.1. Average results (n=133) of sensory evaluation by hedonic scale ofSB-GRSS and SB-TMR biscuits.

Change in letter indicates significant difference ($P < 0.05$).

6.3.2 Temporal profile of shortbread biscuits formulated with grass and TMR butter

Temporal curves for SB-GRSS and SB-TMR biscuits are depicted in Fig. 6.2. a & b. Each stage of the evaluation will be discussed in the following segments. To ensure the quality of the TCATA data, panel agreement for each attribute, and panel repeatability for each TCATA session, was assessed. For the majority of attributes, panellists were in good agreement, producing indices of 0.60 and higher (Poveromo & Hopfer, 2019)

(Table S1). 'Sweet' perception had the lowest agreement indices among panellists. Repeatability is judged on an index closest to 1, with 0 indicating non repeatable. Panellist's repeatability indices were satisfactory, ranging from 0.72-0.84.

6.3.2.1 Orthonasal evaluation

For both biscuits, orthonasal profiles (Fig. 6.2. a&b, 0-20 s) were perceived largely by a 'sweet', 'malty', 'biscuity', 'buttery' aroma. A significant ($P < 0.05$) difference in the citation proportions of 'artificial butter' was identified in SB-TMR between 6-9 s and 13-19 s (Fig 2b). In addition, the AUC value for 'artificial butter' during orthonasal evaluation was also significantly ($P < 0.05$) higher in SB-TMR (Table 6.1a). 'Artifical butter' is often associated with the aroma of 'butter popcorn' (Jinjarak, Olabi, Jiménez-Flores, & Walker, 2006) which is commonly added to some prepared foods in the form of the volatile compound 2,3-butanedione (diacetyl) (Bartowsky & Henschke, 2004; Clark &Winter, 2015). The 'artificial butter' aroma is likely due to the difference in volatile aroma compounds in these butters (Chapter 5), which will be discussed further. SB-TMR also had a significantly ($P < 0.05$) higher AUC value for 'buttery' aroma during orthonasal perception, compared to SB-GRSS. This contradicts the results found in Chapter 5, and O'Callaghan et al. (2016), where FS-GRSS butters were perceived significantly higher for 'buttery' aroma compared to FS-TMR. However, as the butter encompassed in the biscuit is subjected to LO, CR and MR browning reactions during baking (Zamora & Hidalgo, 2005), these factors are also likely impacting the generation of compounds responsible for 'buttery' aroma.

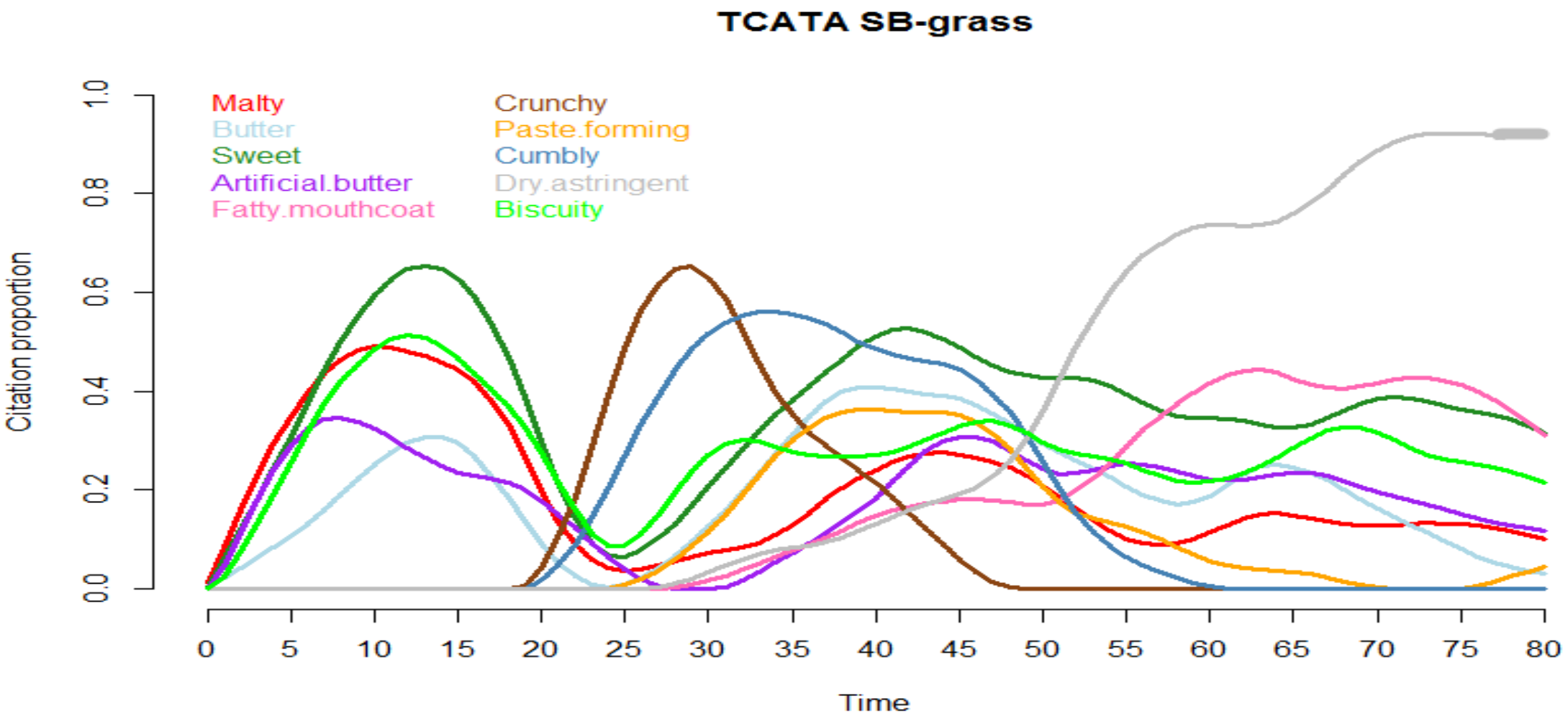


Figure 6.2 a. TCATA curve depicting orthonasal (0-20 s), in mouth (21-50 s) and aftertaste (51-80 s) evaluation of SB-GRSS shortbread biscuit. Bold line overlay represents significant (P <0.05) difference in citation proportion, compared to SB-TMR, for a particular time point.

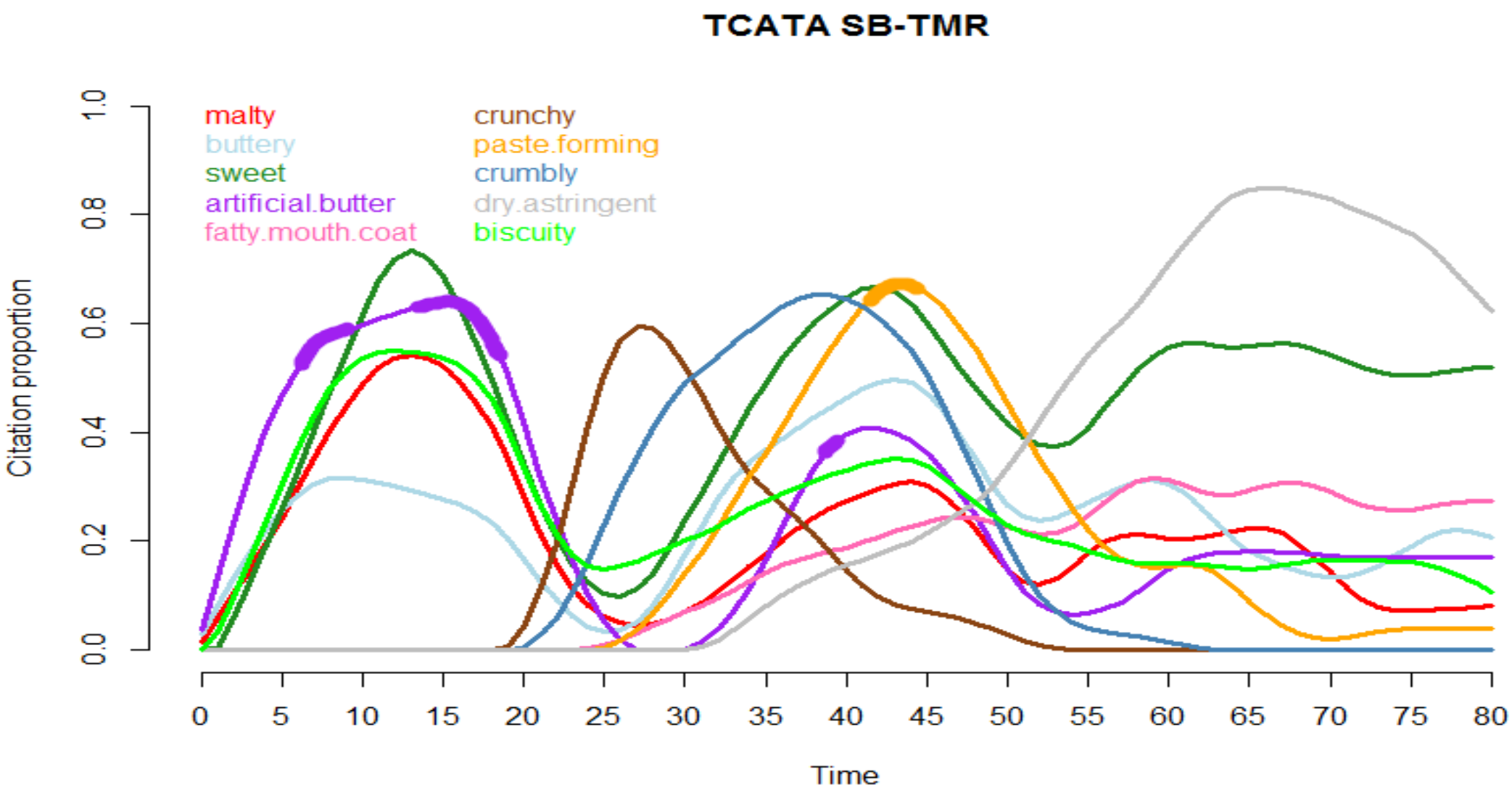


Figure 6.2 b. TCATA curve depicting orthonasal (0-20 s), in mouth (21-50 s) and aftertaste (51-80 s) evaluation of SB-GRSS shortbread biscuit. Bold line overlay represents significant (P <0.05) difference in citation proportion, compared to SB-GRSS, for a particular time point

6.3.2.2 In-mouth evaluation

As seen in Fig. 6.2. a & b (21-50 s), the initial stages of biscuit consumption were characterised by 'crunchy'. As mastication developed and the solid biscuit was deconstructed, 'crumbly' contributed to the temporal profile of both SB-GRSS and SB-TMR, with a similar temporal profile identified by Le Calvé et al. (2019), in biscuits with varying levels of fat composition (butter and margarine) and fat content. Although the FA profile of FS-TMR butter is characterised with a more saturated FA profile (O'Callaghan et al. 2016), yielding a firmer butter at room temperature, there was no significant ($P > 0.05$) difference for perception of 'crunchy' between the SB-GRSS and SB-TMR. This suggests the difference in butter firmness did not translate into biscuits after the baking process. As mastication prolongs, the biscuit crumb is combined with saliva and a bolus, or 'paste' is formed, annotated by 'paste-forming' in this study. As seen in Fig. 6.2. a & b, there was a significant ($P < 0.05$) difference in 'paste-forming' perception, with SB-TMR receiving higher citation proportions at 42-44 s. Although the 'crunchy' attribute is not influenced by the FA profile of FS-GRSS and FS-TMR butters, it can be reasoned that the higher perception of 'paste formation' in the mouth in SB-TMR is likely due to the differences in the melting properties of the butter formulated in the biscuit. Chapter 4 previously identified that Irish consumer's found that FS-GRSS butter melted significantly faster in the mouth compared to FS-TMR, which may explain the behaviour of SB-TMR in this study. The AUC value (Table 6.1b) for 'paste forming' and 'fatty mouthcoat' during in-mouth evaluation was also significantly ($P < 0.05$) higher for SB-TMR compared to SB-GRSS. During paste formation, other flavour and aroma attributes are released concurrently; 'sweet', 'buttery', 'malty', 'artificial butter' and 'biscuity', with 'artificial butter' perceived significantly ($P < 0.05$) higher in SB-TMR, at 39 s.

6.3.2.3 Aftertaste evaluation

Once panellists were signalled to swallow at 50s, they began evaluating the aftertaste of SB-GRSS and SB-TMR. As seen in Fig 6.2 a. & b., the aftertaste of both biscuits was characterised by high citation proportions for 'dry/astringent'. Considering Fig.2.a, the attribute 'dry/astringent' increased after swallowing of SB-GRSS, and then plateaus, with perception at 78-80 s significantly ($P < 0.05$) higher compared to SB-TMR, which appears to drop after ~15 s into aftertaste. Similarly, the AUC value for 'dry/astringent' was significantly ($P < 0.05$) higher in SB-GRSS compared to SB-TMR. Laguna et al. (2013) examined the impact of reformulation (fat reduction, fibre increase) on the temporal profile of biscuits, using TDS. Dry/astringent was not perceived as a dominant sensation in aftertaste of the control biscuits (100% margarine); however, the attribute was identified as significant in the fat reduced/added fibre biscuits. Although some level of dryness will be perceived in biscuits due to the inherent low moisture content (1-4%), it is evident that the fat component also contributes to the perception of 'dryness' and 'astringency'. In this study, the 'dry/astringent' attribute is also impacted by the type of butter utilised, which corresponds to the results obtained by Giarnetti et al. 2015, who identified shortbread biscuits (100% butter) as having significantly higher 'dryness' compared to those reformulated with an inulin/olive oil gel. It appears that cow diet is driving the increased perception of 'dry/astringent' in SB-GRSS compared to SB-TMR.

Table 6.1a

Area Under the Curve (AUC) values obtained for shortbread biscuit attributes during orthonasal temporal check-all-that-applies evaluation for SB-GRSS and SB-TMR biscuits. P-Value of < 0.05 indicates a significant difference in perception during orthonasal evaluation.

	AUC Values Orthonasal Evaluation (0-20 s)		
Attribute	**SB-GRSS**	**SB-TMR**	**P-VALUE**
Malty	0.35 ± 0.15	0.36 ± 0.18	0.80
Buttery	0.18 ± 0.11	0.24 ± 0.09	**0.041**
Sweet	0.43 ± 0.21	0.43 ± 0.26	0.920
Artificial butter	0.24 ± 0.11	0.50 ± 0.19	**<0.001**
Fatty mouthcoat	n.p	n.p	-
Crunchy	n.p	n.p	-
Paste forming	n.p	n.p	-
Crumbly	n.p	n.p	-
Dry/astringent	n.p	n.p	-
Biscuity	0.34 ± 0.17	0.39 ± 0.19	0.147

n.p = not perceived

Table 6.1b

Area Under the Curve (AUC) values obtained for shortbread biscuit attributes during in-mouth temporal check-all-that-applies evaluation for SB-GRSS and SB-TMR biscuits. P-Value of < 0.05 indicates a significant difference in perception during in-mouth evalution.

	AUC Values In-Mouth Evaluation (21-50 s)		
Attribute	**SB-GRSS**	**SB-TMR**	**P-VALUE**
Malty	0.15 ± 0.09	0.18 ± 0.10	0.241
Buttery	0.24 ± 0.16	0.29 ± 0.17	0.171
Sweet	0.33 ± 0.17	0.41 ± 0.21	0.085
Artificial butter	0.14 ± 0.12	0.20 ± 0.15	0.144
Fatty mouthcoat	0.09 ± 0.08	0.13 ± 0.09	**0.048**
Crunchy	0.29 ± 0.24	0.27 ± 0.21	0.645
Paste forming	0.21 ± 0.15	0.34 ± 0.26	**0.045**
Crumbly	0.41 ± 0.15	0.44 ± 0.20	0.197
Dry/astringent	0.10 ± 0.10	0.10 ± 0.11	0.933
Biscuity	0.25 ± 0.10	0.25 ± 0.08	0.663

n.p = not perceived

Table 6.2c

Under the Curve (AUC) values obtained for shortbread biscuit attributes during aftertaste temporal check-all-that-applies evaluation for SB-GRSS and SB-TMR biscuits. P-Value of < 0.05 indicates a significant difference in perception during aftertaste evalution.

n.p = not perceived

	AUC Values Aftertaste Evaluation (51-80+ s)		
Attribute	**FS-GRSS**	**FS-TMR**	**P-VALUE**
Malty	0.13 ± 0.03	0.15 ± 0.07	0.418
Buttery	0.17 ± 0.08	0.22 ± 0.07	0.051
Sweet	0.37 ± 0.05	0.51 ± 0.08	**<0.001**
Artificial butter	0.20 ± 0.05	0.14 ± 0.05	**<0.001**
Fatty mouthcoat	0.37 ± 0.09	0.27 ± 0.05	**<0.001**
Crunchy	n.p	n.p	**-**
Paste forming	0.05 ± 0.06	0.12 ± 0.11	**0.005**
Crumbly	0.02 ± 0.04	0.02 ± 0.03	0.978
Dry/astringent	0.78 ± 0.14	0.70 ± 0.14	**0.035**
Biscuity	0.26 ± 0.05	0.16 ± 0.03	**<0.001**

The aftertaste evaluation of SB-GRSS was also significant for 'fatty mouthcoat' and 'biscuity' attributes. As shown in Fig 6.2.a & b, the biscuit is consumed at ~20s and citations for 'fatty mouthcoat' begin to increase at ~35 s, once the biscuit had been broken down and the bolus formed. After panellists swallowed, the temporal curve illustrates that both butter biscuits (SB-GRSS and SB-TMR) left a fatty or oily residue in the oral cavity. This is a result of the fat content (~67% -expressed as a percentage of flour weight) of the biscuits, having similar 'butter lasting' and 'fatty mouthcoat' results as seen in the temporal evaluation of biscuits (Laguna et al. 2013; Le Calvé et al. 2019). The differences in AUC values (Table 6.1c) for 'fatty mouthcoat' of SB-GRSS and SB-TMR links back to the higher incidence of a unsaturated FAs in the FA profile of butter produced from a pasture diet (O'Callaghan et al. 2016). As reported in Chapter 5, butter produced from grass is perceived to melt significantly faster, compared to butter from an indoor concentrate diet, and it appears even when encompassed in the matrix of a biscuit, the butters exhibit the same behaviour.

Contradictory to results identified in orthonasal and in-mouth evaluation, 'artificial butter' was perceived significantly ($P < 0.05$) more in the aftertaste evaluation of SB-GRSS, compared to SB-TMR. As stated, this aroma is a result of the aroma compounds present in the biscuits. As the 'fatty mouthcoat' is lingering more so in the palate after consumption of SB-GRSS, the volatile compounds responsible for 'artificial butter' are also likely to linger, leading to increased retronasal perception of this attribute. Although the term 'biscuity' is more so related to the biscuit matrix itself, as opposed to the fat content/profile, the higher ($P < 0.05$) AUC value for 'biscuity' in the aftertaste profile of SB-GRSS may also be explained by the differences presented in the volatile profiles. Laguna et al. (2013) demonstrated using TDS how the perception of 'toasted/ biscuit/ caramel' shifts from dominant to not dominant on 50% fat reduction in biscuits.

Corresponding to in-mouth evaluation 'paste forming' was perceived significantly ($P < 0.05$) more in the aftertaste of SB-TMR, compared to SB-GRSS. As stated, due to the denser, cohesive bolus, or paste, formed as a result of the FA profile of TMR butter, it is likely the bolus was more difficult to fully salivate and eradicate from the palate, hence higher incidence of 'paste forming' in aftertaste evaluation. Similar results were also noted by Laguna et al. 2013, where the control biscuit (100% margarine) had the lowest dominance rate for 'pastiness', with significant dominance identified in fat reduced/fibre replaced biscuits. The SB-TMR biscuit was also perceived significantly ($P < 0.05$) more 'sweet' throughout aftertaste evaluation (Table 6.1c). Previous studies have shown no difference in the perception of sweetness of butters produced from outdoor pasture versus indoor TMR (Chapter 5; O'Callaghan et al. 2016), and both shortbread formulas contained equal levels of sucrose. Fat and sweetness perception, in relation to fat and sugar content, have been difficult to establish in biscuits (Biguzzi, Lange, & Schlich, 2015; Biguzzi, Schlich, & Lange, 2014; Giarnetti et al. 2015), indicating the difference in melting behaviour of SB-TMR in the mouth may be impacting on the perception of 'sweetness' during mastication and on the lingering aftertaste.

6.3.3 Volatile profiles of SB-GRSS and SB-TMR biscuits

HS-SPME-GC analysis identified a total of 64 compounds across between SB-GRSS and SB-TMR biscuits (Table 6.2). Aldehydes (14), ketones (14), furans (12), pyrazines (6), lactones (5) and acids (3) were the main chemical classes contributing to the volatile profile of the biscuits. A principle component analysis (PCA) was performed initially to identify associations within the data (Fig. 6.3). The first two components of the PCA account for 84.4% of the total variance among the samples. To further establish the influence of the FS-GRSS and FS-TMR butters in the shortbread biscuits, a difference in means test (independent t-test) was conducted.

Fourteen aldehyde compounds were identified in SB-GRSS and SB-TMR biscuits, with significant ($P < 0.05$) differences identified in levels of 2-methylpropanal, 3-methylbutanal, heptanal, octanal and nonanal. The Strecker aldehydes, 2-methylpropanal and 3-methylbutanal, are derived from the amino acids valine and leucine, respectively. Although these compounds are associated with products of the Maillard reaction in baked confectionery products (Chapter 1), differences in 2-methylpropanal and 3-methylbutanal have been identified when fats of different FA profiles were used in the formulation of sweet bakery products (Giarnetti, Caponio, Paradiso, Summo, & Gomes, 2012; Giarnetti et al. 2015; Maire, Rega, Cuvelier, Soto, & Giampaoli, 2013). As stated, this may be related to the interaction of MR browning and LO reactions occurring in the baked matrix, where LO products, such as epoxyalkenals, are known to participate in Strecker degradation reactions and aid in the formation of Strecker aldehydes (Hidalgo & Zamora, 2004; Zamora & Hidalgo, 2005). The difference in the FA profile of the biscuits is likely contributing to altered epoxyalkenal activity during Strecker degradation reactions, and hence the difference in levels identified. 2-Methylpropanal and 3-methylbutanal have been identified as odour active in sponge cake, imparting 'spicy/cake crust' and 'toffee/sweet' aromas, respectively (Chapter 3). It is possible these compounds

are contributing to the 'sweet' aroma perceived during orthonasal evaluation. Heptanal, octanal and nonanal were also identified as significantly ($P < 0.05$) higher in SB-GRSS compared to SB-TMR. Heptanal is generated from linoleic acid (Fujisaki, Endo, & Fujimoto, 2002) and has been previously identified in shortbread (Chapter 2). The differences in aldehydes between SB-GRSS and SB-TMR appears to be translating directly from the butter, with heptanal significantly more abundant in FS-GRSS butters, compared to FS-TMR (Chapter 5). Heptanal contributes a 'fatty', 'oily' odour to the aroma profile of sponge cakes (Chapter 3), which could be partially responsible for the higher perception of 'fatty mouthcoat' in the aftertaste evaluation of SB-GRSS. Both octanal and nonanal were also significantly ($P < 0.05$) higher in SB-GRSS. Both compounds are derived from the auto-oxidation of oleic acid (Whitfield & Mottram, 1992), however, oleic acid has not been identified significantly higher in similar butters produced from pasture and TMR diets (O'Callaghan et al. 2016). Oleic acid was not quantified in this study but the significant difference in these aroma compounds indicates increased auto-oxidation of oleic acid is taking place during baking. Octanal has been identified as 'orange peel' in sponge cakes by gas-chromatography-olfactometry (Matsakidou, Blekas, & Paraskevopoulou, 2010), whereas nonanal has been described as 'bready/cake crust' (Chapter 3).

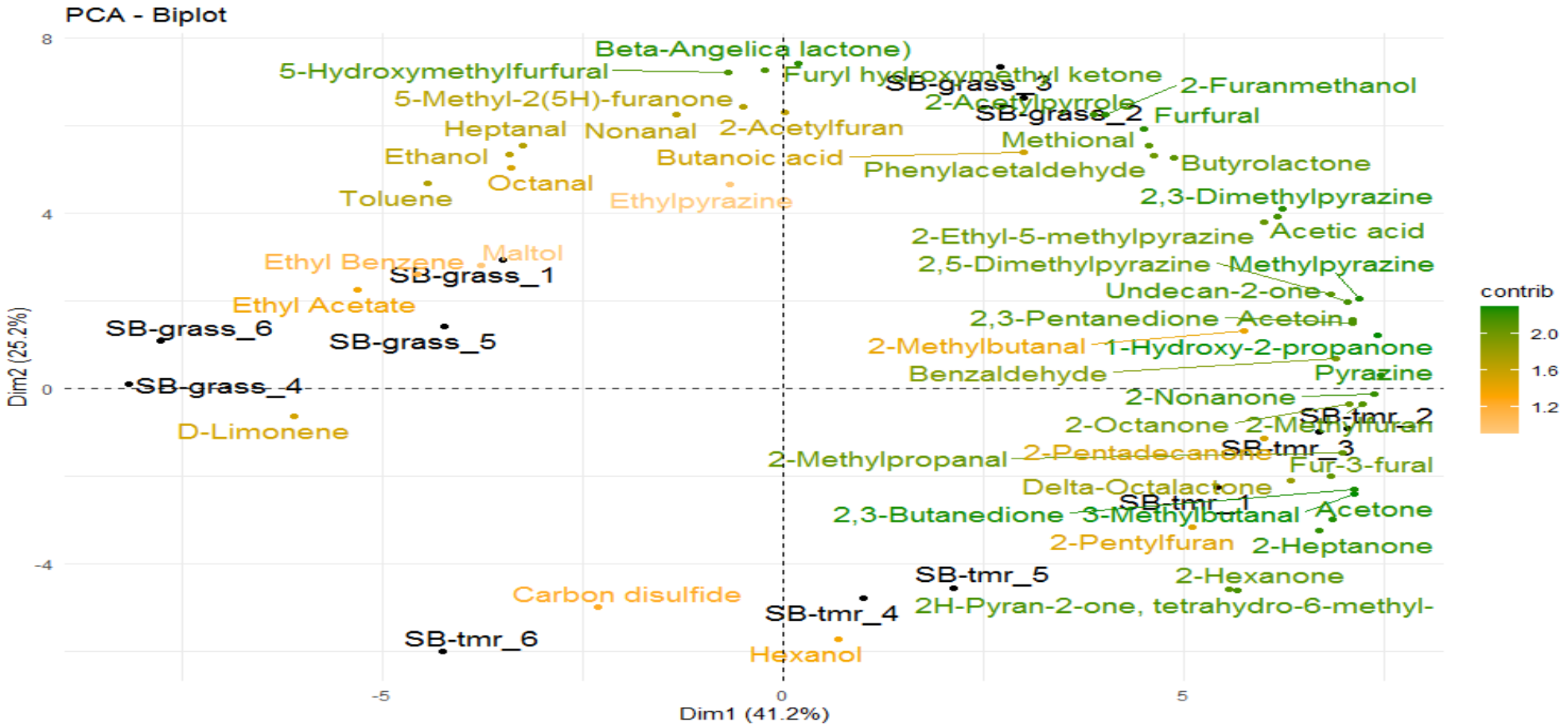


Figure 6.3. Principle component analysis of the volatile compounds associated with SB-GRSS (shortbread biscuit with butter produced from grass/ pasture diet) and SB-TMR (shortbread biscuit with butter produced from a total mixed ration diet) analysed by headspace solid-phase microextraction gas chromatography mass spectrometry. Compounds were recovered from two baking replicates, analysed in triplicate.

Levels of ketones; acetone, diacetyl, 2-hexanone, 2-heptanone and 2-pentadecanone were significantly ($P < 0.05$) higher in SB-TMR compared to SB-GRSS. Acetone can be generated by two means in milk and butter, citrate metabolism (McSweeney & Sousa, 2000) and directly from feed (Marsili, 2003). Incidence of this is shown in O'Callaghan et al. (2016) where similar levels were reported in butters produced from pasture and TMR, whereas in Chapter 5, butters from pasture contained significantly higher levels of acetone compared to TMR. Similarly, diacetyl was also identified in higher abundance in SB-TMR, in contrast to the volatile analysis of FS-GRSS and FS-TMR butters, which demonstrated higher levels of diacetyl in FS-GRSS (Chapter 5). The rationale for the inconsistent levels is ambiguous, however, in baked confectionery products, diacetyl is prominently derived from CR and the primary stages of the MR (Poisson, Auzanneau, Mestdagh, Blank, & Davidek, 2016). The significantly ($P < 0.05$) higher levels in SB-TMR could yet again be related to the intertwined relationship between MR and LO, with the higher levels of PUFA's in SB-GRSS (O'Callaghan et al. 2016) inhibiting the Maillard reaction (Benet, Guàrdia, Ibañez, Solà, Arnau, & Roura, 2016). Diacetyl has been identified as yielding a 'butter', 'fruity', 'caramel' aroma in sponge cakes (Pozo-Bayón, Ruíz-Rodríguez, Pernin, & Cayot, 2007), but could also be contributing to the consensus of 'artificial butter' aroma in SB-TMR orthonasal evaluation. The methyl ketones, 2-hexanone, 2-heptanone and 2-pentdadecanone were also significantly ($P < 0.05$) higher in SB-TMR. Only 2-heptanone was identified in FS-GRSS and FS-TMR butters, with no significant difference in abundance (Chapter 5; O'Callaghan et al. 2016). Through heating of milk fat; in this case, the heating of the butter during baking, it is likely ß-oxidation of saturated FAs (Wong & Patton, 1962) is accelerated and hence the higher levels of methyl ketones in SB-TMR. 2-Heptanone was in particular high abundance (10^7) in both biscuits, and although it has not been reported as odour active in baked confectionery products, it yields a distinct 'blue cheese' aroma,

possibly further contributing to 'artificial butter' aroma (Risner, Tomasino, Hughes, & Meunier-Goddik, 2019).

Furan compounds have been widely identified in biscuits (Giarnetti et al. 2015; Pasqualone, Bianco, Paradiso, Summo, Gambacorta, & Caponio, 2014; Pasqualone, Bianco, Paradiso, Summo, Gambacorta, Caponio, et al., 2015) and impart a range of pleasant odours to baked confectionery products. In this study, butter formulation had a significant ($P < 0.05$) influence on the levels of eight furans. 2-Methly furan, 3-furfural and 2-2-pentylfuran were significantly ($P < 0.05$) more abundent in SB-TMR compared to SB-GRSS. Although previously shown to be influenced by the abundance and profile of simple sugars (Chapter 3), furanic generation in this study seems to also be indirectly impacted by the different FA profiles of the butters. Furan generation is complex with factors such as amino acid content and FA profile found to influence the abundance of 2-methylfuran in a model matrix (Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008). Methylfuran has been previously shown to be influenced by linoleic acid (Märk, Pollien, Lindinger, Blank, & Märk, 2006) content, which has been identified as significantly higher in butter produced from a TMR diet (O'Callaghan et al. 2016). Higher ($P < 0.05$) levels of 2-2-pentylfuran, identified as 'earthy' and 'vegetable' in sponge cakes (Matsakidou, Blekas, & Paraskevopoulou, 2010), also appears to be influenced by the higher abundances of linoleic acid present in butter from a TMR diet (Liu, Li, Cheng, & Liu, 2020), with LO and MR reactions both contributing to its abundance. Conversely, SB-GRSS yielded significantly ($P < 0.05$) more abundant levels of 2-acetylfuran, 5-methylfurfural, 5-methyl-2(5H)-furanone (β-Angelica lactone), furyl hydroxymethyl ketone and 5-hydroxymethylfurfural (HMF). PUFAs have been widely identified as contributing to the generation of HMF and other furanic compounds (Anese & Suman, 2013), with pasture butters having more n-3 FA compared to TMR butter (O'Callaghan

et al. 2016). In addition, Owczarek-Fendor et al. (2011) highlighted that ß-carotene can also be a precursor of furanic compounds, thus potentially further highlighting why differences in furanic compounds exist between these SB-GRSS and SB-TMR. The higher incidence of 'biscuity' aftertaste in FS-GRSS can be explained by the increased levels of these furanic compounds, and due to their low volatility, may indicate their perception after swallowing the biscuit. Although it was postulated that LO is not an important pathway for furan formation in sponge cakes (Cepeda-Vázquez, Rega, Descharles, and Camel, 2018), it is clear that their play a significant role in volatile formation in shortbread biscuits.

Two lactones, γ-hexalactone and δ-octalactone, were also significantly ($P < 0.05$) higher in SB-TMR. Lactones are produced on heating of milk fat, with a diet of hay and silage shown to produce more 'coconut' aromas derived from lactones (Urbach & Stark, 1978; Villeneuve et al., 2013). Lastly, SB-GRSS biscuits contained significantly ($P < 0.05$) higher levels of ethanol, ethyl acetate and toluene, compared to SB-TMR. Ethanol has been postulated to derive from plant diets (Kilcawley, Faulkner, Clarke, O'Sullivan, & Kerry, 2018), and the abundance of ethyl acetate is likely generated from the abundance of ethanol. Toluene is a biomarker of the pasture produced dairy products including butter as its generation pathway is linked to ß-carotene (Villeneuve, et al. 2013), Neither ethanol or toluene are very odour active and thus are less likely to influence sensory perception (Faulkner et al. 2018).

Table 6.2.

Average (n= 6) peak area values (x10^6) of selected volatile compounds identified in SB-GRSS and SB-TMR shortbread biscuits.

Compound	#CAS	SB-GRSS	SB-TMR	P-VALUE
Aldehydes				
2-Methylpropanal	78-84-2	0.02 ± 0.01	0.03 ± 0.004	**0.012**
3-Methylbutanal	590-86-3	0.10 ± 0.02	0.15 ± 0.02	**0.002**
2-Methylbutanal	96-17-3	0.17 ± 0.04	0.19 ± 0.02	0.336
Hexanal	66-25-1	1.60 ± 0.17	1.76 ± 0.17	0.127
(E)-2-Hexenal	6728-26-3	0.02 ± 0.003	0.03 ± 0.005	0.060
Heptanal	111-71-7	0.58 ± 0.06	0.41 ± 0.04	**<0.001**
Methional	3268-49-3	0.05 ± 0.01	0.05 ± 0.01	0.412
(E)-2-Heptenal	18829-55-5	0.16 ± 0.02	0.15 ± 0.03	0.977
Benzaldehyde	100-52-7	0.36 ± 0.04	0.39 ± 0.02	0.130
Octanal	124-13-0	0.25 ± 0.02	0.19 ± 0.02	**<0.001**
Phenylacetaldehyde	122-78-1	0.83 ± 0.11	0.77 ± 0.13	0.468
Nonanal	124-19-6	0.75 ± 0.06	0.61 ± 0.05	**0.001**

(E,E)-2,4-Decadienal	25152-84-5	0.18 ± 0.013	0.20 ± 0.008	0.589
Decanal	112-31-2	0.06 ± 0.02	0.05 ± 0.02	0.603
Ketones				
Acetone	67-64-1	0.43 ± 0.09	0.69 ± 0.10	**0.001**
2,3-Butanedione (Diacetyl)	431-03-8	1.02 ± 0.21	1.46 ± 0.17	**0.002**
2-Butanone	78-93-3	0.14 ± 0.01	0.22 ± 0.07	0.068
2-Pentanone	107-87-9	7.96 ± 8.98	8.22 ± 1.12	0.945
1-Hydroxy-2-propanone	116-09-6	26.27 ± 4.69	30.04 ± 3.72	0.154
2,3-Pentanedione	600-14-6	0.47 ± 0.09	0.53 ± 0.06	0.226
Acetoin	513-86-0	0.24 ± 0.05	0.27 ± 0.03	0.239
2-Hexanone	591-78-6	0.32 ± 0.02	0.54 ± 0.08	**<0.001**
1-Hydroxy-2-butanone	5077-67-8	0.27 ± 0.32	0.57 ± 0.55	0.273
2-Heptanone	110-43-0	37.40 ± 1.65	42.43 ± 1.87	**0.001**
2-Octanone	111-13-7	0.36 ± 0.04	0.42 ± 0.06	0.069
2-Nonanone	821-55-6	14.40 ± 1.51	16.49 ± 1.75	0.051
Undecan-2-one	112-12-9	3.95 ± 0.58	4.31 ± 0.60	0.306
2-Pentadecanone	2345-28-0	0.22 ± 0.06	0.31 ± 0.07	**0.034**

Furans				
2-Methylfuran	534-22-5	0.04 ± 0.01	0.06 ± 0.01	**0.044**
2(5H)-Furanone	497-23-4	0.03 ± 0.01	0.01 ± 0.002	0.945
Dihydro-2-methyl-3(2H)-furanone	3188-00-9	0.03 ± 0.02	0.01 ± 0.002	0.807
3-Furfural	498-60-2	0.08 ± 0.01	0.10 ± 0.01	**0.008**
Furfural	98-01-1	14.88 ± 2.35	13.52 ± 2.51	0.355
2-Furanmethanol	98-00-0	2.00 ± 0.35	1.76 ± 0.29	0.239
2-Acetylfuran	1192-62-7	0.22 ± 0.03	0.18 ± 0.03	**0.018**
2-2-pentylfuran	3777-69-3	1.03 ± 0.09	1.18 ± 0.08	**0.012**
α-Angelica lactone (5-methyl-2(3H)-Furanone)	591-12-8	0.10 ± 0.05	0.08 ± 0.04	0.477
5-Methylfurfural	620-02-0	0.20 ± 0.02	0.14 ± 0.02	**0.002**
β-Angelica lactone (5-Methyl-2(5H)-furanone)	591-11-7	0.24 ± 0.05	0.16 ± 0.03	**0.007**
Butyrolactone	96-48-0	0.40 ± 0.05	0.39 ± 0.03	0.497
Furyl hydroxymethyl ketone	17678-19-2	2.74 ± 0.33	2.09 ± 0.22	**0.002**
5-Hydroxymethylfurfural	67-47-0	3.40 ± 0.58	2.28 ± 0.32	**0.002**

Pyrazine				
Pyrazine	290-37-9	0.24 ± 0.06	0.31 ± 0.05	0.065
Methylpyrazine	109-08-0	0.46 ± 0.10	0.52 ± 0.09	0.280
2,5-Dimethylpyrazine	123-32-0	0.31 ± 0.07	0.36 ±0.09	0.332
Ethylpyrazine	13925-00-3	0.05 ± 0.01	0.04 ± 0.02	0.081
2,3-Dimethylpyrazine	5910-89-4	0.04 ± 0.01	0.04 ± 0.01	0.913
2-Ethyl-5-methylpyrazine	13360-64-0	0.05 ± 0.01	0.05 ± 0.01	0.836
Lactones				
γ-Hexalactone	695-06-7	0.14 ± 0.01	0.14 ± 0.01	0.542
δ-Hexalactone	823-22-3	0.60 ± 0.05	0.95 ± 0.09	**<0.001**
δ-Octalactone	698-76-0	0.39 ± 0.02	0.44 ± 0.04	**0.012**
δ-Decalactone	705-86-2	0.70 ± 0.04	0.74 ± 0.07	0.268
δ-Dodecalactone	713-95-1	0.10 ± 0.03	0.09 ± 0.02	0.459
Acids				
Acetic acid	64-19-7	0.18 ± 0.07	0.19 ± 0.05	0.808
Butanoic acid	107-92-6	0.12 ± 0.07	0.09 ± 0.02	0.349
Hexanoic acid	142-62-1	0.16 ± 0.05	0.15 ± 0.03	0.872

Alcohols				
Ethanol	64-17-5	0.32 ± 0.03	0.21 ± 0.03	**<0.001**
Hexanol	111-27-3	0.24 ± 0.36	0.61 ± 0.09	0.054
Other				
Ethyl Acetate	141-78-6	0.17 ± 0.03	0.09 ± 0.06	**0.013**
Carbon disulfide	75-15-0	0.16 ± 0.14	0.22 ± 0.04	0.363
Toluene	108-88-3	0.33 ± 0.05	0.19 ± 0.02	**0.001**
α-Pinene	80-56-8	0.26 ± 0.21	0.14 ± 0.08	0.202
D-Limonene	5989-27-5	2.37 ± 1.59	1.22 ± 0.79	0.154
2-Acetylpyrrole	1072-83-9	0.04 ± 0.01	0.03 ± 0.01	0.250
Maltol	1072-83-9	0.06 ± 0.02	0.05 ± 0.01	0.078
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	28564-83-2	0.04 ± 0.02	0.04 ± 0.02	0.992

6.4 Conclusion

This research investigated the impact of butter produced from cows grazed outdoors on pasture, versus indoor on concentrates (TMR), as an ingredient to alter the sensory properties and volatile profile of shortbread biscuits. Overall, liking and acceptability was not discriminated in SB-GRSS and SB-TMR biscuits, with the exception of colour, which received significantly higher liking for SB-GRSS, and presumably directly relates to higher ß-carotene in butter produced from FS-GRSS, and therefore, driving consumer liking. It appears that the FA profile of SB-GRSS and SB-TMR is the prominent contributor to differences in the temporal sensory profile of these shortbread biscuits. The higher melting point of FS-TMR butter formulated in SB-TMR led to an increased perception of 'paste formation' during mastication (in-mouth perception), as well as significantly higher perception of 'paste formation' during aftertaste, due to the increased denseness of the bolus. As a result of the grass butter, and the lower melting point of the fatty acids, SB-GRSS was perceived higher for 'fattymouth coat', 'biscuity' and 'dry/astringent' attributes, with 'dry/astringent' of particular interest as it continued to linger in the palate post swallowing. The significantly higher perception of 'artificial butter' and 'buttery' attributes during orthonasal evaluation of SB-TMR may be explained by the results of the volatile analysis, with significantly higher abundance levels of diacetyl, 2-heptanone and 2-hexanone in SB-TMR. In addition, volatile aroma profiling identified the modulation of Strecker aldehydes and furanic compounds as impacted by the difference in fatty acid profiles in the butter. As these compounds have been proposed to be strongly influenced by sugar, this emphasises the complex relationship between MR and LO reactions in baked goods. In addition, it is evident that the volatile compounds in butter, lactones, and specific aldehydes, can translate into the biscuit and further influence aroma. This study highlights the importance of comprehension of flavour

reactions, in addition to temporal sensory methodology, to successfully assist reformulation that will result in an end product of optimum sensory appeal.

6.5 References

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Chapter 7. General Discussion & Conclusion

7. 1 Background

A greater need for innovative concepts and approaches towards new product development is required now more than ever, with greater demands and expectations from consumers in relation to 'clean-label', sustainable and natural ingredients, and need for healthier options. Baked confectionery, cakes, biscuits etc., have been subject to countless reformulation solutions, particularly in relation to reduction of refined sucrose (Luo, Arcot, Gill, Louie, & Rangan, 2019; Struck, Jaros, Brennan, & Rohm, 2014). Similarly, due to the negative associations of consuming trans/ saturated fats, efforts have been made in an attempt to find a suitable alternative for vegetable and dairy based spreads in these products (Curti, Federici, Diantom, Carini, Pizzigalli, Wu Symon, et al., 2018; Giarnetti, Caponio, Paradiso, Summo, & Gomes, 2012; Laguna, Primo-Martín, Varela, Salvador, & Sanz, 2014; Rodríguez-García, Laguna, Puig, Salvador, & Hernando, 2013; Sudha, Srivastava, Vetrimani, & Leelavathi, 2007). This research has highlighted a need for further studies as to why changes in sensory perception of baked confectionery products occurs on reformulation, and to fully elucidate the roles of core ingredients; sugar and fat, to aid in the development of suitable reformulated products that meet consumer expectations. This Doctoral thesis undertook necessary and innovative research required to further enhance our understanding of the contribution of sugar and fat to flavour and aroma formation in baked confectionery products, and to evaluate informed novel formulations on the aromatic and sensory properties of selected baked confectionery products.

7.2 Understanding aroma formation in baked confectionery products- development of an optimised method

The task of comprehending how flavour and aroma is influenced by ingredients proves difficult unless the volatile aroma compounds responsible, are interpreted. Although methods for volatile recovery from baked confectionery matrices have been outlined (Cepeda-Vázquez, Blumenthal, Camel, & Rega, 2017; Maire, Rega, Cuvelier, Soto, & Giampaoli, 2013; Pasqualone, Bianco, Paradiso, Summo, Gambacorta, & Caponio, 2014; Pozo-Bayón, Ruíz-Rodríguez, Pernin, & Cayot, 2007), there was an apparent lack of an optimised, validated, method targeting the wide-spread recovery of volatile compounds from these product types. The development, optimisation and validation of a volatile extraction method targeted at recovering a representative volatile profile from baked confectionery products was conducted (**Chapter 2**). Headspace solid-phase microextraction gas-chromatography mass-spectrometry (HS-SPME-GC-MS) was chosen as the method for optimisation due to the simplicity of sample preparation, and the fact that it is solvent free, rapid, low cost, highly automated, and highly sensitive, especially for low molecular weight volatile compounds. Subsequently fibre type, incubation time, extraction time and extraction temperature parameters were optimised, based on their relevance to the product matrix. In addition, as the aroma and flavour of baked confectionery products are primarily determined by carmelisation (CR), Maillard (MR) and lipid oxidation (LO) reactions, 18 volatile compounds deriving from these reactions were chosen for optimisation of the HS-SPME method. A sponge cake matrix was chosen as the test product.

Optimisation trials can be cumbersome due to the volume of experiments required prior to drawing a valid conclusion. However, with the application of response surface methodology (RSM), a tool consisting of mathematical and statistical concepts,

the HS-SPME-GC-MS was capable of being optimised effectively and efficiently within 20 experimental runs. Fibre type was initially trialled prior to optimisation of the extraction parameters, with a multi- phase 50/30μm DVB/ CAR/PDMS fibre selected based on the diversity of volatiles extracted. RSM enabled the efficient optimisation and validation of HS-SPME analysis of baked confectionery, with an incubation time of 5 min, an extraction time of 60 min and an extraction temperature of 60 °C. This method can then be applied to monitor the changes in volatile profile of reformulated matrices, particularly on sugar and fat manipulation, allowing for a better understanding of the impact of these changes on aromatic volatiles and also on sensory perception.

7.3 Understanding the influence of sucrose replacement, and manipulation, on aroma volatile formation and sensory perception

With the optimised HS-SPME-GC-MS method established, this was subsequently applied to study the impact of sucrose manipulation on volatile generation in sponge cakes, and hence the sensory properties. Due to trends in consumer consumption patterns (Asioli, Aschemann-Witzel, Caputo, Vecchio, Annunziata, Næs, et al., 2017; Milner, Kerry, O'Sullivan, & Gallagher, 2020), **Chapter 3** outlines the incorporation of clean-label sucrose replacers into sponge cake formulations (30% w/w reduction), compared to a control (100% sucrose) in relation to volatile compounds, odour active compounds and sensory perception. The unique aspect of this study involves the application of gas-chromatography- olfactometry (GC-O) to assess the influence of sucrose replacers (oligofructose and apple pomace powder) on aroma perception. Using a combined HS-SPME-GC-MS, GC-O approach and sensory evaluation (liking, ranking descriptive analysis) we were able to assess the impact of

sucrose replacers, (particularly those containing reducing sugars), on the acceleration of MR and CR reactions, and the subsequent influence on sensory perception. Apple pomace powder was negatively perceived by consumers for overall liking and appears to be related to the higher "roasty", "toasty", "off-flavour" attributes generated. GC-O analysis provided in-depth information in relation to the identity and odour activity of key aromatics influencing sensory perception, such as 'potato/damp' methional and 'spicy bready' furfural in these products. In contrast, the control, which had highest perception of "fresh cake" odour, was profiled by 'fatty/ cake crust' heptanal, a compound identified as having lower perception levels in apple pomace and the oligofructose formulas.

The impact of sucrose source and sucrose crystal size on the sensory and volatile properties of sponge cakes was also investigated. Sucrose was sourced from sugarbeet and sugarcane, and different sucrose crystal sizes were prepared using sieves. **Chapter 4**. Richardson, Tyuftin, Kilcawley, Gallagher, O'Sullivan, and Kerry (2018) had explored the influence of sucrose crystal size in chocolate brownies and identified that smaller sucrose crystals imparted a greater sweet taste, were more moist and softer and lighter in colour. To date, the influence of sugar crystal size has not been investigated in relation to volatile formation in sponge cakes. Taking a similar approach to **Chapter 3**, the optimised HS-SPME-GC-MS method was applied, and also utilising GC-O, we identified that sucrose source does not have a significant influence on aroma formation but smaller particle size sucrose crystals positively influenced MR and CR reactions, increasing levels of aromatic furans and pyrazines. Due to the lower melting point of small sugar crystals (< 250 μm), MR and CR reactions were accelerated and thus produced more compounds responsible for the desired aroma of bakery products at a faster rate. Application of GC-O further validated the contribution of 'spicy/ bready' furfural and 'oily/fatty' heptanal to the perceived aroma of baked confectionery products.

7.4 Understanding butter as a raw material, and how manipulation of its production can translate to the sensory properties of butter biscuits (short bread)

Butter is highly coveted for its organoleptic properties and is used as a primary ingredient in many bakery products, particularly premium products. Dairy products from a pasture-based diet are perceived to be more natural by consumers, with the type of feed-system employed shown to influence the sensory properties of butter (O'Callaghan, Faulkner, McAuliffe, O'Sullivan, Hennessy, Dillon, Kilcawley, Stanton and Ross, 2016). However, as butter makes up the component of many biscuits and cookies, translation of feed-type properties to the sensory perception of a bakery product has yet to be explored. In **Chapter 5** we investigated the sensory properties of salted butters produced from pasture diets, versus indoor concentrate (TMR) diets, and its sensory perception by different consumer and descriptive sensory groups in Ireland, Germany and the USA. No significant difference in overall liking of the butters were evident among USA, German and Irish consumers, although cross-cultural preferences were evident, likely influenced by familiarity. Butter profiling through RDA (German and Irish assessors) and DA (trained panel USA) aided in defining specific attributes from cow diet, such as 'creamy' flavour, 'buttery aroma', that drive consumer liking of pasture butters, and also potentially translate and drive consumer liking in a bakery product (**Chapter 6**). Specific volatile aroma compounds, particularly aldehydes and ketones, were significantly impacted by the feed system, also aiding in the reasoning of differences in sensory perception of these butters.

From gaining a thorough understanding of how cow diet can influence the sensory perception of salted butter in **Chapter 5**, the final experimental chapter of this

doctoral thesis explores the inclusion of the butter produced for the same bovine diets, and incorporated it into shortbread biscuits. This thesis attempts to demonstrate the crucial requirement for combined approaches to fully elucidate the changes in sensory perception during reformulation. **Chapter 6** highlights the use of temporal-check-all-that-applies (TCATA) to characterise how the two butters behave within a biscuit matrix, during the dynamics of consumption. The sensory quality of shortbread biscuits produced from different butter can be profiled, with stages of oral processing; orthonasal, in-mouth and aftertaste, differentiating mainly due to the difference in fatty acid profile. The combination of temporal sensory analysis in conjunction with aroma analysis, proved a unique approach to understanding reformation in baked confectionery products.

7.5 Conclusions

This thesis outlines a series of approaches taken to further the knowledge of ingredient application for the reformulation of bakery products. Gaining an in-depth understanding of the volatile aroma profile of products, prior and post reformulation, provides information on the nature of flavour and aroma formation reactions that impact sensory perception. This information can be used to make informed formulation changes that can maintain or even enhance consumer acceptability. On selection of suitable sucrose replacers, the prerequisite of having a particular monosaccharide composition should be considered, based on their ability to enhance the formation of desirable aroma compounds, such as furans and pyrazines, in bakery products. However, the correct balance of volatile aromatic compounds is crucial, to prevent the onset of undesirable off-odours. Reducing sucrose crystal size to small./fine sugar crystals aids in the generation and acceleration of desirable aroma compounds, which offers the potential to

reduce the volume of sugar in baked confectionery products without compromising on sensory experience. Understanding the inherent sensory properties of raw materials also aids in the selection of the most appropriate ingredients for optimum reformulation. This doctoral thesis has highlighted the potential of combining multiple sensory techniques, volatile profiling and olfactometry to best elucidate the impact of formulation changes in baked confectionery products on sensory perception to improve our overall understanding of flavour development in these products.

7.6 Future Recommendations

Additional suggested studies that could be conducted to further enhance the knowledge of aroma and flavour formation of baked confectionery products:

- Investigation of alternative pomace powders for their ability to promote favourable volatile formation in cakes, biscuits etc.
- Determine how a combination of the clean-label sucrose replacers, as identified in this study, could produce a desirable reduced sucrose formula with optimum aroma compound formation.
- Inclusion of fine sucrose particles in reduced sugar sponge cake formula for sensory evaluation, particularly to identify the sweetness profile.
- Extraction optimisation of butter compounds for olfactometry analysis to further delve into the desirable aroma of this commodity.
- Further temporal work on reformulated products

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Appendix

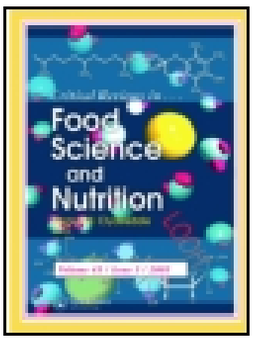
Publications from this thesis and supplementary tables

Chapter 6. Consumer liking, temporal sensory profile and volatile aroma compounds of biscuits formulated with butter produced from a pasture versus total mixed ration diet

Table S1.

Panelist repeatability for the 8 panelists and panel agreement for 10 sensory attributes of SB-GRSS and SB-TMR biscuits.

Panellist	Malty	Buttery	Sweet	Artificial Butter	Fatty mouthcoat	Crunchy	Paste forming	Crumbly	Dry/ astringent	Biscuity	Repeatability
p1	0.77	0.64	0.45	0.67	0.82	0.87	0.79	0.78	0.81	0.48	0.82
p2	0.78	0.71	0.60	0.69	0.61	0.88	0.86	0.84	0.80	0.55	0.79
p3	0.71	0.75	0.57	0.71	0.79	0.84	0.85	0.84	0.80	0.68	0.84
p4	0.41	0.57	0.53	0.67	0.53	0.89	0.80	0.83	0.74	0.65	0.72
p5	0.73	0.71	0.58	0.62	0.69	0.88	0.85	0.82	0.80	0.61	0.77
p6	0.75	0.73	0.56	0.69	0.79	0.82	0.76	0.79	0.71	0.67	0.81
p7	0.69	0.73	0.56	0.73	0.80	0.85	0.83	0.76	0.81	0.59	0.79
p8	0.69	0.63	0.56	0.67	0.75	0.87	0.84	0.81	0.81	0.63	0.79



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REVIEW

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Factors influencing the sensory perception of reformulated baked confectionary products

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ABSTRACT

Baked confectionary products such as cakes, biscuits, cookies, and muffins are consumed globally as they are coveted for their sensory attributes. However, due to their high sugar and fat content, baked confectionary products are also considered major contributors to the prevalence of obesity and the rise of type II diabetes in industrialized nations and in emerging economies. Both sugar and fat have multiple roles in baked confectionary products in terms of structure, texture, shelf-life, aroma, and taste. Considerable efforts have been undertaken to modify product formulations to decrease sugar and fat contents without compromising on product or sensory quality, and this review focuses on relevant research undertaken to date. Aspects addressed include the impact of decreasing sugar and fat content, the impact of sugar or fat substitutes in relation to sensory perception, with a focus on the role of key product constituents, processing parameters, flavor reactions, aromatic compounds, and flavor chemical and sensory techniques.

KEYWORDS

Aroma; baked confectionary; fat; sensory; sugar

Introduction

Baked confectionary is an umbrella term used to categorize a variety of cakes, muffins, biscuits, cookies etc. (O'Sullivan 2016). Globally, these products are highly appreciated by consumers across all populations. They are characterized by their aroma, flavor, texture, and esthetic appeal, having the ability to induce a feeling of satisfaction and happiness when consumed (Poonnakasem et al. 2016). As cakes and other confectionary products are associated with celebrations, they are considered as a "reward" or a "treat" and are anticipated to be of high quality. These products are predominantly comprised of sugar, flour, water, fat, eggs, and a leavening agent. Combined in different ratios, these ingredients produce various products such as cakes, muffins, cookies etc. It is the individual contribution of these raw materials that deliver the desired organoleptic properties and therefore drive consumer liking. Fat and sugar have been identified as the most important contributors to the overall acceptability of sweet bakery products with both contributing to texture, mouthfeel, volume, color, and flavor (Heenan et al. 2010; Manohar and Rao 1999; Zoulias, Oreopoulou, and Kounalaki 2002).

In 2016, 13% of the global adult population was reported obese with 39% of adults aged 18 years and over classified as overweight (WHO 2017). As a result, the food industry have become motivated to modify product formulations through sugar and fat reduction in order to aid consumer welfare, while simultaneously striving to retain the sensory appeal and maintain purchase intent. There is also a demand for "clean label" products that are both nutritious and low in calories, yet consumers still expect a product that is not compromised in sensory quality. However, there is a vast quantity of literature exploring sugar (sucrose)/fat replacement or reduction, with the majority of results correlating sugar and fat reduction with a decrease in consumer acceptability (Cavalcante and Silva 2015; Eslava-Zomeño, Quiles, and Hernando 2016; Giarnetti et al. 2015; Karp et al. 2016; Onacik-Gür et al. 2016; Serin and Sayar 2016; Zahn, Pepke, and Rohm 2010).

Taste and aroma are considered paramount to a consumer's acceptability of a food product. When a food is eaten, a complex mechanism occurs between the taste receptors in the mouth and aroma receptors in the nasal cavity that result in flavor perception (Naknean and Meenune 2010). Although nonvolatile compounds and structural components contribute significantly to the recognition of taste, volatile aroma compounds are considered the major influencer in the overall liking and acceptability of food (Taylor and Linforth 1996). The process of baking induces many changes; structural enhancement, development of the desired texture, and improved digestibility, but the major effect is the transformation of the sensory attributes, specifically aroma formation (Mohsen et al. 2009). Baking promotes thermal reactions and other interactions within the matrix which are thought to be the main precursors of the

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"characterizing" volatile aroma compounds associated with baked goods (Pozo-Bayón, Guichard, and Cayot 2006a). Identification of the most significant compounds responsible for the desired flavor (taste and aroma) of baked confectionary products could be a stepping stone for innovative development of healthier confectionary that possess an integral appeal to the consumer.

The consumption of food is an elaborate process which includes mastication, salivation, tongue movement and swallowing (Piggott 2000), and therefore these events have an impact on the rate and intensity at which an aroma is perceived (Linforth, Baek, and Taylor 1999; Wilson and Brown 1997). In addition, the food matrix can possess a number of factors that influence aroma release; for example, viscosity (Hollowood, Linforth, and Taylor 2002), fat content (van Ruth, King, and Giannouli 2002), and the presence of hydrocolloids and emulsifiers (Koliandris et al. 2008). Different sensory methods can be employed to gain an insight into the consumer's experience during food consumption and aftertaste. Combining instrumental data of volatile compounds with the application of an appropriate sensory methodology can yield important correlations between aroma and flavor perception and therefore, consumer acceptance (Heenan et al. 2009; Lee and Ahn 2009; Quílez, Ruiz, and Romero 2006). Gas chromatography coupled to mass spectrometry (GC-MS) is the separation technique usually applied for the identification and quantification of volatile aromatic compounds in foods (Kilcawley 2017). Although there may be a vast quantity of compounds present in a food product, only a fraction will impact on the flavor perception (Dunkel et al. 2014).

This review aims to provide information on the factors that impact the sensory acceptance of baked confectionary, especially in products where fat and/or sugar has been decreased or replaced.

Raw materials

Although baked confectionaries share many similar ingredients, it is the proportion and ratio of the ingredients that generally defines them on an individual basis. Cakes and muffins are of a similar classification, as the finished products are characterized by a light aerated structure with a moisture content of 20–30% (Fiszman, Sanz, and Salvador 2013). Whereas, biscuits and cookies possess a much lower moisture content (1–4%) and aeration is not as critical as the desired texture of the end product is favorably described as "crispy" or "chewy" (O'Sullivan 2016). Before trying to decipher the complex mechanism of volatile production in baked confectionary products, it is noteworthy to consider the raw materials involved in the process, which act as precursors for the development of the desired aroma and flavor.

Flour

Wheat flour is a predominant ingredient in the bakery industry. Flour is mainly composed of starch and protein and is essentially the "glue" that binds all ingredients of a bakery product together. The functional properties that flour provides are attributed to the quantity and quality of the proteins present. Gluten proteins makeup 80–85% of total wheat protein and are responsible for its unique ability to form a viscoelastic dough. Gluten also plays a role in gas retention and determination of the overall quality of a baked product (Goesaert et al. 2005; Majzoobi et al. 2016). Although these properties are more important in bread manufacture, protein interactions are necessary for an adequate structure in sweet bakery products (Wilderjans et al. 2008).

In terms of its contribution to aroma and flavor production, compounds such as vanillin, 3-hydroxy-4.5-dimethyl-2(5H)-furanone, 4,5-epoxy-(E)-2-decenal and (E)-2-nonenal have been identified as the most odor active compounds in white wheat flour, with odor qualities ranging from vanilla-like to fatty (Czerny and Schieberle 2002). Widely utilized in baked confectionary, white wheat flour yields a soft, somewhat bland taste that allows the other ingredients to command flavor perception. Bakery products produced utilizing grains and plants with nutritional benefits (high in fiber, antioxidant properties etc.) receive a lot more attention in literature due to the presence of celiac disease in populations, and also the increasing demand for low glycaemic products fit for diabetic patients. As flour is usually the most abundant ingredient in a bakery product, replacement with a suitable alternative can be an opportunity to significantly enhance the nutritional profile.

Many flour replacement ingredients have been evaluated. Hedonic assessments by untrained panelists revealed increasing substitution of wheat flour for pea and broad bean derived flours lead to a decrease in organoleptic properties of sponge cakes (Belghith-Fendri et al. 2016). The aroma of "cake like" donuts made with 20% and 30% cowpea meal was described as "slightly beany"; however, untrained panelists did not necessarily rate this as an adverse attribute (McWatters 1982). Similarly, cookies enriched with cowpea flour at 33 and 50% were described by untrained panelists as having a "beany", "nutty" or "fishy" flavor (McWatters et al. 2003). Trained panelists have also described biscuits enriched with soya flour as "beany" (Shrestha and Noomhorm 2002). Addition of resistant starch in muffins led to a significant decrease in the "typical taste" and "typical odor" by descriptive analysis (Baixauli et al. 2008). On replacement of $\geq$20% of wheat flour with β-glucan-rich hydrocolloids from oats, a descriptive sensory panel experienced an increase in "cardboard flavor" and a decrease in "sweetness" (Lee and Ahn 2009).

Chocolate chip cookies containing a mix of barley and wheat flour (30–70% replacement) were perceived by a semi-trained panel, using descriptive sensory analysis, as having an increase in "baked barley" aroma but attributes such as "chocolaty aroma", "sweet flavor" and "chocolaty flavor" were not impacted (Frost, Adhikari, and Lewis 2011). On replacement of 70% wheat flour with almond flour in Chinese moon cakes, quantitative descriptive analysis (QDA) yielded favorable results with trained panelists having appreciated the "almond flavor" derived from methyl-butyraldehyde (Jia et al. 2008).

Although these substitutes demonstrate potential, it is apparent from the literature that none replicate the same sensory experience as traditional formulas made with white wheat flour.

Eggs

Eggs are widely utilized in baking due to their multifunctional composition. Egg white proteins are excellent foaming agents capable of forming a network of air bubbles which coagulate on heating to form a porous aerated stable structure desirable in cakes and muffins (Arunepanlop et al. 1996). However, egg yolk also provides emulsifying capabilities, aids color development, and contributes to the flavor and aroma of baked confectionary products (Yang and Baldwin 1995). Eggs are responsible for the Maillard compounds which produce "roasty", "sweet" and "malty" aromas desirable in cakes and cake-like products. Literature regarding egg replacement in baked confectionary appears to be motivated by a number of factors; the cholesterol content of eggs and its association with cardiovascular disease, utilization of cheaper plant-based alternatives or the growing interest in vegetarian and vegan diets.

Shao, Lin, and Chen (2015) examined creating eggless cakes with the use of hydrocolloids. Sensory evaluation by trained panelists revealed a significant decrease in the intensity of "egg taste" and "egg smell" in eggless cakes compared to the control. Similarly, on evaluation of eggless cakes by QDA, trained panelists allocated a higher rating for "egg flavor" in control cakes compared to the formula without egg (Kohrs et al. 2010). Angel cake and muffins reformulated with lentil protein as an egg/milk replacer were assessed by untrained panelists using a hedonic scale (Jarpa-Parra et al. 2017). The results demonstrated that the cocoa in the muffin formula appeared to mask the direct taste of the lentils (100% replacement of milk and egg), but a "beany" taste was apparent. In the case of the angel cakes, panelists favorably described the flavor as "nutty."

The implementation of soy sources as an egg substitute in baked confectionary has been frequently reported. Muffins produced with soy flour as an egg replacement (Geera et al. 2011) resulted in untrained panelists rating the product as having the highest "off-favor", lowest "overall favor", and the most "intense aftertaste", compared to that of other muffins formulated with egg substitutes. QDA of eggless cakes produced with soy protein isolate (SPI), assessed by trained panelists, yielded significantly different scores for the attributes "beany taste", "eggy taste" and "overall aroma" compared to that of the control (Lin et al. 2017). Corresponding with these results, cakes reformulated with soy alternatives, in place of egg, generally score significantly lower for overall acceptability on hedonic scales, compared to that of the control (Geera et al. 2011; Rahmati and Mazaheri Tehrani 2015).

On replacement of egg with baking powder in sponge cake, Pozo-Bayón et al. (2007) demonstrated that characterizing "malty", "chocolate" (3-methylbutanal), "roasty", "nutty" (2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, trimethylpyrazine), "caramel-like" (5-methylfurfural), and "cherry", "almond" (benzaldehyde) compounds where absent in the formulas made without egg. Similarly, Maire et al. (2013) identified that sponge cakes made without egg yolk were lacking methional ("musty"/"potato"). In addition, the authors noted less lipid oxidation (LO) compounds in the sponge cake made with egg, suggesting that egg phospholipids may as act an antioxidant (Haeyoung and Eunok 2008).

Sensory evaluation of sponge cake, by hedonic scales, found that replacement of egg white with 12.5% and 25% whey protein isolate (WPI) did not significantly impact the odor, flavor or appearance of the cake (Díaz-Ramírez et al. 2016). Although WPI may seem promising as an egg replacer, the incentive for egg replacement is also motivated by cost, which limits the application of WPI. It is evident that eggs contribute to overall flavor acceptability in addition to structural properties in baked confectionary products.

Fat

Fat has a major influence on the overall acceptability of baked confectionary products and is usually present in the form of hydrogenated shortenings or butterfat. In terms of functionality, fat plays a critical role in the incorporation of air bubbles; enabling an increase in volume and the development of a porous structure. Additionally, fat aids in the entrapment of moisture leading to a moist and tender crumb (Conforti 2006; Eslava-Zomeño et al. 2016). Through the interaction with starch in the baked product matrix, fat forms lipid-amylose complexes, which hinder retrogradation; helping to maintain a desirable texture and hence extend shelf-life (Mert and Demirkesen 2016). However, due to the adverse health effects associated with saturated and trans fats, suitable alternatives are desirable.

Fat is a principle contributor to aroma and flavor perception. Fat has the ability to enhance palatability by imparting lubricity and a specific mouthfeel, whilst many aroma volatile compounds are fat soluble and bound within the lipid component of a product (O'Sullivan 2016; Zoulias, Oreopoulou, and Tzia 2002). Due to its unique fatty acid composition, butter is difficult to replace in recipes without having an adverse effect on the organoleptic qualities of the finished product. Compounds such as 2,3-butanedione, acetoin, δ-decalactone, δ-octalactone, and butyric acid are important contributors for the typical flavor/aroma of butter (Mallia, Escher, and Schlichtherle-Cerny 2008; Schieberle et al. 1993). Pastries produced with butter have been characterized by a "sweet" and "coconut" aroma originating from δ-decalactones (Gassenmeier and Schieberle 1994). Giarnetti et al. (2015) explored replacing butter in cookies with a combination of inulin and extra virgin olive oil at different percentages. Descriptive sensory analysis revealed that the reformulated cookies scored much lower in "caramel odor", "buttery odor", "buttery flavor", and lacked a sweet perception, compared to the control. Similarly, 50% butter replacement with prune puree in cookies resulted in a decrease in "butter flavor" intensity and a less desired product (Swanson

and Perry 2007). It appears the amount of butter incorporated into a recipe strongly reflects the intensity of "butter flavor" and "butter aroma" perceived on consumption.

Margarine and shortening blends are more commonly used in bakery products due to their plasticity and lower cost compared to butter. The make-up of margarine is relatively simplistic, consisting of a water in oil emulsion, whereas shortening is comprised solely of an oil blend. Although the characterizing compounds of butter are not as abundant in margarines and shortenings, they are still capable of imparting positive attributes such as "buttery", "fruity" and "sweet" derived from 2,3-butanedione, ethyl butanoate, and δ-decalactone, and δ-octalactone, respectively (Shiota et al. 2011). Shortening replaced with different fat replacers in cookies resulted in significantly lower intensity scores for "vanilla" and "sweet" on a descriptive scale compared to a control (Armbrister and Setser 1994), indicating that the source of these aromatic compounds was bound within the fat matrix. Similarly, biscuits formulated with vegetable shortening were identified by Free Choice Profiling to have stronger intensity in "buttery", "vanilla", "coconut", and "cinnamon" attributes than biscuits with the same percentage of dairy based shortening and liquid oils (Tarancón et al. 2013). Hedonic scales usually reveal lower aroma and flavor acceptability when sensory panelists evaluate sweet bakery products where the fat has been removed or replaced (Psimouli and Oreopoulou 2013; Rodríguez-García, Salvador, and Hernando 2014; Singh and Kumar 2018). However, when hydroxypropyl methylcellulose was used as a fat replacer for margarine in biscuits, it did not appear to adversely affect the sensory properties of biscuits at a substitution rate of 15%, but at 30%, "buttery" flavor was significantly reduced (Laguna et al. 2013).

Carbohydrate fat replacers have been extolled for their ability to replicate the texture of fat in the mouth as their globular structure can somewhat mimic the impression of creaminess (Meyer et al. 2011). However, maltodextrin and polydextrose were found unable to imitate the lubricity, taste, and flavor of fat in short dough biscuits (Sudha et al. 2007). Trained panelists associated an increase in "floury" and a decrease in "buttery" flavors with reduced fat biscuits formulated with N-DULGE® (a mixture of tapioca dextrin and tapioca starch) and resistant starch, by descriptive analysis (Laguna et al. 2012). Partial replacement of oil in chocolate muffins, with soluble cocoa-fiber, has been associated with an increase in "bitterness" by descriptive analysis (Martínez-Cervera et al. 2011). On the contrary, the addition of apricot kernel fiber to replace shortening in cookies, did not adversely impact sensory perception (Seker et al. 2010). Fat reduction can also coincide with a decreased in sweetness perception, which has been reported in biscuits (Biguzzi, Schlich, and Lange 2014; Forker, Zahn, and Rohm 2012).

Butter replacement in cookies corresponded with a significant decrease in the levels of methyl ketones (2-butanone, 2-heptanone, 2-nonanone, and 2-undecanone) (Giarnetti et al. 2015), which are known to impact on "buttery" and sweetness perception. As stated, the unique fatty acid profile of butter is comprised mostly of short and medium length fatty acids, having the capability to generate short chain methyl ketones via oxidation. These compounds contribute to the aroma of cookies and other sweet bakery products. On replacement of margarine with extra virgin olive oil in Madeira cakes, Matsakidou, Blekas, and Paraskevopoulou (2010) found that the alcohols ((Z)-2-pentenol, (Z)-3-hexenol, (E)-2-hexenol and (Z)-2-hexenol) were created from oxidation of the virgin olive oil. Although untrained panelists did not negatively rate the re-formulated sponge cake, the presence of these LO alcohols may have implications for product shelf-life as they can contribute to off-flavors over time.

Overall, there appears a lot more information is required to further understand the role of fat in consumer acceptability of confectionary products.

Sugar

Dominating a large proportion of the ingredient declaration for the majority of commercial cakes, muffins, biscuits etc., sugar or sucrose, is considered the most important raw material incorporated in baked confectionary products. Not only providing the characteristic sweetness, sugar also plays a vital role in creating and maintaining the structure, and texture of baked confectionary products. Sugar also restricts water activity, thus inhibiting microbial growth and contributing to the preservation of the product (Rodríguez, Magan, and Medina 2016). Sucrose is highly recognized in food manufacturing for its ability to impart a clean, sweet taste. However, providing 4 kcals of energy per gram, and usually present in large quantities in baked confectionary, excess sucrose consumption is identified as a major contributor to the prevalence of obesity and type II diabetes worldwide (Hashem, He, and MacGregor 2016).

Sweeteners, both artificial and natural, are widely utilized for their ability to impart a conventional "sweet flavor" with only a fraction of the calorific value to that of sucrose. Although these sweeteners influence the perception of sweetness, they cannot fully imitate the role sucrose plays in structural development, functionality, or color formation (Struck et al. 2014). The sugar alcohol xylitol conjoined with bulking agents, such as oligofructose, has shown potential for reduced sugar cake formulation (Nourmohammadi and Peighambardoust 2016; Ronda et al. 2005), due to the synergistic effect of these substances. Xylitol imparts a high level of sweetness but is unable to partake in the Maillard reaction (MR), whereas bulking agents are less sweet by nature but are capable of aiding in structural and color development, thus resulting in an acceptable product.

Steviol glycosides are widely used as a sucrose replacement with their popularity due to their "clean label" status. Although these sweeteners deliver a high intensity of sweetness, 100–300 times sweeter than sucrose (Cardello, Da Silva, and Damasio 1999), they are unable to meet all the requirements of a sucrose substitute. Steviol glycosides have been shown to perform well with other bulking agents in confectionary systems (Periche et al. 2016; Shah, Jones, and

Vasiljevic 2010). Sucrose reduction of 30% was achieved in muffins with the use of a steviol glycoside (*rebaudioside A*) in addition to inulin and polydextrose (Zahn et al. 2013). Flash sensory profiling revealed these formulas were associated with attributes such as "buttery flavor", "sweet", and "aromatic". However, on evaluation of muffins where sucrose was partially replaced with Stevia (25%), trained panelists identified the control (sucrose), on a hedonic scale, as having the highest acceptability (Karp et al. 2016). Complete replacement of sucrose with stevia does not seem to be well received by consumers in baked confectioneries, but partial replacement shows potential (Wardy et al. 2018).

Although sucrose contributes hugely to the sweet flavor of baked confectionary, it can also play a role in the development of flavor and aroma that is not necessary related to sweetness. Reduced sucrose cookies have shown to have a significantly reduced perception of "buttery" flavor (Laguna et al. 2013). Similarly, on replacement of sucrose with isomaltose, cakes were perceived as having a significantly less "buttery" and "caramel" flavor (Heenan et al. 2010). This may be explained by the interaction sugar has in thermal processes that occur during baking. When sucrose is removed from the equation, volatile compounds may be lost or suppressed due to the lack of monosaccharaides available to partake in the MR and caramelization. Despite the desire for sugar to be eradicated in food formulations, it is evident sucrose directly impacts on the appreciated flavor and aroma of baked confectionaries, as well as playing an important role in functional properties.

Other ingredients

Introduction of non-conventional materials can also favor the production of desired aroma compounds in baked confectionary matrices and offers scope to improve the nutritional quality of a product. Wheat cookies supplemented with SPI at 10% scored significantly higher on a hedonic scale for "aroma" and "taste" compared to the control cookie (Mohsen et al. 2009). The addition of SPI, an additional source of amino acids, favored the generation of 2-ethyl-5-methylpyrazine ("biscuit-like") and maltol ("cotton-candy") with concentrations of these compounds higher than that of the control. Cookies re-formulated with an emulsion gel containing inulin (Giarnetti et al. 2015), showed increased levels of 3-methylbutanal ("malty/chocolate"), methylpyrazine and trimethylpyrazine ("roasty/nutty"). The formation of these compounds can be explained by the degradation of inulin that occurs during baking, producing mono- and di-saccharides that are then available to accelerate the MR. Similar results were found when inulin was added to wheat bread (Poinot et al. 2010). On replacement of whole meal flour with purple wheat flour in biscuits, Pasqualone et al. (2015) saw significantly higher amounts of potent aroma compounds 3-methylbutanal, 2-methylbutanal, benzaldehyde, and the furan compounds furfural, 5-methylfuran, and hydroxymethylfurfural (HMF).

Bi-products of wine fermentation, such as grape marc extract has been shown to increase the level of benzaldehyde ("cherry"/"almond"), phenylacetaldehyde ("floral"/"honey"), and furans 2-methylfuran, 2-acetylfuran, 5-methylfurfural and 2-furanmethanol ("sweet"/"caramel") in biscuits, resulting in enhanced consumer acceptability and purchase intention (Pasqualone et al. 2014). Higher levels of furanic compounds were identified in the grape marc extract biscuits compared to the control. This can be explained by the acidic pH of this material, which is favorable for the formation of these compounds.

Varying yeast amounts have been shown to have an impact on compounds derived from the MR (Birch et al. 2013a; Birch et al. 2013b; Poinot et al. 2008; Zehentbauer and Grosch 1998b), which are associated with "malty", "sweet", and "roasty" attributes, and hence important to the overall aroma of bakery products. The monosaccharide fructose, in the presence of high temperatures, has been shown to have a positive effect on the formation of HMF in cookies and biscuits (Ameur et al. 2007; Nguyen, Van der Fels-Klerx, Peters, and Van Boekel 2016; Zhang et al. 2012). HMF and furfural have also been shown to be influenced by salt (NaCl) content in cookies (Kocadağlı and Gökmen 2016; Van Der Fels-Klerx et al. 2014).

Matrix effect

It is well understood how the removal of key ingredients (fat and sugar) in product formulation can adversely impact on aroma and flavor of baked confectionary (Giarnetti et al. 2015; Struck et al. 2014; Sudha et al. 2007). The food matrix can also significantly influence how flavor and aroma are perceived. On consideration of manipulating the integral high sugar, high fat composition of a confectionary product, it is important to understand how aroma compounds can be retained or released from the matrix when concentrations of these ingredients are altered.

The main function of sucrose in the majority of formulas is to enhance palatability by imparting a sweet, clean taste. Sucrose has proven to have a significant impact on aroma release in sweetened beverages, with studies demonstrating that sugar increases aroma perception (Hansson, Andersson, and Leufvén, 2001; Nahon et al. 1998; Saint-Eve et al. 2009). This effect can be explained by the "salting out" phenomenon, whereby sucrose saturates the solution and as free water is lost due to sugar hydration, aroma compounds are forced into the headspace (Nawar 1971). Headspace analysis of cereal bars showed increasing amounts of glucose solids had a pronounced effect on aroma release for some compounds (acetaldehyde, ethyl butyrate, ethyl methyl butyrate, and limonene) but not others (maltol and methyl cinnamate) (Heenan et al. 2012). As sugar has the ability to increase the aroma intensity of compounds, in theory, when sugar is removed, perception of aroma compounds can also decrease. Aroma addition has been suggested as a tool to compensate for the decline in sensory quality on sucrose reduction in food formulas (Hutchings, Low, and Keast 2018). However, this theory is drawn from liquid and semi-solid models. In order for this concept to apply to sugar reduction in baked confectioneries, more work on

aroma-interactions in soft-solid matrices, as found in bakery products, is required (Poinot et al. 2013).

Sugar reduction is a difficult challenge as it is almost inevitable that sweetness perception decreases concurrently with sugar reduction (Biguzzi et al. 2014; Drewnowski, Nordensten, and Dwyer 1998; Martínez-Cervera et al. 2012), leading to diminished consumer acceptance. Fat and sugar are very much intertwined in the role of sensory perception in baked confectionary products. Fat contributes hugely to the texture and mouth-feel of food products. In addition, the perception of fat on consumption can be somewhat hard to define by consumers, with sweetness impression shown to decrease with a decrease of fat in biscuits (Biguzzi et al. 2014; Forker et al. 2012). Cognizance of the relationship between aroma and perception must be taken into account when sugar and fat are reduced so that consumer desirability is not adversely impacted.

Manipulation of components of the matrix can be an innovative way to enhance aroma perception and even improve the quality of reduced fat/sugar products. On variation of particle size distribution in chocolate, Afoakwa et al. (2009) demonstrated that with finer particle sizes, an increase in favorable compounds associated with "cocoa-chocolate-praline" and "caramel-sweet" notes were released into the headspace. Richardson et al. (2018) employed sugar particle size reduction in a chocolate brownie matrix. Replacing standard sugar crystals with a smaller particle size in the formula produced brownies that retained their conventional "sweet" taste and were identified as significantly sweeter than the control. From these findings, the authors postulated that sucrose of smaller particle size can be used in product formulation to produce sugar reduced brownies of acceptable quality.

Precursors of flavour- volatile formation

Aroma is considered a critical determinant to the overall quality of bakery products as it is one of the initial sensory attributes the consumer encounters. Even in small quantities, low aroma threshold compounds can act as a determinant of product quality and consumer preference (Quílez et al. 2006). Aroma compounds can be produced as a result of enzyme activity, fermentation, or through thermal reactions (Pozo-Bayón et al. 2006a). Although the ingredients contribute immensely to the overall flavor perception of the product, it is the thermal reactions that occur during baking that significantly influence the aroma, and thus flavor. The following reactions are thought to generate the most characterizing compounds associated with baked confectionary products.

The Maillard reaction

Maillard reactions are non-enzymatic reactions that occur on heating and have the ability to completely transform the flavor, aroma, and color of food products. The MR is a complex cascade of chemical reactions and has been extensively studied (Hodge 1953; Nursten 1981). It is generally described as occurring in three main stages. The MR is instigated by a condensation reaction between a carbonyl group of a reducing sugar and a free amino group ($-NH^2$) originating from amino acids, peptides, or proteins, in a low moisture, high temperature environment, to produce amines, N-glycosylamine (aldose sugar) or fructosylamine (ketose sugar) (Parliament 1989). These products are colorless and not odor active. As the temperature increases internally in the food product and moisture is driven off, N-glycosylamine or fructosylamine rearrange to form an Amadori or a Heyns product, respectively. Amadori/Heyns products are inherently unstable and subsequently degrade, impacted by the pH of the matrix; this degradation by means of pH is known as dehydration. At pH $\leq$7, 1,2-enolization is promoted to form furfural and HMF, whereas in an alkaline environment (pH $\geq$7), 2,3 enolization occurs forming highly reactive reductones and dehydroreductones (Martins, Jongen, and Van Boekel 2000; Pozo-Bayón et al. 2006a). The temperature, nature of the reactants (amino acid, peptide and sugar), and water activity also strongly influence the rate at which these reactions occur (Van Boekel 2006). Alternatively, Amadori and Heyns products can also undergo cyclization to produce nitrogen-containing heterocyclic compounds, such as pyrroles or pyridines (Jousse et al. 2002). Sugar fragmentation is another possible route of degradation for these products, a complex mechanism involving retro-aldol, hydrolytic, oxidative and amine-induced carbohydrate cleavages resulting in the production of α-dicarbonyl compounds which can recombine to yield HMF and other furans (Nursten 2007; Smuda and Glomb 2013; Taş and Gökmen 2017). The third potential pathway of Amadori/Heyns degradation is through means of Strecker degradation. In relation to the MR, Strecker degradation is brought about by α-dicarbonyls, and induces deamination and decarboxylation of free amino acids, resulting in the production of volatile aldehydes whose structure mimics that of their amino acid counterpart (Rizzi 2008; Yaylayan and Mandeville 1994). Compounds such as 3-methylbutanal, phenylacetaldehyde, and methional are well established as volatile compounds derived from Strecker degradation of leucine, phenylalanine, and methionine, respectively, and can be considered some of the most important products of the MR (Hofmann, Münch, and Schieberle 2000). In addition to aldehydes, aminoketones are also a result of α-dicarbonyl and amino acid reactions. These compounds have the ability to condense into heterocyclic compounds such as pyrazines, pyridines, thiazoles, pyrroles etc. (Shu 1998). As seen in Figure 1, each one of these pathways is capable of producing volatile intermediates that are important aroma compounds which influence the flavor of baked confectionaries. On further condensation, these compounds form polymers known as melanoidins (Zamora and Hidalgo 2005), yielding the characteristic golden brown color of bakery products.

Carmelization

Although the MR receives a lot of attention for the role it plays in the formation of volatile and nonvolatile

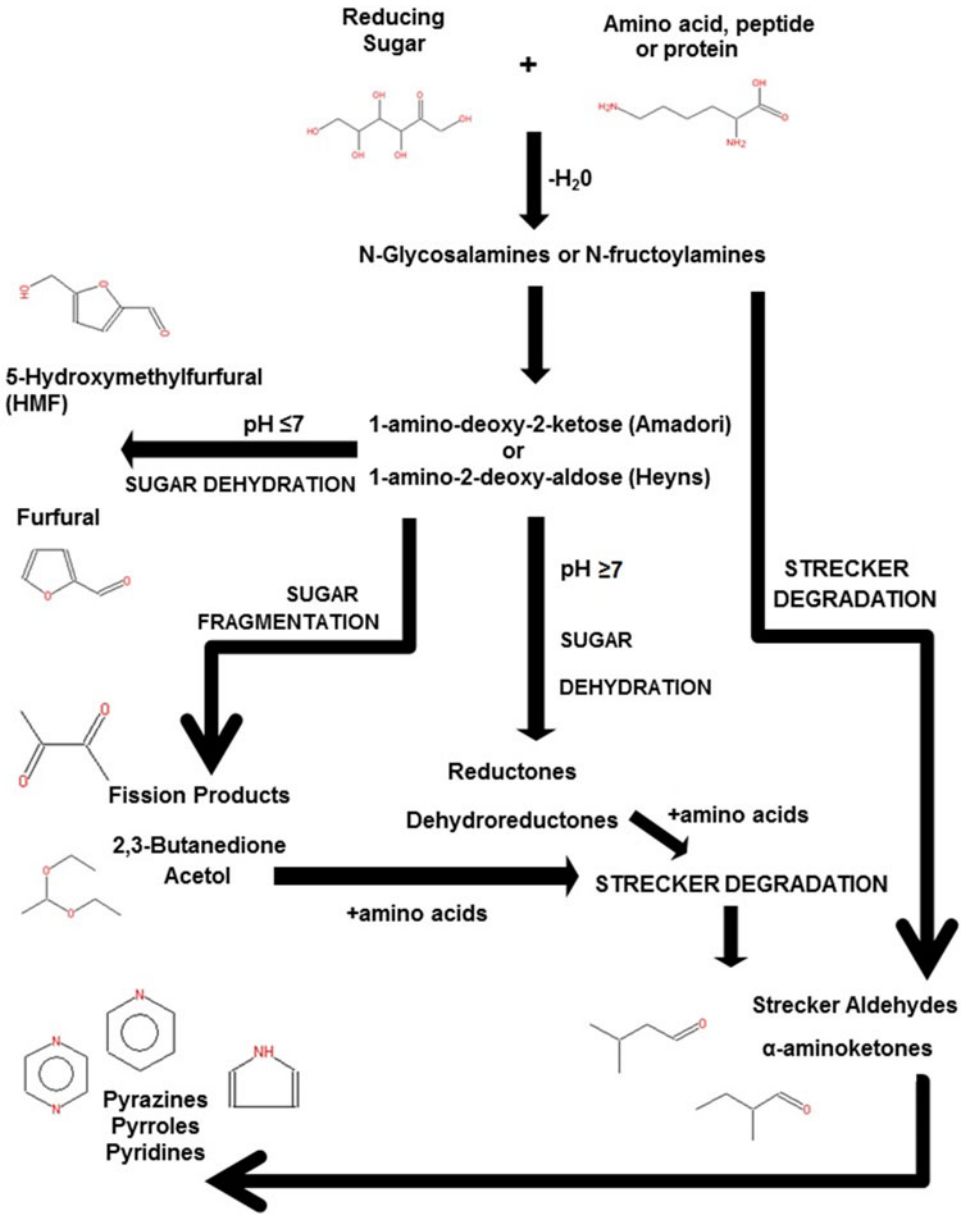


Figure 1. Flavour compound formation. The Maillard reaction(adapted from Pozo-Bayón Guichard, and Cayon 2006a)

compounds during baking, caramelization is also an important contributor to the development of the overall aroma and color of baked products. Caramelization is referred to as the decomposition of sugars and happens at temperatures >120 °C, favored by a pH of <3 or >9, and can be associated with a brown color and "caramel" odor in food (Lee and Lee 1997; Zhang et al. 2012). Isomerization of monosaccharides is generally the initial step in caramelization, where sugar molecules experience enolization, and further degradation reactions lead to the formation of α-dicarbonyls (Kroh 1994). Sugar degradation produces compounds comparable to that of the early stages in the MR, but are produced at a slower rate due to the lack of a catalyst, the amino group (Van Boekel 2006). As the MR relies on the participation of reducing sugars, the extreme temperatures attained on the surface of the product during baking can induce starch and sucrose hydrolysis, thus leading reducing sugars to be available for both MR and caramelization reactions simultaneously (Capuano et al. 2008). As the name suggests, caramelization is associated with aroma compounds associated with a "caramel" odor, which derive from furans, ketones, aldehydes, and lactones, aromatic compounds formed during thermal decomposition of sugars (Paravisini et al. 2015).

Lipid oxidation

Unsaturated lipids are susceptible to LO, a problematic reaction leading to undesirable changes in flavor, nutritional quality, and shelf-life (Waraho, McClements, and Decker 2011). Auto-oxidation is the most common form of LO in bakery products (Maire et al. 2013) and can be described as a free radical chain reaction consisting of three stages; initiation, propagation and termination (Frankel 2014). Margarine and shortenings utilized in baking are an abundant source of oleic, linoleic, and linolenic acid and are thus

prone to secondary oxidation. The formation of various aldehydes, ketones, and alcohols are indicators of LO in bakery products and these LO derived compounds can contribute up to a quarter of the volatile profile of bread (Jacobsen 1999; Pico, Bernal, and Gómez 2015). The main pathways of LO occur on ingredient preparation, in the presence of oxygen, at high temperatures of baking, and on storage, with hexanal being the primary marker of LO in sponge cake and other bakery products (Maire et al. 2013; Purcaro, Moret, and Conte 2008).

Processing factors

Processing factors have been shown to influence the formation of volatile compounds generated through thermal reactions in bakery products. Most work to date has focused on furanic compounds such as HMF and furfural, however, other volatile compounds are likely to be affected.

Compounds important to aroma and color development in baked products are produced via thermal reactions, thus baking times and temperatures will have a pronounced effect on their formation and development. Rega et al. (2009) monitored volatile compounds produced during baking a sponge cake over a period of 0–25 minutes. Strecker aldehydes and pyrazines expressed linear behavior and increased with baking time. However, HMF was formed mainly at the end of the baking processed. Longer baking times coupled with higher baking temperatures were shown to have a positive effect on the formation of HMF in sponge cakes (Zhang et al. 2012). This may be reasoned by the longer period for caramelization to occur, which brings about a pH shift in the matrix (slightly acidic), and therefore promotes the formation of this furanic compound. Varying mixing times, baking times, and baking temperatures have been shown to significantly impact the volatile composition of bread, and manipulation of these parameters can yield greater amounts of MR and caramelization volatile compounds (Sabovics, Straumite, and Galoburda 2014).

Volatile analysis of baked cereal products

Gas chromatography (GC)

Sensory analysis acquires useful information on the perception and acceptance of foods but cannot provide information on the compounds responsible for a given flavor perception. Therefore, combining data from both flavor chemistry and sensory science can help identify the compounds responsible for a desired aroma or taste. Gas chromatography mass spectrometry (GC-MS) is a strategic technique used in food analysis to identify potent compounds with the ability to impact on aroma perception, and this information can be used to establish the impact of processes and raw materials on the overall flavor profile, as well as help predict product quality and market acceptance (Paraskevopoulou et al. 2014). The working principle of GC is separation of analytes based on volatility and affinity to a column phase. The analytes elute depending on characteristics such as volatility, molecular weight, vapor pressure, and polarity, and are detected by Mass selective and flame ionization detectors.

To maximize the efficiency and output of the GC instrument, there are a number of aspects that require optimization depending upon the separation required. The type of column is one of the most important considerations. As seen from Table 1, a range of stationary phase columns of various polarities have been utilized in the analysis of baked cereal products. The criteria for the choice of column should suit the chemistry of the compounds extracted. Traditionally most analysis has been undertaken using one-dimensional chromatography, where a single column of selected polarity is used. However, in complex samples, volatiles may co-elute making identification and quantification difficult. The advent of two-dimensional or, comprehensive chromatography, improves separation using two columns of different polarity. In this case, all or part of the eluent of the first column is directed to a second column using modulation (thermal or flow) to create a three-dimensional output. By employing this approach, Matsakidou, Blekas, and Paraskevopoulou (2010) were able to identify 92 compounds from the volatile fraction of Madeira cake.

Flame ionization detector is a popular detector as it has sensitivity for an extensive range of organic compounds, low noise level, excellent linear range, low cost, and excellent durability (Colón and Baird 2004). However, mass spectrometry (MS) has become the detector of choice due to its selectivity, sensitivity, and versatility (Milman 2015). MS operates as a detector through the mechanism of initial molecule ionization followed by resolution of the ionized molecule based on mass-to-charge (m/z) ratio (Croissant, Watson, and Drake 2011). As a result, a mass spectra is created for each compound and therefore enables the identification of compounds in the sample through comparison of library databases and retention indexes.

Chemistry of extraction

Prior to GC analysis, it is necessary to extract volatiles from the sample of interest. Currently no analytical technique can compare to the human nose in terms of sensitivity, therefore it is necessary to concentrate the volatiles during extraction to ensure an optimum representation of the sample is attained (Kilcawley 2017). In addition, compounds responsible for aroma and flavor perception in food range from a diverse mixture of chemical classes of different molecular weight, polarity, and volatility. Hence, the application of the most suitable extraction technique is crucial for creating an accurate depiction of the volatile profile of the product. Implementation of the appropriate extraction technique needs to take into account; type of analysis (trace, target, untargeted, profiling etc.), labor intensity, robustness, flexibility, cost, sample matrix, time, and sample preparation (Ebeler, Terrien, and Butzke 2000; Hyötyläinen and Riekkola 2008). All extraction techniques have advantages and disadvantages, but also an inherent degree of bias. Extraction

Table 1. Extraction techniques utilized in the volatile analysis of baked cereal matrices.

Sample of interest	Extraction technique	Parameters employed	NaCl used in extraction	GC COLUMN	Number of volatiles extracted	Reference
Cookies	**Simultaneous distillation extraction**	**Sample:** 10 g mixed with 40 mL distilled H20 **Solvent:** Dichloromethane **Concentrated 10 times under nitrogen**	N	HP5 ***Non-polar***	14	Prost et al. 1993
Cookies	**Thermal Desorption**	**Adsorbent Material:** Not stated **Purge Time:** 3 min **Desorption Time/ Temp:** 5 mins at 240 °C **Gas/ Desorption Flow:** 200 mL Nitrogen min^{-1} **Temp of Cold Trap:** −20 °C	N	DB-5 ***Non-polar***	5 (Compounds added and recovered)	Heiderich and Reineccius 2001
Sponge Cake	**SAFE**	**Sample:** 20 g **Extraction Time:** 2 hours **Temp:** 30 °C **Solvent:** Dichloromethane	N	DB-Wax ***Polar***	19 (Compounds added and recovered)	Pozo-Bayón et al. 2006b
Sponge Cake	**SAFE**	**Sample:** 70 g mixed with 150 mL distilled H20 **Time:** 2 hours **Temp:** 30 °C **Solvent:** Dichloromethane	N	DB-Wax ***Polar***	77	Pozo-Bayón et al. 2007
Sponge Cake	**Purge and Trap**	**Adsorbent Material:** Tenax Ground cake **Temp**: 25 °C **Purging Gas:** 25 mL/min with Nitrogen **Purge times:** 5, 15, 30 and 60 min and 14 hour	N	DB-Wax ***Polar***	90	Pozo-Bayón et al. 2007
Altamura Bread	**Purge and Trap**	**Adsorbent Material:** Tenax TA **Temp**: 40 °C **Purging Gas:** 40 mL/min with helium **Purge time:** 15 mins	N	Supclowax ***Polar***	89 in crust 74 in crumb	Bianchi et al. 2008
Wheat Bread	**SPME**	**Fibre:** 75 µm DVB/ CAR/ PDMS **Extraction:** 30 mins at 35 °C (shaken with magnetic bar) Bread sample crushed	N	DB-WAX ***Polar***	46	Poinot et al. 2008
Sponge Cake	**Purge and Trap**	**Adsorbent Material:** Tenax	N	DB-Wax ***Polar***		Pozo-Bayón et al. 2008

(*continued*)

Table 1. Continued.

Sample of interest	Extraction technique	Parameters employed	NaCl used in extraction	GC COLUMN	Number of volatiles extracted	Reference
		Temp: 25 °C **Purging Gas:** 25 mL/min with Nitrogen **Purge times:** 5, 15, 30 60 min +14 h			Amylose Interaction Study (looked at 19 compounds)	
Cookies	**Simultaneous distillation extraction**	**Sample:** 100 g +400 mL distilled H20 **Solvent:** Diethyl ether-pentane	N	DB-5 ***Non-polar***	80	Mohsen et al. 2009
Sponge Cake	**SPME**	**Fibre:** 50/30 μm DVB/ CAR/ PDMS and 75 μm CAR/ PDMS and 100 μm PDMS **Extraction:** 30 mins at 50 °C	N	DB-Wax ***Polar***	49 (between 3 fibers)	Rega et al. 2009
Sponge Cake	**SPME**	**Fibre:** 50/30 μm DVB/ CAR/ PDMS **Extraction:** 60 mins at 60 °C (manual) Cake sample cryogenically ground	N	FFAP ***Polar*** and BP-5 ***Non-polar***	92	Matsakidou, Blekas, and Paraskevopoulou 2010
Oat Cake	**SPME**	**Fibre:** 85 μm CAR/ PDMS **Extraction:** 15 mins (temperature not stated)	N	DB-1701 ***Low/ Mid Polar***	36	Cognat et al. 2012
Oat Cake	**Thermal Desorption**	**Adsorbent Material:** Tenax TA **Purge Time:** 1 min **Desorption Time/ Temp:** 5 mins at 240 °C **Gas/ Desorption Flow:** 200 mL Nitrogen min^{-1} **Temp of Cold Trap:** −10 °C	N	DB1701 ***Low/ Mid-polar***	46	Cognat et al. 2012
Pineapple Breads	**SPME**	**Fibre:** 75 μm CAR/ PDMS **Extraction:** 10 mins at 40 °C	Y	DB-5 ***Non-polar***	59	Ying et al. 2012
Sponge Cake	**SPME**	**Fibre:** 75 μm DVB/ CAR/ PDMS **Extraction:** During Baking	N	DB-FFAP ***Polar***	72	Maire et al. 2013
Sponge Cake	**SPME**	**Fibre:** 75 μm CAR/ PDMS **Extraction:** 37°C for 40 mins (agitated at 600 rpm)	Y	HP-5 ***Non-polar***	31	Petisca et al. 2013
Biscuits	**SPME**	**Fibre:** 75 μm CAR/PDMS **Extraction:** 40 °C for 50 mins	Y	HP-Innowax ***Polar***	60	Pasqualone et al. 2014

(*continued*)

Table 1. Continued.

Sample of interest	Extraction technique	Parameters employed	NaCl used in extraction	GC COLUMN	Number of volatiles extracted	Reference
Triticale Bread	**SPME**	**Fibre:** 85 µm CAR/PDMS **Incubation:** 15 mins at 40 °C **Extraction:** 65 mins at 40 °C	N	Elite-WAX ETR ***Polar***	26	Sabovics, Straumite, and Galoburda, 2014
Shortbread Cookies	**SPME**	**Fibre:** 50/30 µm DVB/ CAR/ PDMS **Extraction:** 15 mins at 35 °C	N	HP-Innowax ***Polar***	24	Giarnetti et al. 2015
Biscuits	**SPME**	**Fibre:** 75 μm CAR/PDMS **Extraction:** 40 °C for 50 mins	Y	HP-Innowax ***Polar***	56	Pasqualone et al. 2015
Crackers	**Thermal Desorption**	**Extraction time/ temp:** 20 mins at 30 °C **Purge Time:** 2 min **Desorption Time/ Temp:** 5 mins and 150 °C followed by 5 mins at 300 °C Gas/ Desorption Flow: 50 mL Nitrogen min^{-1} **Temp of Cold Trap:** 30 °C	N	DB-5 ***Non-polar***	49	O'Shea, Kilcawley and Gallagher, 2017

techniques utilized to profile the aroma of baked confectionary products are as follows.

Simultaneous distillation extraction

Simultaneous distillation extraction (SDE) is one of the oldest, widely used methods of volatile extraction and is based on vapor differences over water (Veith and Kiwus 1977). This technique can recover significant amounts of volatiles of different chemical classes with good reproducibly (Chaintreau 2001). Using SDE, Prost et al. (1993) recovered 14 compounds representative of cookie odor, but the technique poorly recovered compounds such as vanillin, γ-butyrolactone, maltol, and 4-(4-hydroxyphenyl)-2-butanone, which are thought to be important constituents to the characteristic cookie odor. Mohsen et al. (2009) applied the same technique and similar parameters in analyzing wheat cookies. The authors were capable of identifying and quantifying γ-butyrolactone and maltol, as well as another 42 volatile aromatic compounds of diverse chemical classes. Although SDE has been widely used in food research, studies in baked matrices are limited. This is probably due to the elevated temperatures associated with distillation, leading to the formation of artifact compounds, particular those relating to the MR (Cai, Liu, and Su 2001; Engel, Bahr, and Schieberle 1999). In addition, solvents utilized in extraction discriminate against compounds of a similar polarity, and hence the recoveries may not provide a true representation of the sample.

Solvent-assisted flavor evaporation

Designed to overcome some of the short comings of SDE, solvent-assisted flavor evaporation (SAFE) is a well-established technique that is suitable for extraction of volatiles from a range of matrices (Drake, Miracle, and McMahon 2010; Mahajan, Goddik, and Qian 2004; Mayuoni-kirshinbaum et al. 2012; Xu, Fan, and Qian 2007). The practicality of the SAFE apparatus allows for reduced loss of highly volatile compounds as the extraction is contained within a single glassware unit and operates at lower temperatures than SDE, thus minimizing the production of artifacts (Engel et al. 1999). On correct application, this method has demonstrated a higher sensitivity than other extraction techniques for compounds related to perceived aroma (Havemose et al. 2007; Majcher and Jeleń 2009; Murat et al. 2012). However, detailed knowledge of the product composition is beneficial to the successful operation of SAFE, as components such as fat and alcohols can interfere with the extraction process (Reineccius 2007).

Pozo-Bayón et al. (2006b) investigated SAFE as a mechanism for quantifying aroma compounds in sponge cake. Nineteen aroma compounds associated with a "rich" and "sweet" character were added to a sponge cake and SAFE recovered all compounds with quantification achieved for 13. Key volatiles such as acetoin, γ-decalactone, and vanillin were quantified, highlighting the suitability of this technique for baked cereal matrices. In a similar study, Pozo-Bayón et al. (2007) employed SAFE to investigate the contribution

of egg to the aroma of sponge cake. By combining the use of two extraction techniques, SAFE and Purge and Trap (P&T), the authors were capable of recovering an elaborate volatile profile of 100 compounds. Although it stated the two techniques were complimentary, SAFE had the advantage of isolating 1,2-dimethylbenzene, butan-1-ol, limonene, 2-methyl-dihydro-2(H)-furan-3-one, as well as 19 other compounds, which P&T was unable to recover. However, limitations of this technique include the tendency to favor the extraction of high molecular weight compounds (Thomsen et al. 2014). Solvent extraction techniques by nature retrieve most compounds in the sample, without accounting for the retention effect of the matrix; therefore the sample profile reflects heavier compounds that are bound in the matrix, which may not be truly representative (Kilcawley 2017). Other drawbacks include the copious amounts of solvents used during extraction, leading to the generation of hazardous waste, as well as the length of time the process requires, and the lack of automation.

Purge and trap

P&T is a headspace technique that entails purging volatiles from a sample to a highly sorbent material (usually Tenax®) where they are concentrated prior to desorption to the GC (Lee et al. 2001). Some of the attractions to this technique include: a limited sample amount, large volume traps, and a solvent free technique (Pillonel, Bosset, and Tabacchi 2002). P&T has been mainly utilized for the analysis of pollutants in water and air, but has demonstrated successful recoveries in baked cereal matrices (Table 1). Pozo-Bayón et al. (2007) utilized P&T to evaluate the aroma profile of sponge cake, of which 90 compounds were isolated. P&T was capable of identifying 2,3-butanedione (diacetyl), acetoin, 2-ethyl-5-methyl-pyrazine, and δ-decalactone, not detected in SAFE extracts. The aroma of Altumura bread was also successfully characterized using P&T where 89 volatile compounds were identified in the crust, and 78 in the crumb (Bianchi et al. 2008). Purging time is an important parameter in the optimum operation of P&T. Studies in liquid matrices have shown that increasing purging times can actually decrease the rate of compound recovery (Campillo et al. 2004; Salemi et al. 2006). When equilibrium has been reached between sample, headspace, and sorbent material, the sorbent material reaches its full capacity and continuation of purging gas after this point can result in the loss of volatiles.

As seen in Table 1, Pozo-Bayón et al. (2007) utilized a range of different purging times and found 14 hours to be the most effective in extracting volatile compounds from a sponge cake. Similarly, long purging times were effective in studying the interaction of amylose with aroma compounds in a sponge cake (Pozo-Bayón et al. 2008). However, Bianchi et al. (2008) applied a purging time of 15 minutes and retrieved an ample profile of compounds from Altumura bread, comparable to that of Pozo-Bayón et al. (2007).

Complications with this technique can include (i) contamination of the sorbent material from samples (Schmidt 2003), (ii) moisture control, (iii) the catalytic activity occurring on the adsorbent, which can lead to the generation of artifacts compounds (Pillonel et al. 2002), and similarly to SAFE, the length of time needed preform the technique.

Thermal desorption

Similar to the development of P&T, Thermal Desorption (TD) was designed for the analysis of air borne volatiles (Wauters et al. 1979). However, TD is now also widely used to extract aroma compounds from food. The sample is usually incubated and the volatiles are purged dynamically to pre-packed absorbent tubes (usually containing Tenax, or other absorbents such as charcoal or silica gel). The tubes are heated and the volatiles are directly injected into the GC, or further concentrated prior to transfer to the GC. Enhanced sensitivity and efficiency of reusable adsorbent tubes are a significant benefit, but the main appeal is the large adsorption capacity of the tubes (Madruga et al. 2009; Ramírez et al. 2010). This technique has been successful in extracting esters from cookies (Heiderich and Reineccius 2001), characterizing crackers supplemented with barley (O'Shea, Kilcawley, and Gallagher, 2017), as well as differentiating fresh and rancid oat cakes by their volatile profile (Cognat et al. 2012). The main disadvantage associated with TD is moisture control (Pillonel et al. 2002), which may explain the lack of studies utilizing this technique. However, it may be suitable for low moisture biscuit and cookie products, flours etc.

Headspace solid-phase microextraction

Solid-phase microextraction (SPME) is widely utilized for the analysis of volatiles in foods (Cuevas-Glory et al. 2007; Frank, Owen, and Patterson 2004; Ruiz et al. 1998), mainly because it is highly automatable with good reproducibility. The working principle of SPME involves a fused silica fiber that is coated with a stationary phase. The phase can be composed of multiple materials of different polarity to assist in extraction of a wide range of compounds or of single phases for targeted extraction of specific chemical classes, which is accomplished based on polarity, volatility, or molecular weight. The most common types of fibers utilized in literature are comprised of a multi-phase, consisting of a molecular sieve Carboxen (CAR), polar divinylbenzene (DVB), non-polar polydimethylsiloxane (PDMS), or a single phase polyacrylate (PA), which targets very polar analytes. The main application of SPME is in head-space (HS) analysis, where the fiber is exposed to the HS above the sample in a sealed container/vial. Consequently, the volatiles are adsorbed or absorbed onto the fiber through gentle agitation (Kataoka, Lord, and Pawliszyn 2000).

HS-SPME is the most popular technique for volatile extraction of foods, especially in baked cereal analysis (see Table 1). As well as being automatable, HS-SPME is an attractive extraction technique due to the simplicity of sample preparation, solvent free, relatively low cost, and can be targeted towards a wide range of chemical classes (Afoakwa et al. 2009). Rega et al. (2009) evaluated the efficacy of three fibers (50/30 µm DVB/CAR/PDMS, 75 µm CAR/PDMS and 100 µm PDMS) to obtain a representative profile for sponge cake and found that the 50/30 µm DVB/CAR/PDMS

extracted the largest quantity of volatile compounds (See Table 2) and the 75 µm CAR/PDMS was capable of isolating high boiling point compounds. It is essential that the appropriate parameters; extraction time, extraction temperature, suitable fiber for compounds of interest, and sample size, are taken into account to ensure optimum results are obtained in SPME analysis (Kataoka et al. 2000).

HS-SPME has been widely utilized for baked cereal products (Cognat et al. 2012; Giarnetti et al. 2015; Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Pasqualone et al. 2014; 2015; Petisca et al. 2013; Poinot et al. 2007; Raffo et al. 2015; Rega et al. 2009; Sabovics et al. 2014; Ying et al. 2012). Poinot et al. (2007) trialed 27 HS-SPME conditions varying in extraction time, extraction temperature and SPME fiber, to optimize the extraction of volatile compounds most representative of bread odor. By permitting a panel of trained judges to compare the odor qualities of collected HS-SPME volatile extracts, the authors were able to conclude that an extraction time of 30 and 60 minutes at 35 °C, using either 50/30 µm DVD/CAR/PDMS or a 75 µm CAR/PDMS fiber, can yield a volatile profile representative of bread odor. Raffo et al. (2015) found an extraction time of 60 minutes at 50 °C (under agitation) with a DVD/CAR/PDMS fiber beneficial for providing a complete volatile profile of wheat bread. Through preliminary work, Matsakidou, Blekas, and Paraskevopoulou (2010) also identified a 60 minute extraction time at 60 °C favorable for the recovery of volatiles representative of cake odor. It is likely that the extensive extraction time and relatively higher extraction temperature contributed to the wide range of volatile compounds identified (92 compounds). Shortbread cookies were examined with a 50/30 µm DVD/CAR/PDMS fiber for 15 minutes at 35 °C, enabling the recovery and identification of 24 volatile compounds (Giarnetti et al 2015). This result seems rather low compared to Mohsen et al. (2009) who were able to identify 42 compounds in cookies using the SDE technique. Pasqualone et al. (2014) utilized a 75 µm CAR/PDMS fiber for the extraction of compounds from biscuits (enriched with grape marc extract) at 40 °C for 50 minutes, and yielded 60 compounds from a wide range of chemical classes; alcohols, aldehydes, ketones, esters, furans etc. The authors employed the same parameters to analyze biscuits enriched with purple wheat, yielding a similar result of 56 compounds (Pasqualone et al. 2015). However, the authors did consider that this fiber was more sensitive to compounds arising from LO, meaning, perhaps the profile depicted by these extraction conditions, was not a true representative of the sample.

On-line extraction of volatile compounds during the baking of sponge cake has been accomplished with SPME (Maire et al. 2013; Rega et al. 2009). By assembling a glass inlet hood from the oven to a refrigerated extraction chamber, volatile compounds generated during baking were captured at different stages throughout the baking process. Utilising this technique, Rega et al. (2009) monitored the development of compounds associated with LO, and the MR, at different time points. By employing the same technique, Maire et al. (2013) demonstrated how varying the flow rate of vapors from the chamber during baking impacted on the extraction of very volatile and semi-volatile compounds. A flow rate of 7.5 L min^{-1} at 40 °C enabled the extraction of a higher volume of compounds and was particularly beneficial in extracting semi volatiles such as pyrans and furans, however, 1 L min^{-1} at 10 °C yielded the extraction of very volatile compounds.

The major downside to SPME is the limited capacity of the fiber. This leads to competition on the fiber and results in the compounds with a higher affinity for the fiber phase displacing more volatile compounds. Fragility of the SPME fiber and the possible carryover of compounds are also potential issues associated with SPME as an extraction technique (Prosen and Zupančič-Kralj 1999).

Potent aroma volatile compounds in baked confectionary

As baked confectionary products exhibit similar formulations and baking procedures, their qualitative volatile profiles can be similar. However, the ratio of individual volatiles will vary significantly, thus impacting on consumer's perceptions (Table 3). The following covers the key volatile classes associated with baked confectionary products.

Aldehydes

On consumption of baked confectionary products, the perception of "sweet" is un-doubtfully one of the initial attributes perceived during mastication, inherently due to the volume of nonvolatile sucrose present in product formula. However, retronasal olfaction perception of 'sweet' can also result from specific aldehydes, such as benzaldehyde and phenylacetaldehyde, which are associated with "almond", "cherry", "honey", and "floral" notes in biscuit, cookies and cakes (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Mohsen et al. 2009; Pasqualone et al. 2014; 2015; Pozo-Bayón et al. 2007; Rega et al. 2009). Egg yolk provides an abundance of amino acids and when subject to the high temperatures of baking, Strecker degradation occurs, resulting in aldehyde formation. Both benzaldehyde and phenylacetaldehyde are products of Strecker degradation of the amino acid phenylalanine (Chu and Yaylayan 2008). 2-Methylpropanal, 3-methylbutanal, and 2-methylbutanal are also Strecker aldehydes considered important to the aroma of baked goods and derive from valine, leucine, and isoleucine, respectively. 2-Methylpropanal has been described as 'sweet', 'mint', and 'floral' by gas chromatography-olfactory (GC-O) evaluation of cakes (Pozo-Bayón et al. 2007; Rega et al, 2009; Maire et al. 2013), whereas 3-methylbutanal and 2-methylbutanal yield a more 'chocolate', "malty" aroma in baked confectionary, with concentrations particularly high in the crust of cakes (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Pozo-Bayón et al. 2007). "Fatty" and "fruity" odors in cake and biscuits derive from aliphatic aldehydes such as octanal, nonanal and decanal (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010;

Table 2. Comparison of different SPME fibers utilized in the volatile extraction of sponge cake (Rega et al. 2009).

Compound	CAR/ PDMS	PDMS	DVB/ CAR/ PDMS
2-Methylpropanal	x		
2-Methylbutanal	x		x
3-Methylbutanal	x	x	x
2-Pentanone	x		x
2,3-Pentanone	x	x	x
Hexanal	x		x
Heptanal	x		x
2-Pentylfuran	x		x
Pentanol	x		x
2-Methylpyrazine	x		x
Octanal	x	x	x
1-Hydroxy-2-propanone	x		x
2,5-Dimethylpyrazine	x	x	x
2,6-Dimethylpyrazine			x
2,3-Dimethylpyrazine	x		x
Nonanal	x		x
Trimethylpyrazine	x	x	x
(E)-2-octenal	x		x
1-octen-3-ol	x		x
Acetic Acid	x	x	x
Furfural	x		x
Decanal	x		x
Benzaldehyde	x		x
(E)-2-nonenal			x
Octanol	x		x
Undecanal	x		x
Acetylpyrazine	x		x
Phenylacetaldehyde	x		x
Butyric Acid	x		x
Furfuryl alcohol	x		x
Nonanol	x		x
Dodecanal	x		x
2-Undecanal	x	x	x
(E,Z)-2,4-Decadienal	x		x
(E,E)-2,4-Decadienal	x		x
Hexanoic acid	x		x
Dimethylsulfone	x		x
2-Acetylpyrrole	x		x
Maltol			x
Pentadecane-2-one			x
Furaneol	x	x	x
Octanoic Acid	x		x
Tetradecanol		x	x
Nonanoic Acid	x		x
2,3-Dihydro-3,5-dihydroxy-6-methyl- 4(H)-pyran-4-one	x	x	x
5-Hydroxymethylfurfural	x	x	x

Mohsen et al. 2009; Pasqualone et al. 2014; 2015; Pozo-Bayón et al. 2007; Rega et al. 2009), whose presence is as of result of the auto-oxidation of linoleic or oleic acid (Fullana, Carbonell-Barrachina, and Sidhu, 2004; Whitfield and Mottram 1992). Similarly, hexanal, heptanal, and 2,4-decadienal, markers of auto-oxidation of linoleic acid (Fujisaki, Endo, and Fujimoto 2002), have been reported in bakery products as imparting a "fruity", "herbal", "fresh cut grass" aroma (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Mohsen et al. 2009; Pasqualone et al. 2014; 2015; Pozo-Bayón et al. 2007; Rega et al. 2009). Methional has been identified as a key contributor to the "roasty" smell of baguettes (Zehentbauer and Grosch 1998a), and is generated from the Strecker degradation of the amino acid methionine (Escudero et al. 2000). Methional contributes a "dusty", "potato-like" odor and is perceived at very low levels in cake products (Maire et al. 2013; Pozo-Bayón et al. 2007; Rega et al. 2009).

Alcohols

Quite a number of alcohols have been identified in cake and biscuit/cookie products (Table 3). As mentioned, LO of the fat promotes the generation of alcohols through degradation of unsaturated fatty acids, particularly polyunsaturated fatty acids due to the presence of multiple double bonds. Depending on the fatty acid, and the point of cleavage, various alcohols of different odor qualities can be produced. Alcohols positively associated with baked confectionary aroma include fatty 2-ethylhexanol, 1-octanol, 1-nonanol, and 1-decanol, identified as having odor qualities described as "orange", "rose", and "sweet" (Maire et al. 2013; Mohsen et al. 2009; Pasqualone et al. 2014; 2015; Pozo-Bayón et al. 2007; Rega et al. 2009). Other odor descriptions include "cauliflower", "cardboard", "mushroom/fungal", and are associated with alcohols; 1-pentanol, 1-hexanol, and 1-octen-3-ol, respectively (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Pasqualone et al. 2014; 2015; Pozo-Bayón et al. 2007; Rega et al. 2009). Linoleic acid is prone to oxidation and thus yields 1-hexanol and 1-octen-3-ol (Paraskevopoulou, Chrysanthou, and Koutidou 2012). Although these compounds may be perceived as unpleasant at high concentrations, in relatively low concentrations they add to the overall dynamic of baked and cereal products, with 1-octen-3-ol identified as a key compound in oat flakes (Klensporf and Jeleń 2008).

Flour is also identified as a contributor to the alcohol profile of baked confectionary (Maire et al. 2013). The process of milling induces the release of free fatty acids and propagates LO reactions, as well as microbial degradation to produce alcohols (Hansen and Hansen 1994). Wheat flour starch has shown to have high levels of 2-ethylhexanol, a degradation product of LO (Sayaslan et al. 2000). This corresponds to Pozo-Bayón et al. (2007) and Maire et al. (2013) identifying this compound in the dough of sponge cakes, indicating this compound originates from the raw material, but formation is potentially promoted during baking preparation.

Ketones

Ketones are generally associated with favorable aromas. The MR and caramelization can contribute some of the most characteristic volatile compounds associated with bakery products. The decomposition of sugar results in diketones such as 2,3-butanedione (diacetyl) and 2,3-pentadione, responsible for "buttery", "caramel", and "butterscotch" notes in sweetened baked goods (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Mohsen et al. 2009; Pasqualone et al. 2014; 2015; Pozo-Bayón et al, 2007). As previously mentioned the methyl ketones, 2-butanone, 2-heptanone, 2-nonanone, and 2-undecanone have been identified in cookies (Giarnetti et al. 2015) and are associated with "buttery" and "sweet" attributes. These compounds are generated from β-keto acids in milk fat when exposed to heating (Wong and Patton 1962), and contribute to the aroma of butter (Mallia et al. 2008).

Table 3. Volatile compounds identified in baked confectionary products.

Compound	Odour description	Product	Reference
Alcohols			
Ethanol		Biscuit/Cookie	Pasqualone et al. 2014 Pasqualone et al. 2015
Propanol		Biscuit/Cookie	Pasqualone et al. 2014
Butanol		Cake, Biscuit/Cookie	Pasqualone et al. 2014 Pozo-Bayón et al. 2007
1-Pentanol	Foot, cauliflower, pungent, fusel oil,	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
1-Hexanol	Cardboard, solvent, potatoes, fruity, sweet, green	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007
Heptanol	Musty, leafy, violet, herbal, green, sweet, fresh, woody	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
2-Ethylhexanol	Citrus, fresh, floral, oily	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pozo-Bayón et al. 2007
4-Hexen-1-ol		Biscuit/Cookie	Pasqualone et al. 2015
2-Octanol		Cake	Pozo-Bayón et al. 2007
2-Butoxyethanol		Cake	Pozo-Bayón et al. 2007
1-Methoxy-2-propanol		Cake	Pozo-Bayón et al. 2007
1-Octen-3-ol	Mushroom, musty,fungal, earthy	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
2,6-Dimethyl-2,7-octadien-1,6-diol		Cake	Matsakidou et al. 2010
1-(2-Methoxypropoxy)-2-propanol		Cake	Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007
1-Octanol	Waxy, green, orange, aldehydic, fatty, rose	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
1-Nonanol	Fresh, clean, fatty, floral, rose, orange, dusty, wet,	Cake, Biscuit/Cookie	Maire et al. 2013 Mohsen et al. 2009 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Decanol	Floral, fatty, orange, sweet, clean, watery	Cake	Maire et al. 2013
Dodecanol	Earthy, soapy, waxy, fatty, honey, coconut	Cake	Maire et al. 2013
Octadecanol		Cake	Maire et al. 2013
1-Penten-3-ol		Cake	Matsakidou et al. 2010
α-Terpineol		Cake	Pozo-Bayón et al. 2007
Borneol		Cake	Pozo-Bayón et al. 2007
1-(2-butoxyethoxy)ethanol		Cake	Pozo-Bayón et al. 2007
Benzyl alcohol		Cake, Biscuit/Cookie	Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007
2-Phenylethanol		Cake, Biscuit/Cookie	Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007
Hexadecanol	Waxy, floral	Cake	Maire et al. 2013 Pozo-Bayón et al. 2007
Tetradecanol		Cake	Rega et al. 2009
2-Methylcyclopentyl alcohol		Biscuit/Cookie	Pasqualone et al. 2014 Pasqualone et al. 2015
Aldehydes			
Acetaldehyde	Pungent, fresh, aldehydic, refreshing, green	Cake	Maire et al. 2013 Matsakidou et al. 2010

(*continued*)

Table 3. Continued.

Compound	Odour description	Product	Reference
2-Methylpropanal	Fresh, sweet, mint, floral	Cake, Biscuit/Cookie	Maire et al. 2013 Mohsen et al. 2009 Pozo-Bayón et al. 2007 Rega et al. 2009
2-Methylbutanal	Musty, cocoa, coffee, nutty,malty	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Mohsen et al. 2009 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007
3-Methylbutanal	Chocolate, ethereal, aldehydic, peach, fatty, malty	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Mohsen et al. 2009 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Pentanal		Biscuit/Cookie	Pasqualone et al. 2014 Pasqualone et al. 2015
2-Pentenal		Biscuit/Cookie	Mohsen et al. 2009
Hexanal	Floral, fruity, herbal, cut grass, green, sweaty	Cake, Biscuit/Cookie	Giarnetti et al. 2015 Maire et al. 2013 Matsakidou et al. 2010 Mohsen et al. 2009 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Methional	Musty, tomato, potato,earthy, vegetable, creamy	Cake	Maire et al. 2013 Pozo-Bayón et al. 2007 Rega et al. 2009
(*E*)-2-Hexenal		Biscuit/Cookie	Mohsen et al. 2009, Pasqualone et al. 2014 Pasqualone et al. 2015
3-Hexenal		Biscuit/Cookie	Mohsen et al. 2009
Heptanal	Fresh, green, sweet, herbal	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
(E)-2-Heptenal		Cake, Biscuit/Cookie	Maire et al. 2013 Pasqualone et al. 2014 Pasqualone et al. 2015
(Z)-4-Heptenal		Cake, Biscuit/Cookie	Matsakidou et al. 2010 Mohsen et al. 2009
Octanal	Floral, citrus, fruit, orange peel	Cake, Biscuit/Cookie	Giarnetti et al. 2015 Maire et al. 2013 Matsakidou et al. 2010 Mohsen et al. 2009 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
(*E*)-2-Octenal	Fried, Fatty, Unpleasant	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2014 Pozo-Bayón et al. 2007 Rega et al. 2009
Benzaldehyde	Sweet, bitter, almond, sharp, cherry	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Mohsen et al. 2009 Pasqualone et al,2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Phenylacetaldehyde	Rose, honey, floral, flowers, sweet, cocoa	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Mohsen et al. 2009 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Nonanal	Aldehydic, waxy, citrus, orange, green, peel	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010

(*continued*)

Table 3. Continued.

Compound	Odour description	Product	Reference
			Mohsen et al. 2009
			Pasqualone et al. 2014
			Pasqualone et al. 2015
			Pozo-Bayón et al. 2007
			Rega et al. 2009
2-Nonenal	Vegetable, solvent, floral, musty, cucumber, green	Cake, Biscuit/Cookie	Maire et al. 2013
			Matsakidou et al. 2010
			Pasqualone et al. 2015
			Rega et al. 2009
(*E*,*E*)-2,4-Heptadienal	Fatty, green, oily, aldehydic, cake, cinnamon	Cake, Biscuit/Cookie	Maire et al. 2013
			Mohsen et al. 2009
			Pasqualone et al. 2014
			Pasqualone et al. 2015
Decanal	Floral, fruity, sweet, waxy, orange, peel, citrus	Cake	Maire et al. 2013
			Matsakidou et al. 2010
			Pozo-Bayón et al. 2007
			Rega et al. 2009
(*E*)-2-Decenal	Waxy, fatty, earthy, coriander, green, mushroom	Biscuit/Cookie	Giarnetti et al. 2015
			Maire et al. 2013
			Pasqualone et al. 2015
(*E*,*E*)-2,4-Decadienal	Rice, cooked, baked, fried potato, fatty, pumpkin nut, meat	Cake, Biscuit/Cookie	Maire et al. 2013
			Matsakidou et al. 2010
			Mohsen et al. 2009
			Pasqualone et al. 2014
			Pasqualone et al. 2015
			Pozo-Bayón et al. 2007
			Rega et al. 2009
(E,Z)-2,4-Decadienal	Fried oil, cooked, fatty, geranium, green	Cake	Maire et al. 2013
			Pozo-Bayón et al. 2007
			Rega et al. 2009
2,4-Nonadienal		Biscuit/Cookie	Pasqualone et al. 2015
(*E*,*E*)-2,4-Nonadienal		Biscuit/Cookie	Mohsen et al. 2009
			Pasqualone et al. 2014
2-Dodecanal	Vegetable, floral, fatty, clean	Cake, Biscuit/Cookie	Maire et al. 2013
			Pasqualone et al. 2014
			Pasqualone et al. 2015
			Rega et al. 2009
2-Undecanal	Floral, bud, soapy, citrus, green, fatty, fresh laundry	Cake	Maire et al. 2013
			Rega et al. 2009
Methylbenzaldehyde		Cake	Pozo-Bayón et al. 2007
Tridecanal	Fresh, clean, soapy, citrus, petal, waxy, grapefruit peep	Cake	Maire et al. 2013
Octadecanal	Oily	Cake	Maire et al. 2013
Vanillin	Sweet, vanilla, creamy, chocolate	Cake	Maire et al. 2013
Pyrazines			
Pyrazine		Cake, Biscuit/Cookie	Matsakidou et al. 2010
			Mohsen et al. 2009
Methylpyrazine		Cake, Biscuit/Cookie	Giarnetti et al. 2015
			Matsakidou et al. 2010
			Mohsen et al2009
			Pasqualone et al. 2014
			Pasqualone et al. 2015
			Pozo-Bayón et al. 2007
			Rega et al. 2009
2,5-Dimethylpyrazine	Solvent, hospital, perfumed rice, cake crust	Cake, Biscuit/Cookie	Giarnetti et al. 2015
			Matsakidou et al. 2010
			Pozo-Bayón et al. 2007
			Rega et al. 2009
2,6-Dimethylpyrazine	Cake, roasted, bread crust, rice, walnut, praline	Cake	Matsakidou et al. 2010
			Pozo-Bayón et al. 2007
			Rega et al. 2009
Ethylpyrazine		Cake, Biscuit/Cookie	Matsakidou et al. 2010
			Pasqualone et al. 2014
			Pozo-Bayón et al. 2007
2,3-Dimethylpyrazine	Earthy, potatoes, green pea, perfumed rice, cake, crust, nutty, peanut butter, walnut, caramel, leather	Cake	Maire et al. 2013
			Matsakidou et al. 2010
			Pozo-Bayón et al. 2007
			Rega et al. 2009
2-Ethyl-6-methylpyrazine	Roasted, burnt	Cake	Matsakidou et al. 2010
			Pozo-Bayón et al. 2007

(*continued*)

Table 3. Continued.

Compound	Odour description	Product	Reference
			Rega et al. 2009
2-Ethyl-5-methylpyrazine		Cake, Biscuit/Cookie	Matsakidou et al. 2010 Rega et al. 2009
Trimethylpyrazine	Herbal, earthy, potatoes, roasted, cake	Cake	Matsakidou et al. 2010 Pozo-Bayón et al. 2007 Rega et al. 2009
Vinylpyrazine		Cake	Pozo-Bayón et al. 2007
3-Ethyl-2,5-dimethylpyrazine		Cake	Matsakidou et al. 2010 Pozo-Bayón et al. 2007 Mohsen et al. 2009
2-Ethyl-3,5-dimethylpyrazine		Cake, Biscuit/Cookie	Matsakidou et al. 2010 Pozo-Bayón et al. 2007
2-Methyl-6-vinylpyrazine	Vegetables, potato	Cake	Pozo-Bayón et al. 2007
2-Methyl-5-vinylpyrazine		Cake	Pozo-Bayón et al. 2007
3,5-Diethyl-2-methylpyrazine		Cake	Pozo-Bayón et al. 2007
Dimethyl-2-vinylpyrazine (isomer)	Pungent, herbal, potatoes	Cake	Pozo-Bayón et al. 2007
Acetylpyrazine	Hazelnut, praline, cake	Cake	Pozo-Bayón et al. 2007 Rega et al. 2009
2-Methyl-5-(2-propenyl)-pyrazine		Cake	Matsakidou et al. 2010
2-Acetyl-5-methylpyrazine		Cake	Pozo-Bayón et al. 2007
2-Acetyl-6-methylpyrazine		Cake	Pozo-Bayón et al. 2007
Benzopyrazine		Cake	Pozo-Bayón et al. 2007
Ketones			
Acetone		Biscuit/Cookie	Giarnetti et al. 2015
2,3-Butanedione (Diacetyl)	Butter, fruity, caramel, butterscotch	Cake, Biscuit/Cookie	Giarnetti et al. 2015 Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2014 Pozo-Bayón et al. 2007
2-Butanone		Biscuit/Cookie	Giarnetti et al. 2015 Mohsen et al. 2009 Pasqualone et al. 2015
2-Pentanone		Cake, Biscuit/Cookie	Mohsen et al. 2009 Pasqualone et al. 2015 Rega et al. 2009
2,3-Pentanedione	Pungent, sweet, butter, creamy, caramel, nutty	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pozo-Bayón et al. 2007 Rega et al. 2009
Hydroxyacetone (1-Hydroxy-2-propanone)		Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Acetoin (3-Hydroxy-2-butanone)		Cake, Biscit/Cookie	Giarnetti et al. 2015 Pozo-Bayón et al. 2007
2-Heptanone		Cake, Biscuit/Cookie	Giarnetti et al. 2015 Matsakidou et al. 2010 Mohsen et al. 2009 Pasqualone et al. 2015 Pozo-Bayón et al. 2007
1-Octen-3-one	Herbal, mushroom, earthy, musty	Cake	Maire et al. 2013
2-Octanone		Cake	Matsakidou et al. 2010 Rega et al. 2009
3-Octen-2-one		Cake	Matsakidou et al. 2010
2-Nonanone		Cake, Biscuit/Cookie	Giarnetti et al. 2015 Matsakidou et al. 2010 Pozo-Bayón et al. 2007
2-Decanone		Cake	Maire et al. 2013 Matsakidou et al. 2010 Pozo-Bayón et al. 2007
2,3-Methyloctanone		Cake	Matsakidou et al. 2010 Rega et al,2009
2-Pentadecanone		Cake	Rega et al. 2009
2-Undecanone		Cake, Biscuit/Cookie	Giarnetti et al. 2015 Matsakidou et al. 2010
2-Dodecanone		Cake	Matsakidou et al. 2010
6-Methyl-5-hepten-2-one		Cake	Matsakidou et al. 2010 Pozo-Bayón et al. 2007
(*E*,*E*)-3,5-Octadiene-2-one		Cake, Biscuit/Cookie	Pozo-Bayón et al. 2007 Mohsen et al.,2009 Pasqualone et al.,2015

(*continued*)

Table 3. Continued.

Compound	Odour description	Product	Reference
Acetophenone		Cake	Pozo-Bayón et al. 2007
Acids			
Acetic acid	Unpleasant, earthy, sharp, pungent, sour, vinegar	Cake, Biscuit/Cookie	Giarnetti et al. 2015 Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Formic acid	Pungent, vinegar	Cake	Maire et al. 2013
Propanoic acid		Biscuit/Cookie	Pasqualone et al. 2015
Butanoic acid	Sweat, fish, unpleasant	Cake, Biscuit/Cookie	Mohsen et al. 2009 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Pentanoic acid		Cake	Pozo-Bayón et al. 2007
Hexanoic acid	Mild, sour, fatty, sweat, cheese, rancid	Cake, Biscuit/Cookie	Giarnetti et al. 2015 Maire et al. 2013 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Heptanoic acid		Cake	Pozo-Bayón et al. 2007
Octanoic acid	Fatty, acid, sour	Cake, Biscuit/Cookie	Maire et al. 2013 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
2-Hexenoic acid		Biscuit/Cookie	Maire et al. 2013 Pasqualone et al. 2015
2,4-Hexadienoic acid		Biscuit/Cookie	Giarnetti et al. 2015 Pasqualone et al. 2014 Pasqualone et al. 2015
Nonanoic acid	Waxy, dirty, cheese, cultured dairy	Cake, Biscuit/Cookie	Maire et al. 2013 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Decanoic acid	Unpleasant, rancid, sour, fatty, citrus	Cake, Biscuit/Cookie	Maire et al. 2013 Mohsen et al. 2009 Pasqualone et al. 2015 Pozo-Bayón et al. 2007
Dodecanoic acid	Fatty, coconut, bay oil	Cake	Maire et al. 2013 Pozo-Bayón et al. 2007
Benzoic acid	Faint, balsm	Cake	Maire et al. 2013 Pozo-Bayón et al. 2007
Dodecanoic acid	Fatty, coconut, bay oil	Cake	Maire et al. 2013 Pozo-Bayón et al. 2007
Hexadecanoic acid	Slightly fatty, waxy	Cake	Maire et al. 2013
Furans			
2-Methylfuran	Sweet, pungent, caramel, burnt	Biscuit/Cookie	Pasqualone et al. 2014
2-Pentylfuran	Earthy, vegetable, beany, metallic	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Dihydro-2-methyl-3(2H)-furanone	Roasted, biscuit, hazelnut, nutty	Cake, Biscuit/Cookie	Mohsen et al. 2009 Pozo-Bayón et al. 2007
Furaneol (Strawberry Furanone)	Caramel-like, spice, cake, sweet, cotton candy, strawberry, sweet, fruity	Cake, Biscuit/Cookie	Maire et al. 2013 Matsakidou et al. 2010 Mohsen et al. 2009 Rega et al. 2009
2-Furanmethanol	Sweet caramel, burnt	Cake, Biscuit/Cookie	Matsakidou et al. 2010 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009
Furfural	Earthy, potatoes, green pea, parfumed rice, cake, crust, sweet, woody, almond, fragrant, bready	Cake, Biscuit/Cookie	Giarnetti et al. 2015 Maire et al. 2013 Matsakidou et al. 2010 Mohsen et al. 2009 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007 Rega et al. 2009

(*continued*)

Table 3. Continued.

Compound	Odour description	Product	Reference
2-Acetylfuran	Sweet, balsam, almond, cocoa, caramel, coffee	Cake, Biscuit/Cookie	Maire et al. 2013 Pasqualone et al. 2014 Pozo-Bayón et al. 2007
5-Hydroxymethylfurfural (HMF)	Fatty, musty, waxy, caramel	Cake, Biscuit/Cookie	Maire et al. 2013 Pasqualone et al. 2015 Rega et al. 2009
5-Methylfurfural	Biscuit, chocolate, roasted, cake, spice, caramel, maple	Cake, Biscuit/Cookie	Maire et al,2013 Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007
2-Ethyl-5-methylfuran		Biscuit/Cookie	Mohsen et al. 2009
5H-furan-2-one		Cake, Biscuit/Cookie	Giarnetti et al,2015 Matsakidou et al. 2010 Pozo-Bayón et al. 2007
Alkanes			
Octane	Gasoline	Cake	Maire et al. 2013
Decane		Cake	Matsakidou et al. 2010
Dodecane	Alkane	Cake	Maire et al. 2013
Hexadecane		Cake	Maire et al. 2013
Tridecane		Cake	Matsakidou et al. 2010
Tetracosane		Cake	Maire et al. 2013
Tetradecane	Mild Waxy	Cake	Maire et al. 2013 Matsakidou et al. 2010
Pentadecane	Waxy	Cake	Maire et al. 2013
Esters			
Ethyl Acetate		Cake, Biscuit/Cookie	Matsakidou et al. 2010 Pasqualone et al. 2014 Pasqualone et al. 2015
Butyl Acetate		Cake	Matsakidou et al. 2010
Ethyl Butanoate		Cake	Pozo-Bayón et al. 2007
Ethyl Hexanoate	Vegetable, floral, fruity	Cake	Pozo-Bayón et al. 2007
2-Ethylhexanoic acid		Cake	Pozo-Bayón et al. 2007
Methyl Benzoate		Biscuit/Cookie	Pasqualone et al. 2015
Ethyl Benzoate		Biscuit/Cookie	Pasqualone et al. 2015
Methyl Decanoate		Biscuit/Cookie	Pasqualone et al. 2015
Methyl Dodecanoate		Biscuit/Cookie	Mohsen et al. 2009
Ethyl Decanoate		Biscuit/Cookie	Mohsen et al. 2009
Isopropyl Tetradecanoate		Cake	Pozo-Bayón et al. 2007
Ethyl Octanoate	Fruity, wine, waxy, sweet, apricot, banana, brandy	Cake	Maire et al. 2013
Lactones			
γ-Butyrolactone		Cake, Biscuit/Cookie	Giarnetti et al. 2015 Mohsen et al. 2009 Pozo-Bayón et al. 2007
γ-Hexalactone		Cake	Pozo-Bayón et al. 2007
γ-Octalactone		Cake	Pozo-Bayón et al. 2007
γ-Nonalactone		Cake	Pozo-Bayón et al. 2007
γ-Decalactone		Cake	Pozo-Bayón et al. 2007
δ-Decalactone		Cake, Biscuit/Cookie	Mohsen et al2009. Pozo-Bayón et al. 2007
Sulfur Compounds			
Dimethyl Disulphide	Sulfurous, vegetable, cabbage, onion	Cake	Maire et al. 2013 Pozo-Bayón et al. 2007
Dimethyl Trisulfide	Solvent, gas, wastewater, pungent	Cake	Pozo-Bayón et al. 2007
Dimethyl Sulfone		Cake	Pozo-Bayón et al. 2007
2-Acetyl-2-thiazoline		Cake	Pozo-Bayón et al. 2007 Rega et al. 2009
2-Acetylthiazole	Hazelnut, popcorn	Cake	Matsakidou et al. 2010 Pozo-Bayón et al. 2007
Benzothiazole		Biscuit/Cookie	Pasqualone et al. 2014
Aromatic Hydrocarbons			
Toulene		Cake	Maire et al. 2013
Pentylbenzene		Biscuit/Cookie	Pasqualone et al,2014
2-Methyl-propenylbenzene		Biscuit/Cookie	Pasqualone et al. 2014
Hexylbenzene		Biscuit/Cookie	Pasqualone et al. 2014
Octylbenzene		Biscuit/Cookie	Pasqualone et al. 2014
Phenolic Compounds			
Phenol		Cake, Biscuit/Cookie	Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007
2-Methoxyphenol (Guaiacol)		Cake	Pasqualone et al. 2014 Pasqualone et al. 2015 Pozo-Bayón et al. 2007

(*continued*)

Table 3. Continued.

Compound	Odour description	Product	Reference
2-Methoxy-4-vinylphenol		Cake	Pozo-Bayón et al. 2007
Pyrroles			
1-H-Pyrrole		Cake, Biscuit/Cookie	Matsakidou et al. 2010 Mohsen et al2009
2-Acetylpyrrole		Cake	Matsakidou et al. 2010 Mohsen et al2009 Pozo-Bayón et al. 2007, Rega et al. 2009
2-Acetyl-1-pyrroline	Popcorn	Biscuit/Cookie	Mohsen et al. 2009
Terpenes			
Verbenone		Cake	Pozo-Bayón et al. 2007
D-Limonene		Cake, Biscuit/Cookie	Giarnetti et al. 2015 Matsakidou et al,2010 Pasqualone et al. 2014 Pozo-Bayón et al. 2007
Pyran			
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one		Cake, Biscuit/Cookie	Maire et al,2013 Matsakidou et al. 2010 Mohsen et al. 2009 Rega et al. 2009
Maltol	Caramel, sweet, cotton candy, jam, fruity	Cake	Maire et al. 2013 Matsakidou et al. 2010 Rega et al. 2009
Pyridines			
N-acetyl-4(H)-pyridine	Walnut, popcorn	Cake	Matsakidou et al. 2010
Lactams			
N-Methyl-2-pyrrolidine(NMP)		Cake	Pozo-Bayón et al. 2007

Pyrazines

Similar to wheat bread, cake is composed of a crust and a crumb that are distinguishable by the quantitative differences of their volatile profile. The crust of cake is a concentrated source of thermal reactions, and therefore generates a greater quantity of heat derived compounds such as pyrazines; compounds responsible for the "roasted", "caramel", and "nutty" odors in baked confectionary. Pyrazines are formed through the Strecker degradation of α-aminoketones during the high temperatures of baking, with formation being promoted in an alkaline pH (Jousse et al. 2002). A range of pyrazines have been identified in the crust and crumb of cakes (see Table 3), with 2,5-dimethylpyrazine, 2,6-dimethlypyrazine, 2,3-dimethylpyrazine, trimethylpyrazine, and 2-methyl-6-vinylpyrazine having high odor activity and noted to be the main contributors to the characteristic "roasty" and "perfumed rice" aroma of sponge cake (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Pozo-Bayón et al. 2007; Rega et al. 2009). Some pyrazines have high odor thresholds, thus requiring their concentration to be quite high before their "roasty", "nutty" aroma can be perceived in cereal products (Bredie et al. 1998). "Biscuit like" 2-ethyl-5-methylpyrazine has been identified in cookies (Mohsen et al. 2009), as well as odor active 2,5-dimethylpyrazine and trimethylpyrazine (Giarnetti et al. 2015). It appears the abundance of pyrazine compounds is not as prominent in biscuits and cookies, compared to that of cake (Table 3). However, this could be a repercussion of the extraction technique and parameters taken to isolate these compounds (Pasqualone et al. 2015), thus more research is required to understand pyrazine contribution to biscuit/cookie aroma.

Furans

Furan and its derivatives are widespread in foods and beverages, with quantities present depending on heat exposure. These compounds generate interest due to their ability to thrive in low moisture systems, with formation favored in acidic environments (Kroh 1994). The low moisture content of biscuit/cookie structures accelerates caramelization and Maillard reactions, enhancing the concentration of furans (Ameur et al. 2007). Similar to pyrazines, the crusts of cakes reflect higher concentrations of furan compounds compared to the crumb (Matsakidou, Blekas, and Paraskevopoulou 2010; Pozo-Bayón et al. 2007). Furans have low odor thresholds and significantly contribute to the delicate aroma of bakery products. Fresh biscuits have been associated "sweet", "toasted", and "caramel" attributes (Heenan et al. 2009), elucidated by the presence of furfural and HMF. Furanic compounds are described as the most potent compounds in biscuits and cookies, yielding a desirable "bready", "almond", "pungent", and "sweet" aroma (Giarnetti et al. 2015; Mohsen et al. 2009; Pasqualone et al. 2014; 2015). Pyrolysis of hexose and pentose induce the formation of HMF and furfural, respectively (Petisca et al. 2014). Levels of furans have been shown to be significantly higher in fresh cookies compared to those after storage (Mohsen et al. 2009), demonstrating their importance in cookie aroma. Furaneol, 2-pentylfuran, and 2-furanmethanol have been identified in high amounts in the crust and crumb of sponge cakes (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Rega et al. 2009). Furaneol significantly contributes to sweet tasting fruits such as strawberries and pineapples, contributing a sweet taste and "burnt sugar", "caramel" aroma (Chen and Sidisky 2011; Elss et al. 2005; Sanz, Richardson, and Pérez 1995). Lipoxygenase-catalysed oxidation of

linoleic acid can produce 2-pentylfuran which is associated with an "earthy" "beany" aroma (Vara-Ubol, Chambers, and Chambers 2004). Oxidation of flour lipids can also contribute to levels of 2-pentyl furan (Birch, Petersen, and Hansen 2013). "Caramel-like" aroma derives from 2-furanmethanol, a compound associated with products exposed to high temperatures, with significant levels identified in coffee and chocolate (Afoakwa et al. 2009; Nebesny et al. 2007). It is apparent that furan and its derivatives are important to the perceived aroma of baked confectionary products.

Other compounds

Although the above chemical classes may dominate the profile of baked confectionary, many others can impact greatly on the perceived aroma of cakes, biscuits and cookies. Maltol, a pyran compound, is considered important to the aroma of cakes (Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Rega et al. 2009), yielding a "cotton candy" odor at low concentrations. This compound is a well-known product of the MR, with 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one acting as a precursor (Yaylayan and Mandeville 1994). N-Acetyl-4(H)-pyridine and 2-acetylthiazole have been identified in cake crust and associated with a "walnut", "hazelnut", "popcorn" aroma (Matsakidou, Blekas, and Paraskevopoulou 2010), where 2-acetyl-1-pyrroline yields a "popcorn aroma" and has been identified in cookies (Mohsen et al. 2009). This compound is known to give rice its characteristic aroma (Buttery et al. 1983). Ethyl esters of fatty acids, ethyl octanoate, and ethyl hexanoate, have also been identified in cake (Maire et al. 2013; Pozo-Bayón et al. 2007) and offer "sweet", "apricot", "floral", and "fruity" notes.

Baked confectionary in general are associated with having pleasant aroma, however, depending on ingredient preparation, or thermal processes, unfavorable compounds with low odor threshold can form. Although present in low quantities, carboxylic acids can be detected in baked confectionary ranging in a variety of unpleasant odors (Table 3). Hexanoic acid, octanoic acid, and nonanoic acid, auto-oxidation products of their corresponding aldehydes (Paradiso et al. 2008), have been identified in cakes, biscuits, and cookies (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou, Blekas, and Paraskevopoulou 2010; Pasqualone et al. 2014; 2015; Pozo-Bayón et al. 2007; Rega et al. 2009) and yield a "fatty", "rancid", "cheese" aroma, risking deterioration to the sensory properties of these products. LO is the main precursor of off flavors and taints in many foods; therefore it is optimum to manage the cascade of reactions to retain the desirable aroma and flavor of bakery products (Maire et al. 2013).

Relating volatile compounds to sensory data

The aim of sensory analysis is to gain an insight into the way food is perceived by humans using visual, olfactory, taste, touch, and auditory responses. It is beneficial for all those involved in product development to have knowledge and understanding of the types of sensory methodologies available. Application of the most suitable sensory method can aid evaluation of new and reformulated products, and yield insights into product acceptability. Although information on volatiles gives a comprehensive insight into the compounds that may affect aroma and flavor, it can only provide an estimate on how consumers may perceive a product; therefore it is of utmost benefit to use volatile information in conjunction with sensory analysis to obtain a better understanding of the relationship between aroma and sensory perception.

There are many sensory tests available to evaluate a food product, with the most suitable depending on the information required. Considerations such as complexity of the test, cost, resources, and training or commitment from panelists, must be all taken into account when choosing an appropriate sensory test (Lawless and Heymann 2010).

Sensory acceptance testing, through the use of hedonic scales, is a popular choice for consumer research as they are easily understood and panelists do not require in depth training. Hedonic scales normally assess the degree of liking or disliking of sensory attributes such as appearance, odor, taste, aroma, texture, and are popularly utilized to assess food and beverages (O'Sullivan 2016). Hedonic scales have been extensively utilized in many studies to evaluate reformulated baked confectionary (Cavalcante and Silva 2015; Eslava-Zomeño et al. 2016; Giarnetti et al. 2015; Karp et al. 2016; Matsakidou, Blekas, and Paraskevopoulou 2010; Mohsen et al. 2009; Onacik-Gür et al. 2016; Serin and Sayar 2016; Wardy et al. 2018; Zahn et al. 2010). However, this type of sensory method can yield ambiguous information and can be difficult to correlate with volatile information.

Descriptive analysis is the most complete and informative tool for assessing the sensory attributes of food products (Lawless and Heymann 2010). Methodologies under this category include; Flavour Profile Method (Caul 1957), Texture Profile Method (Brandt, Skinner, and Coleman 1963), QDA (Stone et al. 2004), as well as general descriptive analysis. These are extensively utilized for their comprehensive evaluation of food and beverages (Murray, Delahunty, and Baxter 2001). In short, all descriptive analysis techniques involve the same principle steps. Initially, the generation of an agreed list of sensory attributes that best describe the product is developed. This is followed by panelist training; the selected attributes are defined using product references or standards, helping the assessors to distinguish clearly between attributes (O'Sullivan 2016). Subsequently, the panelists are permitted to assess the intensity of each attribute in respect to the product. Training and commitment of panelists is crucial for the success of this technique.

When trying to understand the intricate make-up of flavor, descriptive analysis used in conjunction with volatile analysis can elucidate relationships between aroma compounds and flavor perception. Utilising this strategy, Cognat et al. (2014) identified specific volatiles related to particular off-flavors perceived by panelists when monitoring oat biscuits over time, providing important information regarding product quality throughout shelf-life. Without

complimenting volatile data with sensory analysis, it is impossible to know if the product continues to have approval on the market. The concentrations of volatile compounds that form the aroma fraction of bread are highly susceptible to changes in processes and ingredients, however, combining sensory and chemical information have proven effective in characterizing individual aroma profiles of similar breads (Heenan et al. 2009; Poinot et al. 2007). QDA has also been used to validate volatile information from reformulated biscuits and cookies (Pasqualone et al. 2014; 2015; Giarnetti et al. 2015).

In order to define a true relationship between volatile and sensory data, chemometric methods are often employed. Combining the principle concepts of multivariate statistical techniques, mathematics, and computer science, chemometrics enables important correlations to be realized between sensory attributes and volatile compounds through a simplistic, visual aid (Zielinski et al. 2014). Principal component analysis (PCA) is frequently used and attempts to identify the prominent factors (variables) that best explain the variance in a large data set (Kallithraka et al. 2001). PCA has been utilized to relate volatile compounds in different bread aroma extracts to sensory results (Poinot et al. 2007) as well as relating volatile compounds to color data in biscuits supplemented with grape marc extract (Pasqualone et al. 2014). Partial least square (PLS) analysis another popular technique utilized to make connections between instrumental and sensory data. Depending on the information sought, PLS may be considered superior to PCA as this takes into consideration the correlation between the dependent variable and the independent variables.

GC-O utilizes the human nose as a detection device to aid in the identification of odor active fractions of a chromatograph (Wardencki, Chmiel, and Dymerski 2013). Although compounds may be present in large concentrations, it is dependent on their odor threshold whether they are relevant to the aroma quality of a product. GC-O is a preeminent technique for determining odor thresholds of key volatiles, but has limitations. Sensory perception is often a combination of multiple volatiles rather than individual compounds. Volatiles need to be extracted/concentrated and therefore some compounds may be lost, underestimated or overestimated depending upon procedures used. Extraction methods, SAFE and SPME, have successfully been able to identify the odor active compounds which relate to the traditional aroma of a sponge cake (Matsakidou, Blekas, and Paraskevopoulou 2010; Pozo-Bayón et al. 2007; Rega et al. 2009). GC-O can be time consuming as human assessors require selection and training, with most approaches requiring multiple sessions (Delahunty, Eyres, and Dufour 2006; Zellner et al. 2008). However, on successful of application of this technique, the important volatiles responsible for the characteristic odor in a product can be established.

Conclusions and future work

Characterizing the volatile aroma compounds in baked confectionary provides a basis for improving the quality of reduced fat/reduced sugar formulas. It is evident that the raw materials of baked confectionary have a major impact on flavor perception, and modification of these ingredients can have a significant impact on sensory quality. Although a small percentage of volatiles transfer directly from the raw materials, thermal degradation of components in the formula generates the most potent and characterizing compounds. Aldehydes, alcohols, pyrazines, ketones, and furans are by far the most prominent and potent compounds that appear to influence the sensory appeal of baked confectionary products. LO also appears to be an important contributor to the volatile profile of these products, and therefore reducing fat content or, changing lipid types, is likely to have implications for flavor perception and shelf-life. Further research is required in relation to how the sensory impact of the inclusion or exclusion of the fundamental raw materials influence the volatile profile and sensory character of baked confectionary. This challenge would be best achieved using a chemometric approach to analyze sensory and flavor chemical data. In addition, the application of GC-O to determine the odor activity of key volatile compounds could also be useful in determining their direct impact on sensory perception and how they are influenced by production formulation changes.

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Optimisation of HS-SPME Parameters for the Analysis of Volatile Compounds in Baked Confectionery Products

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Abstract

Optimised extraction methods are required to better understand the impact of volatile compounds on the physical and organoleptic attributes of baked confectionary products (cakes, etc.). This is especially relevant with an increased focus on the reformulation of such products to aid in the reduction of diet-related chronic diseases. Headspace solid-phase microextraction (HS-SPME) has become one of the most widely used extraction techniques for volatile profiling of foods and beverages, mainly because it is very automatable, has a high sample throughput, is solvent-free and multiple fibre phases are available to target a wide range of volatile organic compounds. This study used response surface methodology to optimise HS-SPME parameters for the extraction of volatiles in baked confectionary products. After HS-SPME fibre selection, a central composite design was used to evaluate the effect of incubation time, extraction time and extraction temperature on 18 selected volatile compounds, representative of key volatiles in baked confectionary products, using a sponge cake crumb as the matrix. The most suitable fibre was the divinylbenzene/carboxen/polydimethylsiloxane. The results demonstrated that the final reduced models significantly ($p < 0.0001$) fitted the responses of 18 selected volatile compounds, with R^2 values ranging from 0.8178 to 0.9871. The optimal conditions derived were an incubation time of 5 min, extraction time of 60 min and an extraction temperature of 60 °C. These were subsequently evaluated in three baked confectionary products, highlighting the effectiveness of this approach.

Keywords Baked confectionery products · HS-SPME · Response surface methodology · GC-MS · Aroma

Introduction

Baked confectionery products (cakes, muffins, biscuits, etc.) are consumed across all populations due to their desirable organoleptic properties. However, reformulation of these traditional 'high sugar', 'trans/saturated fat' food commodities has become a priority due to the rising prevalence of chronic diseases, such as obesity and type II diabetes (Richardson et al. 2018; Silow et al. 2018). Reformulation is challenging as sugar and fat significantly contribute to the development of structure, texture and shelf life, as well as playing a key role in creating the desired flavour and aroma. In order to comprehend how aroma is influenced by the raw materials, volatile compounds from the prominent reactions, Maillard reaction (MR), caramelisation (CR) and lipid oxidation (LO), are of interest as they are subject to modulate on reformulation of traditional recipes. Thus, having an optimised method to identify volatile compounds responsible for the desired aroma of baked confectionery products could be useful in relation to the impact of process changes on product quality and assist in the development of higher quality reformulated products (Garvey et al. 2019).

Volatile organic compounds responsible for aroma and flavour perception in baked confectionery products are derived from a range of chemical classes, alcohols, aldehydes, ketones, pyrazines, furans, etc., with over 100 reported (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Pozo-Bayón et al. 2007; Rega et al. 2009). Volatile analysis of cake and cake-like products has been reported utilising different extraction techniques prior to gas chromatography mass spectrometry (GCMS) analysis, such as simultaneous distillation

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extraction (Mohsen et al. 2009), solvent-assisted flavour evaporation (SAFE) (Pozo-Bayón et al. 2007), purge and trap (Pozo-Bayón et al. 2007) and headspace solid-phase microextraction (HS-SPME) (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Rega et al. 2009). HS-SPME is an attractive technique due to the simplicity of sample preparation and the fact that it is solvent-free, rapid, can be highly automated and has a range of single or multiple fibres phases available, varying in polarity and molecular size to assist in efficient extraction of target analytes. The working principle of HS-SPME is based on obtaining equilibrium between the sample matrix and headspace and between the headspace and fibre coating. Factors such as extraction time, extraction temperature, pH and sample concentration can influence the efficiency of the process (Prosen and Zupančič-Kralj 1999). The influence of these factors will vary between sample types due to the changes in sample matrix, and therefore, HS-SPME parameters need to be optimised to achieve the most comprehensive volatile profile possible for baked confectionary products.

In order to achieve precise optimisation, a copious amount of experimental runs may be required in order to assess the combined effects of a range of SPME parameters on volatile response. This can be reduced considerably by employing statistical and mathematical techniques to monitor the effect of these parameters (independent variables) on the volatile response (dependant variable). HS-SPME optimisation has been effectively achieved for various food matrices utilising response surface methodology (RSM) (Chmiel et al. 2017; Ma et al. 2013; Pérez-Palacios et al. 2012). RSM allows not only for the observation of the direct influence of a parameter on volatile response but also the interaction effect of parameters on responses, thus reducing the number of experimental runs required.

Therefore, the objective of this study was to develop an optimised HS-SPME method for the volatile analysis of baked confectionary products by GCMS. Initially, the most appropriate SPME fibre was selected; an RSM approach was used to optimise HS-SPME parameters, using sponge cake crumb as a test sample. The effect of SPME fibre type, incubation time, extraction time and extraction temperature on the extraction of 18 selected volatile compounds (Table 1), widely identified in baked confectionery products (Giarnetti et al. 2015; Matsakidou et al. 2010; Pasqualone et al. 2015; Pozo-Bayón et al. 2007), was explored. The optimised HS-SPME method was subsequently applied to three baked confectionery products (shortbread biscuit, sponge cake and chocolate brownie) to demonstrate its competency in comparison to published studies.

Material and Methods

Sample Preparation

The reference recipe of the sponge cake comprised of 400 g of plain cream flour (Odlums, Ireland), 220 g of caster sugar (Siucra, Nordzucker, Germany), 180 g of free-range egg (local retailer), 180 g of cake margarine (Stork, UK), 140 g of water and 8 g of baking powder (Dr. Oetker, UK). Flour and baking powder were sifted into a bowl followed by the addition of sugar, margarine, eggs and water. The contents were mixed together using a household mixer (Kenwood Mixer, Model KMM710, UK) at minimum speed 1 for 30 s and again at speed 2 for 2 min. The batter was poured into a round cake mould (30.48 cm) and baked at 180 °C for 40 min in a domestic convection oven (Zanussi, Bedfordshire, UK). This process was carried out in triplicate. The cakes were left to fully cool overnight at ambient temperature. The following morning, 1 cm of the outer crust of each cake was removed and the crumbs were broken down with a wooden spoon to form one homogenous bulk crumb. The bulk crumbed cake mixture was frozen at − 20 °C until subsequent analysis.

For the method application part of this study, three different baked confectionery matrixes were chosen—a chocolate brownie, a shortbread biscuit and a sponge cake. The brownie product was prepared as per Richardson et al. (2018). Dark chocolate (85% cocoa, Aldi, Ireland) (175 g) and butter (Kerrygold, Ornua, Ireland) (175 g) were melted together and 250 g of caster sugar was added and hand-stirred for 1 min. Eggs (180 g) were beaten in a separate bowl and added to the mixture. Flour (115 g) was folded in gently and the mixture was stirred by hand until smooth (2 min). The batter was poured into baking trays (16.5 × 24 cm) and batches were baked for 30 min at 180 °C. The shortbread biscuits were prepared by mixing together 200 g of butter and 100 g of sugar until smooth, in a household mixer. Flour (300 g) was gently folded in until incorporated evenly to the sugar/butter mixture. The biscuit dough was compressed and rolled out to 1-cm thickness, and shortbread biscuits were cut out using a cookie cutter (3.81 cm diameter). The shortbread biscuits were baked for 20 min at 160 °C. The sponge cake sample was produced as above. For the application part of the study, the end products were stored in an airtight container at room temperature until subsequent analysis, which took place within 24 h after baking.

HS-SPME Method Development

Fibre Selection

Fibre screening was carried out prior to HS-SPME optimisation. Four HS-SPME fibres, 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 75 μm CAR/PDMS, 85 μm polyacrylate (PA) and 100 μm PDMS, were

Table 1 Volatile compounds chosen for the optimisation of HS-SPME method

Chemical class	Volatile compound	Origin	Odour description*
Aldehyde	Hexanal	LO	Floral, fruity, herbal, cut grass, green
	Heptanal	LO	Fresh, green, sweet, herbal
	Benzaldehyde	MR	Sweet, bitter, almond, cherry
	Phenylacetaldehyde	MR	Rose, honey, floral, sweet, cocoa
	Nonanal	LO	Aldehydic, waxy, citrus, orange, green, peel
	(E,Z)-2,4-Decadienal	LO	Rice, baked, fried potato, fatty
Furan	Dihydro-2-methyl-3(2H)-furanone	MR/CR	Roasted, biscuit, hazelnut, nutty
	2-Furanmethanol	MR/CR	Sweet caramel, burnt
	2-Pentylfuran	MR/LO	Earthy, vegetable, beany, metallic
	Furfural	MR/CR	Sweet, woody, almond, fragrant, bready
Ketone	2,3-Butanedione	MR/CR	Butter, fruity, caramel, butterscotch
	2-Pentanone	LO	Sweet, fruity, woody
Pyrazine	2,5-Dimethylpyrazine	MR	Roast, coffee, peanut, cake crust
	2-Ethyl-5-methyl-pyrazine	MR	Herbal, earthy, potatoes, roasted
Alcohol	1-Hexanol	LO	Cardboard, solvent, potatoes, fruity
	1-Octen-3-ol	LO	Mushroom, musty, fungal, earthy
Lactone	δ-Decalactone	RM	Coconut, fatty, buttery, milky
Terpene	d-Limonene	RM	Fresh, citrus

*Odour qualities taken from www.goodscentcompany.com, origin of compounds from Garvey et al. 2019 and Maire et al. 2013

LO, lipid oxidation; *MR*, Maillard reaction; *CR*, carmelisation; *RM*, raw material

compared for their efficacy of obtaining the most representative volatile profile of baked confectionary products. The HS-SPME fibres were exposed to 3 g of cake crumb (bulk batch produced as above) for a 10-min incubation time and 50-min extraction time at 40 °C for each fibre and analysed in triplicate. Fibres were conditioned according to the manufacturer's instructions prior to use.

Optimisation of HS-SPME Parameters

RSM was employed to optimise the parameters involved in the HS-SPME method for the extraction of volatile compounds from baked confectionary products. Utilising a central composite rotatable design (CCRD, $\alpha = 1.68$), the effect of incubation time (x), extraction time (x_1) and extraction temperature (x_2) on the extraction of volatile aroma compounds from a sponge cake matrix was investigated. The experimental design consisted of a 2^3-factorial design comprised of 20 experimental runs, which included 6 axial points (estimation of curvature) and 6 replicates of the centre point (estimating pure error) (Table 2). Data from individual responses (peak area value of compounds) were inputted into the statistical model and tested for lack of fit (ANOVA) and determination coefficient (R^2). Insignificant model terms were removed. The 'desirability function' in Design Expert allowed for the creation of one optimised method based on the maximum response of the 18 selected volatile compounds.

Volatile Analysis by HS-SPME GCMS

Volatile analysis was carried out utilising a Gerstel MultiPurpose Sampler (GMPS) rail system (Anatune, Cambridge CB3 0NA, UK) connected to a Shimadzu GP2010 plus GC (Mason Technology Ltd., Dublin, Ireland). Cake crumb (3 g) was added to an amber 20 ml screw-capped SPME vial (Apex Scientific Ltd., Co. Kildare, Ireland) and equilibrated for varying times (5–10 min) while exposed to heat with pulsed agitation for 5 s at

Table 2 Experimental conditions applied for the optimisation of HS-SPME for baked confectionery matrices

Run	x: incubation time	x_1: extraction time	x_2: extraction temperature
1	11.7045	40	50
2	10	60	60
3	7.5	73.6359	50
4	5	60	60
5	7.5	40	50
6	7.5	40	50
7	7.5	40	33.1821
8	7.5	40	50
9	7.5	40	66.8179
10	10	20	60
11	5	20	40
12	7.5	40	50
13	10	60	40
14	5	20	60
15	7.5	40	50
16	7.5	40	50
17	7.5	6.36414	50
18	5	60	40
19	3.29552	40	50
20	10	20	40

350 rpm using the GMPS agitator/heater. The SPME fibre was exposed to the headspace above the samples, at a depth of 21 mm, varying incubation times (5–10 min), extraction times (20–60 min) and varying temperatures (40–60 °C), throughout the optimisation trial. The fibre was retracted, injected into the GC inlet and desorbed for 3 min at 250 °C using the GMPS fibre bakeout station. For each experimental run (Table 2), 3 g of cake crumb (bulk batch prepared as described earlier) was analysed in triplicate. An external standard stock solution (1-butanol, dimethyl disulphide, butyl acetate, cyclohexanone) (Sigma-Aldrich, Arklow, Ireland) at 1000 ppm in methanol (Sigma-Aldrich, Ireland) was also analysed at the start and end of the sample set batch, and levels of each external standard were quantified and compared to expected values to ensure that both the SPME extraction and MS detection were performing within specification.

The GC analysis was performed on a Shimadzu 2010 Plus GC (Mason Technology Ltd., Dublin Ireland), equipped with a split/splitless injector, operating in splitless mode with a Merlin Microseal (Sigma-Aldrich, Wicklow, Ireland). The carrier gas was helium held at a pressure of 43.8 psi and a flow rate of 1.2 mL/min. The volatile compounds were separated on a DB-624 UI (60 m × 0.32 mm × 1.80 μm) column (Agilent Technologies Ireland Ltd., Cork, Ireland). The temperature of the column oven was set at 40 °C, held for 5 min, increased at 5 °C/min to 230 °C then increased at 15 °C/min to 260 °C. The total GC run time was 65 min. Compound identification was carried out by a mass spectrometry detector-Shimadzu TQ8030 (Mason Technologies Ltd., Dublin, Ireland) ran in single quad mode. The ion source temperature was 220 °C and the interface temperature was set at 260 °C. The MS mode was electronic ionization (70 eV) with the mass range scanned between m/z 35–250. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, the Shimadzu commercial library FFNSC version 2 and an in-house library created in GCMS Solutions software (Shimadzu, Japan) with target and qualifier ions and linear retention indices for each compound. Spectral deconvolution was also performed to confirm identification of compounds using AMDIS.

Model Validation

Validation of the model was performed by applying the optimised HS-SPME conditions to the bulk sponge cake matrix analysed in triplicate, and comparing the average response values obtained to the values predicted by the regression model. Subsequently, fifteen (replication of the centre point was removed) runs (Table 2) were chosen for repetition using freshly baked sponge cake.

Application of the Optimised HS-SPME Method

Once the HS-SPME GCMS method had been optimised, it was applied to three freshly baked confectionery matrices (sponge cake, shortbread biscuit and chocolate brownie) in triplicate. This was undertaken to prove the competency of the method in volatile recovery of typical baked confectionery products. The number of volatile compounds recovered was then compared to published studies on similar or related products to have an estimate of its effectiveness.

Statistical Analysis

The response surface methodology design and desirability function were accomplished with the aid of Design Expert Version 10 (Stat-Ease Inc. Minneapolis, MN). Statistical analysis for model validation was performed using ANOVA followed by a Tukey post hoc test to compare the difference in means. This was performed at a 0.05 alpha level, using SPSS Statistics Version 25 (SPSS, IBM, Chicago, IL, USA).

Results and Discussion

SPME Fibre Screening

Of the four fibres tested, the DVB/CAR/PDMS fibre recovered the greatest abundance of volatile compounds (70) from the sponge cake matrix. Although the CAR/PDMS fibre was capable of recovering 52 compounds and achieved good recovery of 2,3-butanedione, it was unable to recover high molecular weight aldehydes such as phenylacetaldehyde and (E,Z)-2,4-decadienal (Fig. 1), reported to be important to the aroma of baked confectionery products (Matsakidou et al. 2010; Pozo-Bayón et al. 2007). These results correspond with Rega et al. (2009) who also identified that the DVB/CAR/PDMS fibre was effective in extracting a greater quantity of compounds compared to the CAR/PDMS fibre, but the CAR/PDMS fibre was more suitable for extracting highly volatile compounds. The PA and PDMS fibre only recovered 27 and 20 compounds, respectively. The success obtained by the DVB/CAR/PDMS fibre is due to the chemical makeup of various phases, a molecular sieve carboxen (CAR), polar divinylbenzene (DVB) and non-polar polydimethylsiloxane (PDMS), and hence, has the ability to target a wider range of compounds; thus, this fibre was chosen for HS-SPME optimisation study (Table S1).

Optimisation of HS-SPME Procedure Using Response Surface Methodology

It is important that the most influential factors contributing to the successful extraction of volatile and semi-volatile

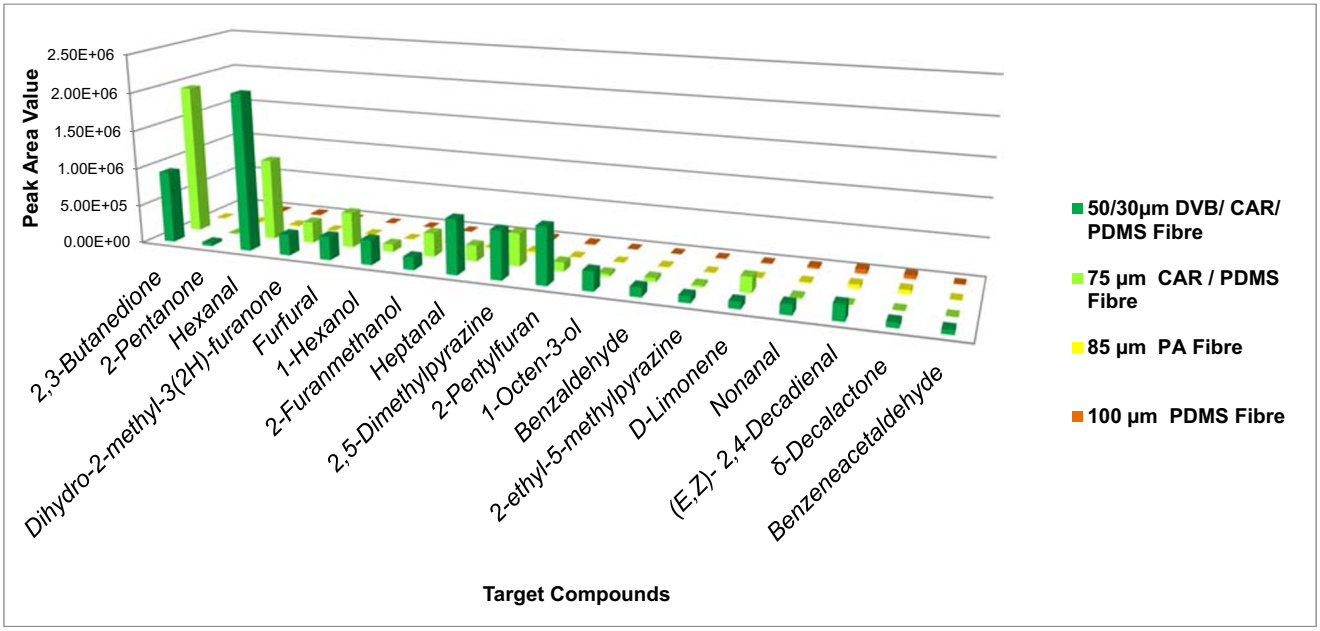


Fig. 1 Efficacy of HS-SPME fibres on extraction of target compounds from sponge cake matrix. Average peak area values ($n = 3$). HS-SPME conditions: incubation time = 10 min, extraction time = 50 min, extraction temp = 40 °C

compounds, from the matrix of interest, are established prior to optimisation of the HS-SPME method. This can be determined through a screening step of all factors that show potential influence on the extraction of volatiles by HS-SPME. In the case of a baked cereal matrix, there have been a number of studies involving HS-SPME GCMS analysis reported in literature (Giarnetti et al. 2015; Maire et al. 2013; Matsakidou et al. 2010; Pasqualone et al. 2014; Pasqualone et al. 2015; Rega et al. 2009). Factors such as NaCl (salting out), pH and sample amount have also been altered to recover volatile profiles in bread products (Raffo et al. 2015). However, as we were undertaking headspace analysis on solid samples in this study, we decided it was unnecessary to asses salting out or pH changes. The authors did not investigate the sample amount as the volume of 3 g of crumbed sample in a 20-mL headspace vial was just below the depth of the SPME fibre after insertion, and we felt that we need to ensure that we saturated the headspace with volatiles without any potential issues with fibre coming into contact with the sample. The parameters, incubation time, extraction time and extraction temperature, were evaluated for the extraction of volatile aroma compounds from the sponge cake matrix in this study.

Preliminary experiments (data not shown) were undertaken to optimise the HS-SPME method by evaluating extraction parameters individually; however, this approach does not take into account possible interactive effects, and therefore, maximum volatile response may not be realised (Ma et al. 2013). The CCRD experimental results demonstrate the main, interaction and quadratic effect of the extraction parameters on volatile response (Table 3) generated using analysis of variance (ANOVA). The final reduced models were satisfactory ($p < 0.0001$) in explaining the variability of responses amongst the selected 18 compounds, with satisfactory determination coefficients (R^2) ranging from 0.8178 to 0.9871. The lack of fit was not significant ($p > 0.05$) for all compounds analysed, indicating the data fitted the regression model adequately. Response surface plots were capable of depicting the behaviour of compounds in relation to the varying extraction time and extraction temperature (Fig. 2a, b).

Incubation time is regarded as the length of time taken for volatile compounds to partition from the food matrix into the headspace. It is considered an important factor in saturating the headspace prior to compound adsorption/absorption onto the fibre (Mondello et al. 2005), and assists in compounds reaching equilibrium in the headspace, leading to a potentially greater recovery. However, incubation time (x) did not have a significant effect ($p > 0.05$) on the recovery of the 18 selected compounds from the sponge cake matrix in the time range studied, 5–10 min. In this study, the time range may have been insufficient to confer an effect. However, longer incubation times were applied in HS-SPME GCMS of honey, 7–23 min (da Costa et al. 2018), and beer, 5–25 min (Moreira et al. 2013), without any significant effect. As this parameter demonstrated no significant effect, an incubation time of 5 min was selected. This incubation time has also been applied in a similar matrix (bread) without prior optimisation (Pico et al. 2018).

However, extraction time had the most pronounced effect on volatile extraction, with the response of all 18 selected compounds significantly ($p < 0.05$) impacted by the length of extraction time. Extraction time is important as it is the time taken for compounds to reach equilibrium on the fibre, including very volatile and semi-volatile compounds that may take

Table 3 Significance of main effect, quadratic effect and interaction effect of extraction time (x_1) and extraction temperature (x_2), regression coefficient (R^2) and lack of fit of final reduced models

Target Volatile Compound	Regression Equation	Main Effect		Quadratic Effect		Interaction Effect	Lack of Fit	R^2
		X_1	X_2	X_1^2	X_2^2	X_1X_2		
2,3-Butanedione	$2102000 + 374135x_1 + 607976x_2 - 262941x_1^2 - 189396x_2^2$	< 0.0001	< 0.0001	< 0.0001	0.0007		0.4826	0.9514
2-Pentanone	$422551 + 85946.5x_1 + 23507.4x_2 - 51628.5\ x_1x_2 - 61329.3x_1^2 - 36641.5x_2^2$	< 0.0001		0.0008	0.0233	0.0187	0.9111	0.8248
Hexanal	$2561670 + 748832x_1 + 892874x_2 – 252297x_1^2$	< 0.0001	< 0.0001	0.0040			0.7634	0.9362
Dihydro-2-methyl-3(2H)-furanone	$235283 + 36331.8x_1 + 6484.85x_2 - 19190.3x_1x_2 - 27963.3x_1^2 - 30040.6x_2^2$	< 0.0001		0.0001	< 0.0001	0.0196	0.7160	0.8812
1-Hexanol	$345755 + 141744x_1 + 162498x_2 + 48046x_1x_2$	< 0.0001	< 0.0001			0.0123	0.7664	0.9463
2-Furanmethanol	$318121 + 129013x_1 + 130818x_2 + 81517.9x_1x_2$	< 0.0001	< 0.0001			0.0002	0.4132	0.9304
Heptanal	$1466000 + 376373\ x_1 + 549193\ x_2 + 160684\ x_1x_2 - 98706.5x_1^2$	< 0.0001	< 0.0001	0.0047		0.001	0.6048	0.9705
2,5-Dimethylpyrazine	$831763 + 310830x_1 + 405832x_2 + 169432x_1x_2 - 48533x_1^2 + 44310.7x_2^2$	< 0.0001	< 0.0001	0.0082	0.0139	< 0.0001	0.2641	0.9871
2-Pentylfuran	$1198900 + 399745x_1 + 571735x_2 + 163799x_1x_2 - 100181x_1^2$	< 0.0001	< 0.0001	0.0111		0.0032	0.7127	0.9638
1-Octen-3-ol	$428208 + 186721x_1 + 259844x_2 + 127385x_1x_2$	< 0.0001	< 0.0001			< 0.0001	0.7377	0.9602
Furfural	$225209 + 81422.3x_1 + 92590x_2 + 38905.4x_1x_2 - 15602.7x_1^2$	< 0.0001	< 0.0001	0.0155	0.1670	0.0001	0.7280	0.9741
Benzaldehyde	$220312 + 85582.5x_1 + 91273.2x_2 + 49945.5x_1x_2$	< 0.0001	< 0.0001			0.0004	0.2280	0.9359
2-Ethyl-5-methyl-pyrazine	$128134 + 48555.3x_1 + 70261.7x_2 + 26613.5x_1x_2 - 6906.43x_1^2 + 8111.93x_2^2$	< 0.0001	< 0.0001	0.0299	0.0132	< 0.0001	0.5064	0.9847
d-Limonene	$103261 + 61644.9x_1 + 37586.3x_2 + 44666.1x_1x_2$	< 0.0001	0.0011			0.0023	0.6609	0.8178
Phenylacetaldehyde	$81688.8 + 37055.3x_1 + 69525.9x_2 + 25867.5x_1x_2 + 21380.6x_2^2$	< 0.0001	< 0.0001		< 0.0001	< 0.0001	0.4495	0.9859
Nonanal	$278989 + 128152x_1 + 220550x_2 + 98340.6x_1x_2 + 55618.4x_2^2$	< 0.0001	< 0.0001		< 0.0001	< 0.0001	0.5555	0.9773
(E,Z)-2,4-Decadienal	$54448.9 + 32784.8x_1 + 65852.3x_2 + 28765.3x_1x_2 + 28758.8x_2^2$	< 0.0001	< 0.0001		< 0.0001	0.0002	0.1505	0.9563
δ-Decalactone	$29521.3 + 14626.2x_1 + 35579x_2 + 11001.8x_1x_2 + 16134.5x_2^2$	< 0.0001	< 0.0001		< 0.0001	0.0080	0.0846	0.9415

Main effect, quadratic effect and interaction effect data for incubation time (x) removed as identified as non-significant variable ($P > 0.05$)

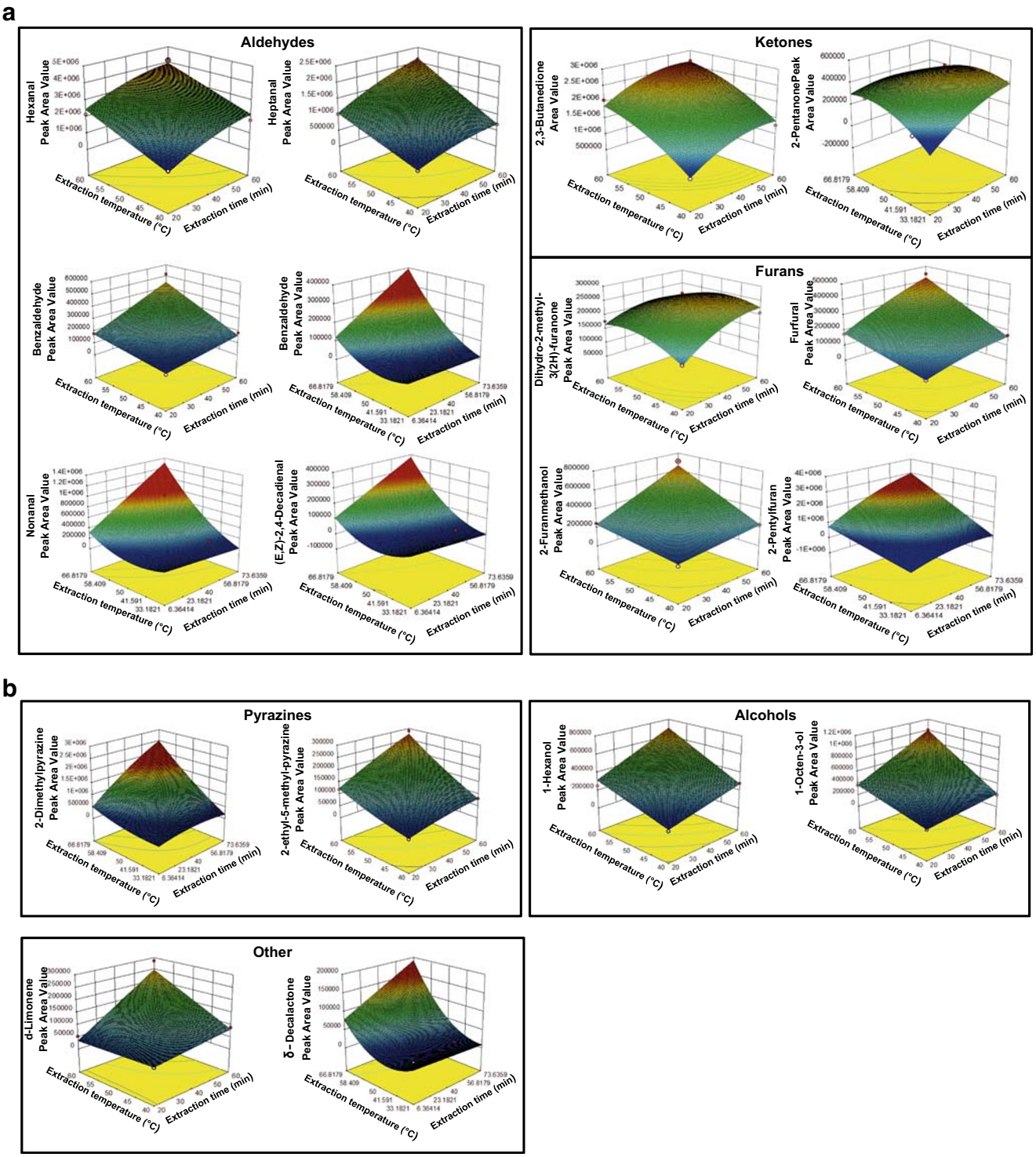


Fig. 2 Response surface plots showing the influence of HS-SPME conditions (extraction time and extraction temperature; incubation time fixed at 5 min) on the response area value of (**a**) target aldehydes, ketones and furans and (**b**) target pyrazines, alcohols and other chemical class (lactone, terpene) in the sponge cake matrix

longer to reach equilibrium (Prosen and Zupančič-Kralj 1999). When the sample was exposed to varying extraction times throughout the study, the peak areas increased concurrently with extraction times (Fig. 2a, b) for most of the 18 selected volatile compounds. Similar results were shown for volatiles in bread (Ruiz et al. 2003).

Extraction temperature was shown to have a significant effect ($p < 0.05$) on the extraction of the majority of the 18

selected aroma compounds (Fig. 2a, b). Depending on the matrix of the sample studied (i.e. solid vs liquid), above-ambient temperatures may be required during HS-SPME GCMS analysis to assist the transition of compounds from the barrier of the sample matrix into the headspace, and subsequently onto the fibre (Prosen and Zupančič-Kralj 1999). For most compounds, an extraction temperature of 60 °C or above demonstrated an increase in the response of the volatile compounds (Fig. 2a, b). However, with any extraction technique, elevating temperatures increase the risk of artefact formation, particularly for furans and other compounds related to the Maillard reaction (Pérez-Palacios et al. 2012). However, previous HS-SPME studies of baked cereal products (Matsakidou et al. 2010; Raffo et al. 2015; Rega et al. 2009) have utilised temperatures of 50 °C and above and have recovered a greater number of compounds compared to studies utilising a temperature of 40 °C and below (Giarnetti et al. 2015; Petisca et al. 2013). In addition, as sponge cakes, and other baked confectionery products, are already exposed to the extreme temperatures of baking, it is unlikely that furan formation would occur during HS-SPME (Wang et al. 2017). Our study found that 2-pentanone and dihydro-2-methyl-3(2H)-furanone were not significantly influenced by extraction temperature and obtained the highest recovery at 40 °C and 50 °C, respectively.

The interaction effect of extraction time and extraction temperature is depicted in the experimental range by the response surface plots (Fig. 2a, b), with a fixed incubation time of 5 min. The interaction effect of these independent variables was significant ($p < 0.05$) on the volatile response of 16 of the selected 18 compounds, with no significant effect ($p > 0.05$) demonstrated on 2,3-butanedione or hexanal. This result is important as it highlights the benefit of employing RSM for optimisation. Single parameter optimisation (data not shown) was unable to achieve maximum volatile response as all experimental conditions (low temperature, long time and vice versa) were not trialled. The interaction effect of time and temperature demonstrates the efficiency of RSM; as with just 20 experimental runs, all extraction conditions were evaluated on the ability to achieve maximum volatile response. Overall, the majority of compounds favoured a higher extraction temperature at longer extraction times. The optimised HS-SPME method was derived using the 'desirability function' on Design Expert version 10, whereby the optimum conditions for each response (volatile compound) are combined to identify a method that will achieve the highest desirability figure between 0 and 1 (ideally closer to 1). In this study, the optimum extraction conditions were identified as an incubation time of 5 min, an extraction time of 60 min and an extraction temperature of 60 °C, using a DVB/CAR/PDMS fibre. The optimisation desirability value of this proposed method was 0.872.

Model Validation

To validate the proposed HS-SPME model, the optimised extraction conditions (incubation time 5 min, extraction time 60 min and extraction temperature 60 °C) were subsequently applied to the same bulk sponge cake sample and analysed in triplicate. The results were compared to those predicted by the regression model and the values obtained for 2-pentanone, hexanal, furfural, hexanol, 2-furanmethanol, heptanal, 2,5-dimethylpyrazine, (E,Z)-2,4-decadienal and δ-decalactone were within the range of predicted values (Table 4). However, values for 2,3-butanedione, dihydro-2-methyl-3(2H)-furanone and benzaldehyde were below the predicted values, whereas 1-octen-3-ol, 2-ethyl-5-methyl-pyrazine, d-limonene, phenylacetaldehyde and nonanal were above the predicted values (Table 4). Differences in real and predicated recoveries, for different chemical class compounds, are not unusual especially when dealing with headspace extractions from complex solid materials (Nongonierma et al. 2006). The efficiency of the adsorbent is impacted by the hydrophobicity, volatility and vapour pressure of the analytes and the sample/adsorbent partition coefficient; therefore, responses are likely to vary more in non-homogenous solid materials than in homogenous fluids (Spietelun et al. 2013). Another possible factor influencing recovery is the inherent instability of the product matrix. Previous work by Pico et al. (2017) demonstrated that cryogenically ground bread crumb stored at −21 °C showed significant decreases in the levels of 2,3-butanedione, d-limonene, 1-octen-3-ol, benzaldehyde and phenylacetaldehyde over 4 weeks when analysed by solvent extraction. Jensen et al. (2011) also found that levels of nonanal and benzaldehyde increased in bread stored at ambient temperature over 3 weeks. Thus, changes in volatiles may also be due to further reactions during frozen storage or thawing, such as lipid oxidation and Strecker degradation reactions (Mohsen et al. 2009; Bueno et al. 2013).

Although 9 out of the 18 compounds studied achieved statically similar levels to the predicted model, further experimental runs were required to fully validate the method. Of the 20 experimental runs devised by the RSM software, 15 (removing replication of centre point) were chosen for repetition using a freshly baked cake, to confirm the performance of the newly established HS-SPME method parameters. The results from experimental conditions are demonstrated in Fig. 3, using the sum of the volatile peak areas, as seen in other HS-SPME optimisation studies (Moreira et al. 2013; Pérez-Palacios et al. 2012). On replication of all individual experimental conditions, the optimised parameters, incubation time of 5 min, extraction time of 60 min and extraction temperature of 60 °C, achieved a significantly ($p < 0.05$) higher volatile recovery compared to all other experimental conditions, therefore validating the defined optimal extraction conditions.

Table 4 Predicted values of the regression models for validation of the optimised HS-SPME method compared to actual values obtained. Average ($n = 3$) compound values with an * did not meet regression model predictions

Compound	Predicted peak area value	95% prediction interval low	Observed mean ($n = 3$)	95% prediction interval high
2,3-Butanedione	2631450	2366000	1887937*	2897000
2-Pentanone	382406	2860000	400405	4788000
Hexanal	3951080	3504000	3639652	4398000
Dihydro-2-methyl-3(2H)-furanone	200905	1648000	154399	2371000
Furfural	422524	3854000	459323	4593000
1-Hexanol	698044	6154000	738606	7807000
2-Furanmethanol	659470	5753000	583883	7436000
Heptanal	2134140	1937000	1985872	2332000
2,5-Dimethylpyrazine	1713640	1608000	1693876	1819000
Pentylfuran	2234280	2005000	2056139	2464000
1-Octen-3-ol	1002160	8942000	1124044*	1110000
Benzaldehyde	447113	3929000	318358*	5014000
2-ethyl-5-methyl-pyrazine	274770	2556000	304142*	2939000
D-Limonene	247158	1874000	325845*	3069000
Phenylacetaldehyde	235518	2190000	284957*	2520000
Nonanal	778009	7214000	906908*	8346000
(E,Z)-2,4-decadienal	210610	1815000	196770	2397000
δ-Decalactone	382406	89223	94554	1245000

Application to Baked Confectionery Matrices

The optimised HS-SPME GCMS method was applied to cake-like matrices to demonstrate its effectiveness. The method was applied to a freshly prepared shortbread biscuit, chocolate brownie and a sponge cake matrix in triplicate. In total, 163 compounds were identified between the three matrices (Table 5). Among the main compounds identified were 25 aldehydes, 20 ketones, 19 esters, 18 furanic compounds and 12 pyrazines, as well as lactones, terpenes and phenols. For the sponge cake matrix, 70 compounds were identified, which is comparable to studies by Maire et al. (2013) who reported 70 volatile compounds in sponge cakes using a thicker HS-SPME fibre (75 μm DVB/CAR/PDMS), and Pozo-Bayón et al. (2007) who recovered 77 compounds using SAFE which was previously shown to recover a larger quantity of

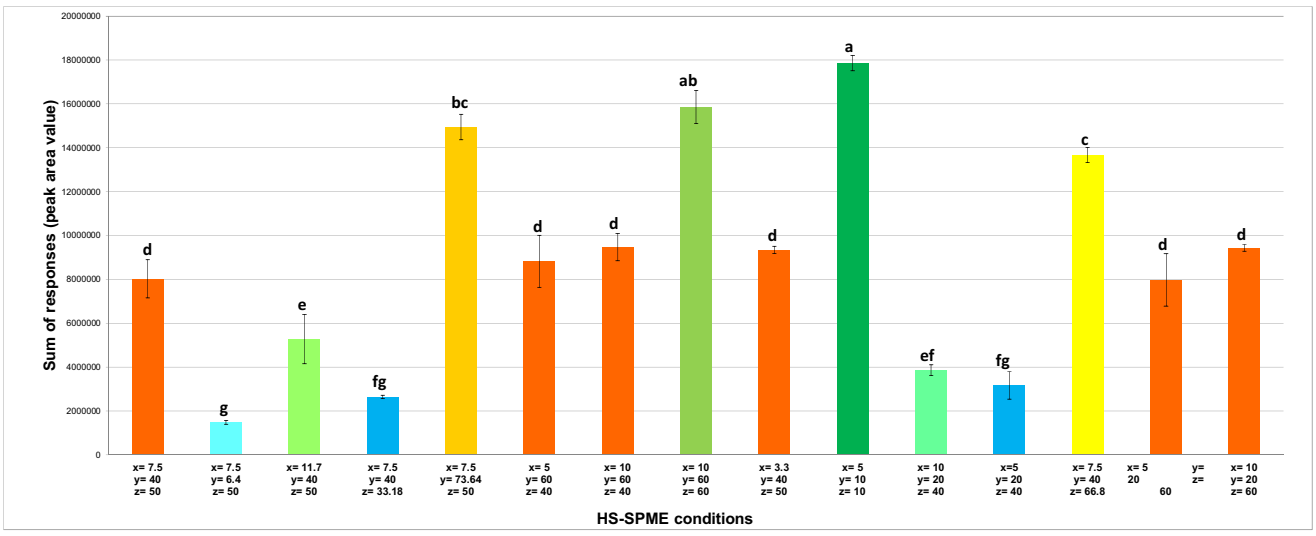


Fig. 3 Validation of HS-SPME method: comparison of experimental conditions for the recovery of volatiles from fresh sponge cake. Mean of peak area values ($n = 3$) with different lowercase letters are significantly different ($p < 0.05$). x = incubation time, y = extraction time, z = extraction temperature

Table 5 Application of the HS-SPME-GC-MS method to baked confectionery matrices. Peak area values obtained ($n = 3$) for the volatile compounds identified in a chocolate brownie, biscuit and sponge cake matrix

Compound	CAS#	rt	RI	REF RI	Chocolate brownie matrix	Shortbread biscuit matrix	Sponge cake matrix	Identification
Aldehydes								
Acetaldehyde	75-07-0	5.43	453	454.9	1.19E + 06	6.54E + 05	9.68E + 04	MS, RI
2-Propenal	107-02-8	8.045	522	524.9	5.15E + 04	7.89E + 04	nd	MS, RI
Propanal	123-38-6	8.185	526	528.9	5.40E + 04	2.43E + 04	nd	MS, RI
2-Methylpropanal	78-84-2	10.615	591	594.2	1.41E + 06	2.09E + 05	3.06E + 04	MS, RI
3-Methylbutanal	590-86-3	15.07	689	692.7	1.21E + 07	2.20E + 06	3.16E + 05	MS, RI
2-Methylbutanal	96-17-3	15.445	697	700.9	3.98E + 06	2.05E + 06	6.12E + 05	MS, RI
Pentanal	110-62-3	17.105	733	737.3	nd	nd	2.46E + 05	MS, RI
(E)-2-Pentenal	1576-87-0	20.295	803		nd	nd	1.03E + 05	MS
Hexanal	66-25-1	21.64	835	839.4	1.81E + 06	2.74E + 06	2.84E + 06	MS, RI
(E)-2-Hexenal	6728-26-3	24.445	901		nd	7.96E + 04	nd	MS
Heptanal	111-71-7	25.855	937	942.8	6.55E + 05	8.12E + 05	1.08E + 06	MS, RI
(E)-2-Nonenal	18829-56-6	27.005	967		4.40E + 05	nd	nd	MS
Benzaldehyde	100-52-7	29.14	1024	1030.3	7.66E + 06	1.41E + 06	1.65E + 05	MS, RI
Octanal	124-13-0	29.715	1040	1046.2	8.48E + 05	9.42E + 05	1.49E + 05	MS, RI
Phenylacetaldehyde	122-78-1	32.245	1112	1118.6	1.82E + 07	4.08E + 06	1.42E + 05	MS, RI
Nonanal	124-19-6	33.26	1142	1145.2	2.20E + 06	2.32E + 06	5.37E + 05	MS, RI
(E)-2-Nonenal	18829-56-6	35.704	1218		nd	nd	1.03E + 05	MS
Decanal	112-31-2	36.53	1245	1253.1	2.24E + 05	1.24E + 05	6.25E + 04	MS, RI
2,4-Nonadienal	6750-03-4	37.725	1284		nd	nd	1.17E + 04	MS
(2E)-2-Decenal	3913-81-3	38.78	1319		3.34E + 05	2.72E + 05	nd	MS
2,4-Decadienal	2363-88-4	40.035	1363		nd	nd	1.67E + 04	MS
(E,Z)-2,4-Decadienal	25152-84-5	40.69	1386		3.44E + 05	3.48E + 05	1.59E + 05	MS
2-Undecenal	2463-77-6	41.695	1422		nd	1.44E + 05	nd	MS
Dodecanal	112-54-9	42.41	1449		nd	4.81E + 04	nd	MS
Vanillin	121-33-5	44.665	1543		nd	2.73E + 04	nd	MS
Alcohols								
Ethanol	64-17-5	7.425	506	508.4	1.97E + 05	1.15E + 05	2.56E + 04	MS, RI
(R)-2-Butanol	14898-79-4	13.015	644		3.66E + 04	nd	1.48E + 04	MS
2-Methyl-3-buten-2-ol	115-18-4	13.5	655		2.02E + 04	nd	nd	MS
1-Butanol	71-36-3	16.035	710	715.4	nd	7.49E + 04	nd	MS, RI
1-Penten-3-ol	616-25-1	16.897	729		nd	nd	8.74E + 04	MS
(R)-(−)-2-Pentanol	31087-44-2	17.43	740		1.39E + 05	nd	nd	MS
3-Methyl-1-butanol	123-51-3	19.23	779		nd	2.87E + 04	nd	MS
2-Hexanol	626-93-7	19.485	785		1.47E + 06	nd	nd	MS
1-Pentanol	71-41-0	20.655	811	816.6	1.53E + 05	2.77E + 05	1.17E + 06	MS, RI
2,3-Butanediol	19132-06-0	22.86	863	862.5	9.64E + 06	nd	nd	MS, RI
3-Hexen-1-ol	544-12-7	24.596	905		nd	nd	9.04E + 04	MS
1-Hexanol	111-27-3	24.78	909	915.6	nd	8.54E + 05	1.84E + 06	MS, RI
2-Propylheptanol	10042-59-8	28.665	1010		3.33E + 05	nd	nd	MS
1-Octen-3-ol	3391-86-4	28.983	1019		nd	nd	8.90E + 05	MS
2-Ethylhexanol	104-76-7	30.79	1070	1076.3	7.79E + 05	4.98E + 05	1.61E + 05	MS, RI
1-Undecanol	112-42-5	37.85	1288		nd	1.43E + 04	nd	MS
Ketones								
Acetone	67-64-1	8.385	531	534.3	2.19E + 06	9.36E + 05	1.17E + 05	MS, RI
2,3-Butanedione	431-03-8	12.28	628	631.7	2.95E + 06	1.30E + 06	7.35E + 05	MS, RI
2-Butanone	78-93-3	12.6	635	638.8	1.05E + 06	4.30E + 05	5.24E + 04	MS, RI
2-Pentanone	107-87-9	16.75	725	729.6	4.51E + 06	2.65E + 06	1.67E + 05	MS, RI
2,3-Pentanedione	600-14-6	17.115	733		nd	nd	2.30E + 05	MS
1-Hydroxy-2-propanone	116-09-6	17.015	731	735.3	1.16E + 07	4.66E + 07	2.32E + 05	MS, RI
Acetoin	513-86-0	18.975	774	778.1	6.23E + 06	8.40E + 05	4.56E + 05	MS, RI
2-Hexanone	591-78-6	21.32	827	832.8	nd	6.44E + 05	2.33E + 04	MS, RI
1-Hydroxy-2-butanone	5077-67-8	21.525	832		4.18E + 05	2.98E + 05	nd	MS
2-Hydroxy-3-pentanone	5704-20-1	23.105	869		7.49E + 05	nd	nd	MS
2-Heptanone	110-43-0	25.515	928	934.6	4.07E + 07	5.13E + 07	1.25E + 07	MS, RI
2,3-Hexanedione	3848-24-6	27.605	982		4.05E + 05	nd	nd	MS
4-Methyl-2-heptanone	6137-06-0	29.43	1032	1038.6	nd	nd	2.60E + 06	MS, RI
(3E,5E) -Octadien-2-one	30086-02-3	32.755	1127		nd	nd	1.11E + 04	MS
2-Nonanone	821-55-6	32.905	1132	1138.9	1.33E + 07	3.55E + 07	nd	MS, RI
Acetophenone	98-86-2	33.07	1137	1144	6.56E + 05	nd	nd	MS, RI
3,5-Octadien-2-one	38284-27-4	33.685	1155		nd	nd	4.23E + 04	MS
2-Undecanone	112-12-9	39.225	1335	1344.2	3.17E + 06	1.25E + 07	nd	MS, RI
2-Dodecanone	6175-49-1	42.055	1436		nd	7.09E + 04	nd	MS

Table 5 (continued)

Compound	CAS#	rt	RI	REF RI	Chocolate brownie matrix	Shortbread biscuit matrix	Sponge cake matrix	Identification
2-Tridecanone	593-08-8	44.865	1553		6.72E + 05	2.71E + 06	4.42E + 04	MS
Furans								
Furan	110-00-9	7.84	517	519.7	1.92E + 04	nd	nd	MS, RI
2-Methylfuran	534-22-5	11.895	620	623.1	3.65E + 04	1.30E + 05	6.24E + 03	MS, RI
2-Ethylfuran	3208-16-0	16.285	715	720	3.20E + 04	2.10E + 04	2.42E + 04	MS, RI
2,5-Dimethylfuran	625-86-5	16.53	720		1.71E + 04	2.42E + 04	nd	MS
2-Vinylfuran	1487-18-9	20.135	799		nd	1.02E + 05	4.51E + 04	MS
Dihydro-2-methyl-3(2H)-furanone	3188-00-9	22.37	852		6.20E + 05	2.90E + 05	nd	MS
Furfural	98-01-1	24.145	894	898.5	6.21E + 06	5.17E + 07	1.07E + 05	MS, RI
2-Butylfuran	4466-24-4	24.65	906	911.6	7.55E + 04	7.12E + 04	3.07E + 04	MS, RI
2-Furanmethanol	98-00-0	25.24	921		4.15E + 06	2.22E + 07	4.91E + 04	MS
Acetylfuran	1192-62-7	27.16	971		9.88E + 05	2.95E + 06	nd	MS
2-Pentylfuran	3777-69-3	28.505	1006	1012.4	1.96E + 06	1.81E + 06	1.98E + 06	MS, RI
5-Methyl-2-furfuryl alcohol	3857-25-8	28.67	1011		nd	4.82E + 05	nd	MS
Butyrolactone	96-48-0	28.995	1020	1026.3	5.40E + 06	nd	nd	MS, RI
2(5H)-Furanone	497-23-4	29.105	1023	1029.5	1.54E + 06	6.23E + 06	nd	MS, RI
Furyl hydroxymethyl ketone	17678-19-2	34.26	1173		nd	2.62E + 07	nd	MS
4-Methyl-5H-furan-2-one	6124-79-4	34.445	1178		2.45E + 05	2.55E + 07	nd	MS
4-Hydroxydihydro-2(3H)-furanone	5469-16-9	39.845	1356		nd	6.35E + 05	nd	MS
5-Hydroxymethylfurfural	67-47-0	40.06	1364		1.58E + 05	2.55E + 07	nd	MS
Pyrazines								
Pyrazine	290-37-9	18.75	769	773.2	3.09E + 05	2.66E + 05	nd	MS, RI
Methylpyrazine	109-08-0	22.65	858		5.38E + 06	nd	1.99E + 05	
2,5-dimethylpyrazine	123-32-0	26.145	945	950.3	7.97E + 06	2.59E + 06	5.38E + 05	MS, RI
2,3-Dimethylpyrazine	5910-89-4	26.56	955	961.1	1.74E + 06	3.32E + 05	nd	MS, RI
2-Ethyl-6-methylpyrazine	13925-03-6	29.385	1031		1.40E + 06	nd	6.96E + 04	MS
Trimethylpyrazine	14667-55-1	29.525	1035	1041	7.72E + 06	4.49E + 05	nd	MS, RI
2,5-Dimethyl-3-ethylpyrazine	13360-65-1	32.11	1108	114.5	2.18E + 06	nd	nd	MS, RI
Tetramethylpyrazine	1124-11-4	32.375	1116	1122.6	1.34E + 07	2.43E + 05	nd	MS, RI
2-Ethyl-3,5-dimethylpyrazine	13925-07-0	32.435	1117		1.36E + 06	nd	nd	MS
2,5-Diethylpyrazine	13238-84-1	32.58	1122		8.51E + 04	nd	nd	MS
2-Ethyl-3,5,6-trimethylpyrazine	17398-16-2	34.64	1184	1192.1	1.43E + 06	nd	nd	MS, RI
2,5-Dimethyl-3-isoamylpyrazine	18433-98-2	39.565	1346		3.73E + 05	nd	nd	MS
Acids								
Acetic acid	64-19-7	14.96	686	690.4	7.55E + 07	2.64E + 07	4.05E + 04	MS, RI
Propanoic Acid	79-09-4	18.955	773	778.4	nd	1.82E + 05	nd	MS, RI
Butanoic acid	107-92-6	22.603	857	862.5	2.67E + 06	5.17E + 06	nd	MS, RI
3-Methylbutanoic acid	503-74-2	24.82	910	916	1.93E + 07	nd	nd	MS, RI
2-Methylbutanoic acid	116-53-0	25.085	917		4.17E + 06	nd	nd	MS
Hexanoic acid	142-62-1	29.835	1043	1049.6	2.68E + 06	5.39E + 06	4.61E + 04	MS, RI
Octanoic acid	124-07-2	36.195	1234	1242.1	5.57E + 05	8.75E + 05	nd	MS, RI
Esters								
Ethyl ether	60-29-7	7.75	515	517.3	6.08E + 04	1.72E + 05	6.54E + 03	MS, RI
Acetic acid, methyl ester	79-20-9	9.15	552		2.03E + 05	5.41E + 04	nd	MS
Vinyl acetate	108-05-4	11.325	608		2.78E + 04	6.79E + 04	nd	MS
Ethyl Acetate	141-78-6	12.775	639	642.6	7.29E + 04	9.93E + 04	nd	MS, RI
Propanoic acid, 2-methyl-	79-31-2	21.38	829		4.36E + 06	nd	nd	MS
Allyl butanoate	2051-78-7	23.6	881		nd	4.49E + 04	nd	MS
Ethylbenzene	100-41-4	23.902	888	892.9	nd	nd	1.53E + 04	MS, RI
Isoamyl acetate	123-92-2	24.285	897	902.5	3.24E + 05	nd	nd	MS, RI
2-Methylbutyl acetate	624-41-9	24.44	901	906.1	7.04E + 04	nd	nd	MS, RI
Isovaleric acid	503-74-2	24.76	909	914.5	nd	3.07E + 05	nd	MS, RI
1-Methoxy-2-propyl acetate	108-65-6	27.945	991		4.05E + 06	nd	nd	MS
Isobutylacetic acid	646-07-1	28.81	1014		2.42E + 05	nd	nd	MS
Ethylhexanoic acid	149-57-5	34.421	1178		nd	nd	1.71E + 04	MS
Ethyl octanoate	106-32-1	35.56	1213	1222	2.28E + 05	nd	nd	MS, RI
Ethyl benzoate	93-89-0	35.865	1223	1231.8	9.77E + 04	nd	nd	MS, RI
Ethyl benzeneacetate	101-97-3	38.11	1296	1305.1	3.14E + 05	nd	nd	MS, RI
β-Phenethyl acetate	103-45-7	38.55	1311	1320.7	1.20E + 06	nd	nd	MS, RI
Ethyl dodecanoate	106-33-2	47.1	1659		5.60E + 04	nd	nd	MS
Lactones								
α-Angelica lactone	591-12-8	25.765	935		nd	6.10E + 05	nd	MS
Butyrolactone	96-48-0	25.515	928		nd	1.55E + 06	nd	MS
δ-Caprolactone	823-22-3	35.575	1213		nd	2.92E + 06	nd	MS

Table 5 (continued)

Compound	CAS#	rt	RI	REF RI	Chocolate brownie matrix	Shortbread biscuit matrix	Sponge cake matrix	Identification
δ-Hexalactone	66-25-1	35.585	1214		1.34E + 06	nd	nd	MS
γ-Heptalactone	105-21-5	37.155	1265	1273.7	nd	1.89E + 05	nd	MS, RI
Caprolactone	502-44-3	37.285	1269		nd	4.62E + 04	nd	MS
γ-Undecalactone (peach lactone)	104-67-6	40.34	1373		nd	1.90E + 05	nd	MS
γ-Octalactone	104-50-7	40.355	1374		2.23E + 05	nd	nd	MS
δ-Octalactone	124-13-0	41.37	1410		4.51E + 05	7.79E + 05	nd	MS
γ-Nonalactone	104-61-0	43.3	1483	1492.9	2.50E + 05	1.81E + 05	nd	MS, RI
δ-Nonalactone	3301-94-8	44.32	1527		nd	1.95E + 04	nd	MS
δ-Decalactone	705-86-2	47.65	1685	1620.9	8.83E + 05	1.70E + 06	1.09E + 05	MS, RI
Terpenes								
o-Xylene*	95-47-6	23.82	886	891.4	nd	1.78E + 05	nd	MS, RI
p-Xylene*	106-42-3	25.29	923	928.1	2.02E + 05	2.78E + 05	nd	MS, RI
Styrene	100-42-5	25.31	923	929.2	nd	7.94E + 04	nd	MS, RI
α-Pinene	80-56-8	26.365	950	955.6	1.17E + 06	1.07E + 06	1.76E + 05	MS, RI
β-Pinene*	127-91-3	28.21	998		1.75E + 05	nd	nd	MS
Sabinene	13466-78-9	28.27	999		1.66E + 05	nd	nd	MS
3-Carene	13466-78-9	29.29	1028	1034.4	8.68E + 05	nd	nd	MS, RI
d-Limonene	5989-27-5	29.985	1047	1053.9	1.24E + 06	1.90E + 06	6.22E + 04	MS, RI
o-Cymene*	527-84-4	30.1	1051	1057.1	3.65E + 05	7.44E + 05	2.83E + 04	MS, RI
Linalool	78-70-6	33.105	1138	1145.2	4.56E + 05	2.99E + 05	nd	MS, RI
Phenols								
Phenol	13127-88-3	31.415	1088	1093.9	7.72E + 05	nd	nd	MS, RI
2-Methoxy-4-vinylphenol	7786-61-0	41.105	1400	1410.6	4.12E + 04	1.97E + 05	5.20E + 04	MS, RI
2,4-Di-tert-butylphenol	96-76-4	46.355	1624		5.15E + 05	6.00E + 05	nd	MS
Other								
Carbon disulfide	75-15-0	8.91	545	548.3	9.69E + 05	1.13E + 07	5.32E + 05	MS, RI
Toluene	108-88-3	19.71	790	794.4	1.10E + 06	3.09E + 05	1.08E + 05	MS, RI
Methyl acetylacetate	105-45-3	25.817	936		nd	nd	8.45E + 06	MS
1-Octene	111-66-0	24.47	901		4.34E + 04	nd	nd	MS
2-Acetylthiazole	24295-03-2	31.14	1080		nd	nd	5.88E + 04	MS
Corylon (Maple Lactone)	80-71-7	31.795	1098	1105	3.59E + 05	nd	nd	MS, RI
Glycerin	56-81-5	32.015	1105		4.11E + 06	7.82E + 06	nd	MS
2-Acetylpyrrole	1072-83-9	33.445	1148		2.76E + 06	nd	nd	MS
2-Pyrrolidinone (γ-Butyrolactam)	616-45-5	34.685	1185	1193.4	1.71E + 06	nd	nd	MS, RI
2-Phenylethanol	60-12-8	34.935	1193	1201.2	6.12E + 06	2.38E + 06	nd	MS, RI
Maltol	118-71-8	35.03	1196		9.09E + 05	4.90E + 06	nd	MS
3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	28564-83-2	36.265	1236		7.08E + 05	1.27E + 07	nd	MS
2-Phenyl-2-butenal	4411-89-6	39.905	1358		2.73E + 05	nd	nd	MS
2,5-Dihydrothiophene	1708-32-3	40.545	1381		6.89E + 04	nd	nd	MS
5,6-Dihydro-2H-pyran-2-one	3393-45-1	40.895	1393		6.59E + 05	nd	nd	MS
Glycerine diacetate	102-62-5	41.225	1405		nd	3.14E + 04	nd	MS
6-Pentyl-5,6-dihydro-2H-pyran-2-one	54814-64-1	47.155	1662		2.25E + 05	nd	nd	MS

rt, retention time; *RI*, retention indexes; *REF RI*, retention index from internal library; *nd*, not detected in sample. Compounds marked with an * indicate isomer identification that is not confirmed

compounds in comparison to HS-SPME in other studies (Murat et al. 2012; Majcher and Jeleń 2009). Similarly, the number of volatiles identified in shortbread biscuits (99) demonstrated the efficacy of the method in comparison to previous studies that found 24 compounds in butter cookies (Giarnetti et al. 2015) and 60 and 56 in wheat biscuits, respectively (Pasqualone et al. 2014; Pasqualone et al. 2015). To our knowledge, no studies have been published on the volatile profile of chocolate brownies to date. Although a direct comparison of the optimised HS-SPME method to other extraction methods using the same samples was outside the scope of this study, the results are at worst comparable but typically better than published studies on similar baked confectionary matrices.

Conclusion

Application of RSM enabled the optimisation of an HS-SPME method to extract a range of aromatic volatiles from baked confectionery products. Fibre type, extraction time and extraction temperature were shown to have the most pronounced effect on the extraction of 18 selected volatile compounds from a sponge cake

matrix. The optimal and validated conditions derived for HS-SPME analysis of baked confectionery were an incubation time of 5 min and an extraction time of 60 min at an extraction temperature of 60 °C. This method is suitable to study the volatile profile of a wide range of baked confectionary products.

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Compliance with Ethical Standards

Conflict of Interest Garvey E.C. declares that he has no conflict of interest. O'Sullivan M.G. declares that he has no conflict of interest. Kerry J.P. declares that he has no conflict of interest. Kilcawley K.N. declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent is not applicable.

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Characterising the sensory quality and volatile aroma profile of clean-label sucrose reduced sponge cakes

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ABSTRACT

The sensory and aroma quality of 30% (w/w) sucrose reduced sponge cakes incorporating clean-label replacers were investigated. The sensory quality of the reformulated sponge cakes varied, with those containing apple pomace powder (APP) showing the greatest difference to the control (SC100). Volatile profiles mainly differed in relation to compounds derived from the Maillard reaction, caramelisation and lipid oxidation. Thrity six aroma active volatile compounds were identified in the SC100, APP and oligofructose (OLIGO) sponge cakes by olfactometry. Furfural 'spicy bready' contributed most to the overall aroma of all samples, with factor dilution values differing the most for heptanal 'fatty cake crust', methional 'potato damp', and 2,5-dimethylpyrazine 'cake crust, nutty'. This study provides an in-depth insight into the impact of sugar reduction reformulation on the sensory perception of sponge cakes and demonstrates how this approach can be used to improve the sensory perception of reduced sucrose sponge cakes.

1. Introduction

With increasing awareness of dietary sugar intake and associated chronic diseases (obesity and type II diabetes), there is a need to reformulate foods to reduce the refined sugar content. Baked confectionery products are a prime matrix to explore alternative sucrose replacers due to the critical functionality of sucrose to the desirable structure and organoleptic properties. Sucrose replacement in these products has been extensively reviewed (Garvey, O'Sullivan, Kerry, & Kilcawley, 2020a; Struck, Jaros, Brennan, & Rohm, 2014). Trends of sucrose reduction/replacement in the past mainly consisted of the incorporation of artificial sweeteners and/ or sugar alcohols (polyols), due to their ability to mimic sucrose in terms of functionality and sweetness (Martínez-Cervera, Salvador, & Sanz, 2014; Ronda, Gómez, Blanco, & Caballero, 2005; Zoulias, Piknis, & Oreopoulou, 2000). However, current trends are shifting towards a more 'clean' mechanism of sucrose reduction, as manufacturers include statements such as 'free from artificial additives' on their products to evoke consumer interest and purchase intent (Asioli et al., 2017).

Although no concrete definition for clean-label ingredients/ products is established, foods of natural origin, organic, or free from additives/ preservatives are generally deemed superior by consumers. Similarly, by-products which contribute specific functional properties (e.g. antioxidant, added fibre) are also highly regarded by consumers due to the attraction of sustainable production practises and potential health benefits (Pasqualone et al., 2014). There has been a wide spectrum of studies involving the use of clean-label ingredients; OptiSol™, a natural ingredient derived from flaxseed, was used to replace 30% fat and was found to be capable of producing a clean-label sponge cake with similar physiochemical and sensory properties to that of a control sponge cake (Eslava-Zomeño, Quiles, & Hernando, 2016). Steviol glycosides, from *Stevia rebaudioside*, were combined with inulin or polydextrose to replace 30% sucrose in a muffin with the resulting products providing similar sensory attributes to a control without sucrose reduction (Zahn, Forker, Krügel, & Rohm, 2013). Fructans such as inulin and oligofructose are also widely used as functional and clean-label ingredients due to their prebiotic classification and are found naturally in the chicory plant. Fructans, due to their level of sweetness, also have potential as sucrose replacers in products. Volpini-Rapina, Sokei, and Conti-Silva (2012) added an Orafti® Synergy1 ingredient, an inulin/ oligofructose mixture, to orange cakes and found it impacted on the crust colour, dough colour and cake hardness, but did not affect the

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'orange aroma', 'orange flavour', or 'sweet taste'. Consumers also preferred the reformulated cake over a comercial cake. Following the addition of the same ingredient to gluten-free chocolate cookies, da Silva and Conti-Silva (2018) reported an increase in 'caramel aroma', 'chocolate aroma', 'sweet taste' and 'caramel flavour' with increasing replacement of rice flour on a w/w basis. This indicates the potential of fructans as a clean-label sucrose replacement ingredient to positively influence the aroma and flavour of baked confectionery products. As stated, food industry by-products are an attractive option as sucrose replacers due to the sustainability of utilising and reducing food waste, the potential of contributing added nutritional value, and the fact they are clean-label. Fruit pomaces are by-products of juice/ alcoholic beverage processing and are comprised mainly of peel and seeds, contributing additional advantages of added fibre and possible antioxidant activity (Ktenioudaki & Gallagher, 2012). The application of apple pomace powder as an ingredient in bakery products has been successfully achieved for biscuits (Alongi, Melchior, & Anese, 2019) and sponge cakes (Sudha, Baskaran, & Leelavathi, 2007).

Sucrose imparts a clean, sweet taste appreciated by consumers and previous studies exploring 100% sucrose replacement in baked confectionery frequently report a decline in sensory quality (Ronda et al., 2005). Identification of odour active aroma compounds in reduced sucrose products, in comparison to a standard sucrose control product, is likely to provide important information on factors impacting sensory quality. For example, Pasqualone et al. (2014) identified higher levels of 'favourable' biscuit compounds; benzaldehyde (cherry/almond), phenylacetaldehyde (floral/honey) and furans; 2-methylfuran, 2-acetylfuran, 5-methylfurfural and 2-furanmethanol (sweet/caramel), after the addition of grape marc (a by-product of wine fermentation). Correlating sensory and volatile data aids in establishing how raw materials influence aroma and thus the flavour of baked confectionery products. This approach in theory, can aid in the development of optimal reduced sugar products from a consumer perspective by potentially manipulating ingredients to evoke a desired sensory response or by eliminating or reducing mal-odours. This is particularly relevant for products of the Maillard reaction (MR) and caramelisation (CR), as they are important to the overall aroma of baked confectionery products (Garvey et al., 2020a). Gas chromatography-olfactory (GC-O) is a technique utilised to aid in the identification of aroma compounds that are actually contributing to sensory perception, and thus can provide very useful additional information in comparative studies. Although the volatile profiles of different baked confectionery products have been explored, to the best of our knowledge, no studies on how sucrose reduction/replacement impacts the volatile aroma profile of sponge cakes have been published.

Therefore, the objective of this study was to explore the impact of clean-label sucrose alternatives (apple pomace, whey permeate, and oligofrucrose) on the sensory properties and volatile profile of 30% w/w reduced sucrose sponge cakes. To achieve this we used an optimised headspace solid-phase microextraction gas-chromatography mass-spectrometry (HS-SPME-GC–MS) method to determine their volatile profile (Garvey, O'Sullivan, Kerry, & Kilcawley, 2020b), with GC-O and ranking descriptive analysis (RDA) to determine key volatile changes that influence sensory perception.

2. Materials and methods

2.1. Analytical standards

Olfactory training standards were of analytical grade; ethyl butyrate, octanal, *p*-cresol, and dimethyl disulphide and heptanal of ≥99% and ≥95% purity respectively (Merck Ireland, Arklow, Co. Wicklow, Ireland), were prepared at 0.3% (w/v) in methanol and stored at −18 °C until required. For each GC-O training session, a stock solution was diluted to 0.03% (w/v) in distilled water to allow the odours to be of adequate potency.

External standards used to confirm the identity of odour active compounds were also of analytical grade; linalool, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol), 2-nonanone, and 2-methylpyrazine (Merck Ireland). For linalool, 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 2-nonanone, a 0.3% (w/v) solution was prepared in methanol, with a 0.5% (w/v) solution in methanol prepared for 2-methylpyrazine. As above, for adequate potency, these compounds were analysed at 0.03% (w/v) and 0.05% (w/v) in distilled water, respectively.

2.2. Sponge cake preparation

Five sponge cake formulations (Table S1) and baking conditions described by Milner, Kerry, O'Sullivan, and Gallagher (2020) were employed. A number of preliminary baking trials, involving incremental reduction of sucrose levels, with the aim of maximum sucrose reduction without adverse changes to sponge cake structure were undertaken. Physiochemical analysis was carried out on the resulting products and the ideal sucrose replacement level was established at 30%, which enables these products to be classified as reduced sugar. A sponge cake formula with 30% reduced sugar was referred to as sucrose reduction 70% (SR70). This formed the base formulation for the clean-label reduced sucrose sponge cakes (replaced by 5% replacer on a flour weight basis). The sucrose replacers incorporated into this base sponge cake formula were apple pomace powder (APP), whey permeate powder (WPP), and oligofrucrose (OLIGO). The control sponge cake (100% sucrose), was referred to as SC100.

Plain flour (200 g) (Odlums, Ireland) and baking powder (4 g) (Dr. Oekter, UK) were sifted into a bowl followed by the addition of caster sugar (110 g for control, 77 g for sucrose reduced) (Súcra, Nordzucker, Germany), sucrose replacer (10 g), cake margarine (90 g) (Stork, UK), free range eggs (90 g) (local retailer), and water (70 g). The contents were mixed using a household mixer (Kenwood Mixer, Model KMM710, UK) at speed 1 for 30 s, scraped and mixed again for a further 2 min at speed 2. Miniature loaf tins (80 mm × 60 mm × 40 mm) were filled with 80 g of cake batter and baked at 180 °C for 45 min in a domestic convection oven (Zanussi, Bedfordshire, UK). The cakes were left to cool and placed in sealed storage bags until subsequent volatile, GC-O or sensory analysis (which took place within 24 h after baking).

2.3. Sensory evaluation

Hedonic sensory evaluation and ranking descriptive analysis were carried out with 30 consumers recruited from Teagasc Food Research Centre (Moorepark, Ireland), aging from 22 to 50. Panellists were chosen based on their frequency to consume sponge cake and familiarity with sensory evaluation, but did not receive formal training. Evaluation took place in accordance to international standards (ISO 11136, 2014), where panellists were presented with the five sponge cake formulations simultaneously. Samples were presented on white paper plates with randomised three digit codes assigned to each sample, alongside water and a saltine cracker for palate cleansing. Panellists were asked to rate their liking of each sample based on the colour, odour, flavour, texture and overall acceptability on a nine-point hedonic scale, which ranged from "9 = extremely like" to "1 = extremely dislike". Once the hedonic portion of the evaluation was completed for a sample, panellists were prompted to evaluate the same sample cake on its sensory attributes, using a ranking descriptive analysis (RDA) method. Attributes were generated by a focus group consisting of 7 people, comprised of members from the Food Quality and Sensory Science department at the Teagasc Food Research Centre. The established list of attributes was chosen based on their relevance to sponge cakes, whilst having descriptors targeted at profiling the formulated samples (Table S2). Panellists were briefly coached on the explanation of each attribute in relevance to sponge cake, and asked to evaluate the intensity of each on a 9 cm continuous scale. Sensory analysis was conducted in duplicate over two separate occasions.

2.4. Volatile analysis

Volatile analysis was carried out as described by Garvey et al. (2020b). Each sponge cake was sliced vertically and 1 cm of the outer crust was removed. Cake crumb (3 g) was added to an amber 20 mL screw capped headspace vial (Apex Scientific Ltd, Co.Kildare, Ireland) and equilibrated for 5 min, at 60 °C with pulsed agitation for 5 s at 350 rpm, using the Gerstal MultiPurpose Sampler (GMPS) agitator/heater. Volatile analysis was carried out utilising a GMPS rail system (Anatune, Cambridge CB3 0NA, UK) connected to a Shimadzu GP2010 plus gas chromatograph (GC) (Mason Technology Ltd, Dublin, Ireland) using headspace solid-phase microextraction (HS-SPME). The SPME fibre; 30/50 μm DVB/CAR/PDMS (Suplco), was exposed to the headspace above the samples, at a depth of 21 mm, for 60 min at 60 °C. The fibre was retracted, injected into the GC inlet and desorbed for 3 min at 250 °C using the GMPS fibre bakeout station. Each sponge cake formula was analysed in triplicate.

2.5. Identification of odour active compounds by gas chromatography-olfactometry

HS-SPME-GC-O analysis was only undertaken on APP and OLIGO sponge cakes, and compared to the control (SC100). APP was chosen as it demonstrated obvious contrasts in sensory results, whereas OLIGO showed similarities to both APP and SC100 sponge cakes. Sponge cake samples were sliced and blitzed in a food processor (NutriBullet 600, Australia) to combine cake crust and crumb uniformly to represent a masticated sample. Three sniffer assessors were chosen for the GC-O analysis based on their performance in an olfactory assessment using three different Sniffin' Sticks tests (identification, discrimination, and threshold) (Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997). Prior to sample analysis, the panellists were exposed to a standard stock solution (as described in 2.1), designed for GC-O training, comprised of 5 compounds; dimethyl disulphide ('*sulphur*','*decomposing*'), ethyl butyrate ('*fruity*', '*pineapple*'), heptanal ('*fatty*', '*green*'), octanal ('*orange*', '*fruity*') and ρ-cresol ('*barnyard*'). This step allowed panellists to familiarise themselves with the GC-O process and software, as well as the range of odours they could potentially encounter during the GC-O analysis of sponge cake samples.

Multiple batches (a minimum of three) of each freshly baked sponge cake formula were produced as required. Volatile extraction was carried out by the HS-SPME procedure (as previously described) using 3 g of the crumb and crust mixture. GC-O analyses was performed on an Agilent 7890 GC with a flame ionization detector, 5973N mass detector (Agilent Technologies, Ltd, Cork, Ireland), and an Gerstel ODP-3 olfactory detector port (Anatune Ltd, Cambridge, UK). The volatile compounds were separated on DB-624 UI (20 m × 1.8 mm × 1 μm) (Agilent Technologies Ltd, Ireland) column. Eluting compounds were split 1:1:1 into the MS detector, flame ionisation detector and the sniffing port simultaneously by means of a column flow splitter. The carrier gas was helium, held at a pressure of 9.8 psi and a flow rate of 1.209 mL min^{-1}. GC conditions consisted of an initial oven temperature of 80 °C, held for 2 min and increased at 10 °C/min to 220 °C. The GC run time was shortened to 21 min to reduce the risk of assessors experiencing fatigue during a sniffing session (however it still encompassed the volatile range of interest). In addition, the transfer line to the sniffing port was conditioned with humidified air to reduce olfactory fatigue and prevent the occurrence of condensation droplets collecting in the nasal port. Panellists conducted GC-O analysis of each sponge cake in duplicate. The ion source temperature was 220 °C and the interface temperature was set at 260 °C. The MS mode was electronic ionization (70 eV) with the mass range scanned between m/z 35–250. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, comparison of LRI to the mid polar column from the previous analysis and to standards where possible. Spectral de-convolution was also performed to confirm identification of compounds using AMDIS. If an aroma was detected by at least 3 out of 6 assessments, it was established as odour active (Koutidou, Grauwet, Van Loey, & Acharya, 2017).

To determine the threshold at which each odour active compound could be perceived, Aroma Extraction Dilution Analysis (AEDA) was carried out by manipulation of the GC injection split ratio (Feng et al., 2015). The operating mode of GC analysis was changed from splitless to split injection and the split ratio was adjusted to 1:1, 1:2, 1:5, 1:10, 1:20, 1:50, 1:100 and 1:150, allowing for adequate dilution to determine the most odour active compounds in the sample. Undertaking AEDA using the splitless approach to dilute removes any potential matrix effects that can occur if the sample itself was diluted. The assessor who demonstrated the highest olfactory perception in the previous analysis was chosen for the AEDA study. The last split ratio at which a compound could be detected was referred to as the factor dilution (FD) for that compound.

2.6. Statistical analysis

Data analysis was handled accordingly based on the normality of the data. Hedonic scale data was analysed using Welch test with post hoc Games-Howell. Analysis of variance (ANOVA) with post hoc Tukey significant test was applied to RDA data; both analyses were conducted working at an alpha level of 0.05. Volatile data was treated with ANOVA or Welch test, based on the result of Levenes test (specified in Table 2), with difference in means identified with Tukey or Games Howell post hoc test, respectively, both working at an alpha level of 0.05. Statistical analysis was performed using IBM SPSS Statistics 24 for windows (SPSS Inc., IBM Corporation, NY, USA). Principle component analysis (PCA) was constructed using the "factoextra" and "FactoMinoR" packages in R (v 3.4.1, R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. Sensory quality of sponge cakes

The average results of the sensory evaluation of reformulated sponge cakes are presented in Table 1. Overall, the sucrose reduced formulas (SR70, WPP, and OLIGO) were not perceived significantly different in terms of liking of colour, odour, flavour, texture and overall liking, compared to SC100. However, the APP sponge cake scored significantly ($P < 0.05$) lower for all attributes. The texture and flavour of the SR70 sponge cake was not significantly different ($P > 0.05$) from the APP sponge cake, highlighting reduced consumer acceptance of the SR70 sponge cake.

RDA was undertaken to further interpret the difference in each formulated sponge cake. Panellists were asked to assess the attributes relative to colour (crumb colour & crust colour), odour (vanilla, fresh cake, nutty & roasty), flavour (sweet, toasty, off-flavour & aftertaste) and texture on chewing. For crust colour and crumb colour, the APP sponge cake was ranked significantly ($P < 0.05$) darker (0 = very light, 9 = very dark), compared to all other samples (Fig. 1). However, both the OLIGO and WPP sponge cakes were also perceived significantly ($P < 0.05$) darker to SC100 and SR70 sponge cakes for crust colour and crumb colour.

Pomace refers to a combination of apple peel, pulp and seed, resulting in a reddish/brown dried powder raw material, thus contributing to the overall darker colour of the APP cake. However, the individual sugars present in apple pomace are comprised mainly of fructose (Milner et al., 2020). This reducing sugar is likely accelerating MR and CR reactions during baking, and contributing to the darker crust and crumb of the APP sponge cake. The darker colour of the APP sponge cake may also explain the low hedonic score achieved for cake colour. Thus, in this study a 5% w/w addition of apple pomace on a flour weight basis was perceived negatively by panellists. However,

Table 1
Average (n = 2) results and standard deviation of Hedonic and Ranking Descriptive Analysis evaluation of control sponge cake and sucrose reduced formulas. Values with a change in letter indicate significant difference ($P < 0.05$).

	SC100	SR70	APP	WPP	OLIGO
Hedonics					
Colour	7.77 ± 0.85 [a]	7.47 ± 0.96 [a]	5.05 ± 1.90[b]	7.40 ± 0.79 [a]	7.72 ± 0.75 [a]
Odour	7.20 ± 0.86 [a]	6.87 ± 1.01 [a]	5.35 ± 1.41[b]	7.10 ± 1.00 [a]	7.13 ± 1.05 [a]
Flavour	7.23 ± 0.86 [a]	6.52 ± 1.20 [ab]	5.35 ± 1.94[b]	6.92 ± 1.04 [a]	7.07 ± 1.14 [a]
Texture	6.73 ± 1.04 [a]	6.17 ± 1.31 [ab]	5.40 ± 1.78[b]	6.83 ± 1.17 [a]	6.77 ± 1.48 [a]
Overall Liking	7.18 ± 0.80 [a]	6.55 ± 1.24 [a]	5.15 ± 1.83[b]	7.05 ± 1.01 [a]	7.13 ± 1.21 [a]
Ranking Descriptive Analysis					
Colour					
Crust Colour (0 = very light, 9 = very dark)	4.4325 ± 1.06[d]	4.33 ± 0.98 [d]	7.53 ± 1.09 [a]	6.12 ± 1.18[b]	5.56 ± 1.38[b]
Crumb Colour (0 = very light, 9 = very dark)	3.27 ± 1.25[c]	3.16 ± 1.34[c]	7.23 ± 0.82 [a]	3.56 ± 1.04[c]	4.42 ± 1.23[b]
Odour					
Vanilla Odour	3.09 ± 2.04 [a]	3.17 ± 1.79 [a]	2.27 ± 1.61 [a]	3.10 ± 1.87 [a]	2.97 ± 1.75 [a]
Fresh Cake Odour	5.28 ± 1.77 [a]	4.88 ± 1.90 [a]	3.57 ± 1.72[b]	4.88 ± 1.70 [a]	4.48 ± 2.05 [ab]
Nutty Odour	2.54 ± 1.52[b]	2.73 ± 1.80[b]	4.21 ± 1.83 [a]	3.07 ± 1.89 [ab]	3.11 ± 1.77 [ab]
Roasty Odour	2.74 ± 1.57[b]	2.78 ± 1.54[b]	4.81 ± 1.95 [a]	3.38 ± 1.95[b]	3.50 ± 1.73[b]
Flavour					
Sweet Flavour	5.27 ± 1.46 [a]	4.45 ± 1.62 [ab]	3.75 ± 1.68[b]	4.70 ± 1.58 [ab]	4.72 ± 1.70 [ab]
Toasty Flavour	2.56 ± 1.47[b]	2.84 ± 1.72[b]	4.30 ± 1.89 [a]	3.40 ± 1.98 [ab]	3.28 ± 1.88 [ab]
Aftertaste	2.65 ± 1.63 [a]	2.81 ± 1.62 [a]	3.68 ± 1.96 [a]	2.87 ± 1.77 [a]	2.98 ± 1.79 [a]
Off-Flavour	1.34 ± 0.71[b]	1.46 ± 0.81 [ab]	2.32 ± 1.51 [a]	1.45 ± 0.72 [ab]	1.56 ± 1.10 [ab]
Texture					
Texture on Chewing (0 = very dry, 9 = very moist)	4.51 ± 1.39 [ab]	4.30 ± 1.70 [ab]	3.62 ± 1.44[b]	4.68 ± 1.33 [a]	4.68 ± 1.45 [a]

SC100 = Sucrose Control, SR70 = Sucrose Reduced, APP = Apple Pomace Powder, WPP = Whey Permeate Powder, OLIGO = Oligofructose

addition of 20% apple pomace powder to sponge cakes (Sudha et al., 2007) and 15% to cookies (Toledo, Nunes, Silva, Spoto, & Canniatti-Brazaca, 2017) did not negatively impact consumer's perception of colour/appearance liking. However, this is likely also related to differences in the apple pomace powders used.

Similarly, oligofructose contains approximately 5–6% free sugars consisting of glucose, fructose and sucrose (Milner et al., 2020), and as lactose is the primary carbohydrate component of whey permeate, it can be reasoned that the reducing sugars present in these formulas are likely responsible for the significant ($P < 0.05$) difference in colour perception, in comparison to the SC100 and SR70 sponge cakes. Similar outcomes were evident when oligofructose was added to orange cakes (Volpini-Rapina et al., 2012) and chocolate cookies (da Silva & Conti-Silva, 2018).

'Fresh cake' odour was rated the highest for the SC100 sponge cake, however, only the APP sponge cake was rated significantly ($P < 0.05$) lower. Again, the low association of 'fresh cake' odour with the APP sponge cake may correspond to the low liking of APP odour in the hedonic scale evaluation. The APP sponge cake scored significantly ($P < 0.05$) higher for 'nutty' and 'roasty' odour compared to SC100 and SR70, which may indicate that these odour qualities may dominate over the 'fresh cake' odour, in this sample. Torbica, Škrobot, Janić Hajnal, Belović, and Zhang (2019) also reported that addition of 10% apple pomace powder to wholegrain wheat bread resulted in trained panellists perceiving the formulated bread to have a lower association with 'cereal aroma'. It is interesting to note that the clean-label reduced sucrose sponge cakes, WPP, and OLIGO were not perceived significantly different to APP for 'nutty' odour. The darker appearance of the formulated cakes APP, WPP and OLIGO, may be also influencing odour perception (Maric & Jacquot, 2013).

The reduced sucrose sponge cakes; SR70, WPP, and OLIGO, were not perceived to be significantly ($P > 0.05$) different to SC100 in terms of 'sweet' flavour. However, the APP sponge cake was perceived significantly ($P < 0.05$) less sweet than the SC100 sponge cake, despite all sucrose replacers containing equal levels of sucrose. Alongi et al. (2019) incorporated APP, 20% w/w on a wheat flour basis, into a shortbread biscuit, without a decline in the perception of sweetness. 'Toasty' flavour was perceived significantly ($P < 0.05$) stronger in the APP sponge cake compared to the SC100 and SR70 sponge cakes. 'Toasty' flavour of the WPP and OLIGO sponge cakes were not statistically ($P > 0.05$) different to the APP sponge cake. It is plausible that the higher scores for 'nutty' odour and 'toasty' flavour are also linked to MR and CR reactions due to the presence of reducing sugars in the formulations.

The APP sponge cake scored significantly ($P < 0.05$) higher for 'off-flavour' compared to SC100, which may be due to a 'fruit flavour' often associated with apple pomace (Alongi et al., 2019) (however we did not capture additional details from the panellists as to the nature of the perceived off-flavour). The aim of including the term 'off-flavour' was to identify any unconventional tastes not usually associated with a traditional sponge cake. No significant differences ($P > 0.05$) were identified for 'aftertaste' or 'vanilla' odour between all the sponge cakes.

In terms of 'texture on chewing' the APP sponge cake was perceived as the driest (closest to "very dry" on scale) compared to the SC100, WPP and OLIGO sponge cakes, however there was no significant difference between the APP and SR70 sponge cakes. As none of the sponge cakes scored higher than 5 on the RDA scale, panellists did not perceive them to be overly moist. Instrumental measurements for moisture content on identical sponge cake preparations (Milner et al., 2020) found that there was no significant difference between the moisture contents of these formulations. The desired soft texture of a sponge cake is partially due to the typical moisture content of 20–30%. As sucrose is known to play an important role in moisture retention (Struck et al., 2014), sucrose replacement can therefore adversely impact texture (Martínez-Cervera et al., 2014; Ronda et al., 2005), leading to a decrease in palatability (Martínez-Cervera et al., 2014). Although there was no significant ($P > 0.05$) difference in the instrumental moisture measurement of the cake formulas, the APP sponge cake was perceived significantly drier during mastication compared to other formulas, which also likely contributes to the low hedonic score for texture (Table 1). Apple pomace powder is often incorporated into bakery products to enhance the nutritional value through added fibre and polyphenols (Sudha et al., 2007). As APP had the highest fibre content out of the five formulated sponge cakes (Milner et al., 2020), it also likely contributes to the increased perception of dryness during

Table 2
Average (n = 3) peak area values (x10^6) of selected volatile compounds identified in control (SC100), and reduced sucrose reduced (SR70, APP, WPP, OLIGO) sponge cakes.

Compound	#CAS	RI	SC100	SR70	APP	WPP	OLIGO
Ketones							
2,3-Butanedione	431-03-8	632	2.98 ± 0.785	2.46 ± 0.189	2.23 ± 0.295	2.28 ± 0.260	3.13 ± 0.316
1-Penten-3-one[1]	1629-58-9	727	0.035 ± 0.005^{ab}	0.039 ± 0.008^{a}	0.017 ± 0.002^{b}	0.021 ± 0.004^{b}	0.016 ± 0.002^{b}
1-Hydroxy-2-propanone (Acetol)	116-09-6	737	5.19 ± 1.796	2.38 ± 1.194	0.823 ± 0.110	4.47 ± 2.981	14.9 ± 9.989
2,3-Pentanedione[1]	600-14-6	736	1.11 ± 0.557^{ab}	0.561 ± 0.212^{b}	0.813 ± 0.110^{b}	0.306 ± 0.063^{b}	1.82 ± 0.302^{a}
2-Heptanone[1]	591-78-6	936	7.97 ± 2.226^{b}	10.5 ± 1.542 ^{ab}	9.15 ± 2.165^{b}	14.1 ± 1.353 ^{a}	8.11 ± 0.814^{b}
2-Nonanone	821-55-6	1141	1.77 ± 0.425	1.87 ± 0.298	1.81 ± 0.336	2.04 ± 0.116	1.86 ± 0.288
3,5-Octadien-2-one	30086-02-3	1163	0.751 ± 0.007	0.0682 ± 0.007	0.0733 ± 0.007	0.0878 ± 0.006	0.0740 ± 0.009
Aldehydes							
2-Methylpropanal	78-84-2	594	0.14 ± 0.045	0.116 ± 0.026	0.0888 ± 0.032	0.112 ± 0.036	0.0582 ± 0.010
3-Methylbutanal	590-86-3	693	1.09 ± 0.279	0.774 ± 0.128	0.713 ± 0.193	0.638 ± 0.135	0.671 ± 0.159
2-Methylbutanal	96-17-3	701	0.866 ± 0.215	0.624 ± 0.101	0.594 ± 0.151	0.596 ± 0.108	0.486 ± 0.072
(E)-2-Pentenal[1]	1576-87-0	807	0.146 ± 0.019^{ab}	0.149 ± 0.006^{ab}	0.161 ± 0.018^{a}	0.135 ± 0.010^{ab}	0.121 ± 0.009^{b}
Hexanal[1]	66-25-1	840	3.39 ± 0.765^{abc}	4.30 ± 0.418^{ab}	3.11 ± 0.631^{bc}	4.76 ± 0.724^{a}	2.72 ± 0.215^{c}
Heptanal[1]	111-71-7	944	10.0 ± 2.874^{b}	21.3 ± 3.657^{a}	9.77 ± 2.603^{b}	22.3 ± 2.503^{a}	12.9 ± 1.135^{b}
Methional[1]	3268-49-3	974	0.0178 ± 0.002^{c}	0.0270 ± 0.017 ^{bc}	0.0902 ± 0.017 ^{a}	0.0551 ± 0.002^{abc}	0.0711 ± 0.028^{ab}
Benzaldehyde[1]	100-52-7	1032	2.27 ± 0.296^{ab}	1.56 ± 0.268^{b}	2.60 ± 0.561^{ab}	2.22 ± 0.408^{ab}	3.25 ± 0.592^{a}
Octanal[1]	124-13-0	1048	0.346 ± 0.077 ^{a}	0.398 ± 0.023 ^{a}	0.277 ± 0.034^{b}	0.418 ± 0.054 ^{a}	0.347 ± 0.039 ^{ab}
(E)-2-Octenal[2]	13019-16-4	1121	0.479 ± 0.035 ^{bc}	0.378 ± 0.022^{c}	0.831 ± 0.018 ^{ab}	0.498 ± 0.025 ^{bc}	0.571 ± 0.119^{b}
Phenylacetaldehyde[2]	122-78-1	1122	0.479 ± 0.035^{b}	0.290 ± 0.033^{c}	0.688 ± 0.031 ^{a}	0.498 ± 0.025^{b}	0.571 ± 0.119 ^{ab}
(E)-2-Nonenal[2]	18829-56-6	1226	0.109 ± 0.016^{b}	0.143 ± 0.025^{b}	0.394 ± 0.052 ^{a}	0.136 ± 0.004^{b}	0.153 ± 0.055^{b}
Vanillin	121-33-5	1544	0.022 ± 0.003	0.0229 ± 0.004	0.0341 ± 0.010	0.028 ± 0.004	0.0361 ± 0.005
Furans							
2-Methylfuran[2]	534-22-5	623	0.017 ± 0.003^{b}	0.021 ± 0.001^{b}	0.014 ± 0.004^{b}	0.072 ± 0.023^{a}	0.013 ± 0.002^{b}
2-Ethyl-5-methylfuran[1]	1703-52-2	817	0.054 ± 0.010 ^{a}	0.059 ± 0.008 ^{a}	0.017 ± 0.006^{c}	0.061 ± 0.012^{a}	0.030 ± 0.002^{b}
Dihydro-2-methyl-3(2H)-furanone[2]	3188-00-9	857	0.382 ± 0.029^{a}	0.137 ± 0.015^{b}	0.294 ± 0.067^{ab}	0.170 ± 0.042^{b}	0.583 ± 0.181^{ab}
Furfural[2]	98-01-1	899	1.38 ± 0.406^{b}	0.513 ± 0.086^{b}	3.33 ± 0.210^{a}	3.86 ± 0.716^{a}	1.40 ± 0.607^{b}
2-Furanmethanol	98-00-0	929	1.23 ± 0.764	1.04 ± 0.804	1.14 ± 0.779	22.7 ± 13.269	2.87 ± 2.602
2-Acetylfuran	1192-62-7	978	0.179 ± 0.038^{c}	0.135 ± 0.016^{c}	0.591 ± 0.031^{b}	1.24 ± 0.178 ^{a}	0.355 ± 0.066^{c}
2-Pentylfuran	3777-69-3	1014	7.22 ± 2.287	6.04 ± 0.615	6.81 ± 2.027	6.98 ± 0.381	4.62 ± 0.892
2(5H)-Furanone[2]	497-23-4	1031	0.717 ± 0.183^{b}	0.694 ± 0.124^{bc}	0.430 ± 0.066^{b}	5.07 ± 0.377 ^{a}	0.780 ± 0.061^{b}
Pyrazines							
Pyrazine[2]	290-37-9	773	0.519 ± 0.020^{a}	0.267 ± 0.002^{bc}	0.215 ± 0.024^{b}	0.543 ± 0.079^{ac}	0.246 ± 0.045^{b}
Methylpyrazine[1]	109-08-0	864	3.07 ± 0.637 ^{a}	1.10 ± 0.069^{b}	1.78 ± 0.317^{b}	0.913 ± 0.146^{b}	3.37 ± 0.659 ^{a}
2,5-Dimethylpyrazine[2]	123-32-0	951	5.94 ± 2.416^{ab}	2.48 ± 0.573^{ab}	4.77 ± 0.974^{a}	0.481 ± 0.052^{b}	25.0 ± 6.466 ^{ab}
2,3-Dimethylpyrazine[1]	5910-89-4	962	0.253 ± 0.099^{a}	0.0941 ± 0.023^{b}	0.0968 ± 0.030^{b}	0.0646 ± 0.019^{b}	0.195 ± 0.053^{ab}
Trimethylpyrazine[1]	14667-55-1	1043	0.646 ± 0.281 ^{ab}	0.234 ± 0.068 ^{bc}	0.266 ± 0.051^{bc}	0.0392 ± 0.006^{c}	0.906 ± 0.232 ^{a}
3-Ethyl-2,5-dimethylpyrazine[1]	13360-65-1	1117	0.078 ± 0.035 ^{ab}	0.021 ± 0.015 ^{bc}	0.0307 ± 0.027^{bc}	0.0102 ± 0.006^{c}	0.0133 ± 0.021 ^{a}
Terpenes							
d-Limonene	5989-27-5	1056	0.717 ± 0.096	0.515 ± 0.036	0.705 ± 0.186	0.587 ± 0.083	0.834 ± 0.249
o-cymene[*1]	527-84-4	1059	0.218 ± 0.035^{ab}	0.163 ± 0.015^{b}	0.226 ± 0.033^{ab}	0.197 ± 0.024^{ab}	0.284 ± 0.056^{a}
p-cymene[*1]	99-87-6	1077	0.0267 ± 0.004 ^{ab}	0.0168 ± 0.003^{b}	0.0278 ± 0.009 ^{ab}	0.0223 ± 0.004 ^{ab}	0.0405 ± 0.010 ^{a}
Linalool[1]	78-70-6	1147	0.528 ± 0.148^{a}	0.436 ± 0.087^{ab}	0.121 ± 0.019^{c}	0.239 ± 0.041^{bc}	0.177 ± 0.035^{c}
Alcohols							
1-Penten-3-ol[1]	616-25-1	732	0.047 ± 0.004^{ab}	0.049 ± 0.005^{ab}	0.036 ± 0.004^{bc}	0.055 ± 0.005 ^{a}	0.024 ± 0.007^{c}
1-Hexanol[2]	111-27-3	919	0.31 ± 0.071^{b}	0.452 ± 0.126^{ab}	0.459 ± 0.486^{ab}	0.673 ± 0.025^{a}	0.501 ± 0.205^{ab}
1-Octen-3-ol	3391-86-4	1025	2.18 ± 0.385	2.00 ± 0.345	2.18 ± 0.295	2.47 ± 0.310	2.61 ± 0.369
2-Ethylhexanol	104-76-7	1079	0.025 ± 0.005	0.018 ± 0.010	0.031 ± 0.020	0.022 ± 0.009	0.037 ± 0.022
Lactones							
γ-dodecalactone[1]	706-14-9	1496	0.0342 ± 0.005^{b}	0.0377 ± 0.003^{b}	0.0376 ± 0.004^{b}	0.0562 ± 0.005^{a}	0.0532 ± 0.007^{a}
δ-decalactone[1]	705-86-2	1638	0.122 ± 0.016^{b}	0.108 ± 0.013^{b}	0.223 ± 0.036^{a}	0.118 ± 0.029^{b}	0.168 ± 0.019^{ab}
Other							
Toluene[2]	108-88-3	785	0.266 ± 0.066^{abc}	0.195 ± 0.011^{b}	0.276 ± 0.023^{a}	0.140 ± 0.013^{c}	0.236 ± 0.019^{ab}
Pyrrole[1]	109-97-7	835	0.0748 ± 0.012 ^{ab}	0.0523 ± 0.007 ^{bv}	0.0441 ± 0.009^{c}	0.0892 ± 0.016 ^{a}	0.0482 ± 0.002 ^{bc}
2-Acetylthiazole[2]	24295-03-2	1086	0.266 ± 0.066^{abc}	0.195 ± 0.011^{b}	0.276 ± 0.023^{a}	0.140 ± 0.013^{c}	0.236 ± 0.019^{ab}
2-Acetylpyrrole	1072-83-9	1158	0.0748 ± 0.012 ^{ab}	0.0523 ± 0.007 ^{bv}	0.0441 ± 0.009^{c}	0.0892 ± 0.016 ^{a}	0.0482 ± 0.002 ^{bc}
Dodecane	112-40-3	1203	0.266 ± 0.066^{abc}	0.195 ± 0.011^{b}	0.276 ± 0.023^{a}	0.140 ± 0.013^{c}	0.236 ± 0.019^{ab}
Maltol[2]	118-71-8	1211	0.101 ± 0.146^{b}	0.0563 ± 0.082^{b}	0.104 ± 0.099^{b}	0.853 ± 0.629^{a}	0.995 ± 1.231^{b}
2,3-Dihydro-3,5-dihydroxy-6-methyl 4(H)-pyran-4-one	28564-83-2	1249	0.0126 ± 0.0146	0.00241 ± 0.000	0.0232 ± 0.012	0.00843 ± 0.0021	0.343 ± 0.318

[1] Values for compound with a change in letter indicate significant difference identified using **ANOVA and Tukey post hoc test**
[2] Values for compound with a change in letter indicate significant difference identified using **Welch test and Games Howell post hoc test** Full list of identified compounds can be found in Supplementary Materials- Table S3. Compounds marked with an * indicate tentative identification due to isomer. SC100 = Sucrose Control, SR70 = Sucrose Reduced, APP = Apple Pomace Powder, WPP = Whey Permeate Powder, OLIGO = Oligofructose.

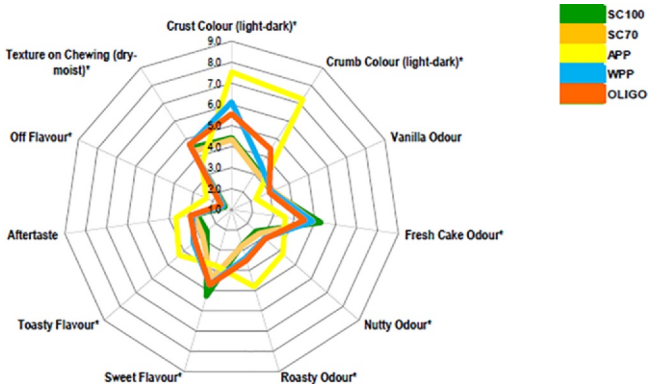


Fig. 1. Average results (n = 2) from ranking descriptive analysis of control sponge cake and sucrose reduced cakes formulated with clean-label sucrose replacers. Attributes annotated with * indicate significant difference ($P < 0.05$) within samples.

mastication (Fig. 1). SR70 was possibly perceived slightly drier than those sponge cakes with the added sucrose replacers (OLIGO and WPP) due to both the 30% reduction in sucrose and the lack of additional hygroscopic ingredients to enhance the perception of moisture.

3.2. Volatile aroma profile of sponge cakes

HS-SPME-GC–MS analysis of the sponge cakes identified a total of 77 volatile compounds across all sponge cakes (Table S3), with the compounds influenced the most by sucrose replacement outlined only in Table 2. Ketones, aldehydes, furans, pyrazine, terpenes and alcohols were the main chemical classes contributing to the volatile profile of the SC100 sponge cake and the reformulated reduced sucrose sponge cakes. To gain an initial insight into the differences, a PCA was undertaken with the strongest associations depicted in Fig. 2. The first two components of the PCA explain ~53% of the total variance among the samples. To gain further information, a difference in means using ANOVA or Welch test was applied. Some distinct trends were observed in relation to the volatiles responsible for the differences between these samples.

Pyrazine compounds, (pyrazine, methylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, trimethylpyrazine) were identified in high levels in the SC100 sponge cake (Table 2). Both the SC100 and OLIGO sponge cakes contained significantly ($P < 0.05$) higher levels of methylpyrazine and 2,3-dimethylpyrazine compared to all other samples. SC100, OLIGO and APP sponge cakes had the highest abundance of 2,5-dimethlypyrazine and trimethlypyrazine. Pyrazines are favourable in baked confectionery products for imparting 'roasty', 'nutty', 'cake crust' aromas (Matsakidou, Blekas, & Paraskevopoulou, 2010; Pozo-Bayón, Ruíz-Rodríguez, Pernin, & Cayot, 2007), and are formed by aminoketone degradation (Martins, Jongen, & Van Boekel, 2000), as a result of α-dicarbonyl and amino acid reactions in the early stages of the MR.

Furan derived compounds are formed primarily through sugar dehydration/ sugar fragmentation during the MR (Martins et al., 2000) or CR through direct decomposition of sugar moieties (Zhang et al., 2012). Their aroma impressions have been described as 'earthy', 'caramel-like', and 'biscuit' in sponge cakes (Matsakidou et al., 2010; Pozo-Bayón et al., 2007). The levels of furan compounds differed immensely amongst the samples (Table 2). The WPP sponge cake had significantly ($P < 0.05$) higher levels of 2-methylfuran, furfural and 2(5H)-furanone compared to all other samples (APP was similar ($P > 0.05$) for furfural). The high level of furans in this sample may be explained by the amount of lactose present in whey permeate, which has been shown to greatly influence furan formation when heated in the presence of protein. For example, when lactose was used to replace 60% sucrose in sponge cakes, 5-hydroxymethylfurfural generation was accelerated

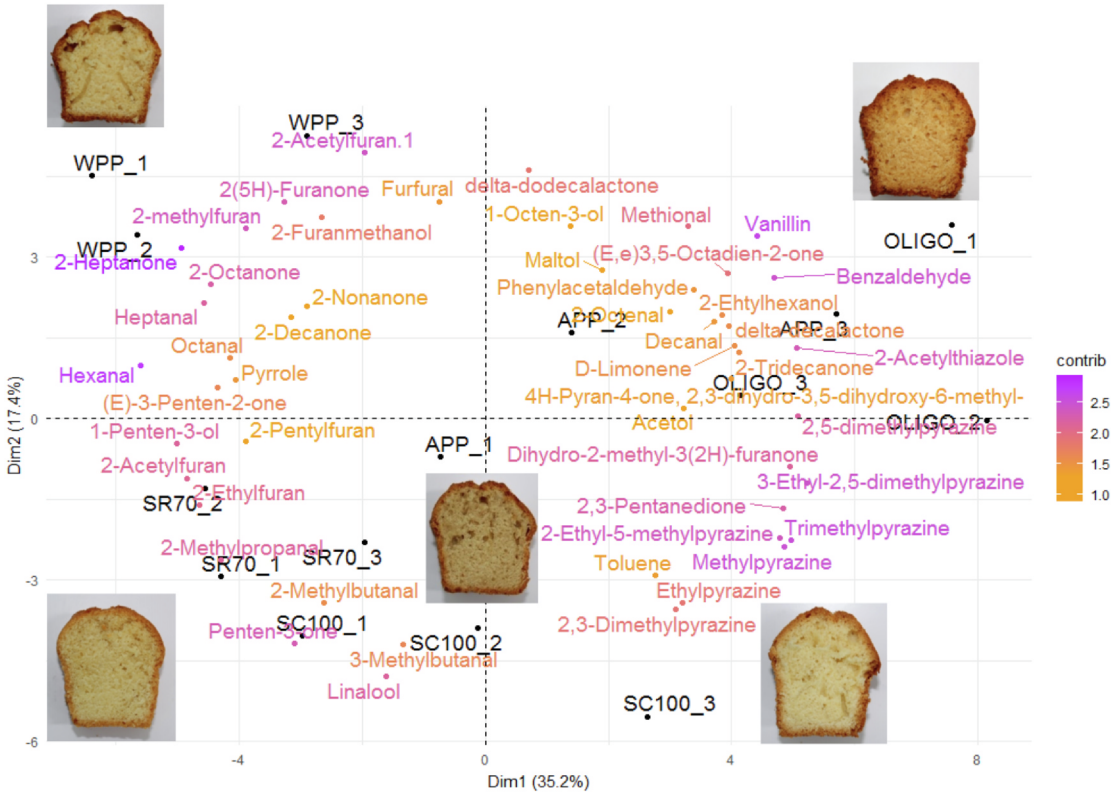


Fig. 2. Principle component analysis of the volatile compounds associated with control (SC100) and reformulated SR70 (30% reduced sucrose), SR-APP (30% reduced sucrose with apple pomace powder), SR-WPP (30% reduced sucrose with whey permeate powder), SR-OLIGO (30% reduced sucrose with oligofructose) sponge cakes analysed by headspace solid-phase microextration gas chromatography mass spectrometry. Score plot represents sample replicates (n = −3).

(Zhang et al., 2012). Lactose can directly be degraded by MR or CR, whereas sucrose has to be exposed to heating for much longer to be hydrolysed to its monosaccharides; glucose and fructose. Furfural was significantly higher in the APP sponge cake compared to all other samples (SC100, SR70 and OLIGO), most likely due to the higher level of fructose and glucose in the apple pomace (Milner et al., 2020). As depicted in Fig. 2., dihydro-2-methyl-3(2H)-furanone (coffee furan) was in highest abundance in the SC100, APP and OLIGO sponge cakes and has been previously been identified as 'caramel-like' in the crust of sponge cakes (Matsakidou et al., 2010). Its formation is likely *via* the degradation of glucose during CR reactions (Umano, Hagi, Nakahara, Shyoji, & Shibamoto, 1995).

Ketone compounds, 2,3-butanedione (diacetyl) and 2,3-pentanedione, are identified as important contributors to the desirable aroma of baked confectionery, with both yielding 'butterscotch', 'caramel' impressions (Garvey et al., 2020a). The abundance of these α-diketones are at risk of being suppressed on sucrose reduction as they are products of sucrose decomposition during CR or sugar fragmentation during MR, and hence cake aroma may be adversely affected. In this study, the level of 2,3-butandione did not significantly ($P > 0.05$) differ across the five sponge cake formulations, however, 2,3-pentanedione was significantly ($P < 0.05$) lower in the WPP sponge cakes compared to SR100 and OLIGO sponge cakes. Poisson, Auzanneau, Mestdagh, Blank, and Davidek (2018) proposed that 2,3-pentanedione can be generated directly from sucrose, whereas 2,3-butanedione can be formed from sucrose fragments, and monosaccharide's contribute to the formation of both, explaining the consistent level of 2,3-butanedione across all samples and the higher abundance of 2,3-pentanedione in the OLIGO and SC100 sponge cakes. Acetol (1-hydroxy-2-propanone) has been previously identified in sponge cakes (Maire, Rega, Cuvelier, Soto, & Giampaoli, 2013; Matsakidou et al., 2010; Pozo-Bayón et al., 2007; Rega, Guerard, Delarue, Maire, & Giampaoli, 2009) and is generated from the decomposition of sugars during CR. The OLIGO sponge cake contained higher levels of acetol compared to all other formulas, with the APP sponge cake containing the least ($P < 0.05$). The abundance of acetol in the OLIGO sponge cake sample indicates CR was accelerated during baking, which may be explained by the susceptibility of fructo-oligosaccharides to degrade when exposed to higher temperatures of baking. 2-Heptanone was significantly ($P < 0.05$) higher (nearly double) in the WPP sponge cake compared to the SC100 sponge cake. Although 2-heptanone has not been reported as odour active in sponge cakes, this compound likely originates from the whey permeate.

Comparing the abundance of aldehyde compounds between the sponge cake samples, the negative component of PC1 (SC100, APP and OLIGO) is more associated with the Strecker aldehydes; phenylacetaldehyde and methional, which are derived from amino acids phenylalanine and methionine, respectively. Phenylacetaldehyde is appreciated for a 'sweet', 'rose', 'honey' aroma and has been shown to contribute to sponge cake odour (Matsakidou et al., 2010; Pozo-Bayón et al., 2007), whereas methional has been identified as having a 'potato' like odour (Pozo-Bayón et al., 2007). Both of these Strecker aldehydes had the highest abundance in the APP sponge cake, with phenylacetaldehyde significantly ($P < 0.05$) higher than the SC100 sponge cake. Both phenylalanine and methionine amino acids have been identified in the flesh of honey crisp apples (Zhang, Li, & Cheng, 2010), which may indicate that the apple pomace is contributing to the higher amounts of these Strecker aldehydes. Benzaldehyde has been characterised as a 'cherry', 'almond' odour in sponge cakes (Maire et al., 2013; Matsakidou et al., 2010; Pozo-Bayón et al., 2007; Rega et al., 2009). Benzaldehyde can also be formed from phenylalanine and there was no significant ($P > 0.05$) difference between the SC100 sponge cake and the reformulated sponge cakes with sucrose replacers, but levels of benzaldehyde in SR70 were significantly ($P < 0.05$) lower compared to the OLIGO sponge cake. The SR70 sponge cake formula had the least simple sugars (Milner et al., 2020), and thus Strecker degradation may not have been as abundant, possibly evident by the fact that it had a significantly ($P < 0.05$) lower abundance of phenylacetaldehyde. Considering Fig. 2., the aldehydes associated with the sponge cake samples SR70 and WPP are primarily lipid derived (hexanal, heptanal & octanal), and were most abundant in the WPP sponge cake sample (Table 2), with heptanal significantly ($P < 0.05$) higher in the WPP and SR70 sponge cakes. This is difficult to interpret, but it may be due the fact that the other samples had more volatiles deriving from MR and CR, which have been shown to exhibit antioxidant activity (Benjakul, Visessanguan, Phongkanpai, & Tanaka, 2005). SC100, APP and OLIGO sponge cakes had significantly ($P < 0.05$) lower amounts of heptanal compared to SR70 and APP, which may be due to the high levels of fructose in these formulas, which has been identified as a powerful oxygen scavenger (Benjakul et al., 2005).

The APP and OLIGO sponge cakes were also found to have the highest levels of 2-acetylthiazole, a compound identified as having a 'hazelnut', 'popcorn' aroma in sponge cakes (Matsakidou et al., 2010; Pozo-Bayón et al., 2007). This compound is a product of the MR and is shown to be accelerated by the presence of the amino acid cysteine (Pripis-Nicolau, De Revel, Bertrand, & Maujean, 2000). The WPP sponge cake also contained significantly ($P < 0.05$) higher amounts of maltol, an odour active compound recognised for its 'sweet', 'cotton candy' odour in sponge cakes (Matsakidou et al., 2010). Although maltol can be created directly from sucrose, in the presence of glycine, lactose is capable of being converted to maltol on heating (Patton, 1950), which may explain its higher abundance in the WPP sponge cake.

3.3. Odour active compounds in the SC100, APP and OLIGO sponge cakes

Thirty six odour active compounds (Table 3) were detected in the SC100, APP & OLIGO sponge cake samples, with the identity of 33 confirmed through comparison of molecular ion matching, RI index's (using the procedure described in Section 2.4) and analytical standards. Co-elution of aroma compounds is common, and in this study, benzaldehyde co-eluted with 1-octen-3-ol, 2-ethyl-5-methylpyrazine with trimethylpyrazine, phenylacetaldehyde with 3-ethyl-2,5-dimethylpyrazine and furaneol with 2-nonanone. The aroma description of the 3 unknown compounds were also included in Table 3. The FD value in Table 3 highlights the intensity of the aroma, thus values of 0 indicate that the aroma could not be perceived by the trained assessor from a splitless injection, and a value of 1 indicates perception operating at splitless, 2 indicates the maximum perception by the trained assessor was from a 2:1 split injection, 10 indicates a 10:1 split injection etc. Thus, the higher the FD value the greater the contribution of that compound to the overall aroma and flavour of the sample.

In total, ten aldehydes were found to be odour active in these samples. Heptanal had the largest contribution to the aroma of SC100 sponge cake due to the large FD value (150) and was described as having a 'fatty, sweet, cake crust' aroma (Table 3). Heptanal had a lower contribution to the aroma of both APP and OLIGO sponge cake samples (FD 50), however, the abundance of heptanal was not significantly different amongst the SC100, APP and OLIGO sponge cakes on volatile analysis of cake crumb (Table 2). Although the difference may be due to other factors influencing perception, the GC-O analysis was undertaken on a combination of crust and crumb sample. Factors such as the matrix effect are also likely contributing (Frank, Eyres, Piyasiri, & Delahunty, 2012), where compositional differences impact on the release of volatiles in food. Previous HS-SPME/Solvent Assistant Flavour Evaporation (SAFE)-GC-O analysis of sponge cakes did not identify heptanal as odour active (Matsakidou et al., 2010; Pozo-Bayón et al., 2007). However, levels are likely to differ considerably in sponge cake formulas dependent upon lipid content and other factors impacting on oxidation.

Strecker aldehydes; 2-methylpropanal, 3-methylbutanal and 2-methylbutanal arise from branch chain amino acids and their contribution to odour activity was similar in the SC100 and OLIGO sponge cakes, but

Table 3
Odour active compounds with corresponding RI value, odour impression and factor dilution value identified in S100, APP and OLIGO sponge cakes.

Order of identification	Retention Index		Volatile compound	Odour Impression	FD Values			Identification*
	DB-624 UI (volatile analysis)	DB-624 UI (GC-O analysis)			SC100	APP	OLIGO	
1	594	606	2-Methylpropanal	spicy, cake crust	2	5	2	MS, RI, Odour
2	632	632	2,3-Butanedione	Butterscotch, sweet, toffee	10	5	20	MS, RI, Odour
3	693	701	3-Methylbutanal	Sweet, toffee, pineapple	50	10	50	MS, RI, Odour
4	701	707	2-Methylbutanal	Spicy, sweet	50	10	50	MS, RI, Odour
5	736	738	2,3-Pentanedione	Woody, spicy	10	2	1	MS, RI, Odour
6	737	755	Acetol	Sweet, fruity, cotton candy	0	1	50	MS, RI, Odour
7	–	805	Unknown 1	Toasted, bready, cake crust	20	0	0	MS, RI, Odour
8	857	857	Dihydro-2-methyl-3(2H)-furanone	Woody, bready	1	1	1	MS, RI, Odour
9	864	864	Methylpyrazine	Butterscotch, sweet, cake crust	10	2	5	MS, RI, Odour, Std
10	899	901	Furfural	Spicy, bready	100	150	100	MS, RI, Odour
11	928	930	2-Furanmethanol	Biscuit, cake crust, caramelized	20	100	50	MS, RI, Odour
12	944	943	Heptanal	Fatty, oily, cake crust	150	50	50	MS, RI, Odour
13	951	950	2,5-Dimethylpyrazine	cake crust, nutty, bready	100	50	100	MS, RI, Odour
14	958	955	Ethylpyrazine	Roasty, bready	5	2	2	MS, RI, Odour
15	962	966	2,3-Dimethylpyrazine	Bready, caramel	100	100	50	MS, RI, Odour
16	974	973	Methional	potato damp	50	150	100	MS, RI, Odour
17	978	977	2-Acetylfuran	Bready, caramel	20	0	10	MS, RI, Odour
18	–	1021	Unknown 2	Sweet, fruity, caramel	20	5	10	MS, RI, Odour
19	1025/1038	1026	Benzaldehyde / 1-Octen-3-ol	Mushroom, mouldy, sweet, almond, fruity	0	10	10	MS, RI, Odour
20	1042/1043	1039	2-ethyl-5-methyl-pyrazine/ Trimethylpyrazine	Musty, mouldy, cake crust	2	10	20	MS, RI, Odour
21	1086	1080	2-Acetylthiazole	Bready, biscuit, cake crust	2	10	50	MS, RI, Odour
22	1117/1122	1113/ 1115	Phenylacetaldehyde/ 3-ethyl-2,5-dimethylpyrazine	Fatty, fruity, cake crust, bready	20	20	50	MS, RI, Odour
23	1121	1119	(E)-2-Octenal*	Damp, earthy, mouldy	20	20	50	MS, RI, Odour
24	1141	1140	Furaneol/ 2-Nonanone	Roasty, cake crust, sweet	50	20	50	MS, RI, Odour, Std
25	1147	1146	Acetophenone	Sweet, cake crust, burnt	20	0	50	MS, RI, Odour
26	1152	1149	Nonanal	Bready, cake crust	20	10	50	MS, RI, Odour
27	1159	1157	2-Acetylpyrrole	cotton candy, fruity, sweet	10	100	50	MS, RI, Odour
28	1163	1161	(3E,5Z)-Octadien-2-one*	Caramel, butterscotch	10	20	50	MS, RI, Odour
29		1192	Unknown 3	Cake crust, sweet, butterscotch	20	50	50	MS, RI, Odour
30	1203	1200	Dodecane	Musty, damp, earthy	5	20	5	MS, RI, Odour
31	1207	1204	Maltol	Sweet, spicy, fruity	20	20	50	MS, RI, Odour
32	1249	1241	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Spicy, bready, roasty	20	10	50	MS, RI, Odour

* Identification by comparison with MS spectra, LRI matching from internal library and volatile analysis, odour comparison to literature and retention time of analytical standard SC100 = Sucrose Control, SR70 = Sucrose Reduced, APP = Apple Pomace Powder, WPP = Whey Permeate Powder, OLIGO = Oligofructose.

different in the APP sponge cakes. Different levels of these aldehydes have been previously identified in the crust of sponge cakes (Maire et al., 2013; Matsakidou et al., 2010; Pozo-Bayón et al., 2007), which explains the difference in FD values. 3-Methylbutanal and 2-methylbutanal, were perceived as 'sweet', 'caramel' and 'spicy, sweet', respectively, whereas 2-methylpropanal was perceived as 'creamy', 'spicy'. Methional is also a Strecker derived volatile and has a distinct 'potato' odour quality and was previously reported as having a high detection frequency in both the crust and crumb of sponge cakes (Pozo-Bayón et al., 2007). In this study, methional had FD values of 50, 150 and 100 in the SC100, APP and OLIGO sponge cakes, respectively, and significantly contributed to the aroma of all three sponge cakes. The abundance of methional across all samples was low (Table 2), but as it has an extremely low odour threshold, reported as 0.09 ppm in water (Giri, Osako, & Ohshima, 2010), highlighting its potential importance. A 'potato-like' odour may not be considered appealing in bakery products, but methional has been identified as having the highest FD in wheat bread (Rega et al., 2009), therefore contributing to the dynamic roasty odour of wheat bread. Methional may be responsible for the significantly lower score for 'odour liking' of the APP sponge cake (Table 1), and also the significantly higher score for the 'roasty' attribute (Fig. 2). Strecker aldehydes benzaldehdye and phenylacetaldehyde, were found to co-elute with other volatiles, making it difficult to discern their true contribution to the odour and flavour perception of these samples. Phenylacetaldehyde appeared to have the biggest impact in the OLIGO sponge cake and the impact of benzaldehdye was similar in both the APP and OLIGO sponge cakes, but absent in the SC100 sponge cake. Nonanal and (E)-2-octenal, are also products of lipid oxidation. The FD values for nonanal were 20, 10 and 50 for the SC100, APP and OLIGO sponge cakes, respectively. Thus, it had the greatest contribution to the OLIGO sponge cake sample. The FD values followed a similar trend for (E)-2-octenal, with a FD 50 value for the OLIGO sponge cake and an FD 20 for both the SC100 and APP sponge cakes.

As mentioned, furans are important volatiles from MR and CR reactions in baked confectionary products. Furfural was described as having a 'spicy', 'sweet', 'caramel', 'bready' aroma (Table 3), and was the most odour active compound in all three sponge cakes samples, with the highest DF (150) in the APP sponge cake and an FD of 100 in SC100 and OLIGO sponge cakes. The abundance of furfural was significantly ($P < 0.05$) higher in APP sponge cake compared to SC100 and OLIGO sponge cakes (Table 2), which explains its perceived odour intensity. Pozo-Bayón et al., 2007 identified furfural in the SAFE extract of sponge cakes but described it as 'earthy', 'potato', although it was co-eluting with 2-ethyl-3,5-dimethylpyrazine, which likely accounts for the different aroma descriptors. Matsakidou et al. (2010) used identical HS-SPME extraction parameters as this study (60 min at 60 °C) and

identified furfural in only the crust of sponge cakes and did not identify it as odour active. Three other furanone compounds also contributed to the aroma in these sponge cakes; 2-furanmethanol (furfuryl alcohol), dihydro-2-methyl 3(2H)-furanone (coffee furanone), and 2-acetylfuran. Of these three furans, furfuryl alcohol had the greatest impact with FD values of 20, 100 and 50 for SC100, APP and OLIGO sponge cakes, respectively. Thus, in this case the sucrose replacers appear to be enhancing the contribution of this compound to the overall aroma and flavour. Table 2 depicts no significant difference between the samples for furfuryl alcohol in the crumb, however, MR and CR reaction compounds, such as furans, are always likely to be higher in the crust (Garvey et al. 2019a). 2-Acetylfuran had FD values of 20, and 10 in SC100, and OLIGO sponge cakes, respectively and was not detected in the APP sponge cake. The reduction in sucrose, or change in composition, appears to have decreased the contribution of 2-acetylfuran to the overall aroma and thus flavour. The impact of coffee furanone was much less with a FD value of 1 achieved for each sample. The contribution of furaneol was also difficult to elucidate as it was not identified in the crumb. The odour of the analysed analytical standard of furaneol corresponded to the intense "sweet" aroma identified by panellists in the sponge cakes (Table 3), therefore potentially demonstrating its presence in sponge cake crust. It has been previously been identified in sponge cake (Pozo-Bayón et al., 2007).

Pyrazine compounds were strongly associated with all of the sponge cake samples. 2,5-Dimethylpyrazine was described as 'cake crust', 'sweet', 'nutty' and was perceived up to a FD of 100 in both SC100 and OLIGO sponge cakes, and at FD of 50 in the APP sponge cake. 2,3-Dimethylpyrazine, described as 'bready', was more odour active in the SC100 and APP sponge cakes (FD 100), compared to the OLIGO (FD 50) sponge cake. Although Table 2 shows similarities in the abundance levels of these compounds, it also appears that differences in composition due to the matrix effect may have also influenced their perception. The prevalence of pyrazines in the crust of sponge cakes (Matsakidou et al., 2010; Pozo-Bayón et al., 2007) likely contributed to the difference. Pozo-Bayón et al. (2007) identified 2,5-dimethylpyrazine in the crust and crumb of sponge cakes with panellists describing the odour as 'solvent', 'hospital'. Similarly, Pozo-Bayón et al. (2007) and Matsakidou et al. (2010) both identified 2,3-dimethylpyrazine in sponge cakes, however, neither were reported as odour active. Methylpyrazine and ethylpyrazine had less of an odour impact, methylpyrazine had GC-O values of 10, 2 and 5, and ethylpyrazine had FD values of 5, 2 and 2, in the SC100, APP and OLIGO sponge cakes, respectively. The odour of methyl- and ethyl-pyrazine was described as 'fruity', 'sweet' and 'bready' (Table 3). 2-Ethyl-5-methyl-pyrazine and trimethylpyrazine co-eluted, but had FD values of 2, 10 and 20 in the SC100, APP and OLIGO sponge cakes, respectively.

Six ketones were identified as odour active in these sponge cakes; 2,3-butanedione, 2,3-pentanedione, 2-nonanone, acetol, and (3,5)-octadien-2-one. The potential sources of these ketones are heat derived MR + CR reactions and lipid oxidation. 2,3-Butanedione was characterised by 'butterscotch', 'sweet', 'toffee' with FD values of 10, 5 and 20 for the SC100, APP and OLIGO sponge cakes, respectively. The higher FD for 2,3-butanedione in the OLIGO sponge cake was likely due to additional CR reactions due to the increased presence of monosaccharides. Although no significant difference in the abundance of 2,3-butanedione was evident between these samples (Table 2), levels were higher in the OLIGO sponge cake (this may be due to differences related to sampling between volatile assessment and GCO as previously stated). 2,3-Pentanedione was described as having 'spicy, woody' character and had lower FD values, at 10, 2 and 1, for the SC100, APP and OLIGO sponge cakes, respectively. Again, the higher perception in the SC100 sponge cake may be explained by slightly higher abundance (Table 2). Another odour active ketone identified was acetophenone, which in this study was described as 'sweet', 'cake crust', and was most odour active in the OLIGO (FD 50) sponge cake, but not perceived in the APP sponge cake, which corresponds with values in Table 2. Acetophenone is found in chicory (Baek & Cadwallader, 1998), which may explain the higher FD value for the OLIGO sponge cake sample. It had a FD of 20 in the SC100 sponge cake. This compound has been identified in star apple fruit as having a 'cherry' aroma (Lasekan, Khatib, Juhari, Patiram, & Lasekan, 2013).

2-Acetylpyrrole was present in all samples, and had the highest perception in the APP sponge cake (FD 100). The FD values for the SC100 and OLIGO sponge cakes were 10 and 50, respectively. 2-Acetylpyrrole is also a MR product, with higher concentrations depending on the amino acid and reducing sugar source. The odour descriptors for 2-acetylpyrrolye, for the three sponge cakes were 'cotton candy', 'sweet' and 'fruity'. Previously 2-acetylpyrrole has been described as having a 'chocolate-like' aroma in soy sauce (Feng et al., 2015). This may be related to the product, or that the odour quality of this compound may differ depending on its concentration. In this study it was relatively low across all the sponge cakes (Table 2). Pozo-Bayón et al. (2007) identified 2-acetylpyrrole in the volatile fraction of sponge cakes, yet did not identify it as being odour active. 2-Acetylthiazole was also present in each sample, the odour activity varied from FD 50 in the OLIGO sponge cake, to 10 in the APP sponge cake, and to 2 in the SC100 sponge cake. Thus the odour activity was much higher in the two clean-label reduced sucrose samples. 2-Acethylthiazole is also a MR product and thus levels appear to be influenced by the inclusion of the apple pomace powder and oligofructose. Maltol and 2,3-dihydro-3,5-dihydroxy-6-methyl4(H)-pyran-4-one were perceived as 'sweet', 'fruity', 'spicy' and 'bready' in all three sponge cakes, with highest odour activity in the OLIGO (FD 50) sponge cake. Maltol is a product of MR and CR, and Matsakidou et al. (2010) identified maltol as 'sweet, caramel' in sponge cakes, highlighting its contribution to the desirable aroma of baked goods. 2,3-Dihydro-3,5-dihydroxy-6-methyl4(H)-pyran-4-one is a precursor of maltol and is also derived from MR/ CR reactions (Rega et al., 2009).

Dodecane is an alkane, and had a pronounced 'earthy', 'damp', 'musty' aroma and is produced by lipid oxidation (Maire et al., 2013). Dodecane was most odour active (FD 20) in the APP sponge cake. 1-Octen-3-ol, a product of lipid oxidation, co-eluted with benzaldehyde but was likely responsible for the mushroom aroma found in both the APP and OLIGO (FD 10) sponge cakes, it was absent in the SC100 sponge cake.

Fig. 3 is a pie-chart of the differences in aroma perception of the ten most odour active volatile compounds in the APP and OLIGO sponge

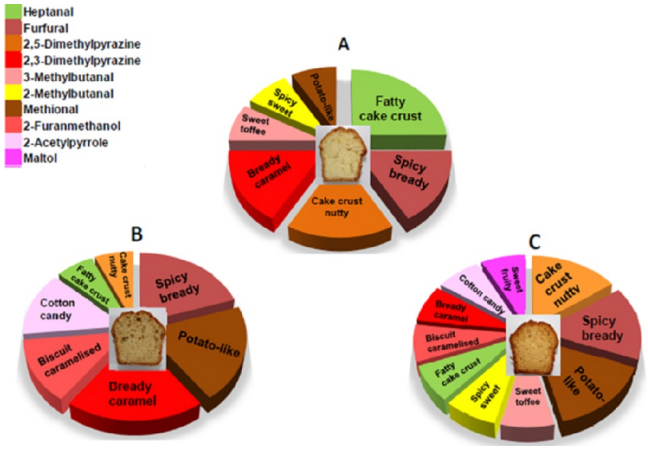


Fig. 3. Graphical representation of the most odour active compounds in the **A** SC100 (control), **B** APP (30% reduced sucrose with apple pomace powder) and **C** OLIGO (30% reduced sucrose with oligofructose) sponge cake formulas. Pie chart segments represent the dilution factor values of the main odour active compounds with larger segments indicating compounds perceived at higher dilutions (Table 3). Colour chart reflects the volatile compounds present in the pie charts. Pie charts contain compounds from ≥ FD 50 only.

cake samples in comparison to the control (SC100). This clearly highlights the significant impact of formulation changes on the odour activity of individual volatile components in the resultant sponge cake.

4. Conclusions

The impact of reducing sucrose with the inclusion of clean-label ingredients on the sensory quality and aroma of sponge cakes was explored. The hedonic liking assessment found no significant differences between the control and reformulated sponge cakes, apart from significantly lower scores for the APP sponge cake, for all attributes, and a reduction for texture and flavour for the SR70 sponge cake. RDA highlighted many significant differences in the perception of crust and crumb colour in the reformulated sponge cakes (APP, OLIGO and WPP), in comparison to the SC100 and SR70 sponge cakes. 'Roasty odour', 'toasty flavour' and 'off-flavour' were significantly higher in the APP sponge cake. 'Nutty odour' was perceived higher in reformulated sponge cakes in comparison to the SC100 sponge cake. 'Fresh cake odour' and 'sweet flavour' were also significantly reduced in the APP sponge cake, in comparison to the SC100 sponge cake. Significant differences in volatile profiles between all the samples were evident, especially in those derived from MD and CR reactions, and lipid oxidation.

Aroma active studies carried out on the SC100, APP and OLIGO sponge cakes, provided insightful information regarding differences in aroma activity between the reformulated samples and the SC100 control sponge cake. Thirty six aroma active compounds were identified, with furfural, methional, heptanal, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-furanmethanol having the greatest impact on sensory perception. Differences in the perception of other branched chain aldehydes, ketones, furans, pyrazine, pyrroles and terpene alcohols were also evident between SC100, APP and the OLIGO sponge cakes, but these had lower aroma intensities. This study has clearly demonstrated a significant deviation in the abundance of aroma active compounds influencing sensory perception in reduced sucrose sponge cakes with added clean-label ingredients in comparison to the control. However, it also highlights that considerable scope exists to manipulate added clean-label ingredients to make the aromatic profile more similar to the control or enhance desired aromas and thus flavour profiles.

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Declaration of Competing Interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128124.

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