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School of Food and Nutritional Sciences



IMPACT OF SUGAR AND FAT REDUCTION STRATEGIES ON THE SENSORY AND QUALITY PROPERTIES OF BAKERY PRODUCTS

Thesis presented by
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For the degree of
Doctor of Philosophy in Food Science and Technology

Under the supervision of,
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Declaration

I hereby declare that this thesis is my own work and contains no material that has been accepted for the award of any other degree in University College Cork or elsewhere.

Signature: _____

Date: _____

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Abstract

Consumption of foods and beverages rich in sugar and fat negatively impacts on human health. The primary objective of this thesis was to examine different strategies for the reduction of sugar and fat in Chocolate brownies, Sponge cake and Shortbread biscuits assessed using sensory (hedonic & intensity) and physicochemical analysis indicators. A survey on attitudes towards the use of sugar replacers indicated consumer caution regarding the use of artificial sugar replacers. Therefore, a clean-label approach was adapted when formulating sugar reduction strategies. Sugar particle size manipulation was examined as a potential sugar and fat reduction strategy. Commercial brown (200-5181μm) and white (102-378μm) sugar were separated into different size fractions by grinding and sieving. Chocolate brownies (CB) and Shortbread biscuits (SB) containing various sugar size treatments were formulated. Sensory analysis indicated that for SB, samples containing the coarser white sugar fraction (228-377μm) were perceived as the sweetest ($p<0.05$) and had higher scores for flavour liking ($p<0.05$) and overall acceptability (OA) ($p<0.05$). For CB, samples containing the smallest brown sugar fraction (459-972 μm) were perceived as the sweetest samples ($p<0.05$) and had the highest perceived moisture ($p<0.05$) and soft ($p<0.05$) texture. Fat perception in foods is strongly related to texture and mouthfeel, therefore sugar particle size reduction may also facilitate fat reduction. Natural fat (pureed black beans (PBLB)) and sugar replacers (inulin and steviol glycosides) were examined in brownie formulations to determine the levels of fat and sugar necessary for sensory acceptance. The smallest sugar fraction (459-972μm) permitted a higher level of fat replacement (75%) using PBLB as a fat replacer. Inulin and rebaudioside A replacing sucrose is feasible at 25% in fat-replaced (75%) brownies prepared with small sugar particles. Utilising the same sugar replacers, the level of sugar and fat required for sensory acceptance of Sponge

cakes was determined using pureed butter beans (PBRB) as a fat replacer. PBRB replacing fat is feasible at 50% in sucrose-replaced (30%) cakes using inulin and rebaudioside A. Finally, optimised sugar and fat-reduced products (OSFR) (Chocolate brownie and Sponge cake) were compared to products commercially available on the Irish market and preference of OSFR samples was observed.

Publications and Presentations

Publications

Richardson, A.M., Tyuftin, A.A., Kilcawley, K.N., Gallagher, E., O' Sullivan, M.G and Kerry, J.P., (2018). The impact of sugar particle size manipulation on the physical and sensory properties of chocolate brownies. *LWT-Food Science & Technology*, 95, 51-57. https://doi.org/10.1016/j.lwt.2018.04.038.

Richardson, A.M., Tyuftin, A.A., Kilcawley, K.N., Gallagher, E., O' Sullivan, M.G and Kerry, J.P., (2019). Impact of sugar size fraction and sucrose level on the sensory and physical properties of Shortbread biscuits. (Submitted for publication to *LWT-Food Science & Technology*, July 2019).

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Abbreviations

a* - Redness

ANOVA - Analysis of variance

b* - Yellowness

CB - Chocolate brownies

FA - Fatty acids

FDA - Food and Drug administration

FR - Fat replacement

FSAI - Food Safety Authority Ireland

HD - Higher degree

HIS - High intensity sweeteners

L* - Lightness

LC - Leaving Certificate

LPR - Large sugar particle size replacement

MBP- Mungbean paste

MPR - Medium sugar particle size replacement

MRD - Maximum recovery diluent

NAS - Non-caloric artificial sweeteners

NSR - Natural sugar replacers

OA - Overall acceptability

OSFR - Optimised sucrose and fat reduced

PBLB - Pureed black beans

PBRB - Pureed butter beans

PCA- Principal component analysis

PD - Primary degree

PG - Postgraduate degree

PLS - Partial least squares regression analysis

QDA - Quantitative descriptive analysis

RDA - Ranking descriptive analysis

Reb A - Rebaudioside A

SAT - Sensory acceptance testing

SB - Shortbread biscuits

SC - Sponge cake

SPR - Small sugar particle size replacement

SR - Sucrose replacement

SRB - Sugar rich beverages

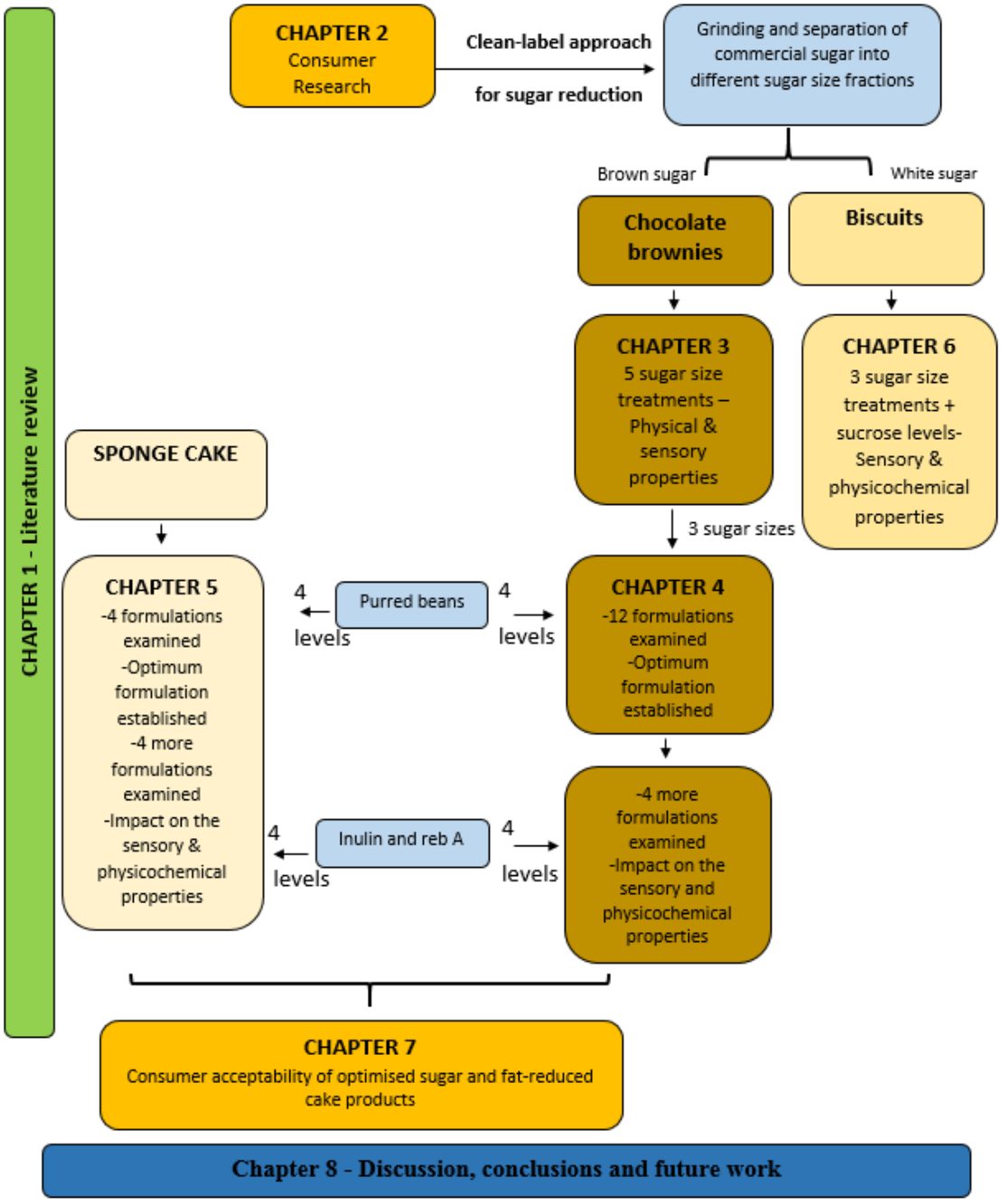
SRF - Sugar rich foods

SSB - Sugar sweetened beverages

TPA - Texture profile analysis

WHO - World Health Organisation

Schematic overview of thesis chapters



Research approach

The literature review, presented in Chapter 1 provides a background for the experimental work carried out in Chapters 2 to 7. After carrying out consumer research through the use of two online surveys which are presented in Chapter 2, a clean label approach for sugar reduction was implemented for subsequent chapters. Brown and white commercial sugar was ground and sieved into different size fractions. In Chapter 3, the impact of brown sugar particle size, using five sugar size treatments, on the physical and sensory properties of Chocolate brownies (CB) was investigated, as a potential approach for sugar reduction. Results from this study showed that small sugar particles increased the perceived moisture and softness of CB. As fat perception is strongly dependant on texture a new approach was developed for fat reduction in CB in Chapter 4, by manipulating sugar particle size. Three sugar sizes were selected and for each sugar size, four levels of fat replacement using pureed black beans were investigated. Once the optimum formulation was established through sensory analysis, four more formulations were manufactured containing increasing levels of sugar replacement using inulin and Reb A. These samples were characterised by sensory and physicochemical analysis. In chapter 5, the same sugar replacers employed in Chapter 4 were used to manufacture Sponge cakes containing four levels of sugar replacement. Once the optimum formulation was established through sensory analysis, four more formulations were manufactured containing increasing levels of fat replacement using pureed butter beans. These samples were characterised by sensory and physicochemical analysis. In Chapter 6, the impact of white sugar size fraction and sucrose level on the sensory and physical properties of Shortbread biscuits was determined. Finally, in Chapter 7, consumer acceptability of optimised sugar and fat reduced cake products was determined. Overall thesis conclusions and future work are presented in Chapter 8.

Chapter 1.
Literature review

1.1 Introduction

According to the World Health Organisation (WHO) in 2016 more than 1.9 billion adults were overweight. Of these, over 650 million were obese (WHO, 2018). Dietary imbalances caused by increased intake of energy rich and nutrient poor food products combined with a decrease in physical activity are the main causes of overweight and obesity. Overweight and obesity are known risk factors for other non-communicable diseases such as diabetes, coronary heart disease and certain cancers (National Taskforce on Obesity, 2005).

Sweet bakery products such as cakes and biscuits are typically energy rich and nutrient poor food products as they contain high amounts of sugar (15-40%) and fat (10-25%). The preference for foods high in sugar and fat is innate and overconsumption is highly likely due to positive hedonic responses overriding metabolic responses such as satiety (Blundell & Macdiarmid, 1997).

Sweet products such as cakes, biscuits, puddings, honey and ice-cream made the highest contribution to added sugar intake among adults (36- 61%) and children (40 to 50%) in a review of the total and added sugar intakes in Europe (Azaïs-braesco et al., 2017). Added sugars can be defined as sugars or syrups that are added to foods or beverages during processing or preparation. Free and refined sugars include; monosaccharides and disaccharides added to foods and beverages by the manufacturer, cook or consumer, and sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates, as defined by the World Health organisation (WHO, 2015). According to IUNA (2011), Irish diets contain 14.6% energy from free sugars and 34.1% energy from fat. Bakery products such as cakes and biscuits are responsible for 6 and 7% of the total intake of fat and carbohydrates respectively among Irish adults (IUNA, 2011). Thus,

reducing sugar and fat in bakery products could be a significant development in reducing the dietary intake of both fat and sugar.

However, the desirable physicochemical and sensory properties of cakes are largely owing to the presence of sugar and fat in the dough and during baking (Wilderjans et al., 2013). Thus, reducing sugar and fat in these products presents a huge challenge for food researchers and industry professionals alike.

This review explores the rationale behind sugar and fat reduction in bakery products and the factors to consider when formulating sugar and fat reduction strategies. Different approaches to reduce sugar and fat in bakery products are also presented in this review, with particular attention to the utilisation of natural sugar and fat replacers. A new strategy to reduce sugar, by optimising the physical form of sucrose is also proposed. Finally, important methodologies to assess the impact of sugar and fat reduction on the overall quality of baked goods are presented.

1.2 Classification of bakery products

The term ‘bakery products’ applies to a broad range of foodstuffs such as breads, cakes, muffins, pastries, cookies, crackers and biscuits. Bakery products are typically made from wheat flour and undergo a heating step to transform into light, aerated and palatable foods. Bread is a fermented food product that has the highest consumption rates among all bakery goods. According to IUNA (2011) breads along with potatoes, meat and dairy products are staple foods in the Irish diet. Breads are prepared from bread wheat flour, yeast, water and salt by mixing, kneading, shaping, fermenting, baking and slicing. Bread typically contains 38-41% moisture (Wade, 1988) and is characterised by a soft crumb with a degree of resilience. Breads typically contain low amounts of fat (5%) and sugar (5%) (Ulziijargal et al., 2013; Normahomed and Capellas, 2010). As a result, breads are characterised by subtle flavour characteristics compared to other bakery products that are enriched with these ingredients.

Cakes can be classified into three categories based on their formulation and mixing methods; batter type, foam type and chiffon type. The classification of cakes is presented in Table 1.1. Cakes contain less moisture than breads (20-30%) and typically contain higher levels of sugar (15-30%) and fat (10-20%). Cakes are typically characterised by a soft and delicate texture due to the enrichment of sugar and fat in the formulation. Flavour and texture characteristics of cakes can vary significantly and are dependent on the recipe, enrichment of ingredients and the presence of fruit, nuts, or cocoa (Cauvin & Young, 2010).

Chocolate brownies, a different type of cake product have been described as cake-like bars. Chocolate brownies contain high amounts of sugar (30%) and fat (20%) (Uruakpa and Fleischer, 2016). They are characterised by unique flavour characteristics due to adequate amounts of chocolate/cocoa (20%) in the formulation.

1.1 Classification of cakes

Types	Major Ingredients	Mixing Method	Examples
Batter type (high-fat cakes)	Flour, sugar, egg, milk; usually containing high fat; if fat < 0.6 flour (w/w), baking soda or baking powder is needed for leavening		
High-ratio type	Sugar $\geq$ flour	Creaming method; two-stage method; flour-batter method	Yellow layer cake; white layer; devil cake; butter cake; pound cake; marble cake
Foam type (low-fat cake)	Egg, flour, sugar; no solid fat		
Meringue type	Using egg white for leavening	Angel food method	Angel food cakes
Sponge type	Using whole egg or the mixture of yolk and whole egg for leavening	Sponge method	Sponge cakes
Chiffon type	The combination of batter type and foam type	Chiffon method	Chiffon cakes

Adapted from Zhou, (2014

Chocolate brownies are also characterised by unique texture properties such as ‘fudgy’ or dense, as a result of no leavening agents in the formulation.

Biscuits, cookies and crackers contain very low levels of moisture (1-5%) (Manley, 2011a), are low in weight (15g/unit) (Cauvin and Young, 2010) and have thin structures (10 mm thick). These products are therefore characterised by unique texture properties such as crispy and hard (Manley, 2011b). Biscuits, cookies and crackers can be classified into groupings based on their name, method of preparation and enrichment of ingredients such as fat and sugar (Wade, 1988). The types of biscuits are shown in Table 1.2. Biscuits can be further classified by the presence of cream or chocolate. Short dough biscuits are a specific type of biscuit, characterised by the considerable presence of fat and/or sugar in the dough. Shortbread biscuits are an example of short dough biscuits and typically contain a higher content of fat (20-40%) than sugar (15-25%). The fat present in the dough of shortbread biscuits impedes the formation of a strong gluten network (Manley et al., 2011). Hence, the dough is short. Biscuits that contain very high levels of sugar are characterised by hard and crunchy textures due to the recrystallisation of sucrose after baking.

Other types of Bakery products include pastries that can be divided into two main types; laminated and non-laminated. Laminated bakery products such as croissants contain high levels of fat (0-40%). Due to the presence of fat in the dough and roll-in fats, these Bakery products are characterised by unique texture characteristics such as ‘flaky’.

Table 1.2 Classification of biscuits

	Crackers	Semisweet	Short		Soft
			High fat	High sugar	
Added water in dough (to 100 units flour)	33%	21%	5%	15%	13%
Moisture in biscuit	3–4%	1–2%	2–3%	2–3%	3+%
Temperature of dough	30–38°C	40–42°C	18–20°C	18–20°C	c. 21°C
Critical ingredients	Flour	Flour	Fat	Fat and sugar size	Fat and sugar size
Baking time	3 min.	5–6 min.	15–25 min.	7 min.	12+ min.
Oven band type	Wire	Wire	Steel	Steel	Steel

Adapted from Manley et al., (2011)

1.3 Rational for sugar and fat reduction in bakery products

1.3.1 Consumption trends of bakery products within the food sector

The global market for baked goods is expected to rise by 2.6% by the year 2024 (Morder Intelligence, 2019). The industry consists of four main sectors; retail, wholesale, in-store and food service. According to Martínez-monzó et al., (2013) health, pleasure and convenience are the basic trends in bread, bakery and pastry innovation and consumption. In recent years, consumer demand has increased for whole wheat, gluten-free, ancient grain and additive free baked products because of the trends towards natural nutrition and health (Morder Intelligence, 2019). According to findings from the Irish Food Board, general trends that dominate the Irish grocery market are health and wellness, sustainability & plastics and origin & provenance (McKeown, 2019).

National food and nutrition consumption surveys are an extremely important research method in establishing consumption trends of food products, and also establishing correlations between food intake and related health outcomes. Dietary recommendations are usually based off extensive review of this research. In Ireland, several National food & beverage consumption surveys have been carried out. In 2001, the North/South Irish food consumption survey (NSIFCS) was carried out by the Irish Universities Nutritional Alliance (Irish Universities Nutrition Alliance (IUNA, 2001)). Findings from this survey showed that biscuits and cakes were consumed by 76% & 60% of the population, respectively and the combined intake of these products among consumers was 48g/day. Findings from the National children's survey showed that 87% & 60% of children aged between 5 and 12 were consumers of biscuits and cakes respectively and children within this age demographic were eating an average of 35g/day (IUNA, 2004). Teenagers were less likely to consume these products with 69% and 48% of teens eating biscuits and cakes respectively (IUNA, 2006). However,

among consumers the average intake of these products was higher at 42g/day. In 2011, the Irish Universities Nutrition Alliance conducted another adult survey entitled the National Adult Nutrition Survey (IUNA, 2011) which reported a decrease in the consumption of cakes and biscuits from the survey carried out by the organisation in 2002 (NSIFCS). However, the combined daily intake of these products was higher among consumers than in 2002 at 59g/day. More recently in 2014, findings from the Healthy Ireland Survey, conducted by the department of Health (Healthy Ireland, 2015) showed that 65% of participants consumed snack foods and sugar sweetened beverages on a daily basis.

1.3.2 Contribution of bakery products to total sugar and fat intake

According to data obtained from the North/South food consumption survey, Irish adults are exceeding recommendations for both fat and sugar intake (IUNA, 2001). Findings from this survey showed that cakes and biscuits were one of the four main sources of fat and carbohydrates in Irish diets. These products collectively contributed to 10% of the total intake of carbohydrates and 9% of the total intake of fat among Irish adults (IUNA, 2001). For children aged between 5 and 12 years, cakes and biscuits accounted for 7.6% of the mean daily intake of fat and 6.8% of the mean daily intake of carbohydrates (IUNA, 2004). In 2011, the Irish Universities Nutrition Alliance reported a decrease in the contribution of cakes and biscuit consumption to the mean daily intake of carbohydrate and fat in adults. Results from this survey showed that the contribution of these products to total intake of carbohydrate and fat had decreased to 7 & 6% respectively (IUNA, 2011). However, cakes and biscuits were still one of the four main sources of fat in the Irish diet and confectionery products were one of the main sources of carbohydrate, contributing 9% to total carbohydrate intake.

In a study conducted by Azaïs-braesco et al., (2017) on the total and added sugar intakes in Europe, all confectionery-type products such as cakes, pies, puddings, sugar, honey, ice cream and biscuits were classified as ‘sweet products’. Sweet products made the biggest contribution to added sugar intake among adults (36-61%) and children (40-50%), with cakes and cookies contributing the most to total sugar intake among children in France, Italy and The Netherlands.

1.3.3 Health implications of a diet high in sugar and fat

The presence of fat and sugar in food products yields positive hedonic responses to attributes such as aroma, texture and flavour (Drewnowski et al., 1989). The risk of passive overconsumption of foods containing a mixture of fat and sugar is high as positive hedonic responses may override metabolic responses such as satiety (Blundell & Macdiarmid, 1997). Bakery products are high in fat and sugar and are typically energy rich and nutrient poor food products (Schirmer, et al., 2012). Thus, the consumption of these products can lead to dietary imbalances which have been associated with diseases such as obesity (WHO, 2003).

Intake of sugars is a determinant of body weight, with a clear positive association between higher intakes of sugars, body fat and long-term weight gain in adults (Te Morenga,et al., 2013). A review of the findings from 28 clinical trials that studied the effects of a reduction in the amount of energy from fat in the diet showed that a reduction of 10% in the proportion of energy from fat was associated with a reduction in weight of 16 g/d (Bray and Popkin, 1998). In Ireland 37% of adults are overweight and a further 23% are obese according to the Healthy Ireland survey (2015). Obesity in adults is increasing by at least 1% every year (National Taskforce on Obesity, 2005). According to the WHO, over half a million people died in 2002 from obesity related complications (WHO, 2003). Excess weight in humans is a major risk factor for the development of type 2 diabetes. Roughly 90% of type 2 diabetes cases worldwide are due to excess weight (Hossain et al., 2007). Findings from an Irish longitudinal study on ageing found that the prevalence of diagnosed diabetes was 8.6% in adults, over 50 years of age (Leahy et al., 2015). In a survey carried out on the lifestyle, nutrition and attitudes of Irish adults the estimated prevalence of pre-diabetes among over 45 year olds in Ireland was 19.8% (Morgan et al., 2008). Other serious implications of obesity

include increased risk of hypertension, cardiovascular problems, osteoarthritis, gall bladder disease and certain cancers such as breast and colon cancer (National Obesity Task Force, 2005). Obesity is also associated with a higher risk of complications during pregnancy (Andreasen et al., 2013). Furthermore, as a result of the stigma attached to overweight and obesity, there is a higher risk for the development of mental health problems among those who are overweight and obese (Puhl and Brownell, 2003). Lastly, the intake of saturated fats has been shown to be an independent risk factor for the development of heart disease by increasing LDL cholesterol (Keys et al., 1965) and the intake of dietary sugar is the most important risk factor for dental caries (Moynihan & Kelly 2014).

1.3.4 Sugar tax

A 10% tax on sugar sweetened beverages (SSB) was proposed in Ireland in 2011, which officially came in to force in May, 2018. The tax applied to the following; water and juice based drinks with an added sugar content of over 5g/100ml, with 20c/L applying to drinks with 5-8 grams of added sugar per 100 millilitres and 30c/L applying to drinks with 8 grams or more of added sugar per 100 millilitres (Department of Health, 2018). The introduction of this sugar tax has generated a greater interest in strategies built around sugar reduction/replacement with supermarkets reducing the sugar content of own-brand beverage products with the primary purpose of avoiding this tax (Duffy, 2018). A potential tax on bakery/confectionery products containing high amounts of free sugar could generate even more of an interest in strategies built around sugar in food products and provide food researchers and industry professionals with more motivation and rationale to reduce sugar in these products.

1.4 Factors to consider when formulating sugar and fat reduction strategies in bakery products

The important factors to consider when formulating sugar and fat reduction strategies are;

- Functionality of sugar and fat in baked goods.
- Consumer attitudes towards the use of replacers.
- Financial cost of replacers.

In order to formulate sugar and fat reduction strategies in bakery products it is important to understand the multiple functions performed by these ingredients and how these functions contribute to the important physicochemical and sensory properties associated with liking and acceptability of these products. As the use of replacers is a very common strategy for sugar and fat reduction, it is important to understand consumer's knowledge and belief towards the use of these replacers. The financial cost of these replacers is also a very important factor to consider when formulating viable sugar and fat reduction strategies.

1.4.1 Sugar and fat functionality

1.4.1.1 Classification and physical form of Sugar

Sugars are low-molecular weight carbohydrates. The simplest form of sugars are called monosaccharides because they contain one monomer molecule. The most naturally abundant monosaccharides are glucose and fructose which are present in a wide variety of fruits. Sucrose is a disaccharide of glucose and fructose with a glycosidic link (Fig 1). Sucrose is present in the tissues of many plants but it is only extracted from sugar cane and sugar beet on a commercial basis. This review will focus on sucrose, as

sucrose is the most abundant commercialised sugar and the most commonly used sugar in cake baking.

Sucrose is a non-reducing sugar and is available in the form of white crystals. The chemical specification for crystalline white sucrose is displayed in Table 1. Today, between the production of refined sucrose from both cane and beet, the Food and agricultural organisation of the United Nations estimates global sucrose production to be 179.3 million tonnes in 2018/19 (FAO, 2019). Sucrose is available in liquid state, where water has been added and is also be available in light to dark brown colours depending on the level of refinement. As well as being available in different colours, commercial sucrose is available in different sizes and shapes and these sizes and shapes can vary between different refineries.

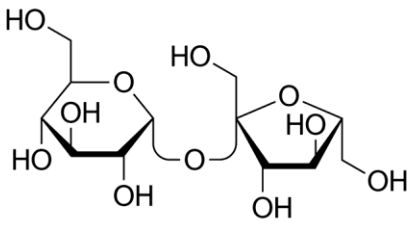


Fig 1.1 Chemical structure of sucrose

Table 1.3
Typical chemical specification for crystalline white sugar

Polarisation	99.8	min
Invert sugar	0.3%	max
Moisture content	0.04%	-
Sulphated Ash	0.04%	-
Copper	1.0 ppm	-
Lead	0.5 ppm	-
Arsenic	1.0 ppm	

Adapted from Manley, (2011c).

Sucrose available in liquid form is called syrup and the concentration of sucrose is usually 70 to 80% (Manley, 2011c). Syrups when added to foods and beverages dissolve instantaneously and are therefore important to the cold beverage industry. Liquid sugars bind more moisture in cakes and biscuits than crystalline white or brown sugar and can prevent the loss of water during storage (Pancoast & Junk, 1980). Small amounts of reducing sugars such as fructose and glucose can also be added easily to sucrose syrups. In this way, syrups can enhance colour formation by enhancing the Maillard reaction (Pancoast & Junk, 1980).

Commercial sucrose is sold in different colours, from white to dark brown. Brown sucrose is a variant of white sucrose in which some molasses are still present. The colour of brown sugar is dependent on the amount of molasses present, for example light brown sucrose contains roughly 3-5% molasses and dark brown sucrose contains roughly 6.5% molasses (Bennion & Bamford, 2013). Handling of brown sugars can be difficult as large variations in particle sizes and shapes exist. As a result, sucrose is often refined into white sugar and then blended with the desired amount of molasses to produce different variants of brown sugar, which makes handling much easier and results in the standardisation of the product. Owing to the presence of molasses, brown sugar has a higher moisture content (2-4%) and can be sticky. Due to the natural

hygroscopic properties, brown sugar used for baking is referred to as soft brown sugar (Pancoast & Junk, 1980). Brown sugar imparts important textural properties in baking by binding more water than white sucrose. As well as adding moisture, brown sucrose also adds unique flavour properties associated with molasses such as caramel flavour. As reducing sugars are present in molasses, brown sugar can also enhance colour formation by enhancing the Maillard reaction (Manley, 2011c).

White and brown commercial sucrose products are sold in a range of different particle sizes which are determined after crystallization. Particle size ranges of sucrose products vary, depending on the refinery in which they are formed however, typical particle size ranges according to Manley, (2011c) are as follows; 940-1000 µm for coarse granulated sugar, 570-635 µm for granulated sugar and 276-300 µm for caster sugar. The British sugar company provide information on the particle size ranges of the wide variety of sucrose products they have to offer (Speciality Crystalline White Sugar, 2015). Sucrose products supplied by the British sugar company range in size from powdered sugar (7-250 µm) which is typically used to coat pharmaceutical tablets, to sugar pearls (2.0 - 4.0 mm) which are typically used for decoration purposes in cakes and biscuits. Thus, commercial sucrose products perform a variety of functions depending on their particle size.

1.4.1.2 Classification and physical form of Fat

Fats are naturally occurring molecules that belong to a larger group of compounds named lipids which also include waxes, sterols and fat-soluble vitamins. Lipids typically share a nonpolar and hydrophobic character, rather than a specific structure. The simplest form of lipids are fatty acids which consist of a hydrocarbon chain and a carboxyl group (−COOH) at one end. They are typically bound to a glycerol molecule which consists of 3 hydroxyl groups (-OH), each bound to a carbon atom (Fig 2). Fatty acids that contain only single bonds are called saturated whereas, fatty acids that contain a double bond in the structure are called unsaturated. Furthermore, fatty acids containing more than one double bond are called polyunsaturated fatty acids. Plant sources of fat include seeds such as peanut and soybean, and vegetable sources such as avocado. Animal sources of fat include meat, milk and eggs.

Fats used in baking can be grouped into two categories; liquid fats and solid fats. Liquid fats include oils such as canola vegetable and grapeseed, whereas solid fats include butter and shortenings (lard, margarine). Shortenings and margarine are modified fat systems which were developed as alternatives to butter due to the high prices associated with the latter (Ghotra et al., 2002). Shortenings are fats formulated from oil and base oil (often with a plasticizer and an emulsifier). Liquid fats do not solidify after cooling and therefore produce baked products that are very tender. However in a study conducted by (Zhou et al., 2011) cakes prepared with liquid oils showed a more rapid increase in firmness over time. Butter is the most common type of solid fat used in baked good formulation and unlike other fats it provides flavour to the final product (Laguna et al., 2014). Butter is mainly composed of triglycerides and contains a high amount of saturated fat (65%) and low amounts of water (16%). A triglycerides is an

ester derived from glycerol and three fatty acids. Triglycerides are the main constituents of body fat in humans and other vertebrates, as well as vegetable fat.

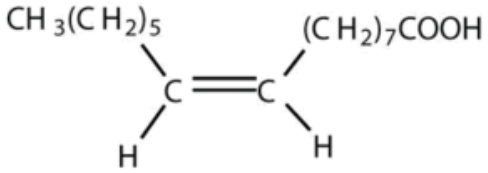


Fig 1.2 Chemical structure of fatty acid (*cis*-isomer)

1.4.1.3 Function of sucrose and fat in bakery products

When considering the multiple functions performed by sucrose and fat in cakes and biscuits, it is logical to group the functions into different stages. For example, Wilderjans et al., (2013), divided the functions of sucrose and fat in cakes into two stages; during batter/dough mixing and during cake baking. In the present review, the functions of sucrose and fat in bakery products will be divided into three stages; during dough/batter mixing, during baking and after baking.

1.4.1.3.1 Functions of sucrose during dough/batter mixing

For both cakes and biscuits, sucrose facilitates the incorporation of air during creaming which lightens the batter (Shepard & Yoell, 1976). Air pockets formed during creaming expand and lift the batter, causing it to rise during baking. In foam-type cakes sucrose interacts with egg proteins to stabilize the foam, so that the air cells can expand (Yang and Foegeding, 2010). Thus, at the mixing stage sucrose affects final volume and bulk of cakes (batter and foam type).

Sucrose is partially responsible for the tenderisation of cakes by binding water during the mixing stage and competing with gluten for hydration, therefore preventing the development of a strong gluten network. (Bennion & Bamford, 2013). Sucrose also binds water in biscuit dough and prevents the development of a strong gluten network. However, biscuits containing very high amounts of sugar may have crunchier and harder textures than biscuits containing low amounts of sugar, as a result of sugar recrystallization after baking (Manley, 2011b).

1.4.1.3.2 Function of sucrose during baking

During cake baking, sucrose increases the temperature of starch gelatinisation and protein denaturation by binding water and therefore limiting water availability to starch granules and protein molecules (Ureta et al., 2016). The delay in starch gelatinisation and protein denaturation permits the rising of cakes during baking. Thus, as well as affecting the volume and bulk of cakes during batter mixing, sucrose also affects the volume and bulk of cakes during baking. During biscuit baking, sucrose dissolves in the dough causing the dough to spread (Kulp et al., 1991). Thus, sucrose affects biscuit width and length dimensions. Sucrose also contributes to browning by the process of caramelisation during baking (Varzakas et al., 2012).

1.4.1.3.3 Function of sucrose after baking

The most commonly known function of sucrose in bakery goods is providing sweetness (Bennion & Bamford, 2013). Understanding the human preference for sweet foods and beverages has been the focus of many studies. In a review written by Drewnowski et al., (2012) it was concluded that the preference for sweet foods is both innate and global. Sucrose does not only provide sweetness, it also provides caramel and aroma flavour

through the process of caramelisation which also contributes to browning (Varzakas et al., 2012). On top of providing sweetness and caramel flavour, sucrose also act as a flavour enhancer. In a study conducted by Oliveira et al., (2015), the perception of chocolate flavour declined with the reduction of sucrose in chocolate milk. Finally, as mentioned, sucrose has the ability to bind water and decrease water activity, in this way sucrose can prolong the microbial stability and shelf life of baked goods (Nip, 2007).

1.4.1.3.4 Function of fat during dough/batter mixing

Fat entraps air and retains it in the batter during the creaming step which provides a structure for leavening gases (Shepard & Yoell, 1976). By doing so, fat affects volume and bulk at this stage.

1.4.1.3.5 Function of fat during baking

During baking, fat melts and fat droplets coat protein and starch particles, thereby interfering with the protein and starch matrix. By doing so, fat tenderises and adds lubricity to the texture of cakes (Pyler, 1988). Fat also emulsifies large amounts of liquid during baking which adds further moisture and softness to cakes and biscuits (Wade, 1988). Thus, fat acts as a tenderiser, adds moisture to baked goods and contributes to mouthfeel.

1.4.1.3.6 Function of fat after baking

No single sensory attribute can be correlated with fat content Drewnowski, (1998). The preference for foods containing high amounts of fat is related to the palatability of these foods. Similar to sweet foods, the preference for foods high in fat begins at an early age (Johnson et al., 1991). The function of fat after baking will depend on the fat source

used, for example using butter will provide the cake with a butter flavour. In a study conducted by Laguna et al., (2014) butter flavour declined with increasing levels of butter replacement using inulin. Fat can also carry lipophilic flavour compounds, in this way fat can also act as a flavour enhancer. Similar to sucrose, fat can also prolong the shelf life of baked goods by coating gas cells and therefore acting as a barrier to moisture loss (Shepard & Yoell, 1976).

1.4.2 Consumer attitudes towards the use of replacers

As briefly mentioned in section [1.3] consumer demand has increased for whole wheat, gluten-free, ancient grain and additive free baked products because of the trends towards natural nutrition, healthy living and organic food products (Morder Intelligence, 2019). Considering these trends when formulating sugar and fat replacement/reduction strategies is important.

In a study conducted by Suez et al., (2014), artificial sweeteners, in particular saccharin, sucralose and aspartame altered the intestinal microbiota and drove the development of glucose intolerance in mice fed commercial formulations of these sweeteners. According to Yang (2010) there was an increase of over 6000 products containing non-nutritive sweeteners in the US between 1999 and 2004. However, in recent years, consumers are leaning more and more towards clean label natural food products (Bizzozero, 2017). A recent US Mintel survey indicated that 64% of its respondents were concerned about the safety of "artificial" sweeteners (Gardner et al., 2012) and in a study conducted by Patterson et al., (2012), "no added sugar" claims were preferred to "reduced sugar" claims on products, with consumers assuming that sweeteners would be added to reduced-sugar products. Furthermore, consumers are more likely to accept foods with ingredients or additives that are made from natural, whole food products (Aschemann-Witzel et al., 2019) which has given rise to the use of whole food products (beans, fruit and vegetable purees) as fat replacers in bakery products.

Thus, caution and controversy towards the use of artificial sweeteners and other additives, perhaps justifies the need for a clean label approach when formulating sugar and fat reduction strategies.

1.4.3 Cost of replacers

The wide variety of sugar and fat replacers used in reformulation studies to reduce sugar and fat in bakery products will be discussed in detail in the next section [1.5]. However, before formulating sugar and fat reduction strategies it is necessary to consider the cost of potential and suitable sugar and fat replacers.

High intensity sweeteners (HIS) such as sucralose, saccharin and stevia are 4% or less the cost of sugar (Sugar and sweetener guide, 2013). However intense sweeteners are often combined with bulk replacers such as polyols and other low calorie carbohydrates to replace sugar in bakery products which adds to cost. The rise in consumer demand for healthy and functional foods justifies functional food ingredients such as fibres as potential bulk sugar replacers in bakery products. However, fibres (inulin) are roughly 3 times the cost of sugar (Sugar and sweetener guide, 2013) and polyols are also much more expensive (Zumbé et al., 2001). However, as consumer demand is high for healthy foods, bakery products that contain these ingredients could be marketed for the added benefits they bring. As a result of the high cost associated with sugar replacers, it is necessary to focus on other strategies to reduce sugar in bakery products such as optimisation of the physical from of sucrose which will be discussed in section [1.5.3].

1.5 Sugar and fat reduction strategies in bakery products

Current approaches to reduce sugar and fat in bakery products include;

-Use of replacers.

-Use of lower quantities of sugar and fat.

-Addition of thickening agents, emulsifiers and flavour enhancers.

The use of replacers is the most widely used approach and the most successful strategy for the reduction of sugar and fat in bakery products (Colla et al., 2018;Struck et al., 2014). Due to the cost associated with replacers and the caution towards the use of artificial sweeteners, it is necessary to focus on other viable approaches to reduce sugar in bakery products. Reducing salt particle size has been shown to increase the perception of saltiness in crisps (Rama et al., 2013). Therefore, the following approach will be discussed as a potential strategy for sugar and fat reduction in bakery products;

- Optimisation of the physical form of sugar and fat

1.5.1 Use of replacers

1.5.1.1 Sugar replacers in bakery products

Sugar replacers can be classified based on their composition; carbohydrate-based (polydextrose), protein based (thaumatin). Sugar replacers can also be classified based on their relative sweetness to sucrose and the physicochemical properties they possess. Fibres (inulin) and polyols possess water binding abilities and have a low relative sweetness, they are therefore used as bulk replacers. Sucralose is 600 times sweeter than sucrose and is used as a high intensity sweetener (HIS). Sugar replacers are natural or synthetic/artificial. The majority of HIS are artificial with the exception of thaumatin and steviol glycosides, whereas the majority of bulk replacers (polyols and carbohydrate-based replacers) are natural. In the present review, the use of inulin and

steviol glycosides as sucrose replacers in bakery products will be discussed in detail. The inclination for combination of intense sweeteners with bulk replacers such as fibres to produce sensory acceptable, low-calorie bakery products has increased, with intense sweeteners providing sweetness and bulk replacers providing the bulking properties lost due to sugar reduction. Other bulk and intense sweeteners used for sucrose replacement in bakery goods will also be discussed in this section.

1.5.1.2 Classification of Inulin

Plant storage polysaccharides called fructans, are composed of fructose units connected with β-(2→1) linkages (Fig 3) (Anadon et al., 2016). Fructans with 10 or more fructose units are classified as inulin (Mitmesser & Combs, 2017). Inulin-type fructans resist being broken down in the human small intestine by intestinal digestive enzymes, which are specific for a-glycosidic bonds (Roberfroid & Slavin 2000). They are therefore, classified as 'non-digestible' oligosaccharides. Inulin is present in over 36,000 plant species (Carpita et al., 1989) and it is naturally present in a wide variety of vegetables and fruits such as onions, asparagus and bananas. According to Van Loo et al., (1995) the average consumption from natural sources in Europe was estimated to be between 3 g and 11 g per day. On a commercial basis, inulin is extracted exclusively from the roots of chicory plants and sold in the form of a fine white powder (Shoaib et al., 2016). 'High performance' (HP) inulin is also available commercially. The average degree of polymerization (DP) of HP inulin is 25, whereas the DP of regular inulin is 10-12 (Niness, 1999). Thus, all residual sugars are removed from HP inulin.

1.5.1.2.1 Physicochemical properties of inulin

Fibres such as inulin impart important textural attributes and bulking properties to food products by binding water (Meyer et al., 2011). Inulin has high affinity for water (Gennaro et al., 2000) and is thought to compete with other constituents in the batter for available moisture. By this process, inulin may prevent full hydration of gluten proteins, thus interfering with the development of a strong gluten network. Inulin may also provide bulking properties by binding water during baking and delaying starch gelatinisation and protein denaturation, thus allowing the cake to rise more during baking.

Standard inulin has high level gel forming abilities (>25%). When dissolved in water or other aqueous solutions and thoroughly mixed, a white creamy structure is formed (Imenson, 2010). As a result, inulin can also be used as a fat replacer and as a texture modifier in foods (Meyer et al., 2011; Devereux et al., 2006). As well as contributing important textural attributes to reformulated bakery products, inulin imparts some sweetness to the final product as its sweetness level is 10% that of sucrose (Shoaib et al., 2016).

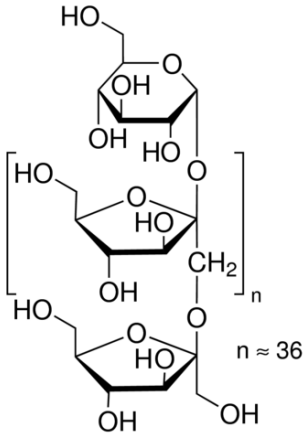


Fig 1.3 Chemical structure of inulin

1.5.1.2.2 Functional properties of inulin

Insightful reviews have been carried out on the full health benefits and functional properties of inulin (Shoaib et al. 2016; Roberfroid, 2005). Inulin-type fructans resist digestion in the gastrointestinal tract and are consequently fermented by bacteria in the colon (Cherbut, 2002). As inulin-type fructans are also carbohydrate components of edible plants as mentioned, these fructans meet the criteria to be considered dietary fibre and are labelled as such on food items (Roberfroid, 2005). A known functional component of dietary fibre is increasing stool rate and frequency by binding water in the colon therefore improving bowel habits. Furthermore, fibres such as inulin possess prebiotic properties by promoting the growth of Bifidobacteria and Lactobacilli in the colon (Shoaib et al., 2016). Due to the functions performed by inulin in the colon, the consumption of theses fibres has been shown to have positive effects regarding colon cancer prevention (Roberfroid, 2005) and other GI diseases such as Crohn's disease (Lindsay et al., 2006). According to (Shoaib et al., 2016) other functional properties of inulin include its role in lipid metabolism, enhancing the absorption of magnesium and iron, regulating food intake and appetite and stimulating the immune system (Shoaib et al., 2016). The incorporation of dietary fibre into foods is now considered as a principal prevention strategy against the risk of non-communicable disease (Stephen et al., 2017). Inulin-type fructans are therefore considered functional food ingredients (Roberfroid, 2005).

1.5.1.2.3 Studies using inulin as sucrose replacers in bakery products

A substantial amount of research has been carried out to assess inulin as a potential fat replacer in baked goods due to its ability to form a gel when mixed with water. O'Brien et al., (2003) investigated the impact of replacing fat with inulin in bread and Rodríguez-garcía et al., (2014) examined inulin as a potential fat replacer in cakes. The following studies have assessed inulin as a fat replacer in biscuits (Laguna et al., 2014; Rodríguez-garcía et al. 2013; Zbikowska and Ruthkowska, 2008; Lourencetti et al., 2013; Zoulias et al., 2002;Zoulias et al., 2002b).

Fewer studies have examined the impact of replacing sucrose with inulin in bakery products. These studies are depicted in Table 1.4. Information, including specific products tested, parameters investigated, level of replacement achieved, and the quality effects established are also summarized in this table. In a study conducted by Roßle et al., (2011) the impact of using a combination of inulin and oligofructose as fat and sugar replacers in scones was evaluated, using a mixture design approach. Sugar was reduced from 10% of the formulation to 0.55% using inulin and oligofructose. The complete substitution of sugar and fat for inulin and oligofructose increased the volume of the final product. This was surprising, considering the role sucrose plays in contributing to increased volume of baked products. Utilisation of both oligofructose and inulin for fat and sugar replacement may have increased starch content of the dough considerably, thus increasing dough viscosity and decreasing water absorption. This possibly contributed to a delay in starch gelatinisation as more starch was present and there was less water for the starch to bind. The presence of sugars in inulin and oligofructose combined may also have contributed to a delay in starch gelatinisation by binding water and thus promoting greater expansion during baking. Samples containing higher levels of inulin and oligofructose had a darker crust and crumb colour which can be attributed

to the presence of reducing sugars in inulin and oligofructose which may have accelerated the Maillard reaction and contributed to the deeper browning of samples. In a study conducted by Laguna et al., (2013) cookie hardness decreased after 50% of the sucrose was replaced with inulin. Although sucrose has a tenderising effect on biscuit dough, if present in high amounts it may also have the opposite effect when sugar recrystallises after cooling causing increased sample hardness (Manley, 2011b). However, full replacement (100%) of sucrose with inulin was found to increase hardness of muffins (Gao et al., 2016). Findings from this study also reported a decrease in muffin springiness with increasing sucrose replacement. However, partial sucrose replacement (50%) with inulin had no negative impact on hardness. Sucrose has a tenderising effect on the dough by binding water and competing with gluten proteins thus, interfering with the development of a strong gluten network. It is therefore no surprise that a complete substitution of sucrose for inulin would increase crust firmness of muffin samples. Furthermore, a reduction in volume is typically observed with the reduction of sucrose which means fewer air cells and much smaller air cells are present in the matrix. A reduction in volume typically results in the increased firmness of cakes as the products are more compact and dense. Hence, sample springiness was also affected after sucrose replacement with inulin.

Moisture mouthfeel was higher for cookies that contained a combination of sucrose and inulin in a study carried by Laguna et al., (2013) and similar results were reported by Zahn et al., (2013) who reported an increase in moisture content for muffin samples containing inulin. Perceived butter flavour was negatively affected with the substitution of 25% of sucrose for inulin in cookies (Laguna et al., (2013), however, a 30% substitution of sugar for inulin in muffins did not negatively affect butter flavour perception (Zahn et al., 2013). In this study however, rebaudioside A was used in

combination with inulin which may have positively affected butter flavour perception by increasing the perception of sweetness.

Therefore, inulin may increase volume when used in combination with other fibres to replace sugar and fat by increasing starch content, decreasing dough water absorption and delaying starch gelatinisation, thus promoting an increase in dough expansion. Insulin has a softening effect on cookies when used to partially replace sucrose but has a hardening effect when used to fully replace sucrose in muffins. Thus, the effect inulin has on texture is dependent on the product and the level of replacement. Combining inulin with intense sweeteners such as rebaudioside A increases the perception of flavour attributes such as butter flavour.

Table 1.4

Sucrose replacement studies in bakery products using inulin

Studies	Bakery Product type	Parameters investigated	Replacement level achieved (%)	Effects on Quality
Rößle et al., (2011)	Scones	Physical analysis (colour, texture, volume, image analysis), Compositional (moisture).	-	Increased volume. Darker crust colour.
Laguna et al., (2012)	Cookies	Sensory analysis (descriptive & consumer testing), Compositional (moisture & water activity), Physical analysis (texture & sound emissions).	25%	Reduction in perceived butter flavour at 25% SR. Moisture mouth-feel higher for sucrose/inulin cookies. Decreased sample hardness at 50% SR.
Zahn et al., (2012)	Muffins	Sensory analysis (descriptive), Physical analysis (height, texture & colour).	30%	Increase in moisture content.
Gao et al., (2016)	Muffins	Physical analysis (texture), Compositional (starch) *In vitro* predictive glycaemic response.	50%	Increased sample firmness at 100% SR. Decreased sample springiness at 100% SR. Reduced predictive glycaemic response at 50% SR

1.5.1.3 Classification of steviol glycosides

Approved for use in the European Union in 2011, steviol glycosides are natural constituents of *Stevia rebaudiana* Bertoni, a plant native to South America (Scardigli, 2011). The leaves of the stevia plant have a sweet taste and steviol glycosides are the constituents of stevia leaves responsible for the sweet taste. Steviol glycosides are extracted from the plant, purified, concentrated and then dried. Individual steviol glycosides and mixtures of steviol glycosides that are intended for use in food are required to have a minimum purity of 95%. The FAO/WHO Joint Expert Committee on Food Additives (JECFA) determined an ADI of 4mg steviol equivalents/kg body weight/day (European Food Safety Authority (EFSA), 2015). Several steviol glycosides such as stevioside, rebaudioside A, B & C are present in stevia leaves and they vary in their taste profiles and sweetness potencies (Perrier et al., 2018). Steviol glycosides are a special class of intense sweeteners as they are considered natural, unlike most other HIS. This family of HIS are generally 250 to 300 times sweeter than sucrose (Kroger et al., 2006). In a study conducted on the effects of stevia on food intake, satiety and postprandial glucose and insulin responses in humans, stevia significantly lowered insulin levels compared with sucrose and aspartame and had significantly lower postprandial glucose responses (Anton et al., 2010). Furthermore, Stevia did not promote compensation of calories at lunch or dinner meals and similar satiety levels were reported for all 3 sweeteners.

1.5.1.3.1 Studies using Steviol glycosides as sucrose replacers in bakery products

Several studies have been carried out examining the feasibility of steviol glycosides as sucrose replacers in bakery products, on their own or in combination with bulk replacers (Kulthe et al., 2014; Miller et al., 2017). Studies are displayed in Table 1.5. Products examined, bulk replacers used, parameters investigated and the quality effects established are also summarized in this table. Walter & Soliah, (2010) found that replacing sucrose with stevia in shortbread and cake had opposite effects on texture. For shortbread an increase in tenderness was observed after a partial replacement of sucrose with stevia (50% SR) however, an increase in hardness was observed after a full replacement of sucrose for stevia in cake and cookies (100% SR). Similar results were reported by (Gao et al., 2016;Gao et al. 2017) who determined that full replacement of sucrose with stevia in muffins increased hardness. Furthermore, samples displayed a reduction in springiness and in the latter study samples containing 100% SR with stevia were perceived as significantly drier as determined by sensory analysis. In a study conducted on Iranian sweet bread a decrease in hardness was observed for bread containing stevioside (Vatankhah et al., 2017). Hence, the impact of stevia on the texture of bakery products varies significantly depending on the type of bakery product. Sucrose is used in high amounts in cakes and biscuits and has a tenderising effect except when used in very high amounts in biscuits where it can have a hardening effect when the sucrose recrystallises after cooling (Manley, 2011b). Hence, this may be why stevia had a softening effect on shortbread and a hardening effect on cakes and muffins. Sugar recrystallisation also occurs in bread after cooling, hence why sweet Iranian breads containing stevioside were softer than sucrose breads (Vatankhah et al., 2017).

In relation to hedonic sensory properties and overall acceptance, Kulthe & Pawar (2014) reported an improvement after a 20% substitution of sucrose for stevia in

cookies. A partial replacement of sucrose with stevia (20%) has been shown to increase the perception of sweetness and cocoa tastes in muffins (Karp et al., 2017). In cake, partial substitution of sucrose with stevioside (25%) in combination with bulk replacers impaired perceived sweetness and overall quality (Manisha et al., 2012). However, sweetness intensity and overall quality improved with increasing levels of sucrose replacement (25-100%). In muffins, perceived sweetness did not discriminate between samples containing different levels of sucrose replacement with stevianna (Gao et al., 2017). However, 100% stevianna muffin samples were significantly drier and thus obtained significantly lower scores for overall liking. For Iranian sweet bread a partial replacement of sucrose with stevioside (50%) improved overall acceptability (Vatankhah et al., 2017). However, taste, texture and flavour liking were negatively affected after a full replacement of sucrose for stevioside. In a study conducted by Zahn et al., (2013) sweet taste discriminated between muffin samples containing stevia and a variety of different bulk replacers.

Thus, sucrose replacement with stevia appears to improve acceptability of a variety of different bakery products (cookies, cakes & muffins) by enhancing perceived sweetness. Perceived sweetness and overall acceptability depend on the level of sucrose replacement in cakes and breads (Manisha et al. 2012;Vatankhah et al. 2017) and the type of bulk replacer used (Zahn et al. 2013). Perceived sweetness however, did not depend on the level of sucrose replacement in muffins as sweetness intensity did not discriminate between samples containing increasing levels of replacement (Gao et al., 2017). However, overall acceptability was affected as a result of changes to textural properties (hard & dry) at 100% SR.

Also, in relation to flavour properties, a metallic aftertaste was reported in high ratio layer cakes containing stevia (Miller et al., 2017). However the addition of vanilla and

cocoa powder was found to mask off-flavour and bitterness of muffins containing stevia (Gao et al., 2017).

The substitution of stevia for sucrose can also have opposite effects on colour properties depending on the type of bakery product. For Iranian sweet bread, an increase in crust lightness, and crust yellowness and a decrease in crust redness was observed after sucrose replacement with stevia (Vatankhah et al., 2017). For muffins, a decrease in crust yellowness was observed with sucrose replacement while an increase in crust redness was reported (Gao et al., 2017).

Finally, in a study conducted on sponge cake, stevia based sweeteners containing maltodextrin were found to be ineffective in decreasing water activity of samples and this contributed to the accelerated fungal growth observed for these samples. Hence, samples containing stevia based sweeteners had a shorter shelf life than sucrose samples. This was not surprising as sucrose is known to contribute to the extended shelf life of cakes because of its ability to bind water and decrease water activity

Table 1.5

Sucrose replacement studies in bakery products using steviol glycosides

Studies	Product type	Bulk replacer	Parameters investigated	Effects on Quality
Walter & Soliah, (2010)	Cake, cookies and shortbread	-	Physical (texture, volume), Compositional (moisture loss)	Increased sample tenderness at 50% SR in shortbread. Increased sample hardness of cake at 100% SR. Decreased volume and tenderness of cookies at 100% SR.
Kulthe et al., (2011)	Cookies	-	Physical analysis (weight, diameter, thickness, spread ratio and texture), Sensory analysis,	Flavour, taste and overall acceptability improved after a 20% SR.
Manisha at el., (2012)	Cake	Liquid sorbitol. Addition of emulsifiers and hydrocolloids	Rheological analysis, Batter; microscopy, specific gravity & viscosity, Physical analysis (colour, texture, volume), Sensory analysis (descriptive), Scanning electron microscopy, Compositional analysis (moisture, ash, protein &fat)	Decrease in sweetness intensity at 25% SR. Overall sensory quality and perceived sweetness intensity increased with higher levels of SR (25-100%).
Zahn et al., (2013)	Muffins	Different fibres	Sensory analysis (descriptive), Physical analysis (height, texture & colour).	'Sweet taste' differentiated among samples containing different bulk replacers.
Rodríguez et al., (2016)	Sponge cake	Erythitol & Maltodextrin	Water activity, humectant properties and relative colonisation rates.	Two commercial stevia-based sweeteners containing maltodextrin were ineffective in decreasing a_w of cakes. Fungal growth faster in cakes containing these sweeteners. Shelf life was significantly shorter for cakes containing these treatments.

Gao et al., (2016)	Muffins	-	Physical analysis (texture), Compositional (starch) In vitro predictive glycaemic response	Increased sample firmness and springiness at 100% SR. Reduced predictive glycaemic response at 50% SR
Karp et al., (2016)	Muffins	-	Physical analysis (texture, colour, cooking yield & porosity) Compositional (dietary fibre & total phenols, total calories) Sensory analysis (descriptive & acceptance)	Partial replacement (20%) of sucrose with stevia enhanced sweetness and cocoa tastes
Miller at al., (2017)	High ratio white layer cakes	Polydextrose & maltodextrin	Physical analysis (volume, texture), Sensory analysis (descriptive & acceptance)	Metallic aftertaste
Vatankhah et al., (2017)	Iranian traditional sweet bread	-	Physical analysis (diameter, thickness, volume, density, texture & colour) Compositional analysis (moisture, protein, fat, ash) Sensory analysis (acceptance)	Decrease in bread hardness. Increase in crust lightness and yellowness and decrease in redness with SR. Increase in moisture content and pH. A 100% SR significantly negatively affected the following hedonic sensory properties; taste, flavour and texture.
Gao et al., (2017)	Muffins		Physical analysis (colour, texture) Sensory analysis	Increase in crust redness. Decrease in crust yellowness at 100% SR. Increase in crumb yellowness. Increase in sample firmness and decrease in springiness. Samples containing 100% stevianna were perceived as significantly harder with a drier mouthfeel. Samples containing 100% stevianna obatined significantly lower scores for overall liking.

1.5.1.4 Alternative bulk sugar replacers

A number of other bulk replacers have been examined as potential sugar replacers in bakery products, which can be loosely grouped into different categories. For the purpose of the present review, other bulk sugar replacers were grouped into the following categories; alternative carbohydrate-based bulk sugar replacers and polyols. Although polyols are technically carbohydrate based, they are chemically defined as saccharide derivatives in which a ketone or aldehyde group is replaced by a hydroxyl group (Zumbé et al., 2001). Carbohydrate-based bulk sugar replacers include complex carbohydrates and fibres.

1.5.1.4.1 Alternative carbohydrate-based sugar replacers

Polydextrose which is a glucose polymer, containing 1 kcal/g has also been used as a bulk sugar replacer in bakery products. Polydextrose is not fully digested by the body and similar to other carbohydrate-based sugar replacers it is an effective bulk replacer in bakery products because of its ability to absorb and retain water. Polydextrose increases starch gelatinization temperature (Rosenthal, 1995). Studies that have investigated the feasibility of polydextrose as a sucrose replacer in bakery products are listed in Table 1.6.

Maltodextrins, which have similar calories to sucrose (4kcal/g) have also been examined as bulk replacers in sugar replacement studies (Zahn et al., 2013). However, maltodextrins are usually considered bulk sweeteners as opposed to bulking agents because of their relatively high sweetness. Unlike inulin, oligofructose and polydextrose, maltodextrins provide little nutritional benefit.

Fructo-oligosaccharides, (FOS) also referred to as oligofructose is a subgroup of inulin, made up of polymers with a degree of polymerization ≤10 (Niness, 1999).

Oligofructose is produced from inulin by partial hydrolysis which involves the use of an inulinase enzyme. The sweetness of oligofructose is 30% that of sucrose (Shoaib et al., 2016). Similar to inulin, oligofructose is also considered as fibre and can be used as a bulk sugar replacer in bakery products because of its ability to absorb and retain water (Niness, 1999). Studies investigating the feasibility of oligofructose as sucrose replacers in bakery products are listed in Table 1.6.

As mentioned, fibres have the ability to bind water and therefore add to the viscosity of the batter which contributes to the volume and texture of the final product (Arocha et al., 2012). In recent years, the feasibility of a variety of different fibres have been investigated in their ability to act as bulk replacers in bakery products, which include pea, oat, apple and wheat fibre (Struck et al., 2016;Zahn et al. 2013).

1.5.1.4.2 Polyols

Polyols or sugar alcohols, are saccharide derivatives defined by having an alcohol group (>CH-OH) in place of the carbonyl group (>C=O) in the aldose and ketose moieties of mono-, di-, oligo- and polysaccharides (Livesey, 2003). Their limited use is permitted in the EU provided that their laxative effects are taken into consideration (WHO, 2000). The poor absorption of polyols is the main reason they provide fewer calories than sugar, with polyols providing roughly 2.4kcal/g (Zumbé et al., 2001). Most common polyols include Erythritol (E968), Isomalt (E953), Lacitol (E966), Mannitol (E421), Sorbitol (E420) and Xylitol (E967) (Mortensen, 2006).

Polyols provide the bulk properties (texture and volume) lost due to sugar reduction in cakes and similar to other carbohydrate-based replacers they are typically used in combination with intense sweeteners as they have a neutral flavour (Sicard & Le Bot, 1994). Xylitol has been shown to induce a reduction in water activity, which delays

starch gelatinisation during baking and increases gelatinisation temperature (Torres et al., 2013). Thus, xylitol has the capacity to provide the bulk properties associated with sucrose in bakery products.

Extensive research has been carried out investigating the feasibility of polyols as sugar replacers in bakery products. A list of reviews summarizing the research carried out to date on the use of polyols as sugar replacer in bakery products is displayed in Table 1.6

1.5.1.4.3 Alternative high intensity sweeteners

High intensity sweeteners (HIS) also called non-nutritive sweeteners (NNS) or non-caloric artificial sweeteners (NAS) offer an alternative to traditional and natural sweeteners in foods and beverages. A list of reviews summarizing the research carried out to date on HIS as sugar replacer in bakery products is displayed in Table 1.6. High intensity sweeteners can be divided into two categories; natural or artificial. Artificial high intensity sweeteners are defined as synthetic organic compounds, whereas natural intense sweeteners are defined as a substances that exists naturally in the environment that are extracted and subjected to purification (Struck et al., 2014). High intensity sweeteners that are currently approved in the EU include aspartame (E951), saccharin (E954) acesulfame-K (E950), cyclamate (E952), neohesperidin DC (E959), sucralose (E955) thaumatin (E957) and Steviol glycosides (E960) (Mortensen, 2006). Obtained by chemical synthesis, Saccharin (E954) is the oldest of all the artificial high intensity sweeteners and was discovered by Constantine Fahlberg in 1879 (Tarbell & Tarbell 1978). Saccharin is 200-700 times sweeter than sugar and is currently permitted in a wide range of products such as desserts, baked goods, yoghurts, sauces and ice-cream. However, in a recent study conducted by (Feijó et al., 2013) it was found that rats, that were fed yoghurt supplemented with saccharin showed increased weight gain compared

to rats that were fed yoghurt with sucrose. Aspartame is the sweetener most like sucrose with regards to sensory profile (Cardello et al., 2003) albeit it is 200 times sweeter. Unlike saccharin which has been found to have a more metallic taste than all the other sweeteners, (Šedivá et al., 2006) aspartame has a desirable flavour profile (Anton et al., 2010). Aspartame is a controversial sweetener, it's consumption has been associated with migraines in human subjects (Newman & Lipton 2001) and seizures in rats due to increased phenylalanine levels in the brain (Maher & Wurtman, 1987). However a review of the correlation between aspartame intake and seizures concluded that present aspartame consumption on or below the ADI (50mg/kg) does not cause brain damage or seizures (Fisher, 1989). Acesulfame K is used in sweetener blends as it has a bitter taste when present in quantities needed to develop desired sweetness (Kuhn, 2004). It is widely used in combination with aspartame or sucralose at permitted levels of 350-1000mg/kg depending on the food category. Although Acesulfame K has been deemed safe and is permitted by the FDA, controversy exists over the original tests conducted on Acesulfame K to determine the possible carcinogenicity of the sweetener (Karstadt, 2006). This sweetener has been nominated twice for retesting in the National Toxicology program in 1996 and 2006 and is yet to be exposed to such testing. Sucralose, a derivative of sucrose and marketed under the brand name Splenda® is 600 times sweeter than sucrose and is permitted in a wide range of products. In a recent review of its potential toxicity conducted by Brusick et al., (2010) it was deemed safe. Permitted levels of Sucralose range from 10mg/l to 1000mg/kg depending on the type of food and has been promoted for use among children (International Food Information Council Foundation, 2010). Neohesperidin is a bitter flavanone present in inedible citrus fruits and is the substrate for the commercial production of neohesperidin dihydrochalcone (NHDC) (Baêr et al., 1990). NHDC is 1500 times sweeter than sucrose

and like Acesulfame K is usually used in sweetener blends (Frydman et al., 2005). Previous studies have observed NHDC as a hepatoprotective agent in mice and rats (Xia et al., 2015) (Shi et al., 2015). Thaumatin is a protein sweetener derived from the arils of the fruit of the African plant katemfe (*Thaumatococcus daniellii* Benth.) (Firsov et al., 2016). Thaumatin is extremely sweet and can be up to 100,000 times sweeter than sucrose on a molar basis (Van der Wel & Loeve, 1972). Thaumatin is considered a natural intense sweetener and is used in the pharmaceutical industry to mask bitter tastes of tablets and pills at a concentration of 20-400 ppm (Priya et al., 2011). No research has been carried out to date investigating the feasibility of thaumatin as a sugar replacer in bakery products.

Thus, controversy exists over the use of artificial intense sweeteners in Bakery products, which justifies a clean-label approach when formulating sugar reduction strategies.

Table 1.6
Sugar replacement studies in bakery products using alternative bulk replacers and HIS

Class		Type	Studies
Carbohydrate-based	Complex carbohydrate	Polydextrose	Ronda et al., (2004) Schirmer et al., (2012) Psimouli & Oreopoulou, (2011) Zahn et al., (2012)
	Fibres	Fructo-oligosaccharides (FOS)/Oligofructose	Gallagher at al., (2003) Rößle et al., (2011) Ronda et al., (2004) Handa et al., (2012) Lecerf et al., (2015) Psimouli & Oreopoulou, (2011)
		Other fibres; Pea, wheat, oat, apple, cellulouse	Zahn et al., (2012) Struck at al., (2016)
Polyols		Maltilol Mannitol Sorbitol Lacitol Xylitol Isomalt Erythritol	Struck et al., (2014) Zumbé et al., (2001) Luo et al., (2018) Monaco et al., (2018) Ghosh & Sudha (2011)
High intensity sweeteners		Aspartame Saccharin Acesulfame-k Cyclamate Sucralose Thaumatin (Natrural)	Struck et al., (2014) Luo et al., (2018)

1.5.1.5 Fat replacers in bakery products

Fat replacers can be divided into three groups based on their composition; carbohydrate-based, protein-based and fat-based (Lucca & Tepper, 1994). Carbohydrate and protein-based fat replacers are known as fat mimetics as they mimic the function of fat in food products. Within each group, various types of fat replacer, with different structures and functions exist. The most abundant group of fat replacers used in bakery products are carbohydrate-based. In a recent review written by Colla et al., (2018) fat replacers in bakery products were categorised into the four following groups; complex carbohydrates, gums & gels, whole foods and combination. In the present review, the use of legumes as fat replacers in bakery products will be examined and other carbohydrate-based fat replacers including complex carbohydrates, gums, gels and fibres will also be discussed briefly.

1.5.1.6 Classification and functional properties of Legumes

Legumes can be broadly defined by their structure, podded fruit, and the ability of 88% of the species to form nodules with rhizobia (Graham & Vance, 1991). Some important legumes include peas, beans lentils and peanuts. Chickpea, cowpea, lentil and green peas are high in protein (25%), low in fat (1.5-5.2%) and high in minerals, such as potassium, phosphorus, calcium, copper, iron, and zinc (Iqbal et al., 2005). Legumes such as beans can be classified as carbohydrate-based fat replacers as they are a source of complex carbohydrates roughly containing up to 20% total carbohydrates. Beans can also be classified as whole food fat replacers. Other whole food fat replacers include fruit and vegetable purees.

Black beans and butter beans are legumes that belong to the '*phaseolus vulgaris'* variety of beans, also known as the common bean type. These legumes contain complex

carbohydrates (20%), are low in total sugars (1.4%), high in protein (8.2%), fibre (6.4%), low in fat (0.8) saturated fat (0.2%), and salt (0.05%) and high in moisture (73.2%). They are also a source of B vitamins, zinc, potassium, magnesium, calcium, iron and folate.

Beans are good source of dietary fibre (Messina, 1999). As previously mentioned, the incorporation of dietary fibre into foods is considered as a principal prevention strategy against the risk of non-communicable disease. Beans are low-glycaemic (Jenkins et al., 1980) and as a result of a good nutritional profile, their consumption has been associated with a decreased risk of coronary heart disease in US men and women (Bazzano & Ogden, 2001). The consumption of legumes such as soyfoods may also decrease the risk of hormone-dependant cancers by influencing oestrogen metabolism (Kushi et al., 1999).

1.5.1.6.1 Physicochemical properties & studies using legumes as fat replacers in bakery products

Carbohydrate-based fat replacers such as beans are used as fat substitutes as the physical and chemical structure of carbohydrates are similar to triglycerides (Zheng et al., 2015). Carbohydrate-based fat replacers imitate the functional and sensorial properties of fat by binding water therefore contributing to volume, texture, lubricity and mouthfeel (Nonaka, 1997). Beans possess a neutral flavour, furthermore the dark colour of black beans blend well with the colour of dark bakery products such as chocolate brownies and chocolate muffins and white or butter beans blend well with light coloured bakery products such as biscuits, chiffon cake and sponge cake. Furthermore, as beans are a whole food, consumers are more likely to accept this

product as opposed to products that contain carbohydrate compounds that have been extracted and purified.

Studies investigating the feasibility of legumes as fat replacers in bakery products are depicted in Table 1.7. Bakery product type, parameters investigated, replacement level achieved and effects on quality are also outlined in this table. A 25% fat replacement using pureed white beans in oatmeal cookies was not found to affect hedonic (colour, flavour, texture, appearance liking) sensory properties (Rankin & Bingham 2000). Higher replacement levels (50%) however, negatively affected hedonic properties and OA. Adair & Knight, (2001) looked at the feasibility of using mung bean paste (MBP) as a fat replacer in peanut butter cookies. Similar results were reported in this study, where acceptability of samples was not negatively affected by a 25% FR, but was negatively affected by a 50% FR. A reduction in cookie spread and a decrease in hardness was observed for samples containing MBP. However, among the samples containing increasing levels of MPB, (25-100% FR) no difference was observed in relation to hardness. A decrease in peanut butter flavour and an increase in 'different' flavour was observed for samples containing MBP however, 'different' flavour did not increase with increasing levels of FR. In a study conducted by Szafranski et al., (2005) pureed cannellini beans were employed to replace fat in chocolate brownies. In this study beans were successful in maintaining hedonic sensory properties and OA of brownies up to a level of 50% FR. However, all hedonic properties were negatively affected at a level of 75% replacement. Brownies containing 50% beans had 21 fewer calories and 2.6 g less fat. Similar results were reported by Uruakpa & Fleischer (2016) who found that replacing fat with pureed black beans (PBLB) reduced total energy and total fat content of chocolate brownies. Although most hedonic properties were affected by a 60% FR using PBLB, all chocolate brownie treatments (0-90% FR) had a hedonic

score of 20 which was adequate enough to be considered acceptable. Thus, a replacement level of 90% was achieved in this study using PBLB as a fat replacer.

Table 1.7
Fat replacement studies in bakery products using legumes

Studies	Bakery product type	Parameters investigated	Replacement level achieved	Effects on Quality
Rankin & Bingham, (2000)	Cookies	Sensory analysis (hedonic)	25%	Decrease in colour, flavour, texture and appearance liking and OA at 50% FR
Adair & Knight, (2001)	Cookies	Physical analysis (cookie spread) Sensory analysis (consumer hedonic, intensity)	25%	Reduction in cookie spread, decrease in hardness, decrease in peanut butter flavour. Increase in butter flavour, increase in different flavour, and acceptability negatively affected at 50% FR
Szafranski et al., (2005)	Chocolate Brownies	Sensory analysis (hedonic) Nutritional analysis	50%	All hedonic properties and OA negatively affected after 75% FR. Reduction in energy and total fat
Uruakpa & Fleischer, (2016)	Chocolate Brownies	Sensory analysis (hedonic), Nutritional analysis	90%	Most hedonic properties negatively affected at a 60% FR with the exception of Appearance liking. Reduction in energy and total fat.

1.5.1.7 Alternative carbohydrate-based fat replacers

Other carbohydrate-based fat replacers used in bakery products range from gums to non-digestible fibres to whole fruit purees. Carbohydrates bind water to form a paste and can therefore mimic the texture and mouthfeel of fat in foods (Lucca & Tepper, 1994). Complex carbohydrates such as polydextrose provide fewer calories than fat (1kcal/g), whereas fibres such as inulin and oligofructose are non-digestible and promote the growth of healthy bacteria in the colon. New and alternative sources of fibre such as coffee silverskin (Ates & Elmaci, 2018) and chitosan (Rios et al., 2018) have been used in recent years to replace fat in bakery products. Studies investigating the feasibility of complex carbohydrates and fibres as fat replacers in bakery products are listed in Table 1.8.

Gums work similarly to complex carbohydrates in that they bind water to form a gel and thus mimic the texture and mouthfeel of fats (Lucca & Tepper 1994). Some studies investigating the feasibility of gums as fat replacers in bakery products are listed in Table 6. A recent review conducted by Colla et al., (2018) concluded that gums are not very successful as fat replacers.

Another family of carbohydrate-based fat replacers include fruit and vegetable purees such as avocado (Wekwete & Navder, 2007) and green banana puree (Souza et al., 2018). Studies investigating the feasibility of fruit and vegetable purees as fat replacers in bakery products are listed in Table 1.8.

Table 1.8
Fat replacement studies using other fat substitutes

Class			Studies
Carbohydrate-based	Complex carbohydrates	Polydextrose	(Zoulias et al., 2002) (Sudha et al. 2007)
		Maltodextrin	(Zoulias et al., 2002) (Sudha et al. 2007)
	Fibres	Inulin	(Zoulias et al., 2002) (Zahn et al. 2010) (Rodríguez-garcía et al., 2013) (Laguna et al., 2014) (Giarnetti et al., 2015) (Sayed & Khalil 2017)
		Oligofructose	(Roßle et al. 2011)
		Peach dietary fibre	(Grigelmo-Miguel et al., 2001)
		Cocoa fibre	(Martínez-cervera et al., 2010)
		Chia mucilage	(Felisberto et al., 2015)
		Coffee silverskin	(Ates & Elmaci, 2018)
		Chitosan	(Rios et al. 2018)
	Gums	Oatrim	(Inglett et al., 1994)
		Pectin	(Confortiv et al., 1997)
	Fruit & Veg Purees	Applesauce	(Swanson & Munsayac, 1999) (Hayek & Ibrahim 2012)
		Prune puree	(Swanson & Munsayac, 1999)
		Pawpaw	(Holben & Bremner 2001)
		Avacado	(Wekwete & Navder 2007)
		Green banana	(Souza et al., 2018)
Protein-based	Whey proteins	Simplesse Dry Simplesse	(Zoulias et al., 2002) (O' Brien et al., 2003)

1.5.2 Use of lower quantities of sugar and fat

Initiatives supporting the gradual reduction of salt in foods have been very successful in EU countries, particularly in the UK where a 20-30% reduction of salt in many processed foods was achieved in three years (Inguglia et al., 2017). This strategy works by removing salt over a long period of time so that the difference is not detected by consumers. However, a recent sugar reduction policy in the UK aiming to reduce 20% of sugar in a variety of products by the year 2020 has not been as successful to date with industry not meeting the 5% reduction planned for 2017 (Belc et al., 2019).

Reducing sugar without substitution in bakery products affects physicochemical and sensory properties in cookies at low levels of reduction (Drewnowski, 1998). In a recent study sugar was reduced by 20-40% without substitution in pounds cakes that utilised green banana puree to replace butter by 25% (Souza et al., 2018). Perhaps sweetness of green banana compensated for the reduction of sucrose without substitution. A recent review of food reformulation in baked products concluded that less than 10% of sucrose can be removed from bakery products without negatively affecting sensory and physicochemical properties (Luo et al., 2019).

Reducing fat without substitution by the food industry has been less successful than replacing fat with fat replacers. Very few studies have been carried out on fat reduction, without substitution in bakery products. In a study conducted by (Drewnowski, 1998) fat was reduced by 50% in cookies without affecting physicochemical and sensory properties.

1.5.3 Optimising the physical form of sugar and fat

As mentioned, sucrose is available in different forms, either in liquid form or as solid crystals. As a solid crystalline structure, sucrose particles can typically range in size from fine (276- 300 μm) to coarse (940-1000 μm) (Manley, 2011c). In a study conducted by Kweon et al., (2009) the impact of sugar particle size on the thermal and starch pasting behaviour and the physical properties of short dough biscuits was measured. Using an ultra-fine and a fine granulated sugar in cookie baking, it was determined that the greater rate of dissolution of smaller sucrose crystals during batter mixing and baking resulted in greater surface crack for sugar-snap cookies, and lower height for wire-cut cookies. In a separate study carried out to determine the effects of sugar particle size on the evaluation of soft wheat flour quality, cookie spread and top-grain scores increased with decreasing sugar particle size (Kissel et al., 1973). Thus, sugar particle size affects the physical properties of biscuits. From extensive review of the scientific literature, the impact of sugar particle size on the sensory properties of bakery products does not appear to have been investigated to date.

However, the impact of sugar particle size on the sensory properties of chocolate has been investigated. Kuster, (1984) hypothesised that fine particles (large surface area) in chocolate would increase the perception of sweet taste, as small crystals dissolve more rapidly than larger ones. In a study conducted by Ziegler et al., (2001) it was found that as the mean particle size of non-fat solids in chocolate decreased so did the ‘effort’ to melt, manipulate and swallow the chocolate as determined by sensory analysis. Thus, particle size affected the perceived texture of chocolate. Findings from this study showed that sweetness perception may be greater at smaller particle size, but only in the presence of adequate viscosity.

In a study carried out to measure the impact of salt crystal size on the perceived saltiness of crisps, results showed that the smallest crystal size dissolved and diffused faster in the mouth than the larger ones (Rama et al., 2013). As a result, perceived saltiness was higher for the crisps that contained the smallest salt particle size.

For fat, in a study conducted by Mela et al., (1994) it was found that a reduction in fat particle size, caused a slightly enhanced perception of fat content in oil in water emulsions. Fat particle size distribution has been found to make a contribution to the perceived creaminess of milk, but again, only in the presence of adequate viscosity (Frøst et al., 2001).

Hence, reducing sugar salt and fat particle size has been shown to increase the sensory perception of sugar, salt and fat, respectively in food products. Optimising the physical form of sugar and fat could therefore be used as a strategy to permit the reduction of these ingredients in bakery products.

1.5.4 Addition of thickeners, emulsifying agents and flavourings

Emulsifiers promote foam formation and also the dispersion of fat by assisting the incorporation and division of air into the batter, thus providing more sites for the expansion of gas, giving rise to a greater volume and soft texture (Pyler, 1988). The addition of hydrocolloid thickeners, increases the viscosity of the batter, thereby providing even crumb expansion on baking. The utilisation of emulsifiers and hydrocolloids could aid higher sugar and fat reduction/replacement levels in bakery products. In a study conducted by Manisha et al., (2012) hydrocolloids and emulsifiers increased the batter viscosity, decreased hardness and increased cohesiveness and lightness of cakes containing 100% sucrose replacement with liquid sorbitol.

The perception of sugar content in foods is strongly related to sweetness intensity whereas the perception of fat in foods is not strongly related to actual fat content (Drewnowski & Schwartz, 1990). The perception of fat in foods is more dependent on textural properties and a combination of fat and replacers used. As the perception of fat is more reliant on textural properties it is logical to focus on ways to create the illusion of fat content. Creating the illusion of a higher fat content can be achieved by adding thickeners to make products more viscous (Drewnowski & Almiron-Roig, 2010). The use of thickeners in prepared milk products and chocolate drinks have been shown to aid in creating the illusion of a higher fat content (Iqbal & Mido, 2005).

Furthermore, the addition of flavourings and aroma compounds to bakery products could increase the perceived intensity of sugar and fat content. In a review on the strategies to reduce sugar in foods conducted by Monaco et al., (2018) a new multisensory approach was proposed to reduce sugar in foods by the addition of appropriate aromas.

1.6 Assessing the impact of sugar and fat reduction/replacement: Methodologies

There are several important methods employed to determine the impact of sugar and fat reduction/replacement strategies on the overall quality of bakery products. These methods can be divided into the following four main categories of analysis;

-Sensory

-Physical

-Compositional

-Microbiological composition

1.6.1 Sensory analysis

A wide variety of sensory evaluation methods exist. When choosing the type of sensory method to use, the first factor to consider is exactly what question you are seeking to answer. Sensory analysis can be divided into two main categories; Sensory acceptance testing and Descriptive profiling. Discrimination testing can be considered as a separate category of sensory analysis, but this method can be used to either determine differences between two or more products in relation to liking of certain attributes, or the differences between two or more products in relation to the intensity of sensory attributes. Thus, in this way discrimination testing can be used as a sensory acceptance method to determine acceptability of products and/or as a descriptive method to determine the descriptive profile of samples.

Food researchers and industry professionals use sensory analysis as a method to assess the impact of using different ingredients and different processing techniques on the acceptability and descriptive profile of food and beverage products. Sensory analysis can be used to benchmark newly developed products against commercially available

standards and to determine sensorial shelf life of products during storage periods. Sensory analysis is also used in industry as a method of quality control (Munoz, 2002).

1.6.1.1 Sensory Acceptance testing (SAT)

Sensory acceptance testing (SAT) also known as affective testing or hedonic testing is a sensory method employed to measure the degree of acceptability and liking/preference of products or its specific sensory properties (Cardello and Jaeger, 2010). Sensory acceptance testing can take place in a laboratory environment or in a more central location. A smaller number of panellists is required (n=25) when SAT takes place internally in a laboratory environment as internally recruited participants are familiar with the products being tested. Consumer testing typically takes place in a central location using panellists that are unfamiliar with the products being tested, for these reasons consumer testing requires a larger number of participants (n=100) (Stone et al., 2012). Consumer testing is typically carried out at the end of product development as a way to validate newly developed products, whereas SAT is carried out throughout the product development process (Sullivan, 2016). Methods employed for SAT include the following; paired comparison test, hedonic scale, alternative number scale testing, ranking, the face or smiley scale and other scales such as the semantic, Likert and the just about right (JAR) scales (Stone et al., 2012).

The 9 point hedonic scale is the most useful and internationally recognised, reliable and easy to use method for sensory research on hedonics and it traditionally comprises of a series of nine verbal categories ranging from ‘extremely dislike’ to ‘extremely like’ (Amerine et al., 1965). A ‘numbers only’ 9 point hedonic scale may also be employed for sensory research however, results from both variations have been shown to be incomparable (Nicolas et al., 2010). Using a 9 point hedonic scale, sensory participants

are typically asked to rate their degree of liking for specific sensory properties such as appearance, colour, flavour, texture and overall acceptability (Laguna et al., 2014;Rodríguez-García et al., 2012;Handa et al., 2012). Other variations of the 9 point hedonic scale have been used to measure sensory acceptance, such as 5 point hedonic scale (Jeddou et al., 2017;Wekwete and Navder, 2007) for cakes and cookies. In recent years unstructured line scales have been used to measure the sensory acceptance of products such as processed meat (Fellendorf et al., 2016) and coffee (Stokes et al., 2016).

1.6.1.2 Descriptive sensory analysis

Descriptive analysis has been described as the most sophisticated tool used for sensory research and several different methods are described in the literature. The first formal method for descriptive profiling was the flavour profile method which is a qualitative method, whereby screened participants discuss and agree to a product description in an open session and a report is generated (Stone et al., 2012). Quantitative descriptive analysis (QDA) is a well-known descriptive method which allows for the description of products to be fully quantified. It involves an initial screening process where participants are tested on their ability to detect and describe differences between products. Several training sessions are required for vocabulary development, definitions of agreed vocabulary and also development of a reference sample which represents both extremities of the scale (Richter et al., 2010). Training sessions are also carried out for participants to familiarise themselves with measurement techniques such as scales. Participants are tested on their ability to discriminate between samples before eventually taking part in descriptive tests. Although QDA is a very successful method for the characterisation of products it is expensive and time consuming. As a result of

this, several rapid sensory profiling methods have been developed (Sullivan, 2016). A recent review of the novel methodologies employed for product description was carried out by Varela & Ares (2012). In this review, the theory and implementation, data analysis and applications and modifications for the following four novel product characterization methods are described; sorting, flash profiling, projective mapping, napping and check all that apply (CATA). The review also includes a section describing alternative methodologies, which included methods such as intensity scales.

A specific intensity scale method was proposed by Richter et al., (2010) called ranking descriptive analysis (RDA) which was shown to produce similar results to QDA and Free choice profiling (FCP). To account for panellists being less trained, a larger number of participants was required for RDA (n=21) compared to QDA (n=12). RDA involves some training for attribute development and agreement and also for familiarisation with intensity scales. All samples are ranked on the same scale for each individual intensity descriptor. RDA has been successfully used to characterise processed sausage and pudding style products (Fellendorf et al., 2016) and coffee (Stokes et al., 2016).

1.6.1.3 Sensory test design

Limiting variability between participants is very important when conducting sensory analysis tests, therefore, controlling the testing environment is imperative to obtaining reproducible results. The international organisation for standardisation offers general guidance for the design of test rooms irrespective of the type of sensory method being carried out (ISO 8589:2007). Sensory analysis typically takes place in a facility designed specifically for the purpose of analysis. These facilities are the same whether they are located in research institutions, universities or industry. Sensory facilities, which are referred to as sensory science laboratories are divided into two spaces; the preparation area and the individual booths. Sample preparation takes place in the preparation area. To limit variability between samples, all samples are portioned out identical to one another for example all samples are cut into the same size/shape. Samples are placed on plates and given individual randomised 3 digit codes to avoid bias, before being passed through individual dividing doors that separate the preparation area from the individual booths. In most test facilities, booths are equipped with various types of lighting which in some cases may be used to limit the effect of the colour/appearance of samples on preference or perceived intensity of certain attributes (Stone at al., 2012). In order to obtain reliable and reproducible sensory results one must consider the psychological errors that may occur. A total of 9 psychological errors are depicted and explained by Stone et al., (2012). Appropriate training will minimise the impact of the following psychological errors; error of central tendency (participants avoiding extremes of the scale), error of habituation & anticipation (participants reporting perception of stimulus before threshold is reached) and logical & leniency error (panellists unfamiliar with the task and self-determine how to complete it). Serving samples in random or counterbalanced order will minimise the

impact of the following error; time error order (scoring the first product higher than any other product). Appropriate product selection will minimise the effect of the following error; contrast and convergence effect (where differences between products may be exaggerated if they are very different, also large differences between products may mask smaller differences). However, this error cannot be avoided if the objective is to compare experimental samples with commercial gold standards, however serving samples in randomised or counterbalanced order will minimise the effect of the error. Randomising the order in which sensory attributes appear on the scorecard is also important to minimise the proximity error which is attributes that are closer to each other on the score card tend to obtain similar scores. Finally, panel selection is very important to minimise the effects of the stimulus error which occurs when panellists are too familiar with the products being tested, as a result participants score products based on knowledge and not on the perception of a specific stimulus. This error occurs when highly trained panellists take part in preference or acceptance tests. Appropriate panel selection can also minimise the impact of the halo effect. The halo effect occurs when consumers rate their degree of liking of sensory properties in an acceptance test and then proceed to rate the intensity of those attributes in a descriptive test. The initial response in the hedonic test will affect subsequent responses in the intensity test.

Therefore, the design of test rooms, controlling the environment, appropriate product and panel selection, training and order of samples are very important factors to consider when conducting sensory analysis trials.

1.6.1.4 Multivariate data analysis for sensory evaluation

Multivariate data are data with many variables, from minimum of six variables to millions. For sensory, such data usually includes control variables (sample treatments) and/or characteristics/properties (sensory responses). Multivariate data analysis techniques can be used to model sample treatments and sensory responses and find the relationship that exists between all factors and responses and can extract useful information from multivariate data. Multivariate methods allow for the analysis of data with more variables than samples and can be used to reveal 'latent' information. Multivariate methods provide several tools for interpretation such as plotting which allows for the easy detection of outliers in the dataset. Unscrambler software is a very useful tool for analysing multivariate data. Multivariate data analysis techniques include Principal Component Analysis (PCA), Multivariate Curve Resolution (MCR) and Cluster analysis. These techniques can be used to find main patterns and variations in the data and only involve one data matrix. PCA is a dimension reduction technique used to determine how samples relate to each other. Regression methods such as Partial Least Squares Regression (PLSR) and Principal Component Regression (PCR) are used to establish linear relationships between two sets of variables (Martens et al., 2000). Raw data from sensory evaluation is typically coded into Microsoft excel and brought through a series of pre-processing techniques, such as data normalisation (taking the logarithm to achieve uniform precision over the whole range of variation) and standardisation (dividing each variable by its corresponding standard deviation). To validate the model and to achieve significant results for the relationships determined in quantitative PLSR, coefficients can be analysed by jack-knifing which is based on custom cross-validation and stability plots (Martens & Martens, 1999). In a study carried out by Bower, (1999) PLSR analysis was used to identify sensory variables

which were most effective in predicting consumer liking of cereal bar snack foods. Fellendorf et al., (2018) used PLSR to assess the impact of replacers on the sensory and physicochemical properties of reduced salt black puddings and in a study carried out by Stokes et al., (2016) PLSR analysis was used to assess the effect of temperature profiles on the sensory (hedonic & intensity) attributes of black coffee.

1.6.2 Physical analysis

Physical analysis is a broad term used by food researchers and industry professionals to describe the measurement of the physical properties of food and beverage products. The physical properties of food and beverages can be measured using assessment by a sensory panel or by instrumental analysis. Similar to sensory analysis, physical analysis is used as a method to assess the impact of using different ingredients and different processing techniques on the overall quality of food and beverage products. Physical analysis can be used to benchmark newly developed bakery products against commercially available standards and to determine shelf life of products during storage periods (staling). Physical analysis is also used in industry as a method of quality control.

The physical parameters of bakery products that are most commonly assessed in food reformulation studies include the following; dough/batter rheology (Sudha et al., 2007;Zoulias et al. 2000;Struck et al. 2016), cake volume and height (Rodríguez-garcía et al., 2014;Ronda et al. 2005), biscuit spread (Handa et al. 2012;Kweon et al. 2009), texture (Roßle et al., 2011; Miller et al., 2017) and colour (Karp et al., 2017;Vatankhah et al., 2017). These physical parameters can be measured using various instrumental techniques. This section of the review will focus on instrumental methods for texture and colour analysis.

1.6.2.1 Texture analysis

The acceptability of cakes and biscuits is dependent on important textural attributes. The instrumental analysis of texture offers a rapid and inexpensive alternative to the sensory analysis of texture (Fiszman, 2013). Food researchers and industry professionals may use both methods to determine textural properties of bakery products during food reformulation studies, quality control and shelf life studies. There are several different tests used to assess the instrumental texture of bakery products. The most common of which are Texture profile analysis (TPA) for cakes and breads and the three-point bend test for biscuits.

TPA and three-point bend tests are carried out using an instrument called a texture analyser. Instrumental TPA was developed by Szczesniak, (1963) who introduced five basic independent textural parameters (hardness, cohesiveness, adhesiveness, viscosity, and elasticity) and three dependent parameters (brittleness, chewiness, and gumminess). During TPA the sample is placed on a platform and a cylindrical probe is used to compress the sample twice (first and second bite). The peak force during the first compression cycle represents the hardness of the sample, springiness is the height to which the food recovers during the time between the end of the first compression and the start of the second, and cohesiveness is the ratio of the positive force area during the second compression to the positive force area during the first compression. During TPA, measurements of resilience (area during the withdrawal of the first compression divided by the area of the first compression) and chewiness (hardness × cohesiveness × springiness) are also determined (Pons and Fiszman, 1996).

The 3-point bend test is used to measure the maximum force required to break biscuit-type bakery products. Biscuits are placed on two parallel fulcrums and the third fulcrum is lowered. The maximum force required to fracture the biscuit is obtained.

Irrespective of the type of texture test being carried out, it is important to define the measurement parameters, such as the loading speed of the sample, sample shape and dimensions, degree of deformation and for TPA specifically, the time interval between the two compression cycles, in order to apply instrumental TPA properly (Pons & Fiszman 1996).

1.6.2.2 Colour analysis

Colour is an important visual cue which influences the consumer's decision to purchase. Typically, the colour of bakery products is assessed on the product surface (crust) and on a cut transverse section (crumb). The colour of bakery products can be measured using visual assessment by a sensory panel or by using instrumental colour analysis which is generally measured using a chroma meter. Chroma describes the intensity of a fundamental colour with respect to the amount of white light in the background (Boakye and Mittal, 1996). The CIE system for the description of colour was developed by the International commission on Illumination and is based on using standard sources of illumination and a standard observer (Robertson, 1997). By integrating the object spectrum with the colour matching functions of the standard observer and the spectral power distribution of the illuminant, the CIE system transforms an object's reflectance into three-dimensional space (Macdougall, 1994). CIE tristimulus values are based on the visible spectrum. By a set of three imaginary red (X), green (Y), and blue (Z) primaries any colour is uniquely specified (Giese, 1995). These tristimulus values are usually converted to another colour scale such as the CIE Lab or Hunter Lab scales to

make colour data more intuitive and easier to interpret, The Hunter L, a, and b and CIE L, a, b colour scales are commonly used in the food industry. These scales measure the degree of lightness (L), the degree of redness (+a) or greenness (-a) and the degree of yellowness (+b) or blueness (-b) (Giese, 1995).

1.6.2.3 Other instrumental analysis techniques

Microscopic analysis involves the visual assessment of the internal structure of biscuit and cake systems which can be used to improve our understanding of the relationship between structure and texture (Fiszman et al., 2013). This method of analysis can be used to determine bubble distribution (Kocer et al., 2007) and cellular structure of the crumb (Rodríguez-garcía et al., 2013). Techniques used include, confocal laser scanning microscopy and scanning electron microscopy.

Acoustic properties and sound emissions of biscuits can also be measured during fracturing using a texture analyser inside an isolated acoustic chamber with a microphone in combination with a preamplifier microphone (Laguna et al., 2013;Laguna et al., 2014)

1.6.3 Compositional analysis

Food compositional analysis includes the quantification of total moisture, fat, protein, ash and carbohydrate. Mandatory compositional data on food labels include; energy value, fat, of which saturates, carbohydrates, of which sugars and protein and salt (FSAI, 2019). The composition of foods is the foundation of diet planning for those who suffer from non-communicable diseases such as diabetes and pancreatitis (Resman et al., 2019). The chemical analysis of food is therefore very important in industry as a method of quality control and also in research during food reformulation studies. Furthermore, food compositional data is very important to nutrition and public health. Several methods of chemical analysis exist for the assessment of moisture, fat, protein, ash and carbohydrate. Details of the various methods including methods approved by the Association of Official Analytical Chemists (AOAC) and the International Organisation for Standardisation (ISO) are depicted in Nielsen (2003).

Traditionally oven drying methods approved by the AOAC were used for moisture determination, however as these methods are time consuming, rapid methods for moisture determination based on infrared and microwave drying are becoming more popular. Ideally, AOAC methods for moisture determination should be carried out in combination with rapid methods of moisture determination.

Several methods exist for the determination of total fat content such as the soxhlet method which involves standard solvent extraction and acid hydrolysis (Nielsen, 2003). This method, however, is also time consuming and requires highly skilled personnel which leaves room for error. Rapid instrumental techniques for the determination of fat such as nuclear magnetic resonance (NMR) are becoming more popular. The technique is based on measurement of the NMR response obtained from fat in the product, and

quantification of the fat content by simple and direct calibration without the use of chemometrics (Nielsen, 2003).

Other compositional properties such as ash (muffle furnace 500-600 ˚C) protein (kjeldahl) and carbohydrate content (calculation) can be quantified using AOAC approved methods.

1.6.4 Microbiological composition & spoilage of bakery products

Food microbiology is a major determinant of quality, safety and shelf-life. Microbiological analysis is therefore very important during food reformulation programmes carried out by industry and research institutions. The stability of baked products depends on both their moisture contents and water activities (Cauvin and Young, 2010). Moisture content and water activity are not identical but as moisture content increases water activity increases. Baked goods with a high water activity (<0.85) are susceptible to microbiological spoilage by bacteria and yeasts and moulds as more moisture is available for their growth (Smith et al., 2004). As biscuits have a low moisture content (2-5%) and a low water activity (<0.6a_w) they are not very susceptible to microbial spoilage (Cauvin and Young, 2010). Moist cakes and breads have higher water activities (0.90-0.97a_w) and are therefore more prone to microbiological spoilage.

Total plate count (TPC) is a method used for the measurement of total or viable bacteria growth. Plating techniques include; spread plate or pour plate. TPC involves the following steps; serial dilution, plating, incubation and enumeration. As mould growth is the major microbial spoilage agent for bakery products, measurement of yeast and mould growth is also important and can be determined using the same techniques as used for TPC. Standard approved methods for TPA and Yeast & mould analysis are

depicted in the FDA's Bacteriological Analytical Manual (FDA-BAM, 2001). The Food Safety Authority of Ireland (FSAI) sets guideline microbiological limits for pathogens, microbial toxins, hygiene indicators and aerobic colony counts in ready-to-eat foods placed on the market (FSAI, 2019).

The physicochemical changes that occur in bakery products over time affect quality and sensory acceptability and thus these changes, or the rate at which these changes occur determines the sensory shelf life of bakery products. According to Del Nobile et al., (2003) the main factors that cause deterioration in baked goods are staling and lipid oxidation. According to Luyts et al., (2013) staling of cakes is a result of moisture migration from crumb to crust and starch retrogradation.

1.7 Conclusion

This review examined the use of natural replacers as a strategy for the reduction of sugar and fat in bakery products, with particular attention to inulin and steviol glycosides as sugar replacers, and legumes as fat replacers. From extensive review of the literature, no work has been carried out on the simultaneous replacement of sugar and fat in bakery products using these natural replacers. Furthermore, few studies have measured the impact of sugar and fat replacement on both the descriptive sensory profile and sensory acceptance of these products. Thus, review of the literature, highlighted the need to measure the impact of a simultaneous replacement of sugar and fat in bakery products, using natural replacers, in order to determine the levels of fat and sugar needed for acceptance.

A potential new approach for sugar reduction in bakery products, which involves the optimisation of the physical from of sugar was also highlighted. As discussed, optimising the physical form of salt (reducing salt particle size) has been used as an approach to reduce salt in crisps. From extensive review of the literature, no work has been carried out on the use of this approach for the reduction of sugar in bakery products. Furthermore, no work has been carried out to measure the impact of sugar particle size on the descriptive profile and sensory properties of these products. Thus, this review highlighted the need for a novel approach to reduce sugar in bakery products by optimising the physical form of sugar.

This review also examined the important factors, which should be considered before formulating sugar and fat reduction strategies, with particular attention to consumer attitudes towards the use of sugar replacers. Results from several surveys have highlighted a feeling of caution towards the use of artificial sweeteners and also a trend towards the consumption of clean-label products. From extensive review of the

literature, we found that no data had been collected on the attitudes, knowledge and consumption behaviour of Irish consumers on the intake of sugar and associated replacers.

Thesis objectives

This thesis was carried out with the following objectives;

- Obtain consumer perception data pertaining to sugar and specific classes of sugar replacers and to determine consumption patterns of products containing these ingredients **(Chapter 2).**
- Examine the feasibility of sugar particle size manipulation as a strategy for sugar reduction in Chocolate brownies (**Chapter 3)** and Shortbread biscuits (**Chapter 6)** using sensory and physicochemical analysis.
- Use sensory analysis to investigate sugar particle size manipulation as a potential strategy for fat reduction in chocolate brownies **(Chapter 4)**.
- Determine levels of fat and sugar required for acceptance of Chocolate brownies using PBLB and inulin & steviol glycosides as fat and sugar replacers respectively **(Chapter 4)**, employing sensory and physicochemical analysis.
- Use sensory and physicochemical analysis to determine levels of fat and sugar required for acceptance of Sponge cake using PBRB and inulin & steviol glycosides as fat and sugar replacers respectively **(Chapter 5)**.
- Assess the consumer acceptability of optimised sugar and fat reduced bakery products (Chocolate brownies and Sponge cakes) compared to products commercially available in the Irish market using consumer sensory acceptance tests **(Chapter 7)**.
- Determine microbial stability of developed products (Chocolate brownies and Sponge cake) (**Chapter 7**).

Chapter 2.

A study on the consumption patterns and behaviour of Irish consumers on the intake of sugar and associated replacers

Abstract

The purpose of the present study was to gain an understanding of the consumption behaviour of Irish consumers and to get an insight into their knowledge and belief towards sugar and sugar replacers in food and beverage products. The study was conducted through the use of two separate surveys, circulated online and analysed independently. Data was summarized as frequencies for each question and presented in contingency tables. Significance was determined using Chi-square analysis, and where statistical differences existed, were identified using Chi square post-hoc tests. A total of 53.7% of participants were wary of sugar replacers. While most participants consumed sugar rich foods (SRF) on a daily basis (54.3%) only 4.2% drank sugar rich beverages (SRB) this regularly. Young adults (18-24) and those with a leaving cert (secondary level) education were the least likely demographic groups to know the difference between a non-caloric artificial sweetener (NAS) and a natural sugar replacer (NSR) ($p<0.019$). The majority of participants indicated that if a sugar tax was introduced in Ireland in 2018, this would influence their purchase of SRB (66%). Participants were much more likely to purchase food and beverages containing NSR, with the inclination to purchase foods and drinks containing NAS decreasing with advancing education. Responses obtained were insightful and inform the debate around sugar reduction in foods and beverages for regulators, the industry and the general consuming public.

2.1 Introduction

Food and nutrition consumption surveys are an extremely important research tool in establishing consumption trends of food products and also establishing correlations between food intake and related health outcomes. Several national adult consumption surveys have been carried out in Ireland including the North/South Irish Food consumption survey (NSIFCS) (IUNA, 2001), the National adult Nutrition survey (NANS) (IUNA, 2011) and the Healthy Ireland survey (Healthy Ireland, 2015). Findings from the National adult survey reported that Irish people ranked nutrition and health as the second most important determinant of food choice after taste (IUNA, 2011). According to a report by Bord Bia, the trends that dominate the Irish grocery market are health and wellness, sustainability & plastics and origin & provenance (Irish Food Board, 2019). Although there are trends towards nutrition and health, recent findings from the Healthy Ireland survey showed that 65% of Irish people consumed snacks and sugar sweetened beverages on a daily basis (Healthy Ireland survey, 2015) Furthermore, sweet foods are major contributors to the intake of dietary sugars (15-25%) in Europe (Azaïs-braesco et al., 2017).

The implications of a high-sugar diet have been well documented. The intake of sugars is a determinant of body weight, with a clear positive association between higher intakes of sugars, body fat and long-term weight gain in adults (Te Morenga et al., 2013). One in four Irish adults is obese (Morgan et al., 2008). Excess weight in humans is a known risk factor for many other diseases, including; high blood pressure, abnormal blood fat level, increased blood-clotting tendency and raised blood insulin levels and type-2 diabetes mellitus (National taskforce on Obesity, 2005). Tooth decay is another implication of a high sugar diet and lower intakes of sugar have been shown to reduce the risk considerably (Moynihan and Kelly, 2014).

Thus, the area of sugar reduction is very topical nationally, as well as in a global context. The introduction of a new sugar tax in Ireland has generated an even greater interest in food and beverage companies in strategies built around sugar replacement/reduction in high sugar products.

Important factors to consider when formulating sugar reduction strategies are consumer trends, purchasing patterns and consumer knowledge and beliefs. In recent years, consumers are leaning more and more towards clean label natural food products (Bizzozero, 2017). However, the use of artificial sweeteners as sugar replacers in products by industry is escalating. According to Yang, (2010) there was an increase of over 6000 products containing non-nutritive sweeteners in the US between 1999 and 2004. However, controversy exists over their use. Artificial sweeteners, in particular saccharin, sucralose and aspartame altered the intestinal microbiota and drove the development of glucose intolerance in mice fed commercial formulations of these sweeteners (Suez et al., (2014). Rats fed yoghurts supplemented with saccharin showed increased weight gain compared to rats that were fed yoghurts containing sucrose in another study conducted by Feijó et al., (2013). The consumption of aspartame has also been associated with migraines in human subjects (Newman and Lipton, 2001) and seizures in rats due to increased phenylalanine levels in the brain (Maher and Wurtman, 1987). Furthermore, a recent US Mintel survey indicated that 64% of its respondents were concerned about the safety of "artificial" sweeteners (Gardner et al., 2012).

Thus, understanding the knowledge, belief and consumption patterns of Irish consumers on the intake of sugar and their associated replacers is very important if Irish companies are to successfully implement sugar reduction/replacement strategies. From review of the scientific literature to date, the consumption patterns of Irish consumers

on the intake of sugar and associated replacers does not appear to have been investigated.

This study involved the distribution and analysis of two separate surveys. The primary objective of the first survey was to obtain data relating to the perception of sugar replacers among Irish adults. Gaining insight into the consumption patterns of sugar rich foods (SRF) and beverages (SRB) and sugar replaced food and beverage products was another aim of this survey. The second survey was undertaken with the primary purpose of obtaining consumer perception data pertaining to specific classes of sugar replacers such as, non-caloric artificial sweeteners (NAS) and natural sugar replacers (NSR). Data relating to the consumption patterns of products containing these specific ingredients was also obtained. Consumer understanding of food labels was also assessed and the impact of a sugar tax on the purchasing of sugary products was investigated. Demographic information was gathered to establish whether age, gender, or education level affected the knowledge, belief and consumption patterns of these products.

2.2 Materials and Methods

2.2.1 Survey design and questions

In September, 2016 a survey with the title ‘Sugar in Foods and Beverages’ was designed and developed. In January, 2017 a second survey was designed with the following title “NAS versus NSR in foods and beverages”. Only consumers who took part in the first survey were invited to participate in the second survey.

The first page of both surveys (Page 1) provided respondents with definitions and examples of terms which were presented as follows;

Balanced diet - a diet consisting of a variety of different types of food and providing adequate amounts of the nutrients necessary for good health.

Sugar replacers - Food additive that provides a sweet taste like that of sugar while containing significantly less food energy than sugar, making it a zero-calorie or low-calorie sweetener.

Sugar rich foods - For the purpose of this survey, sugar rich foods were defined as food products that contain adequate amounts of natural, ‘added’ or ‘free’ sugars. Examples such as non-calorie reduced yoghurts, milk, cream, non-calorie reduced cereal products, cakes, biscuits, jams, honey, savoury sauces, dairy desserts, chocolate and sweets were given.

Sugar rich beverages - For the purpose of this survey, sugar rich beverages were defined as beverages that contain high amounts of ‘added’ or ‘free’ sugars. Examples such as carbonated drinks, fruit juices and energy drinks were given.

Non-caloric artificial sweeteners (NAS) - Synthetic organic compounds which can be several times sweeter than sucrose. Examples: Aspartame, sucralose, saccharin, cyclamate, acesulfame-k

Natural sugar replacers (NSR) - Substances with a low energy content that exist naturally in the environment which can be extracted and purified. Examples: Carbohydrates (polydextrose, maltodextrins), Fibres (inulin, oligofructose) polyols (maltitol, xylitol) and steviol glycosides

Sugar replaced/reduced foods - 'Sugar free' or calorie reduced food products. Example; sugar free yoghurts, calorie reduced sauces, no added sugar cereal products, sugar reduced bakery goods.

Sugar replaced/reduced beverages - 'Sugar free' or calorie reduced beverage products. Example; diet carbonated drinks, no added sugar fruits juices.

Participants were asked to become familiar with the terms and definitions on Page 1 before proceeding to page 2, which contained socio-demographic-based questions such as age, gender and education level. Education level selections were as follows; leaving cert/secondary level education (high school, post-primary education) (LC), primary degree (PD), post graduate degree (PG) or higher degree (HD). The middle section of both surveys contained knowledge and belief-based questions which are outlined, with their respective responses in Table 2.2. The last section of both surveys included consumption-based questions which focused on the eating and purchasing habits of respondents. Questions pertaining to consumption habits and their respective responses are outlined in Table 2.3.

2.2.3 Distribution and statistical evaluation

Surveys were circulated online through University mailing lists and social media websites. Completed questionnaires were exported into a Microsoft Excel worksheet and transferred into IBM SPSS statistics 20 for windows to carry out statistical analysis. Surveys were analysed independently. Data was summarized as frequencies for each question and presented in contingency tables. Significance was determined using Chi-square analysis, and where statistical differences existed, were identified using Chi square post-hoc tests. A significance level of $p<0.05$ was set and this was adjusted to control type 1 error rates. The adjusted p value = 0.05/number of analyses performed.

2.3 Results and Discussion

2.3.1 Respondent demographic

Respondent demographics are presented in Table 2.1. A total of 1,166 responses were collected from the first survey. The majority of respondents were female (76.2%) which was expected as women are more likely to partake in surveys (Curtin et al., 2000; Porter and Whitcomb, 2005; Curtin et al., 2007; Fellendorf et al., 2018) Most respondents were aged between 18 and 24 (30.4%) followed by 25-34 (23.5%). The majority of participants had achieved a PD level of education (29%) followed by a PG degree (28.1%). A total of 600 people who participated in the first survey took part in the second survey. The first survey was distributed in September which is the start of the academic year when people are generally more positive and willing to take part in studies. Similar to demographic results obtained from the first survey, most of the participants were women (76.2%). Unlike the first survey however, most participants were aged between 25 and 34 (35.5%) followed by 18-24 (25.6%). A PD level of education was also the highest level of education achieved by the majority of respondents who took part in the second survey (36.1%) followed by a PG degree (26.4%).

Table 2.1

Respondent demographic

Survey 1			Survey 2	
	n	%	n	%
Age				
18-24	354	30.4	154	25.6
25-34	274	23.5	212	35.5
35-44	211	18.1	97	16.1
45-54	191	16.3	79	13.2
55+	136	11.7	58	9.7
Gender				
Female	889	76.2	459	76.2
Male	277	23.8	141	23.3
Education[a]				
LC	289	24.8	132	21.9
PD	338	29.0	216	36.1
PG	328	28.1	159	26.4
HD	211	18.1	93	15.6
TOTAL	1166	100	600	100

[a]Education level abbreviations; Leaving Certificate (LC) Primary degree (PD) Post-graduate degree (PG) and Higher Degree (HD).

2.3.2 Knowledge and belief of consumers on the intake of sugars and associated replacers

Findings from the first survey showed that the majority of participants cared about having a balanced diet (95%) (Table 2.2). Young adults (18-24) cared less ($p<0.000$) about having a balanced diet than any other age group. With the exception of the 'over 55' age group, caring about having a balanced diet increased with advancing age. Research on the nutrition-related attitudes of Canadian students within this age bracket (18-24) has shown that students fail to see the effect of their current actions on their long-term health status (National Institute of Nutrition, 2002). Young adults underestimate their risk of heart disease and findings from a study conducted by Green et al., (2003) reported that 68% of college aged men and women viewed their risk of a heart attack as lower than that of their peers. This documented youth optimism could be a reason why young Irish adults were found to care less about having a balanced diet in the present study. Caring about having a balanced diet increased with advancing education with the exception of those in the HD education bracket.

Results from the first survey showed that the majority of participants (53.7%) were wary of sugar replacers with a higher percentage ($p<0.000$) of people in the 45-54 age group being cautious of them. Patterns show that the belief that sugar replacers are safe decreased with advancing age and education, with the exception being determined for those with a HD level of education. This caution towards sugar replacers has previously been reported by Patterson et al., (2012) who found that "no added sugar" claims were preferred to "reduced sugar" claims on products, with consumers assuming that sweeteners would be added to reduced-sugar products.

Findings from the second survey showed that the majority of consumers (65.2%) know the difference between NAS and a NSR, as they appear on food and beverage product

labels. However, 18-24 year olds were the least likely age group ($p<0.019$) to know the difference between these two classes of sugar replacers (45.8%). Trends show that the ability to discriminate between the two classes of sugar replacers increased with advancing age. More females than males claimed to know the difference. Education level made the largest impact on participant's knowledge of the different classes of replacers. Consumers with a HD were the education group most likely ($p<0.000$) to know the difference (89.3%) and participants with a LC level of education were the least likely ($p<0.000$) to know the difference. Respondents with a PD had the second lowest number of respondents ($p<0.000$) admitting to know the difference (57.4%). These results were expected as people with a lower level of education have less experience and exposure to this kind of knowledge.

When asked whether they understood food and drink product labels, a total of 50.9% of people said 'Yes' and 49.1% said 'No'. Within the education demographic respondents with a HD level of education were the group most likely to understand product labels. Research on consumer attitudes towards food labelling in Europe has revealed that consumers find information on food products confusing, and although the majority of respondents usually answer "yes" when asked if they understand food labels, respondents also usually answer "yes" when asked whether food labels need to improved (Grunert and Wills, 2007).

The majority of respondents who participated in the second survey stated that a sugar tax would influence their intent to purchase SRF and SRB (66%). A higher percentage ($P<0.001$) of people within the 18-24 age bracket (66%) said that a sugar tax would influence their intent to purchase SRF and SRB, whereas people in the 'over 55' age group were the least likely ($p<0.001$) to be affected by a sugar tax. The influence of a sugar tax on the purchasing of these products decreased with advancing education with

more people with a HD level of education ($P<0.001$) saying a sugar tax would not influence their intent to purchase these products (64.5%).

Table 2.2
Knowledge and belief of consumers on the intake of sugars and associated replacers

	Age (%)					p value[a]	Gender (%)		p value[a]	Education[b] (%)				p value[a]	Total
	18-24	25-34	35-44	45-54	55+		M	F		LC	PD	PG	HD		
Survey 1															
Q. Do you care about having a balanced Diet? (Fruit, vegetables, fibre)															
Yes	91.5[0.00006]	94.9	97.6	99.5[0.003]	96.3	0.000	94.0	96.0	0.200	92.7	94.7	97.2	96.7	0.044	95.5
No	8.5[0.00006]	5.1	2.4	0.5[0.003]	3.7		6.2	4.3		7.3	5.3	2.8	3.3		5.0
Q How do you feel about sugar replacers?															
Safe	12.8	9.5	9.1	5.8	13.2	0.000	11.7	9.8	0.612	12.5	9.6	7.9	12.0	0.423	10.2
Unsafe	10.2	12.0	9.1	15.2	12.5		10.3	11.9		10.4	11.9	12.8	10.5		11.5
Wary	48.0	52.6	57.2	66.0[0.002]	48.5		52.0	54.3		48.8	54.9	56.1	55.0		53.7
Other	29.0	26.0	24.5	13.1[0.00006]	25.7		26.0	24.0		28.4	23.6	23.2	22.5		24.7
Survey 2															
Would you know the difference between an artificial sweetener and a natural sugar replacer as they appear on food labels?															
Yes	54.3[0.0009]	67.0	69.8	70.9	72.4	0.019	62.1	66.2	0.383	55.0[0.005]	57.4[0.002]	70.2	89.3[0.000]	0.000	65.2
No	45.8[0.0009]	33.0	30.2	29.1	27.6		37.9	33.8		45.0[0.005]	42.6[0.002]	29.8	10.8[0.000]		34.8
Q Do you understand food & beverage labels?															
Yes	55.6	49.5	49.0	48.1	51.7	0.756	55.7	49.6	0.203	55.7	49.1	44.3	60.1	0.058	50.9
No	44.4	50.5	51.0	51.9	48.3		44.3	50.4		44.3	50.9	55.7	39.8		49.1
Q Would a sugar tax on products influence your intent to purchase?															
Yes	66.0[0.0002]	53.6	47.9	46.8	36.2[0.006]	0.001	57.9	51.9	0.213	61.8	56.5	52.2	35.5[0.000]	0.001	66.0
No	34.0[0.0002]	46.5	52.1	53.2	63.8[0.006]		42.1	48.1		38.2	43.5	47.8	64.5[0.000]		34.0

[a] P value; level of significance within a group. Superscript values indicate where significance lies.
[b] Education level abbreviations; Leaving Certificate (LC) Primary degree (PD) Post graduate degree (PG) and Higher Degree (HD)

2.3.3 Consumption patterns of consumers

Findings from the first survey showed that the majority of participants consumed SRF on a daily basis (54.3%) (Table 2.3). Trends showed that respondents in the 'over 55' age group and respondents with a HD level of education were the least likely demographic groups to eat these foods on a daily basis.

When asked how often SRBs were consumed, the majority of respondents said on an occasional basis (38%). As little as 4.2% of participants said they drank SRB daily which is low considering the high percentage (54.3%) of respondents consuming SRF this regularly. With that said, the likelihood of never drinking SRB increased with advancing age and education. It was observed that 18-24 year olds were the most likely age group ($p<0.0000$) to consume these drinks on a daily and weekly basis. A lower percentage of people (17.2%) ($p<0.0000$) in the 18-24 age cohort said they never drank SRB. Only 22.9% of respondents in the 25-34 age bracket said they never drank SRB ($p<0.0000$). A higher percentage of people (66.9%) within the 55+ age bracket said they never drank SRB ($p<0.0000$). Significant differences were also observed between genders, with more men ($p<0.020$) drinking SRB on a daily basis than women. This result is similar to findings from the Heathy Ireland survey where males aged between 15 and 24 were the highest daily consumers of sugar-sweetened drinks (Healthy Ireland, 2015). In a study conducted by West et al., (2006) more male students drank SRB on a daily basis than female students. Within the education demographic, participants with a HD were the most likely educational group to never drink SRB ($p<0.000$). A higher percentage of people ($p<0.0000$) with an LC level of education drank SRB on a weekly basis. Only 18.7% of respondents ($p<0.0000$) with this level of education said they never drank SRB

Findings from the first survey show that the majority of respondents purchase sugar-replaced foods (61.4%), with a higher ($p<0.003$) percentage of respondents in the 18-24 age bracket purchasing these products (69.2%). The majority of respondents, however, claimed not to purchase sugar-replaced drinks (52%). More female respondents claimed to purchase sugar-replaced food and beverages then male respondents however results were not significant. The majority of respondents from every educational grouping indicated that they purchase sugar-replaced foods, with the LC educational cohort of respondents being the most likely to purchase these products ($p<0.0017$). The majority of respondents from every educational category claimed that they would not purchase sugar-replaced beverages with the exception being for participants with a LC level of education.

Results from the second survey showed that participants were much more likely to purchase foods containing NSR (78.3%) then they were to purchase foods containing NAS (43.9%). This finding is in agreement with results obtained from Sensus (2016), where it was observed that natural replacers were preferred to artificial sweeteners with 55% of respondents saying that the type of sweetener used in reduced-sugar food products influenced their decision on purchasing those products (Kenward, 2016). In the present study, trends showed that age and education level influenced consumption of NAS-containing foods. People in the youngest age category were the most inclined to buy foods containing NAS, whereas people within the oldest age bracket (55+) were the least inclined to buy these products. Similar trends existed in the educational groupings, with purchasing of these products decreasing with advancing educational level. Although trends were shown through the data generated, no significant differences were determined.

Similar results were obtained when participants were asked if they purchased drinks containing NAS with 45.5% of people claiming to purchase them compared to 70.9% of respondents who claimed to purchase drinks containing NSR. Trends also show that as respondent age increased, the likelihood of purchasing drinks containing NAS decreased. Trends also showed that males were more inclined to purchase drinks containing NAS than women.

Within the age demographic a higher % of people ($p<0.023$) over 55 admitted to not buying foods containing NSR. Respondents with a PG level of education were the most inclined to purchase foods containing NSR, while respondents with a LC level of education were the least inclined to buy these products

Table 2.3
Consumption patterns of consumers

	Age (%)					P value[a]	Gender (%)		P value[a]	Education[b] (%)				P value[a]	Total
	18-24	25-34	35-44	45-54	55+		M	F		LC	PD	PG	HD		(%)
Survey 1															
Q How often do you eat sugar rich foods?															
Daily	55.8	52.8	60.2	55.5	42.7	0.000	52.4	54.9	0.012	55.9	55.5	57.6	49.7	0.099	54.3
Weekly	32.3	37.7	27.5	27.8	28.7		29.2	32.2		31.9	32.3	30.5	31.3		31.5
Monthly	1.7	1.1	1.9	0.5	2.9		0.7	1.8		2.1	2.7	0.6	0.5		1.5
Occasion	9.4	7.7	9.9	15.2	23.5[0.0000]		15.5	10.5		9.7	11.9	10.1	16.6		11.7
Never	0.9	0.7	0.5	1.1	2.2		2.2	0.6		0.4	0.6	1.2	1.9		0.9
Q How often do you drink sugar rich beverages?															
Daily	5.4	5.1	3.8	3.1	1.5	0.000	7.6[0.001]	3.1[0.001]	0.020	5.9	4.1	3.1	3.8	0.000	4.2
Weekly	24.9[0.0000]	21.2	11.9	6.3[0.0000]	3.7[0.0000]		16.9	15.9		21.8[0.0024]	17.8	13.4	9.9		16.1
Monthly	12.4[0.001]	10.2	6.2	6.8	0.7[0.0005]		7.2	8.9		11.4	8.6	7.6	5.7		8.5
Occasion	40.1	40.5	40.8	35.1	27.2		37.9	38.0		42.2	38.8	35.7	34.6		38.0
Never	17.2[0.0000]	22.9[0.0000]	37.4	48.7[0.0000]	66.9[0.0000]		30.3	34.1		18.7[0.0000]	30.8	40.2[0.0013]	45.9[0.0000]		33.2
Q Do you purchase sugar replaced/reduced food products?															
Yes	69.2[0.0002]	57.3	58.8	56.3	60.5	0.032	59.2	62.1	0.582	68.9[0.002]	61.8	57.5	55.5	0.017	61.4
No	30.8[0.0003]	42.3	41.2	43.7	39.6		40.8	37.8		31.1[0.002]	38.2	42.5	43.1		38.5
Q Do you purchase sugar replaced/replaced beverages?															
Yes	53.6	41.8	52.6	46.1	41.9	0.013	44.9	49.0	0.236	53.3	47.9	44.5	46.5	0.168	48.0
No	46.4	58.2	47.4	53.9	58.1		55.1	51.0		46.7	52.1	55.5	53.6		52.0
Survey 2															
Q Do you buy foods containing NAS?															
Yes	48.4	47.2	40.6	38.0	32.7	0.005	44.3	43.8	0.672	47.3	45.8	41.8	37.6	0.016	43.9
No	34.0	38.2	52.1	50.6	60.3		40.7	43.9		33.6	40.3	48.1	54.8		42.9
Don't look	17.7	14.6	7.3	11.4	6.9		15.0	12.5		19.1	13.9	10.1	7.5		13.3
Q Do you buy drinks containing NAS?															
Yes	52.3	47.2	45.3	35.4	34.5	0.006	47.9	44.6	0.682	48.1	43.5	47.8	41.9	0.248	45.5
No	32.0	36.3	47.4	55.7	51.7		37.9	42.0		34.4	41.7	40.8	49.5		40.9
Don't look	15.7	16.5	7.4	8.9	13.8		14.3	13.4		17.6	14.8	11.5	8.6		13.6
Q Do you buy foods containing NSR?															
Yes	77.8	81.1	82.3	74.7	65.5	0.023	71.4	80.1	0.068	75.6	76.4	82.9	77.4	0.137	78.3
No	9.2	8.5	11.5	13.9	25.9[0.0003]		16.4	10.1		10.7	11.1	9.5	17.2		11.3
Don't look	13.1	10.4	6.3	11.4	8.6		12.1	9.8		13.7	12.5	7.6	5.4		10.4
Q Do you buy drinks containing NSR?															
Yes	73.9	72.2	78.1	62.0	58.6	0.079	72.9	70.3	0.786	74.1	68.1	72.8	69.9	0.170	70.9
No	15.0	16.0	15.6	27.9	27.6		16.4	19.0		12.2	19.4	19.0	23.7		18.4
Don't look	11.1	11.8	6.3	10.1	13.8		10.7	10.7		13.7	12.5	8.2	6.5		10.7

[a]P value; level of significance within a group. Superscript values indicate where significance lies.
[b]Education level abbreviations;Leaving Certificate (LC) Primary degree (PD) Post graduate degree (PG) and Higher Degree (HD)

2.4. Conclusion

Young adults (18-24) and those with a LC level of education drink sugar rich beverages more regularly than any other demographic group assessed within this study and are therefore at a higher risk from overconsumption of free-sugar through consumption of these beverages. According to this study however, respondents within these demographic groups are the most likely to be affected by the implementation of the sugar tax, which could affect their purchasing habits and therefore significantly reduce their intake of free-sugar from these products. Although respondents within these demographic groups were very likely to say "Yes" when asked if they understood food labels these groups were also the most likely group to say "No" when asked if they would know the difference between NAS and NSR. These conflicting answers suggest confusion among the younger generation and also highlights that much more needs to be done to educate young adults about food and nutrition.

The caution observed towards sugar replacers in this study, with consumers much more likely to purchase foods and drinks containing natural sugar replacers, is also a finding to be considered by the Irish food industry when formulating sugar replacement strategies. This coupled with the inclination to purchase foods and drinks containing NAS specifically decreasing with advancing age and education perhaps justifies the need for a clean label approach.

The difference between respondent's likelihood to consume sugar rich foods (54.3%) and sugar rich beverages (4.2%) on a daily basis could suggest a growing awareness of the excess amount of free sugar in sugar sweetened beverages. These findings stress that more awareness needs to be made of the excess amount of free sugar also present in food products, perhaps through media/health promotion outlets or taxation.

Chapter 3.

The impact of sugar particle size manipulation on the physical and sensory properties of chocolate brownies

Abstract

The overall objective of this research was to assess the effect of sugar particle size on the physical and sensory properties of chocolate brownies. A commercially available brown sugar was employed for this trial (200-5181 µm) and four of its sieved sugar separates (mesh size of 710, 500, 355 and 212 µm) were determined by grinding and sieving. The particle diameter and diameter distributions of the control sugar and each sugar fraction were measured. As a result, five sugar treatments were determined for chocolate brownie formulations; Control ($C_{200\text{-}5181\ \mu m}$), Large-particle replacement ($LPR_{924\text{-}1877\ \mu m}$), Medium-particle replacement ($MPR_{627\text{-}1214\ \mu m}$), Small-particle replacement ($SPR_{459\text{-}972\ \mu m}$) and a known MIX sample. Samples were tested using sensory (hedonic & intensity), physical (texture and colour) and compositional analyses (moisture and fat). Brownie samples containing the smallest sugar fraction ($SPR_{459\text{-}972\ \mu m}$) were perceived as sweeter than any other sample ($p<0.05$). Brownies containing this fraction were also the softest and moistest samples ($p<0.05$). Texture liking was significantly associated with the $LPR_{924\text{-}1877\ \mu m}$ brownie ($p<0.05$). Darkness of brownie samples increased ($p<0.05$) as sugar particle size decreased. Therefore, sugar particle size alteration affects the physical and sensory properties of chocolate brownies and could be used as a viable approach to reduce sugar in bakery products.

3.1 Introduction

The consumption of free and refined sugar in the diet is one of the main causes for obesity (Hu and Malik 2010; MacGregor and Hashem, 2014). Free and refined sugars include; monosaccharides and disaccharides added to foods and beverages by the manufacturer, cook or consumer, and sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates, as defined by the World Health organisation (WHO, 2015). Intake of sugars is a determinant of body weight, with a clear positive association between higher intakes of sugars, body fat and long-term weight gain in adults (Te Morenga et al., 2013). Findings from the National Adult Survey on Nutrition reported that free sugars account for 14.6% of the total energy intake of Irish adults (IUNA, 2011). According to results from the Healthy Ireland survey (2015), 37% of adults were overweight and a further 23% were obese. Obesity is a strong risk factor for type 2 diabetes (Chan et al., 1994; Rosner et al., 1997). A guideline published by the WHO in March, 2015 strongly recommends that adults and children reduce their daily intake of free sugars to less than 10% of their total energy intake. A conditional recommendation was also made to further reduce free sugar intake to below 5% of total energy intake (WHO, 2015).

Cakes and biscuits account for 7% of carbohydrate intake in Irish adults, therefore reducing sugar in these products would be a significant development in reducing the dietary intake of sugar (IUNA, 2011). However, sugar has multiple functions during batter mixing and baking and thus, reducing this ingredients presents a great challenge for food researchers and industry professionals. Sucrose is the most commonly used sugar in cake making and it is universally known for providing sweetness to cakes and biscuits (Bennion and Bamford, 2013). Sucrose facilitates the incorporation of air during creaming which lightens the batter (Shepard and Yoell, 1976). Air pockets

formed during creaming expand and lift the batter, causing it to rise during baking. Thus, sugar affects final volume and bulk of bakery products during the mixing stage. Sugar also inhibits or reduces gluten development during cake batter mixing by competing with gluten for water and thus, acts as a tenderiser of baked goods (Bennion and Bamford, 2013). During baking sucrose increases the temperature of starch gelatinization and protein denaturation by binding water and therefore limiting water availability to starch granules and protein molecules (Ureta et al., 2016). Thus, sucrose effects final volume and bulk. Furthermore, sugar binds moisture and moisture content varies between the different types of sugar, for example liquid sugars contain more moisture than brown sugar and brown sugar contains more moisture than crystalline white sugar (Manley, 2011). Therefore, sugar is not only responsible for the sweetness of cakes, but contributes significantly and positively to the sensory and physical properties of cakes.

Recent reports indicate that the global sugar substitutes market is valued at around $11.5 billion and it is expected to grow up to $14 billion by 2019 (Markets and markets, 2015). The inclination for combination of high intensity sweeteners (HIS) with bulk sugar replacers to produce a low-calorie bakery product has increased, with HIS such as aspartame and sucralose providing sweetness and bulk replacers providing the bulking properties. However, controversy exists over the use of artificial sweeteners in foods and beverages (Suez et al., 2014; Azad et al., 2016). In a recent US Mintel survey, it was found that 64% of respondents indicated they were concerned about the safety of “artificial” sweeteners. (Gardner et al., 2012). This is an important finding for the food industry if companies are to implement replacement strategies using artificial sweeteners. Therefore, it is necessary to pursue other strategies for sugar reduction/replacement in such products.

In this study, a new strategy of sugar reduction based on the manipulation of sugar particle size is proposed. From extensive review of the scientific literature, we have not been able to detect research investigating the effect of sugar particle size on sweetness perception and overall acceptability of cakes. Sugar particle size has been shown to affect flour cookie quality (Kissel et al., 1973) and in a study conducted by Kweon et al., (2009) ultra-fine sugar in cookie baking, resulted in greater surface crack for sugar-snap cookies, and lower height for wire-cut cookies as a result of a greater rate of dissolution for smaller sucrose crystals. Manley (2011), reported that sucrose crystal size and their rate of dissolution affects the appearance and crunchiness of baked biscuits. Rama et al., (2013) conducted a study on salt particle size manipulation and found that smaller salt crystals increased salt perception in fried sliced potato crisps in a controlled chewing environment. This proves that salt size manipulation can be used to reduce salt in crisp products. Based on these findings, we hypothesise that smaller sugar particles increase sweetness perception in chocolate brownies.

The primary objective of this study was to determine the effect of sugar particle size on the sensory (hedonic, intensity) and physical properties of chocolate brownies and to determine if this approach might constitute an effective strategy for reducing sugar levels in bakery products

3.2 Materials and Methods

3.2.1 Materials

Food ingredients used in this trial, included; light golden soft brown sugar (1.1% moisture, 98% sucrose, cane molasses and invert sugars, Siucra brand, UK); creamery butter (81% total fat, 65.4% of which were saturated, supermarket-own brand, Ireland); cream plain flour (1.4% fat, 82.7% carbohydrate, 2% of which sugars, 3.4% fibre, 11.7% protein and 0.81% salt, Odlums, Ireland); eggs (Upton, Ireland); dark chocolate (34.7% total fat, 55.8% carbohydrate, 97.2% of which sugars, 3.6% protein and 0.1% salt, Homecook wonder bar, Ireland). Food products were all purchased from a local supermarket and stored under refrigerated or cool, dry conditions where appropriate prior to sample preparation.

3.2.2 Preparation of brown sugar particle size fractions; grinding and sieving

Sugar was stored at ambient temperatures of 20ºC prior to grinding and sieving. Sugar was dried at 70º C for one hour (h) in an oven (Binder, ED 115, Germany) to reduce moisture content for more effective sieving. Moisture content was obtained for the sugar, both before and after drying, using methodologies described below. Moisture content (%) was kept constant at 0.5% for all sugar fractions. Dried sugar was ground by hand using rolling pins, in order to obtain adequate amounts of each fraction and mechanically sieved through a sequence of sieves (90, 180, 212, 355, 500, 710, 1,180 and 2,360 µm) set in a mechanical sieve shaker (Endeotts Octagon 200 London, England). Sieving was carried out in batches of 200g of sugar for 10 minutes (min) at 5-mm amplitude and particle size distributions of the sugar, both before and after grinding, were obtained using this method. For the purpose of the baking trials, four sugar-sieve separates were established; 212, 355, 500, and 710µm. The un-ground, un-

sieved, commercially-available parent sugar was used as the control. Several separations were carried out until 1kg sugar quantities were available for all size ranges.

3.2.2.1 Measurement of sugar particle size

Commercial brown sugar was analysed by microscopy using a light microscope (Olympus BX-61 Tokyo, Japan) and cellSens™ standard software (version 510_UMA_Database_cellsens19-Krishna-en_00). Particle size ranges and the average particle size of the commercial sugar and sugar separates captured by different sieve apertures (212, 355, 500 and 710 µm) were determined by obtaining and recording the 2D longest diameter of 100 particles per fraction in transparent light mode. Particle images within each fraction were captured using a microscope digital camera lens (Olympus DP73 Tokyo, Japan).

3.2.3 Chocolate brownie preparation

A standard Chocolate brownie recipe was utilised in this study containing sucrose (28%), butter (19.5%), eggs (20%), flour (13%) and chocolate (19.5%). Five brownie formulations were manufactured containing five sugar size fractions (commercial unground brown sugar ($C_{200\text{-}5181\mu m}$), large sugar ($LPR_{924\text{-}1877\mu m}$), medium sugar ($MPR_{627\text{-}1214\mu m}$), small sugar ($SPR_{459\text{-}972\mu m}$) and a known mix sample of 50% SPR, 40% MPR and 10% of a finer sugar captured by the 212 µm sieve mesh size (MIX). For each formulation, dark chocolate and butter were melted in a heat stable bowl for one min in a microwave oven. The melted mixture was stirred for 30 seconds before sugar was added and stirred manually. Eggs were beaten in a separate bowl and added to the mixture. Ingredients were stirred manually for one min before the flour was sifted into the mixture. Mixture was stirred by hand until smooth. The brownie batter was poured

into tinfoil trays (16.5x24cm) and batches were baked for 30 min at 180̊C in a Zanussi convection oven (C. Batassi, Conegliano, Italy). Brownies were left to set for 30 min in the tray before being removed and cut into individual pieces (45x45mm). Following baking, chocolate brownies were placed on a rack for cooling for one hour before being removed and placed into plastic containers for storage prior to testing.

3.2.4 Sensory analysis

3.2.4.1 Sensory acceptance testing

Sensory acceptance testing (SAT) was carried out in the panel booths of the sensory science laboratory, at University College Cork according to International Standards (ISO 2014). Using untrained assessors (n=25), who were familiar with the products being tested, SAT took place over three separate tasting sessions (Stone et al., 2012a). Samples were assigned a randomised three-digit code, presented in duplicate and sessions were carried out at room temperature under white light. Participants were instructed to use the water provided to cleanse their palates between tastings and used the following hedonic descriptors to rate their degree of liking of biscuit samples; appearance, flavour, texture, colour and aroma. Assessors were asked to indicate their degree of liking for samples on a 9-point, numbers only hedonic scale. Overall acceptability (OA) of samples was also determined using this scale.

3.2.4.2 Ranking descriptive analysis

Ranking descriptive analysis (RDA) was also carried out in the panel booths of the sensory science laboratory at University College Cork. A separate panel of assessors (n=21) who were regular consumers of Chocolate brownies and had received prior training in descriptive analysis were trained and participated in RDA according to the

method of Richter et al., (2010). RDA sessions ran concurrently with SAT sessions and therefore, took place over three sessions. Sensory descriptors were selected from the panel discussion as the most appropriate and reflective of variation in samples profiled. The consensus list of intensity descriptors (Table 3.1) were measured on a 10 cm continuous line scale. The terms 'none' and 'extreme' were used on the 0cm and 10cm anchor points on the scale respectively (unless stated otherwise in Table 3.1). All samples were ranked on the same scale for each intensity descriptor. Samples were served coded in randomised order and presented simultaneously to assessors (Stone et al., 2012).

Table 3.1
Consensus list of sensory descriptors and definitions selected by panel and used in ranking descriptive analysis of Chocolate brownies

Attribute	Definition
Appearance	
Crust darkness	Degree of darkness of crust
Texture	
Hardness	Force needed to compress the sample
Moisture	Moist/wet texture in mouth
Flavour	
Sweet taste	Flavour sensation associated with sucrose
Brownie flavour	Characteristic chocolate brownie flavour
Off flavour	Off-flavour (rancid)
Aftertaste	A taste remaining in the mouth after eating

3.2.5 Chocolate brownie images

Photographs were taken of the chocolate brownie samples portioned out for sensory analysis using a digital camera (Nikon D3200, Japan).

3.2.6 Physical analysis

3.2.6.1 Texture profile analysis

Texture profile analysis (TPA) was used to measure the texture properties of Chocolate brownies. TPA was conducted using a Texture analyser 16 TA-XT2I (Stable Micro Systems, Surrey, UK) and was carried out as described by Martínez-Cervera et al., (2012). Two chocolate brownies (45x45mm) from the centre of each batch tray were used for texture analysis. A 50% double compression test was carried out on each sample with a 75mm diameter flat-ended cylindrical probe (P/75), at a speed of 1mm/s with a 5 sec waiting time between both cycles.

3.2.6.2 Colour measurement

Crust and crumb colour characteristics were assessed using a Minolta CR-200B Chroma meter (Minolta Camera Co. Ltd., Osaka, Japan). Colour measurements were recorded on the CIE (L*a* b*) scale where L* represents lightness (L*=0 [Black], L*= 100 [White]), a* signifies green-redness and b* represents blue-yellowness. Brownies were measured at two separate points. Samples were cut horizontally to remove the crust and crumb colour was measured directly at two separate points.

3.2.7 Moisture and fat

Moisture content was measured using the method of Bostian et al., (1985). Samples were homogenised for analysis using a Büchi Mixer B-400 (Büchi Labortechnik AG, Switzerland). Moisture content was determined using the CEM SMART system and fat was determined using the SMART Trac system (CEM GmbH, kamp-Lintfort, Germany). Two fibreglass pads were placed in the drying chamber of the CEM SMART system and the instrument was tared. Pads were removed and the homogenised samples (2-4g) were weighed accurately on the pads. Following this, one pad was placed over the sample, pressed together and placed back into the drying chamber to begin drying. The moisture (%) was displayed. Fat (%) was determined by wrapping the fibreglass pads with the sample in a sheet of Smart Trac film. Wrapped samples were placed in Smart Trac tube and positioned in the Smart Trac NMR unit. Percentage fat was displayed after roughly 5 Min.

3.2.8 Statistical analysis

3.2.8.1 Sensory data

Three independent trials were carried out for all experimental treatments. Raw data obtained from sensory (hedonic & intensity) evaluation was coded into Microsoft excel and analysed using ANOVA- Partial Least Squares Regression (APLSR) using Unscrambler software version 10.3 (CAMO ASA, Trondheim, Norway). The X-matrix was defined as the different sample treatments. The Y – matrix contained the sensory variables of the design. To achieve significant results for the relationships determined in the quantitative APLSR, regression coefficients were analyzed by jack-knifing which is based on cross-validation and stability plots (Martens and Martens, 2000). Statistical significance for sensory data was defined as $p<0.05$-0.01 (significant), $p<0.01$-0.001 (highly significant) and $p<0.001$ (extremely significant).

3.2.8.2 Physicochemical data

Raw data obtained from physicochemical analysis was coded into Microsoft excel. As three independent trials were carried out for all experimental treatments, data presented represents a mean of six values, unless stated otherwise. As two measurements for crust and crumb colour were taken for each individual sample and two samples were tested for each individual trial, of which there were three, crust and crumb colour values represent a mean of twelve measurements (2 x 2 x 3). One-way ANOVA was used to compare the means of the data obtained from physicochemical analysis. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA).

3.3 Results and Discussion

3.3.1 Particle size distribution

Particle size distribution (PSD) of brown sugar, before and after grinding, is displayed in Fig 3.1. Large differences in sugar particle sizes existed within the parent sugar employed for this trial. Sugar particles captured by 710, 500 and 355µm sieves increased by 34.1%, 17.4% and 7.1%, respectively after grinding. No particles >2,360 µm were present after grinding and particles captured by the sieve with the second largest aperture (1,180µm) decreased by 21.31%. Finer sugar particles (<212µm) were present after grinding. A visual representation of the particle diameter distribution of the commercial sugar and individual sugar separates is displayed in Fig 3.2. Particle size diameter differences between the commercial sugar and individual sugar separates were evident. The commercial sugar had the widest particle size distribution as expected with particles ranging from 200-5181 µm. After grinding and separation, particle size distribution within each fraction became smaller, in the range of 924-1877 µm for LPR, 627-1214 µm for MPR and 459-972 µm for SPR. Particle size ranges and mean sizes for commercial sugar and each sieved sugar-separate are shown in Table 3.2. Microscopic images for brown sugar particles with 2D diameters for different mesh sizes are represented in Fig 3.3.

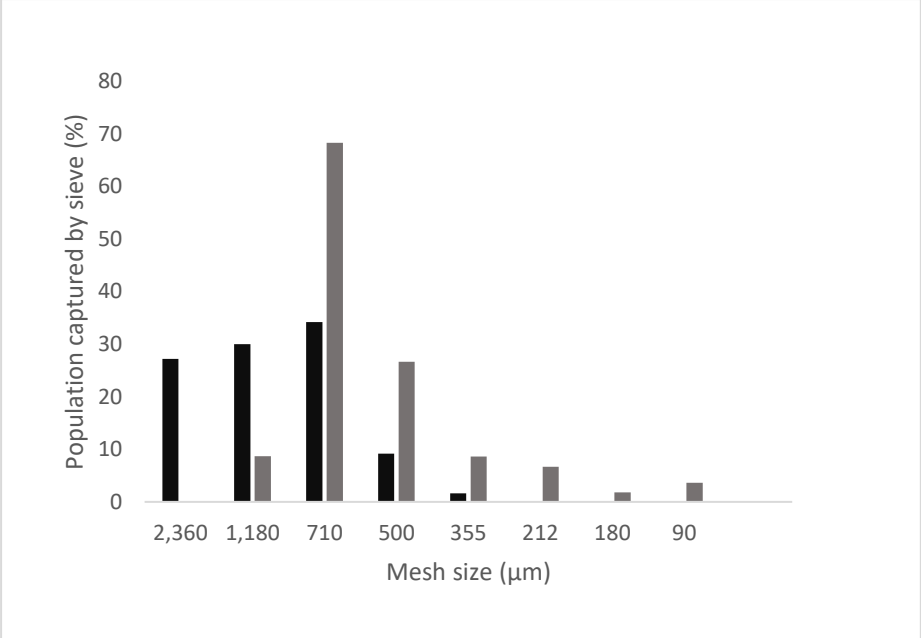


Fig 3.1. Particle size distribution of dried brown sugar (200g, 0.5% moisture) before (■) and after (■) grinding.

Table 3.2
Particle size ranges of parent sugar and sugar sieve separates (µm)

Sugar-sieve separates (µm)	Sample	Particle size ranges (µm)	Average particle size (µm)
Parent sugar	Control (C)	200-5181	1533
710	LPR	924-1877	1276
500	MPR	627-1214	930
355	SPR	459-972	652
212	10% of MIX[a]	330-700	479

[a]MIX sample; SPR (50%), MPR (40%) & a finer particle size captured by the 212µm sieve mesh size (10%)

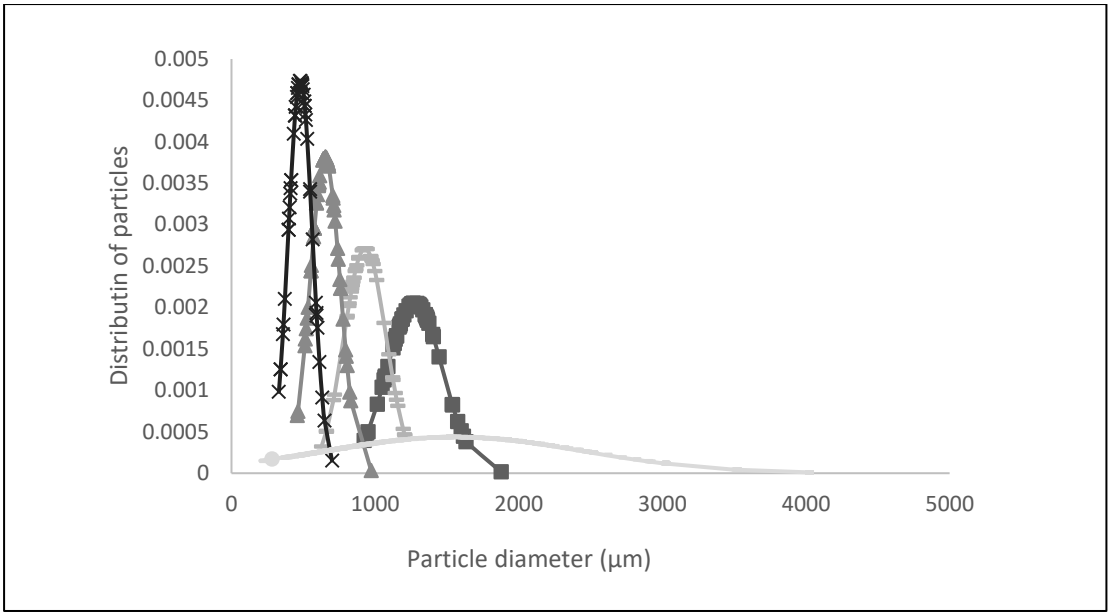


Fig 3.2. Particle diameter distribution of parent brown sugar before grinding: control ( ) and sugar-sieve separates; 710 ( ), 500 ( ), 355 ( ) and 212 µm ( ) after grinding.

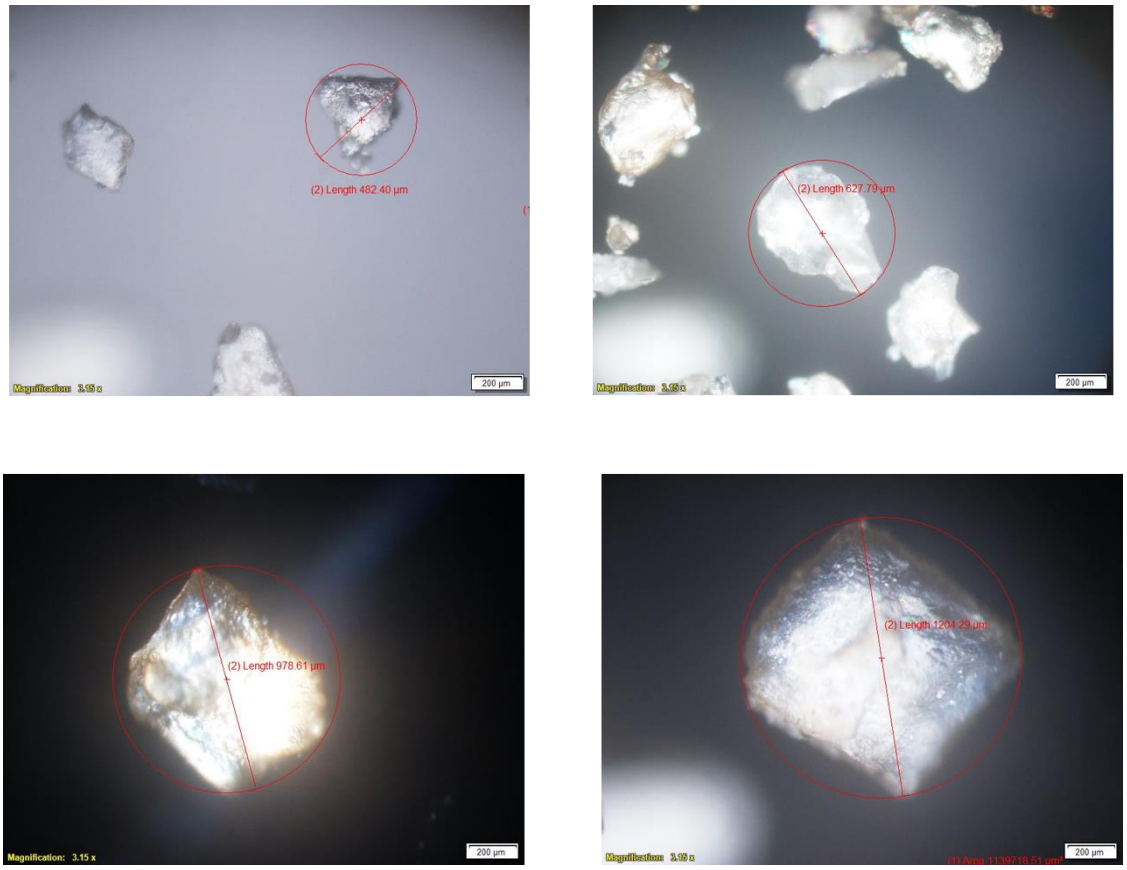


Fig 3.3 Microscopic images of brown sugar particles captured by different sieve apertures after grinding. From top left, 212µm and 355µm apertures and from bottom left 500µm and 710µm apertures. Red line across particle indicates diameter of the particle.

3.3.2 Relationship between Chocolate brownie treatments and sensory properties

A total of 54.8% of the sensory evaluators who participated in this study were female and 45.2% were male. Ages of assessors ranged from 18-45. Significance of estimated regression coefficients for the relationship of sensory terms and chocolate brownies are presented in Table 3.3.

The commercial brownie sample ($C_{200\text{-}5181\ \mu m}$) was negatively associated with crust darkness, ($p<0.001$). Brownie samples containing $LPR_{924\text{-}1877\ \mu m}$ were significantly positively associated with colour liking ($p<0.05$) whereas brownie samples containing $SPR_{459\text{-}972\ \mu m}$ were negatively associated ($p<0.05$).

Chocolate brownie samples with $LPR_{924\text{-}1877\ \mu m}$ were positively correlated with texture liking ($p<0.01$). Samples containing $SPR_{459\text{-}972\ \mu m}$ were associated with having a moist texture ($p<0.001$). These samples were also significantly negatively associated with texture hardness ($p<0.01$). Therefore brownie samples containing this fraction ($SPR_{459\text{-}972\ \mu m}$) were perceived as the softest and moistest samples. In contrast control samples ($C_{200\text{-}5181\ \mu m}$) were perceived as the hardest samples ($p<0.05$). Replacement of sucrose with different fibres has been shown to increase crumb firmness in muffins (Struck at al., 2016). The authors cite air cell incorporation as a contributing factor to mechanical resistance. The presence of larger sugar particles in the $C_{200\text{-}5181\ \mu m}$ sample in the present study could have impacted upon air cell incorporation and could therefore be contributing to the increased hardness observed in samples.

The chocolate brownie samples containing the smallest sugar fraction ($SPR_{459\text{-}972\ \mu m}$) were perceived as significantly sweeter than any other sample ($p<0.05$). This finding is in agreement with results obtained for salt crystal size manipulation, with smaller salt particles being shown to increase the perceived of crisps (Rama et al., 2013).

Images of chocolate brownie samples divided out for sensory analysis can be seen in Fig 3.4. Visual variation in brownie texture was evident. In agreement with the sensory data presented in this study, brownie samples containing $SPR_{459\text{-}972\ \mu m}$ had the greatest moist appearances.

Table 3.3
Significance of estimated regression coefficients (ANOVA values) for the relationship of sensory terms (hedonic & intensity) and Chocolate Brownies prepared with different sugar size fractions

Samples[a]	Appearance Liking	Flavour Liking	Texture Liking	Colour Liking	Overall acceptability	Crust darkness	Hardness	Moisture	Sweet taste	Brownie Flavour	Off flavour	Aftertaste
$Control_{200\text{-}5181\ \mu m}$	0.289	0.478	0.366	0.669	0.463	-0.003**	0.021*	0.061	0.519	0.695	0.574	0.886
$LPR_{924\text{-}1877\ \mu m}$	0.041*	0.186	0.012**	0.050*	0.359	0.809	0.134	0.507	0.433	0.431	0.897	0.530
$MPR_{627\text{-}1214\ \mu m}$	0.607	0.102	0.439	0.939	0.258	0.104	0.662	0.602	0.432	0.192	0.422	0.449
$SPR_{459\text{-}972\ \mu m}$	0.187	0.657	0.134	-0.012**	0.877	0.413	-0.002**	0.001***	0.045*	0.850	0.370	0.502
MIX	0.521	0.996	0.720	0.439	0.655	0.595	0.980	0.544	0.105	0.272	0.805	0.413

Significance of regression coefficients; $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***).
(–); indicates whether the correlation is negatively correlated.
[a]LPR; large particle replacement MPR; medium particle replacement and SPR; small particle replacement and a known MIX sample.



Fig 3.4. Cross section images of chocolate brownies (2 x 2 x 2 cm). Samples were taken from the upper right midsection of each batch tray. From left: Control, LPR, MPR, SPR and MIX sample.

3.3.3 Texture and colour properties of Chocolate brownies

Results from Texture profile analysis are displayed in Table 3.4. In agreement with sensory data, chocolate brownie samples containing $SPR_{459\text{-}972\ \mu m}$ were the softest samples with the force (45.1 ± 2.42 N) required to compress brownie samples being lower ($p<0.05$) than determined for any other sample. Contradictory to the sensory data, brownie samples containing $LPR_{924\text{-}1877\ \mu m}$ were the hardest samples (69.2 ± 2.12 N) ($p<0.05$). As sugar particle size decreased, hardness values decreased significantly ($p<0.05$) with the exception of the Control and Mix samples. These results are similar to mean cake strength results obtained by Dozan et al., (2014), who found that cake strength increased with increasing sugar particle size due to the force required for crystal breakage, as well as cake breakage. This study also found that the force required to compress cakes with larger crystals was greater than the force required to compress cakes with smaller crystals. In our study, chewiness values (N-mm) varied significantly between samples. Chocolate brownies containing $SPR_{459\text{-}972\ \mu m}$ were found to have the lowest value (4.2 ± 0.23 N-mm) for chewiness and were different ($p<0.05$) from all other samples. Brownie samples containing $MPR_{627\text{-}1214\ \mu m}$ presented the second lowest value ($p<0.05$) for chewiness (5.0 ± 0.50 N-mm) and samples containing a mix of sugar particle sizes (MIX) obtained the third lowest value ($p<0.05$) for chewiness 6.2 ± 0.13 N-mm). $Control_{200\text{-}5181\ \mu m}$ and $LPR_{924\text{-}1877\ \mu m}$ brownie samples were not significantly different from each other with regards chewiness, but both samples presented the highest values ($p<0.05$). The slightly higher chewiness values (9.8 ± 0.12 N-mm) associated with chocolate brownie samples containing $LPR_{924\text{-}1877\ \mu m}$ could be why these samples were liked so much in terms of texture and also may be a reason why these samples were not perceived correctly as the hardest samples as determined during sensory evaluation (Table 3.3). No significant differences were observed between

brownie samples with respect to other physical product properties such as adhesiveness, springiness, cohesiveness, or resilience.

In accordance with sensory data, control brownie samples had the lightest crust, and were different ($p<0.05$) from all other samples (Table 5). Trends showed that as sugar particle size decreased, darkness of crust colour increased. The control sample also had the lightest crumb colour ($p<0.05$) compared to all other brownie samples, with the exception of those samples containing $LPR_{924\text{-}1877\ \mu m}$. Trends showed that as sugar particle size decreased, darkness of crumb colour increased, with samples containing $SPR_{459\text{-}972\ \mu m}$ having the darkest crumb colour (24.4 ± 1.81).The darker crumb and crust colour may be due to the lower melting point of smaller sugar crystals which caramelize quicker than larger sugar crystals. The darker crumb observed for the $SPR_{459\text{-}972\ \mu m}$ could be why this sample was negatively associated with colour liking as determined by sensory evaluation.

3.3.4 Moisture and fat content of Chocolate brownies

As anticipated, fat (%) did not vary between sample treatments. The average fat content determined in brownies ranged from 26.24 to 27.64% as shown in Table 3.6. However, moisture content varied significantly between samples. As sugar particle size decreased, moisture content increased in brownie samples, with the exception being that of the MIX sample. $Control_{200\text{-}5181\ \mu m}$ and $LPR_{924\text{-}1877\ \mu m}$ brownie samples had the lowest moisture content and were different ($p<0.05$) from samples containing $MPR_{627\text{-}1214\ \mu m}$ and $SPR_{459\text{-}972\ \mu m}$, but not significantly different from the MIX sample. Chocolate brownie samples containing $SPR_{459\text{-}972\ \mu m}$ had the highest ($p<0.05$) moisture content (13.0 ± 0.84) compared to all other brownie samples, with the exception of samples containing $MPR_{627\text{-}1214\ \mu m}$ which had the second highest moisture content ($p<0.05$).

Table 3.4
Texture profile analysis (TPA) values for Chocolate brownies formulated with different sugar particle sizes.

	TPA					
Sample	Hardness (N)	Adhesiveness	Springiness (mm)	Cohesiveness (n/a)	Chewiness (N-mm)	Resilience (n/a)
$Control_{200\text{-}5181\mu m}$	54.5 ± 1.45^{b}	-0.0 ± 0.73^{a}	0.5 ± 0.74^{a}	0.3 ± 0.05^{a}	9.1 ± 0.80^{d}	0.1 ± 0.01^{a}
$LPR_{924\text{-}1877\mu m}$	69.2 ± 2.12^{c}	-0.0 ± 0.59^{a}	0.5 ± 0.06^{a}	0.3 ± 0.04^{a}	9.8 ± 0.12^{d}	0.1 ± 0.01^{a}
$MPR_{627\text{-}1214\mu m}$	52.0 ± 2.75^{b}	-0.0 ± 0.01^{a}	0.3 ± 0.04^{a}	0.3 ± 0.03^{a}	5.0 ± 0.50^{b}	0.1 ± 0.01^{a}
$SPR_{459\text{-}972\mu m}$	45.1 ± 2.42^{a}	-1.0 ± 1.17^{a}	0.3 ± 0.07^{a}	0.3 ± 0.05^{a}	4.2 ± 0.23^{a}	0.1 ± 0.02^{a}
MIX	53.4 ± 1.72^{b}	-0.0 ± 0.81^{a}	0.3 ± 0.04^{a}	0.3 ± 0.05^{a}	6.2 ± 0.13^{c}	0.1 ± 0.02^{a}

^{abc} mean values (± standard deviation) in the same row bearing different superscripts are significantly different, $p<0.05$.

Table 3.5
Colour lightness values (L*) for Chocolate brownies formulated with different sugar particle sizes

Sample	Colour (L*)	
	Crust	Crumb
$Control_{200\text{-}5181\mu m}$	39.8 ± 1.85^{a}	29.0 ± 1.51^{a}
$LPR_{924\text{-}1877\mu m}$	34.2 ± 0.93^{b}	27.1 ± 1.82^{ab}
$MPR_{627\text{-}1214\mu m}$	33.2 ± 1.22^{b}	25.1 ± 0.80^{b}
$SPR_{459\text{-}972\mu m}$	33.1 ± 1.95^{b}	24.4 ± 1.81^{b}
MIX	32.6 ± 0.80^{b}	25.4 ± 1.73^{b}

^{abc} mean values (± standard deviation) in the same row bearing different superscripts are significantly different, $p<0.05$.

Table 3.6
Moisture and fat of chocolate brownie samples prepared with decreasing particle size

Sample	% Moisture	%Fat
$Control_{200\text{-}5181\mu m}$	9.2 ± 0.80^{a}	26.3 ± 1.87^{a}
$LPR_{924\text{-}1877\mu m}$	9.9 ± 1.08^{a}	27.6 ± 1.68^{a}
$MPR_{627\text{-}1214\mu m}$	11.8 ± 1.03^{bc}	26.6 ± 1.95^{a}
$SPR_{459\text{-}972\mu m}$	13.0 ± 0.84^{c}	26.9 ± 1.28^{a}
MIX	10.3 ± 0.70^{ab}	26.2 ± 0.77^{a}

^{abc} mean values (± standard deviation) in the same row bearing different superscripts are significantly different, $p<0.05$

3.4 Conclusions

This work demonstrates that sugar particle size manipulation has a significant impact on the physical and sensory properties of chocolate brownies. Chocolate brownies formulated with $LPR_{924\text{-}1877\ \mu m}$ received the highest scores for liking of texture, appearance and colour. Thus, replacement of the commercial sugar with this experimental fraction improved acceptance of the final product. Therefore, sugar within this size range could be used to improve the texture and appearance of low-sugar or partially-replaced sugar in bakery products. Chocolate brownies prepared with the smallest sugar particle size ($SPR_{459\text{-}972\ \mu m}$) were the softest and moistest of all samples as supported by sensory, instrumental and compositional analysis. This is an important finding as sugar within this size range could be employed to retain moisture and softness in low sugar/low fat bakery products. Chocolate brownies formulated with the smallest sugar particles were perceived as the sweetest samples. Based on these findings sugar particle size reduction would permit sugar reduction as sweetness perception is increased in samples prepared with smaller sugar particles. Further research needs to be carried out to demonstrate this finding further. In conclusion, sugar particle size reduction increases the sensory perception of sweetness in chocolate brownies and could be used as a viable technological approach to effectively reduce the sugar content of bakery products and be of benefit to the baking industry in the formulation of low-calorie, clean-label baked goods.

Chapter 4.

Investigating the impact of sugar particle size and utilisation of natural substitutes for replacement of fat and sucrose in Chocolate brownies, employing sensory and physicochemical analysis

Abstract

As the perception of fat in foods is strongly related to texture and mouthfeel, it is logical to focus on ways to create the illusion of fat by manipulating texture. Utilisation of small sugar particles (459-972µm) has been shown to increase the perceived moisture and softness of Chocolate brownies. The first objective of this study was to investigate the impact of sugar particle size, using three sugar fractions (Commercial (UC), large (L) and Small (S)) on the sensory (hedonic & intensity) properties of brownies containing four levels of fat replacement (FR) (0, 25, 50, 75%). Twelve formulations were manufactured (UC_0, UC_{25}, UC_{50}, UC_{75}, L_0, L_{25}, L_{50}, L_{75} S_0, S_{25}, S_{50}, S_{75}). Pureed black beans (PBLB) were used as fat replacers. Sensory analysis was employed to establish the best formulation that achieved the maximum level of fat reduction. Four more formulations containing increasing levels of sucrose replacement (SR) (0-75%) using inulin and rebaudioside A were manufactured. These brownies were characterised by sensory and physicochemical (texture, colour, compositional) analysis. The S_{25} sample had the highest correlation with overall acceptability (OA) ($p<0.01$). Utilisation of the (S) sugar fraction permitted higher levels of FR (75%) without negatively affecting OA, compared to other sugar fractions. SR of 25% in PBLB brownies showed the highest acceptance. Perceived sweetness did not discriminate between samples containing increasing levels of SR. Brownies with increasing levels of SR presented higher crumb hardness ($p<0.05$). Results indicate that inulin and rebaudioside A replacing sucrose is feasible at 25% in fat-replaced (75%) brownies prepared with small sugar particles, using PBLB.

4.1. Introduction

Bakery products such as cakes and biscuits account for 6% of the total fat intake of Irish adults (IUNA, 2011). Sweet foods (cakes, biscuits, puddings, pies) also contribute highly to the intake of dietary sugars (36-61%) in Europe (Azaïs-Braesco et al., 2017). According to data generated from the North/South food consumption survey, Irish adults are exceeding recommendations for both fat and sugar intake (IUNA, 2000). Irish adults consumed a total of 61.9g of added sugar/day, which is high considering the recommendations made by organisations such as the World Health Organisation (WHO) (<50g/day for the average adult) and the American Heart Association (AHA) (<25g/day for women and <37.5g/ day for men) for added sugar intake (WHO, 2015; Johnson et al., 2009).

Chocolate brownies can be described as cake-like bars, classified by a high sugar (20-30%), fat (15-25%) and chocolate (20%) content. Typically, no leavening agents are added to the batter, creating a fudge-like, dense texture. Foods rich in fat and sugar are highly palatable (Drewnowski & Greenwood, 1983; Drewnowski et al., 1992). The presence of fat and sugar in food yields positive hedonic responses to attributes such as aroma, texture and flavour (Drewnowski et al., 1989). The risk of overconsumption of foods containing a mixture of fat and sugar is high as positive hedonic responses may override metabolic responses such as satiety (Blundell & Macdiarmid, 1997). Cake-like products are typically energy-rich and nutrient-poor food products (Schirmer, et al., 2012). Thus, consumption of these products can lead to dietary imbalances which have been associated with diseases such as obesity (WHO, 2003).

In Ireland, 37% of adults are overweight and a further 23% are obese according to findings from the Healthy Ireland survey (2015). Excess weight is a known risk factor for type-2 diabetes and it is estimated to be responsible for 90% of type 2 diabetes cases

worldwide (Hossain et al., 2007). Other known negative health implications of obesity, include; increased risk of cardiovascular problems, certain cancers and gall bladder disease (National Obesity Task Force, 2005). According to the WHO, over half a million people died in 2002 from obesity related complications (WHO, 2003).

Therefore, reduction of sugar and fat in cake-like products such as Chocolate Brownies, could be a significant development in reducing the dietary intake of both sugar and fat. Furthermore, replacement of fat and sugar with functional ingredients such as low-calorie carbohydrates or fibres could increase the nutritional quality of these well-liked products.

However, successful replacement of fat and sugar presents a great challenge for researchers because of the multiple functional properties of these ingredients in the dough and during baking. Fat plays a vital role in the tenderisation of cakes and also adds lubricity to the texture by coating the protein and starch particles, thereby interfering with the protein matrix (Pyler, 1988). Fat emulsifies large amounts of liquid during baking which adds more moisture and softness to the final product (Wade, 1988). The most common function of sugar in cakes is providing sweetness to the final product (Bennion and Bamford, 2013). Sugar binds water (Manley, 2011) and inhibits or reduces the development of a strong gluten network, by competing with gluten proteins for available water. Thus, sugar acts as a tenderiser of baked goods (Martínez-Cervera et al., 2012). Sugar also delays starch gelatinisation during baking and in this way contributes to final volume and bulk.

The perception of fat in foods is not strongly related to fat content and is more dependent on textural properties (Drewnowski & Schwartz, 1990), because of this, it is logical to focus on ways to create the illusion of fat content in foods by manipulating texture. The use of thickeners in prepared milk products and chocolate drinks aid in

creating the illusion of a higher fat content (Iqbal & Mido, 2005). In previous related work, the utilisation of small brown sugar particles (459-972µm) increased the perceived moist and soft texture of chocolate brownies (Chapter 3). Given what is known about the functions of fat in bakery goods (provides lubricity, tenderness), it was hypothesised that Chocolate brownies prepared with small sugar particles would retain moisture and softness better than those prepared with commercial sugar and thereby, permit higher levels of fat replacement. Purred black beans (PBLB) were used to replace fat as legumes have been established as effective fat replacers while also increasing the nutritional value of bakery products (Uruakpa and Fleisher, 2016;Rankim and Bingham, 2000). The impact of sugar particle size as a means of facilitating fat replacement in chocolate brownies merits investigation. Therefore, the first objective of this study was to investigate the impact of three brown sugar fractions (including the commercial sugar) on the sensory properties (hedonic & intensity) of Chocolate brownies containing increasing levels of fat replacement (0, 25, 50 & 75%). Sucrose replacement as a strategy for sugar reduction in bakery products has been the focus of numerous studies (Ronda et al., 2004; Manisha et al., 2012; Gao et al., 2017; Martínez-Cervera et al., 2012). The value of using fibres (inulin) as sucrose replacers in cakes has been demonstrated (Zahn et al., 2013; Rodríguez-García et al., 2013). Inulin, a plant derived storage polysaccharide, imparts important textural attributes and bulking properties to cakes by binding water (Meyer et al., 2011). It can be considered a prebiotic dietary fibre as it stimulates the growth of bifiobacteria and lactobacilli (Shoaib et al., 2016). Furthermore, the incorporation of dietary fibre into foods is considered a principal prevention strategy against the risk of non-communicable disease (Stephen at al., 2017). As inulin has a low relative sweetness compared to sucrose (10%), combining inulin with intense sweeteners such as steviol glycosides is

necessary. Approved for use in the EU in 2011, individual steviol glycosides, such as rebaudioside A are considered natural intense sweeteners (Scardigli, 2011). The impact of inulin and rebaudioside A on the sensory and physical properties of PBLB Chocolate brownies merits investigation. Therefore, the second objective of this study was to examine the effect of sucrose replacement (SR) (0, 25, 50 & 75%) using inulin and rebaudioside A on the sensory (hedonic & intensity) and physicochemical (texture, colour, compositional) properties of PBLB chocolate brownies, in order to determine the levels of sucrose needed for sensory acceptance

4.2. Materials and Methods

4.2.1 Materials

Food ingredients used in this trial were; light golden soft brown sugar (1.1% moisture, 98% sucrose, cane molasses & invert sugars, Siucra brand, UK); butter (81% total fats, 65.4% of which were saturated & 15.1% moisture, supermarket own-brand, Ireland); black beans (18% carbohydrate, 0.5% of which were sugars, 8.2% protein, 6.4% fibre, 0.8 % fat, 0.2% of which were saturated, 0.02% sodium, 0.05% salt & 73.2% moisture (after being drained and pureed), Suma brand, UK); inulin (89% fibre & 8% sugar, Bioglan brand, Austrailia); rebaudioside A (Bulk powders brand, Ireland); cream plain flour (82.7% carbohydrate, 2% of which were sugars, 11.7% protein, 3.4% fibre, 1.4% fat, & 0.81% salt, Odlums, Ireland); eggs (Upton brand, Ireland); dark chocolate (55.8% carbohydrate, 97.2% of which sugars, 34.7% fats, 3.6% protein & 0.1% salt, Homecook wonder bar, Ireland). Food products were all purchased from a local supermarket unless stated otherwise and stored under refrigerated or cool, dry conditions where appropriate prior to sample preparation.

4.2.2 Preparation of brown sugar particle size fractions; grinding, sieving and measurement

Preparation of brown sugar was carried out according to the methods described in Chapter 3. To obtain adequate amounts of different sugar sizes, commercial sugar was ground manually using rolling pins. Sugar was then separated into different size fractions by sieving, using sieves of varying apertures, (90, 180, 212, 355, 500, 710, 1,180 and 2,360 µm) set in a mechanical sieve shaker (Endeotts Octagon 200 London, England). The particle diameter distribution of the commercial sugar and two of its sieved separates were determined by obtaining and recording the 2D longest diameter

of 100 particles per fraction in transparent light mode (Olympus BX-61 Tokyo, Japan) and cellSens™ standard software (version 510_UMA_Database_cellsens19-Krishna-en_00). Two sugar fractions collected in different sieve apertures (710µm) (large) and 355µm (small) were selected for inclusion in the Chocolate brownie formulation.

4.2.3 Chocolate brownie formulation for fat replacement

A standard Chocolate brownie recipe was utilised in this study. Twelve Chocolate brownie formulations were manufactured containing three sugar size fractions (unground commercial sugar (UC), large sugar (L) and small sugar (S) and four fat replacement levels (0, 25, 50 & 75%). For each formulation, (UC_0, UC_{25}, UC_{50}, UC_{75}, L_0, L_{25}, L_{50}, L_{75} S_0, S_{25}, S_{50}, S_{75}) dark chocolate and butter were melted in a heat stable bowl in a microwave oven. The melted mixture was stirred before sugar was added. Eggs were beaten in a separate bowl and added to the mixture. Ingredients were stirred until flour was sieved into the mixture and stirring continued manually until smooth. Preparation was carried out as described in Chapter 3 and adapted for fat replacement. Black beans were drained and pureed in a Stephan mixer (UMC-5 Stephan u. Sohner & Co, Hameln, Germany) at 21 RPM for 5 Mins before being added to the batter in partial replacement of butter. The batter was poured into tinfoil trays and baked for 30 Min. Brownies were left to set for 30 Min before being removed and cut into individual pieces. Samples were placed on a rack for cooling for one hour before being removed and placed into plastic containers for storage prior to testing.

4.2.4 Chocolate brownie formulation for sugar replacement

Before manufacture of Chocolate brownies, a ranking test was used to determine the concentration of rebaudioside A necessary to replace the sweetness concentration of sucrose, ensuring iso-sweetness. Ranking tests were carried out twice using 21 assessors. Concentration adjustments for rebaudioside A were carried out according to the method of (Zahn et al., 2013) using similar concentrations of stevia (0.06-0.16g/l) and 24g/L sucrose. One-way ANOVA was used to compare the means of the data obtained for each solution. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA). Following this, four formulations were manufactured containing four sugar replacement levels (0, 25, 50 & 75%). For each formulation; $S_{75/0}$, $S_{75/25}$, $S_{75/50}$, $S_{75/75}$ (where the first letter denotes the sugar particle size and the first and second digits represents the FR and SR level respectively) chocolate brownies were manufactured as previously described in (5.2.3) and adapted for sugar replacement. Inulin and rebaudioside A were mixed together in a separate bowl before being added to the brownie batter in partial replacement of sucrose.

Table 4.1

Formulation (%) of the different Chocolate brownie treatments

Chocolate brownie samples [ab]	Sucrose	Butter	PBLB	Eggs	Flour	Dark chocolate	Inulin	Reb A
UC_0 L_0 S_0	27.9	19.6	0	20.1	12.8	19.6	0	0
UC_{25} L_{25} S_{25}	27.9	14.7	4.9	20.1	12.8	19.6	0	0
UC_{50} L_{50} S_{50}	27.9	9.8	9.8	20.1	12.8	19.6	0	0
UC_{75} L_{75} S_{75}	27.9	4.9	14.7	20.1	12.8	19.6	0	0
$S_{75/0}$	27.9	4.9	14.7	20.1	12.8	19.6	0	0
$S_{75/25}$	20.9	4.9	14.7	20.1	12.8	19.6	7.0	0.018
$S_{75/50}$	14.0	4.9	14.7	20.1	12.8	19.6	13.9	0.03
$S_{75/75}$ -	7.0	4.9	14.7	20.1	12.8	19.6	20.9	0.05

[a] Unground commercial sugar fraction (UC) (200-5181µm), Large sugar fraction (L) (924-1877µm), Small sugar fraction (S) (459-972µm).
[b]The first digit represents the FR level and the second digit represents the SR level.

4.2.5 Sensory analysis

4.2.5.1 Sensory Acceptance testing

Sensory acceptance testing (SAT) was carried out in the panel booths of the sensory science laboratory, at University College Cork according to International Standards (ISO 11136:2014). Using untrained assessors, (n=25) who were familiar with the products being tested, SAT took place over six separate tasting sessions. To accommodate for the analysis of a large number of treatments for fat replacement (12), in duplicate, SAT sessions took place over three days so that all participants tasted every sample twice. According to Stone et al., (2012a), having participants return to evaluate all products produces better results than balanced incomplete block designs. For sucrose replacement, SAT sessions were carried out in one day as only four treatments were tested in duplicate. Samples (2 x 2 x 2cm) were assigned a randomised three-digit code and sessions were carried out at room temperature under white light. Participants were instructed to use the water provided to cleanse their palates between tastings and used the following hedonic descriptors to rate their degree of liking; appearance, flavour, texture, colour and aroma liking. Assessors were asked to indicate their degree of liking for samples on a 9-point, numbers only hedonic scale. The terms ‘extremely dislike’ and ‘extremely like’ were used to anchor the far left and far right end of the scales respectively. Overall acceptability (OA) of samples was also determined using this scale.

4.2.5.2 Ranking descriptive analysis (RDA)

Ranking descriptive analysis (RDA) was also carried out in the panel booths of the sensory science laboratory, at University College Cork. A separate panel of assessors, (n = 21) all of whom had previous experience with descriptive analysis, were trained

and participated in a separate RDA according to the method of Richter et al., (2010). RDA sessions ran concurrently with SAT and therefore took place over six tasting sessions. Sensory descriptors were selected from panel discussion as the most appropriate and reflective of variation in samples profiled. The consensus list of intensity descriptors (Table 4.2) were measured on a 10cm continuous line scale. The terms 'none' and 'extreme' were used on the 0cm and 10cm anchor points on the scale respectively. All samples (2x2x2cm) were served coded in randomised order and presented simultaneously to assessors (Stone et al., 2012b). All samples were ranked on the same scale for each intensity descriptor.

Table 4.2

Consensus list of sensory descriptors and definitions selected by the panel (n=21) and used in the ranking descriptive analysis of Chocolate brownie treatments

Attributes	Definition
Touch	
Springiness	The impact of applying physical force to the original shape of the chocolate brownie sample.
Appearance	
Crust darkness	Degree of darkness of crust
Texture	
Hardness	Force needed to compress sample in mouth
Moisture	Wet texture in mouth
Dense	Heavy, rich, wet-like texture
Flavour	
Sweetness	Taste sensation associated with sucrose
Butter flavour	Flavour sensation associated with butter; creamy mouth-feel and buttery aroma
Chocolate flavour	Intensity of cocoa flavour
Off flavour	Flavour not associated with Chocolate brownies

4.2.6 Physicochemical analysis

The following samples were characterised by physicochemical analysis; S_0, $S_{75/0}$, $S_{75/25}$, $S_{75/50}$ and $S_{75/75}$.

4.2.6.1 Texture profile analysis (TPA)

Texture profile analysis (TPA) was used to measure the texture properties of Chocolate brownies. TPA was conducted using a Texture Analyser 16 TA-XT2I (Stable Micro Systems, Surrey, UK) and was carried out as described by Martínez-Cervera et al., (2012). Two Chocolate brownies (45 x 45 x 30mm) from the centre of each batch tray were used for texture analysis. A 50% double compression test was performed on each sample with a 75mm diameter flat-ended cylindrical probe (P/75), at a speed of 1mm/s with a 5 Sec waiting time between both cycles.

4.2.6.2 Colour measurement

Crust and crumb colour characteristics were assessed using a Minolta CR-200B Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan). Colour measurements were recorded on the CIE (L*a* b*) scale where L* represents lightness, a* signifies green - redness and b* represents blue - yellowness. Crust colour was measured at two separate points. The brownie samples were cut horizontally to remove the crust and crumb colour was measured directly at two separate points.

4.2.6.3 Moisture and fat

Moisture content was measured using the method of Bostian et al., (1985). Samples were homogenised for analysis using a Büchi Mixer B-400 (Büchi Labortechnik AG, Switzerland). Moisture content was determined using the CEM SMART system and fat

was determined using the SMART Trac system (CEM GmbH, kamp-Lintfort, Germany). Two fibreglass pads were placed in the drying chamber of the CEM SMART system and the instrument was tared. Pads were removed and the homogenised samples (2-4g) were weighed accurately on the pads. Following this, one pad was placed over the sample, pressed together and placed back into the drying chamber to begin drying. The moisture (%) was displayed. Fat (%) was determined by wrapping the fibreglass pads with the sample in a sheet of Smart Trac film. Wrapped samples were placed in Smart Trac tube and positioned in the Smart Trac NMR unit. Percentage fat was displayed after roughly 5 Min.

4.2.6.4 Protein

Protein content was determined using the Kjeldahl method which was carried out using the method of Jeddou et al., (2017). Before testing commenced the digestion block (Foss Tecator Digestor, Hillerød, Denmark) was pre-heated to 410˚C. Samples (0.5-2.0g) were weighed accurately into digestion tubes. Two "kjeltabs" were added to each sample in the fume hood followed by 15ml of sulphuric acid and 10ml hydrogen peroxide. Two controls containing no sample were prepared in the same way. Tubes were placed in the heated digestion block and left there for roughly 30-40 Mins until they became colourless. At this point the distillation unit was turned on and rinsed out by connecting a blank tube and receiver flask to the unit and pressing the steam button. Distilled water (50ml) was added to each digested sample in the fume hood after the samples had cooled thoroughly. Samples were placed one by one into the distillation unit (Foss Kjeltec 2100, Hillerød, Denmark) along with a receiver flask containing 50mls of 4% Boric acid with indicator. After the distillation process was complete the contents of the receiver flask was titrated with 0.1N hydrochloric acid until the green

colour reverted back to the original red colour. Nitrogen content was converted to protein content using the factor 6.25.

4.2.6.5 Ash

Ash content was determined using the muffle furnace. Homogenised samples (5g) were weighed into small silica dishes and placed into the muffle furnace. The samples were heated up to 600̊ C until only the inorganic material was left which was indicated by the light-white colour of the samples. The silica dishes containing the samples were then placed in a desiccator to cool and the dishes were weighed carefully. Ash (%) was calculated using the following equation;

$$\% \text{ Ash} = \frac{weight\ of\ Ash \times 100}{\text{weight of sample}}$$

4.2.6.6 Carbohydrate

Carbohydrate content was determined using the following calculation;

% Carbohydrate = 100 - (Moisture % + Fat % + Protein % + Ash %)

4.2.6.7 Dietary fibre

Total dietary fibre was established according to AOAC 985.29 method. Chocolate brownie samples went through sequential enzymatic digestion using a heat-stable α-amylase (95–100 °C, pH 6.0, 15 Min), Subtilisin A (60 °C, pH 7.5, 30 Min), and amyloglucosidase (60 °C, pH 4.5, 30 Min) to remove digestible carbohydrates and protein. To establish total dietary fibre content, the enzyme digests were precipitated with four volumes of 95% (v/v) ethanol, filtered and, then, washed with 78% (v/v) ethanol, acetone before being dried in an oven at 110°C.

4.2.6.8 Total sugars and Sucrose content

Total sugars and sucrose content of samples was determined by ion chromatography, using a method developed internally by an independent accredited laboratory based in Ireland.

4.2.7 Statistical analysis

4.2.7.1 Sensory data

Three independent trials were carried out for all experimental treatments. Raw data obtained from sensory (hedonic & intensity) analysis was coded into Microsoft excel. The significance of sensory properties in discriminating between the samples was analysed using ANOVA and Tukey's post-hoc test (SPSS statistics 20 software (IBM, Armonk, NY, USA). For fat replacement, the relationship between the set of samples (12) and the set of significant sensory variables was determined by partial least squares (PLS) regression using Unscrambler software (Unscrambler 10.3 CAMO software ASA, Trondheim, Norway). In the PLS regression only sensory properties that discriminated significantly between samples were used. The X-matrix was defined as the different sample treatments. The Y – matrix contained the significant sensory variables of the design. For sucrose replacement, the relationship between the set of sample treatments (X) and the set of sensory & physicochemical variables (Y) was examined by PLS regression. Again, only sensory and physicochemical properties that discriminated significantly between samples were used. Both the sensory and physicochemical data were normalised during pre-processing of the data by taking the logarithm to achieve uniform precision over the whole range of variation. Data was also standardised by dividing each variable (sensory & physicochemical) by the corresponding standard deviation. To achieve significant results for the relationships

determined in quantitative PLS, regression analysis, coefficients were analysed by jack-knifing which was based on custom cross-validation and stability plots (Martens and Martens, 1999). Statistical significance for the relationships analysed by PLS were defined as P<0.05-0.01 (significant), P<0.01-0.001 (highly significant) and P<0.001 (extremely significant).

4.2.7.2 Physicochemical data

Raw data obtained from physicochemical analysis was coded into Microsoft excel. As three independent trials were carried out for all experimental treatments, physicochemical data presented represents a mean of six values, unless stated otherwise. As two measurements for crust and crumb colour were taken for each individual sample and two samples were tested for each individual trial, of which there were three, crust and crumb colour values represent a mean of twelve measurements (2 x 2 x 3). One-way ANOVA was used to compare the means of the data obtained from physicochemical analysis. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA). As previously stated, for sucrose replacement, the relationship between the set of sample treatments (X) and the set of sensory & physicochemical variables (Y) was examined by PLS regression, described in (5.2.7.1)

4.3. Results and Discussion

4.3.1 Particle diameter distribution

Particle diameter distributions of the commercial sugar and two of its sieved sugar fractions are displayed in Fig 4.1. The UC sugar fraction had the widest particle diameter distribution as expected, with particles ranging from 200-5181µm. The (L) sugar fraction had a particle diameter distribution of 924-1877µm and the smallest sugar fraction (S) had the narrowest particle diameter distribution of 459-977µm.

4.3.2 Rebaudioside A concentration adjustment for sucrose replacement

The sweetness rankings of six different concentrations of rebaudioside A and one standard solution of sucrose are presented in Table 4.3. The perceived sweetness of the solution containing 0.069 g of rebaudioside A was not different from the standard sucrose solution. Thus, a sucrose-to-rebaudiosideA-ratio of 1/350 was appropriate, meaning that the sweetness of 62.5 g sucrose (25% of the sugar in the formulation) was achieved by 0.17 g rebaudioside A.

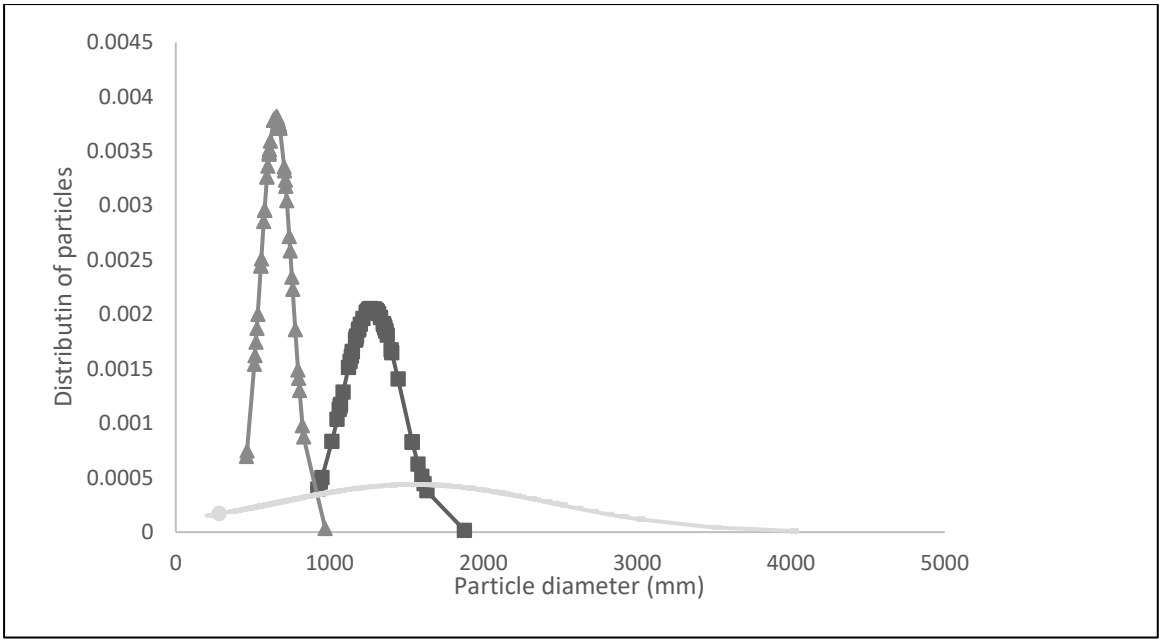


Fig 4.1 Particle diameter distribution of unground control sugar fraction UC; ( ) and sugar-sieve separates; L ( ) and S ( )

Table 4.3
Iso-sweetness of rebaudioside A in aqueous solutions

Sweetener	Concentration (g/L)	Mean scores	Dilution factor
	0.060	1.2[a]	400
	0.069	5.4[b]	350
Reb A.	0.080	6.2[c]	300
	0.096	6.3[c]	250
	0.120	7.4[d]	200
	0.160	9.4[d]	150
Sucrose	24.0	5.6[b]	n/a

[abcd] mean values (± standard deviation) in the same column bearing different superscripts are significantly different, $p < 0.05$.
Concentrations and dilution factors obtained from (Zahn et al. 2013)

4.3.3 Sensory analysis

4.3.3.1 Relationship between sensory variables and Chocolate brownies prepared with different sugar sizes and with different levels of fat replacement

The relationship between sensory properties (hedonic & intensity) (Y) and Chocolate brownies prepared with three different sugar sizes (UC, L & S) and with increasing levels of fat replacement (0, 25, 50 & 75%) (X) is visually represented by a Partial least squares regression plot (PLSR) shown in Fig 4.2. The following sensory (intensity) terms were omitted from PLSR analysis because they did not significantly discriminate between samples; hardness, springiness & off flavour. The hedonic term 'aroma Liking' was also excluded from PLSR analysis. Most of the variation is shown in Factor-1 where 23% of the X data explains 44% of the data in Y. All intensity and hedonic sensory attributes are positioned in the inner circle of the upper and lower right-hand quadrants. A high correlation between all intensity attributes, is evident by their close proximity to each other on the plot. The following intensity attributes were highly correlated with hedonic sensory properties, also evident by their close proximity to each other on the plot; butter flavour, chocolate flavour, sweetness, perceived moisture, dense texture, and crust darkness.

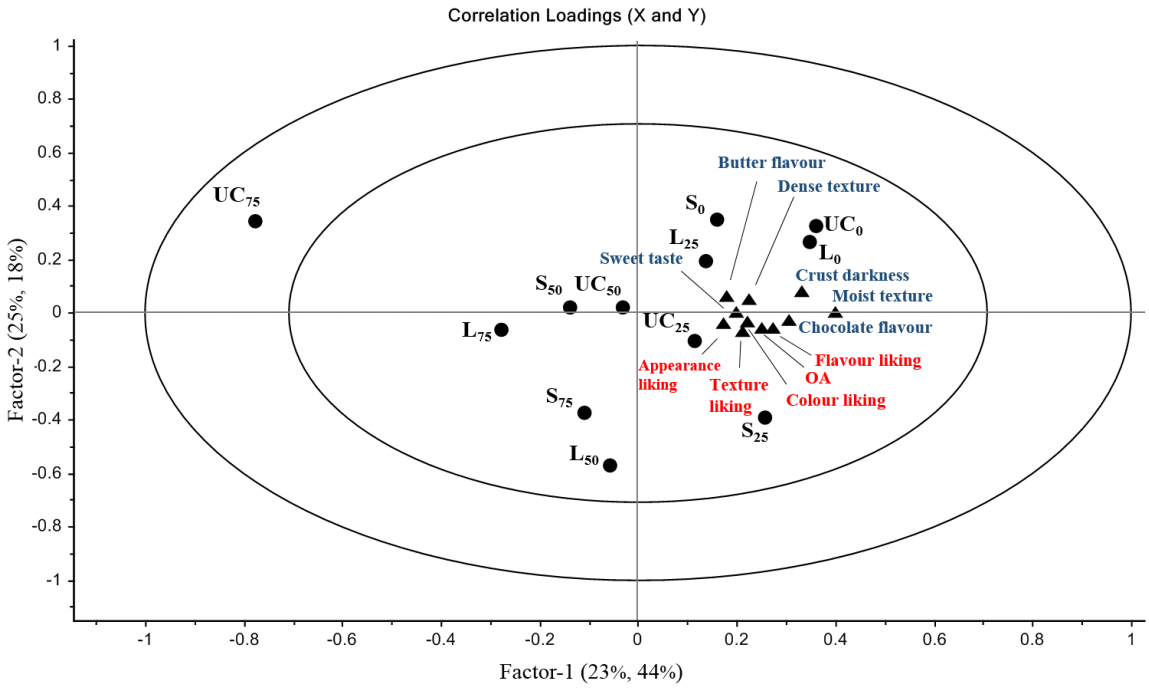


Fig 4.2 Partial least squares regression (PLSR) plot for the relationship between Chocolate brownie samples prepared with three different sugar sizes (UC, L & S) and with increasing levels of fat replacement; 0, 25, 50 &75% (●) and sensory terms (▲). Hedonic (▬) and Intensity sensory terms (▬)

4.3.3.1.1 Chocolate brownies (UC, L & S) containing 0% fat replacement

All samples containing 0% fat replacement (UC_0, L_0 & S_0) are positioned in close proximity with each other in the inner circle of the upper right quadrant of the PLS plot. These samples were highly correlated with intensity and hedonic sensory properties. To aid in further understanding of the relationship between sensory terms and Chocolate brownie treatments, significance of estimated regression coefficients for the relationship between these two sets of variables can be seen in Table 4.4. All Samples containing 0% fat replacement (UC_0, L_0 & S_0) were positively correlated with crust darkness, perceived moisture, dense texture and butter & chocolate flavour ($p<0.001$). The L_0 and S_0 samples had the highest correlation with sweetness ($p<0.001$). Results

obtained for perceived sweetness intensity were therefore in agreement with findings from Chapter 3 (Richardson et al., 2018) where it was determined that sugar particle size influenced the perceived sweetness of Chocolate Brownies. Irrespective of this, UC_0 was the sample most correlated with flavour liking ($p<0.001$). Previous research has shown that preference scores rise and then decrease with increasing levels of sucrose (Drewnowski and Greenwood, 1983). Although sucrose content remained constant, a higher perceived sweetness observed for L_0 and S_0 samples may have negatively affected flavour liking. Although the S_0 sample was highly correlated ($p<0.001$) with intensity sensory attributes (butter flavour, moist texture) this sample was not significantly associated with hedonic sensory properties or OA. These findings are in agreement with results obtained in Chapter 3 where although Chocolate brownies containing the smallest sugar fraction were perceived as the sweetest samples with the moistest and softest textures, these samples were not significantly associated with liking properties or OA.

4.3.3.1.2 Chocolate brownies (UC, L & S) containing 25% fat replacement

All 25% PBLB brownies (UC_{25}, L_{25} & S_{25}) are situated in the upper and lower inner quadrants on the right-hand side of the plot (Fig 4.2). Samples were associated with intensity and hedonic sensory attributes. The S_{25} sample was associated with crust darkness ($p<0.05$), perceived moisture ($p<0.01$) and chocolate flavour ($p<0.001$) (Table 4.4). Consequently, this sample was most correlated with OA ($p<0.001$), appearance liking ($p<0.01$) and texture & colour liking ($p<0.001$). Unlike samples containing 0% fat replacement (UC_0, L_0 & S_0), S_{25} samples were not correlated with perceived sweetness or butter flavour. Regardless, this sample had the highest correlation with

OA. Therefore, the utilisation of smaller sugar particles improved OA of samples while permitting fat replacement up to a level of 25%.

4.3.3.1.3 Chocolate brownies (UC, L & S) containing 50% fat replacement

All 50% PBLB brownies (UC_{50}, L_{50} & S_{50}) are situated in the upper and lower left quadrants of the plot (Fig 4.2). These samples made no significant contribution to the plot and were not well described. The UC_{50} and L_{50} samples were not significantly different from UC_{25} and L_{25} samples respectively, in relation to sensory properties (hedonic & intensity) and OA (Table 4.4).

4.3.3.1.4 Chocolate brownies (UC, L & S) containing 75% fat replacement

The UC_{75} sample made a significant contribution to Factor-2 on the PLSR plot and is positioned in the outer circle of the upper left quadrant. This sample was anti-correlated with the intensity and hedonic sensory properties situated on the right hand side of the plot. Similar to findings visually represented in Fig 4.2, the UC_{75} sample was negatively correlated with all intensity sensory attributes, ($p<0.001$) liking properties ($p<0.001$) and OA ($p<0.001$) (Table 4.4). L_{75} was also negatively associated with these properties ($p<0.05$). However, the S_{75} sample was only negatively correlated with crust darkness ($p<0.001$). Hence, a 75% fat replacement in Chocolate brownies containing the smallest sugar fraction (S) did not negatively affect any other sensory attributes, liking properties or OA compared to samples containing UC and L sugar fractions.

As previously mentioned, sensory attributes were highly correlated with one another. This was expected, due to the difficulty to detect fat in foods as no single attribute can be correlated with fat content (Drewnowski et al., 1998). Hence, the majority of intensity sensory attributes (6/9) were negatively affected by a 75% fat replacement

using PBLB. However, this was only observed for samples containing the UC & L sugar fractions. Laguna et al., (2012) also reported a reduction in perceived butter flavour with increasing levels of butter replacement for inulin in biscuits. A reduction in perceived chocolate flavour at 75% FR may be due to less fat being present in the sample as fats carry lipid-soluble flavour compounds (Akoh, 1998). A reduction in perceived sweetness was expected at high levels of fat replacement as the correlation between perceived sweetness and fat content in food products has been demonstrated by Drewnowski and Greenwood, (1983) and more recently by Biguzzi et al., (2012) who found that perceived sweetness intensity declined with fat reduction in biscuits. Regarding the decrease in perceived moisture, Lillford (2011) reported that higher levels of fat increase the perceived intensity of moisture as the presence of fat reduces the need for saliva absorption.

4.3.3.1.4.5 Summary

The application of small sugar particles (459-972µm) in the manufacture of chocolate brownies permitted higher levels of fat replacement (75%) in terms of overall acceptance, compared to other sugar fractions investigated in this study. S_{25} samples had the highest acceptances. As the utilisation of this sugar fraction has previously been shown to increase the perception of moisture in chocolate brownies (Chapter 3), perhaps perceived moisture was adequate in samples containing 75% FR to maintain the perception of other sensory properties (butter flavour, sweetness) associated with liking and OA. PBLB have been used to replace shortening in brownies up to a level of 90% FR without negatively affecting OA (Uruakpa and Fleisher, 2016). In the present study, since a 75% fat replacement in brownies containing the smallest sugar particle size had no negative impact on OA compared to other sugar fractions, this

combination of particle size and fat replacement level were chosen for proceeding with tests on sucrose replacement.

Table 4.4

Significance of estimated regression coefficients (ANOVA values) for the relationship of sensory terms (Y) and Chocolate Brownies prepared with different sugar sizes (UC, L & S) and with increasing levels of FR using PBLB

	Hedonics					Attribute intensity					
						Appearance	Texture		Flavour		
Sample	Appearance	Flavour	Texture	Colour	Overall acceptability	Crust darkness	Moist	Dense	Sweet taste	Butter flavour	Chocolate flavour
UC_0	0.088	0.001***	0.044*	0.007**	0.002**	0.000***	0.000***	0.000***	0.018*	0.001***	0.000***
UC_{25}	0.889	0.829	0.987	0.882	0.777	0.978	0.506	0.491	0.163	0.399	0.751
UC_{50}	0.500	0.192	0.380	0.327	0.411	0.193	0.273	0.762	0.644	0.944	0.449
UC_{75}	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***
L_0	0.018*	0.091	0.051*	0.022*	0.003**	0.001***	0.000***	0.000***	0.000***	0.001***	0.001***
L_{25}	0.688	0.257	0.555	0.484	0.321	0.032*	0.025**	0.082	0.487	0.107	0.242
L_{50}	0.524	0.788	0.795	0.364	0.978	-0.001***	0.137	-0.039*	0.940	-0.047*	0.652
L_{75}	-0.022**	-0.001***	-0.012**	-0.005**	-0.002**	-0.000***	-0.000***	-0.000***	-0.016*	-0.000***	-0.000***
S_0	0.374	0.402	0.699	0.232	0.502	0.006***	0.000***	0.000***	0.000***	0.000***	0.000***
S_{25}	0.003**	0.231	0.001***	0.000***	0.001***	0.013*	0.006**	0.107	0.684	0.623	0.001***
S_{50}	0.120	0.998	0.188	0.145	0.168	0.569	0.329	0.095	0.219	0.292	0.088
S_{75}	0.764	0.400	0.984	0.438	0.922	-0.001***	0.098	0.185	0.527	0.184	0.735

Significance of regression coefficients; (*) = $p \leq 0.05$, (**) = $p \leq 0.01$, (***) = $p \leq 0.001$

(–) indicates whether the relationship is negatively correlated.

4.3.3.2 Relationship between sensory & physicochemical variables and PBLB Chocolate brownies prepared with increasing levels of SR

The second part of this study involved the sequential replacement of sucrose using a combination of inulin and rebaudioside A, in PBLB Chocolate brownie samples. The relationship between sensory & physicochemical properties (Y) and samples prepared with 0, 25, 50 and 75% SR (X) is visually represented by a PLSR plot in Fig 4.3. The sensory term 'sweet taste' was left out of PLSR analysis because it did not discriminate between samples, which demonstrates the value of inulin and rebaudioside A as sucrose replacers in PBLB brownies, in terms of maintaining sweetness intensity. The following physical and compositional properties were also excluded from PLSR analysis; springiness (mm), cohesiveness, crust & crumb redness (a*) yellowness (b*), moisture, fat, protein, ash and carbohydrate content (%). The physicochemical characteristics of samples ($S_{75/0}$, $S_{75/25}$, $S_{75/50}$ & $S_{75/75}$) are discussed in this section in terms of their correlation with sample treatments. Physicochemical properties will be discussed in detail in subsequent sections; 4.3.4 & 4.3.5. Most of the variation in the PLS plot is shown in Fator-1, where 33% of the X data explains 56% of the data in Y. Hedonic sensory properties, which are positioned in close proximity with one another in the inner circle of the upper right quadrant, were all correlated (aroma, flavour, appearance, texture, colour liking and OA). The following intensity sensory attributes are shown in close proximity with hedonic properties and were therefore driving factors for liking and acceptability; crust darkness, chocolate flavour, butter flavour, perceived moisture and springiness. The $S_{75/0}$ sample, which is positioned in the outer circle of the lower right quadrant makes a significant contribution to Factor-2. The $S_{75/0}$ sample, was negatively correlated with perceived hardness ($p<0.05$) which means these samples

were perceived as the softest samples (Table 4.5). $S_{75/0}$ was also negatively correlated with hardness (N) ($p<0.05$) which means sensory results obtained for perceived hardness correlated with instrumental results. All other sensory (hedonic & intensity) and physicochemical properties did not discriminate between PBLB brownie treatments containing 0% ($S_{75/0}$) and 25% ($S_{75/25}$) sucrose replacement. The $S_{75/75}$, sample which is situated in the outer circle of the lower left quadrant makes a significant contribution to Factor-1 (Fig 4.3). This sample was anti-correlated with springiness ($p<0.01$), perceived moisture ($p<0.05$), sugar content ($p<0.05$) and consequently all hedonic properties and OA ($p<0.05$) (Table 4.6).

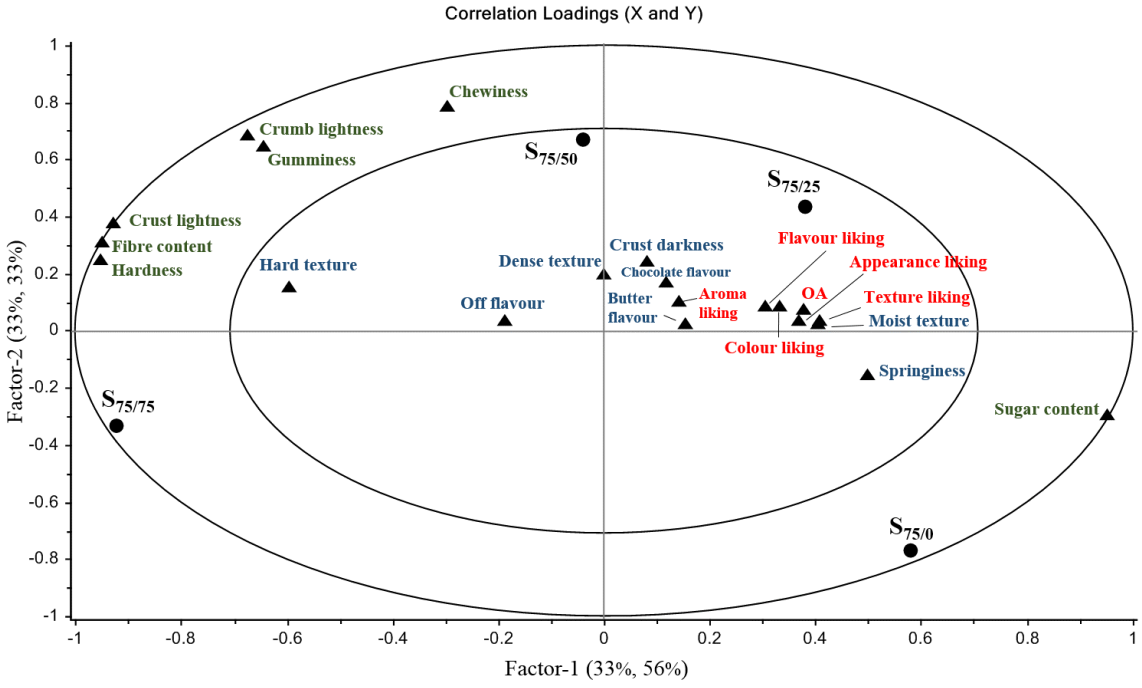


Fig 4.3 Partial least squares regression (PLSR) plot for the relationship between fat-reduced Chocolate brownie samples (75%) prepared with the smallest sugar size and with increasing levels of sucrose replacement; 0, 25, 50 & 75% (●) and sensory & physicochemical variables (▲). Hedonic (▬) and intensity (▬) sensory terms and physicochemical properties (▬).

The $S_{75/75}$ sample was positively associated with fibre content (%) ($p<0.05$), hardness (N) ($p<0.05$), crust and crumb lightness (L*) ($p<0.05$) and perceived hardness ($p<0.05$). An increase in sample hardness was expected after substitution of sucrose, as sucrose plays a vital role in the tenderisation of baked goods, furthermore, an increase in hardness, with the addition of inulin has previously been reported by O'Brien et al., (2005) and Volpini-Rapina et al., (2012) in bread crumbs and orange cakes, respectively. The $S_{75/75}$ was negatively associated with perceived moisture ($p<0.05$) as was the $S_{75/50}$ sample ($p<0.05$) which means these samples were perceived as significantly dryer than any other samples. As mentioned previously, actual moisture content did not discriminate between samples, and was consequently omitted from the PLS plot. Thus, sensory results obtained for perceived moisture did not correlate with instrumental results. Similarly, the instrumental results obtained for sample springiness (mm) did not correlate with sensory results with no significant difference being determined between samples after physical analysis. The sensory results obtained for perceived springiness in this study however, are in agreement with results obtained from a study conducted on muffins, where springiness (mm) decreased with SR using a combination of fibres and rebaudioside A (Zahn et al., 2013).

The $S_{75/75}$ sample was significantly positively associated with crust and crumb lightness ($p<0.05$). A lighter crust and crumb colour as a result of SR has been reported by Ronda et al., (2010) who found that SR using polyols and other non-digestible oligosaccharides increased crust lightness of sponge cake. However, in a study conducted by Martínez-Cervera et al., (2012) replacing sucrose with a combination of sucralose and polydextrose was found to increase crust darkness of muffins.

The $S_{75/75}$ sample was significantly associated with actual fibre content ($p<0.05$) which can be attributed to the amount of dietary fibre present in inulin. This sample was significantly negatively associated with total sugar and sucrose content ($p<0.05$) as expected. Perceived flavour (sweetness, butter flavour, chocolate flavour) did not discriminate between PBLB brownies containing increasing levels (0-75%) of SR, using inulin and rebaudioside A. However, as a result of significant changes in texture and colour (perceived and instrumental) all liking properties and OA were negatively affected by a 75% SR ($S_{75/75}$) ($p<0.05$) with texture liking and OA being extremely negatively affected ($p<0.001$). It is no surprise that all hedonic properties were affected as all hedonic properties were closely correlated. The $S_{75/50}$ sample however, was only significantly negatively associated with texture liking ($p<0.05$) and consequently OA ($p<0.05$).

Table 4.5

Significance of estimated regression coefficients (ANOVA values) for the relationship of sensory & physicochemical properties (Y) and reduced fat Chocolate brownies prepared with increasing levels of SR using inulin and reb A (X) .

	Hedonics						Attribute intensity								Colour		TPA			Proximate composition	
							Touch	Colour		Texture			Flavour		L*		(N)			(%)	
Chocolate brownie sample	Aroma	Appear-ance	Colour	Texture	Flavour	OA	Springi-ness	Darkness	Hard	Moist	Dense texture	Butter	Chocolate	Off-flavour	Crust	Crumb	Hard	Gummi-ness	Chewi-ness	Sugar	Fibre
$S_{75/0}$	0.92	0.80	0.84	0.79	0.82	0.84	0.77	0.77	-0.03*	0.99	0.94	0.88	0.85	0.91	0.46	0.60	-0.05*	0.56	0.64	0.57	0.85
$S_{75/25}$	0.84	0.93	0.47	0.81	0.73	0.75	0.68	0.73	0.89	1.00	0.93	0.92	0.81	0.92	0.79	0.70	0.75	0.62	0.61	0.78	0.62
$S_{75/50}$	0.66	0.57	0.76	-0.05*	0.71	-0.04*	-0.05*	0.82	0.87	-0.04*	0.69	0.68	0.81	0.96	0.78	0.61	0.83	0.47	0.44	0.71	0.87
$S_{75/75}$	-0.01**	-0.03*	-0.04*	-0.001***	-0.01**	-0.001***	-0.01**	0.82	0.02*	-0.03*	0.94	0.84	0.60	0.71	0.02*	0.03*	0.05*	0.57	0.58	-0.04*	0.05*

Significance of regression coefficients; (*) = p≤0.05, (**) = p≤0.01, (***) = p≤0.001
(–) indicates whether the relationship is negatively correlated.

Table 4.6

Physical properties of Chocolate brownie samples

		S_0	$S_{75/0}$	$S_{75/25}$	$S_{75/50}$	$S_{75/75}$
TPA	Hardness (N)	54.4 ± 0.44^{a}	8.2 ± 0.21^{b}	25.8 ± 0.52^{c}	30.4 ± 0.41^{d}	50.4 ± 0.52^{a}
	Gumminess (N)	-0.0 ± 0.43^{a}	1.8 ± 0.51^{b}	6.4 ± 0.67^{d}	5.6 ± 0.68^{c}	6.7 ± 0.58^{d}
	Chewiness (N-mm)	9.1 ± 0.55^{a}	0.8 ± 0.40^{b}	3.7 ± 0.60^{c}	2.7 ± 0.53^{d}	2.7 ± 0.80^{d}
	Springiness (mm)	0.5 ± 0.61^{a}	0.4 ± 0.12^{a}	0.6 ± 0.22^{a}	0.5 ± 0.22^{a}	0.4 ± 0.11^{a}
	Cohesiveness	0.3 ± 0.29^{a}	0.2 ± 0.06^{a}	0.2 ± 0.03^{a}	0.2 ± 0.02^{a}	0.2 ± 0.02^{a}
Crust colour	L*	39.8 ± 0.67^{a}	25.1 ± 0.50^{b}	29.2 ± 0.61^{c}	32.0 ± 0.92^{cd}	34.9 ± 0.82^{d}
	a*	11.1 ± 0.55^{a}	11.5 ± 0.86^{a}	10.5 ± 0.86^{a}	10.6 ± 0.30^{a}	11.5 ± 0.57^{a}
	b*	12.9 ± 0.64^{a}	13.0 ± 0.80^{a}	12.6 ± 0.77^{a}	12.4 ± 0.83^{a}	14.8 ± 0.70^{a}
Crumb colour	L*	29.0 ± 0.55^{a}	26.0 ± 0.58^{b}	27.3 ± 0.29^{bc}	30.9 ± 0.76^{c}	31.6 ± 0.52^{c}
	a*	7.6 ± 0.81^{a}	7.1 ± 0.46^{a}	8.0 ± 0.80^{a}	8.5 ± 0.39^{a}	8.4 ± 0.93^{a}
	b*	11.8 ± 0.37^{a}	11.6 ± 0.60^{a}	12.7 ± 0.55^{a}	12.4 ± 0.60^{a}	12.5 ± 0.56^{a}

^{abcd} mean values (± standard deviation) in the same row bearing different superscripts are significantly different, ($p<0.05$).

4.3.4 Physical properties of Chocolate brownies

Results from physical analysis on the following treatments of Chocolate brownies are displayed in Table 4.5.6; S_0, $S_{75/0}$, $S_{75/25}$, $S_{75/50}$, & $S_{75/75}$. Fat replacement (75%) using PBLB significantly reduced hardness ($p<0.05$). Similar findings were reported by (Confortiv et al., 1997) where crumb firmness decreased with the substitution of fat for carbohydrate-based fat replacers derived from gums. However, sample hardness significantly increased with increasing levels of SR in PBLB brownies (0-75%) ($p<0.05$). As mentioned, hardness results obtained from instrumental analysis are in agreement with the sensory results obtained in this study. Chewiness (N-mm) also decreased with fat replacement (75%) ($p<0.05$) and values significantly discriminated between brownie samples containing varying levels of sucrose ($p<0.05$) however uneven trends were observed.

Fat replacement (75%) significantly increased samples darkness ($p<0.05$). This result however did not correlate with sensory results, as perceived darkness was correlated with samples containing 0 or 25% FR (Fig 4.2). However, samples containing low levels of FR were perceived as moister, and may have had 'wetter' looking appearances, which could explain why these samples were perceived as darker in colour. Instrumental colour results obtained for samples during sucrose replacement, correlated with sensory results. Crust lightness values increased significantly with increasing levels of SR ($p<0.05$) and similar results were reported for crumb lightness.

4.3.5 Proximate composition of brownies

Results from compositional analysis on the following treatments of Chocolate brownies are displayed in Table 4.7; S_0, $S_{75/0}$, $S_{75/25}$, $S_{75/50}$, & $S_{75/75}$. Fat replacement (75%) increased moisture content of samples ($p<0.05$). Similar findings were reported by (Confortiv et al. 1997) who found that using carbohydrate-based fat replacers increased the moisture content of biscuits. Although moisture content (%) increased with fat replacement, the perception of moisture decreased as determined by RDA and depicted in Fig 4.2 and Table 4.4. This finding highlights the importance of fat/butter content to the perception of moisture in Chocolate brownies. As mentioned, moisture content (%) did not discriminate between samples with increasing levels of SR and ranged from values between (22.3-23.2%). Perceived moisture did discriminate between samples however, with samples containing higher levels of SR ($SC_{75/50}$ & $SC_{75/75}$) being perceived as dryer ($p<0.05$). This finding highlights the importance of sugar content to the perception of moisture. Fat content (%) decreased significantly with fat replacement (75%) using PBLB ($p<0.05$) from 26% of the formulation to 9.9%. This equated to a 62.4% reduction in fat, which according to standards set by the FSAI would permit the use of the claim 'reduced fat' (Food Safety Authority, 2016). Carbohydrate content (%) did not discriminate between samples (58.6-61.2%). The type of carbohydrate however, did vary with a total of 39.5% and 19.3% of the carbohydrate present in the S_0 and $S_{75/75}$ samples respectively, being present as sugars. Total sugars and total sucrose content decreased significantly with sucrose replacement (0-75%) ($p<0.05$). Fat replacement (75%) using PBLB significantly increased the fibre content of samples ($p<0.05$). Dietary fibre increased significantly with each level of SR using inulin and rebaudioside A ($p<0.05$). Fibre content was adequate in samples ($S_{75/50}$ and $S_{75,75}$) to permit the claim 'source of fibre'(Food Safety Authority 2016). Fat replacement (75%) reduced the

energy content of samples by 137Kcal/100g, which equated to a 28% total energy reduction. Samples containing different sucrose replacement levels did not discriminate in relation to energy content (kcal/100g). Therefore, reduced energy of the samples can only be attributed to the replacement of fat (75%) for PBLB.

Table 4.7

Proximate composition (%) of Chocolate brownie samples

	S_0	$S_{75/0}$	$S_{75/25}$	$S_{75/50}$	$S_{75/75}$
Moisture	9.2 ± 0.42^{a}	22.3 ± 0.76^{b}	23.1 ± 0.11^{b}	23.2 ± 0.10^{b}	22.3 ± 0.94^{b}
Fat	26.3 ± 0.55^{a}	9.9 ± 0.40^{b}	9.9 ± 0.23^{b}	9.8 ± 0.56^{b}	9.9 ± 0.15^{ab}
Protein	5.1 ± 0.90^{a}	6.1 ± 0.20^{b}	6.0 ± 0.15^{b}	5.7 ± 0.27^{b}	5.8 ± 0.14^{b}
Ash	0.8 ± 0.50^{a}	1.1 ± 0.32^{a}	0.8 ± 0.15^{a}	0.8 ± 0.07^{a}	0.8 ± 0.04^{a}
Carbohydrate	58.6 ± 0.32^{a}	60.3 ± 0.41^{a}	60.2 ± 0.55^{a}	60.4 ± 0.23^{a}	61.2 ± 0.22^{a}
Sucrose	38.4 ± 0.34^{a}	40.5 ± 0.42^{a}	32.7 ± 0.65^{b}	26.5 ± 0.71^{c}	18.6 ± 0.55^{d}
Total Sugars	39.5 ± 0.51^{a}	41.5 ± 0.66^{a}	33.6 ± 0.58^{b}	27.3 ± 0.61^{c}	19.3 ± 0.18^{d}
Dietary Fibre	1.5 ± 0.55^{a}	2.3 ± 0.91^{b}	3.0 ± 0.65^{bc}	3.7 ± 0.54^{c}	5.6 ± 0.88^{d}
Energy (Kcal/100g)	491	354.7	353.9	352.6	357.1

^{abcde} mean values (± standard deviation) in the same row bearing different superscripts are significantly different, ($p < 0.05$).

4.4. Conclusions

A 25% SR with inulin and rebaudioside A in fat-replaced (75%) Chocolate brownies prepared with small sugar particles and PBLB was feasible with no negative impact on sensory properties or acceptance. Although these modifications were found to affect the physicochemical properties of brownies, sensory acceptance was not negatively affected. SR (0-75%) using inulin and rebaudioside A, did not impair the flavour properties (sweetness, butter, chocolate) of PBLB Chocolate brownies and consequently flavour acceptance was not negatively affected. However texture and colour properties of PBLB brownies were negatively affected at higher levels of SR and consequently liking properties and OA. The addition of humectants and emulsifying agents in the formulation could improve texture and therefore, permit higher levels of sucrose replacement, using this combination of replacers. Further studies are necessary to demonstrate this. A simultaneous reduction in fat (62.4%) and sugar (15%) and an increase in fibre (3g/100g) was achieved. Overconsumption of developed products would be less likely due to increased satiety as a result of increased fibre content which would contribute to a better caloric balance. The proposed modifications to Chocolate brownies could be applied by industry to other cake-like products which could be a significant development in reducing the dietary intake of fat and sugar while increasing the dietary intake of fibre. Further studies are necessary to demonstrate this.

Chapter 5.

The application of pureed butter beans and a combination of inulin & rebaudioside A for the replacement of fat and sucrose respectively in Sponge Cake, employing sensory and physicochemical analysis

Abstract

Determining minimum levels of fat and sucrose needed for sensory acceptance of Sponge cake while increasing nutritional quality, was the main objective of this study. Inulin and rebaudioside A were used to replace sucrose at different levels (SR) (0, 25, 50 & 75%) and four formulations were manufactured (SC_0, SC_{25}, SC_{50} & SC_{75}). Sensory acceptance tests (SAT) were employed to establish the best formulation that achieved the maximum level of sucrose reduction. Four more formulations containing increasing levels of fat replacement (FR) (0-75%) using pureed butter beans (PBRB) were manufactured ($SC_{30/0}$, $SC_{30/25}$, $SC_{30/50}$ & $SC_{30/75}$). These cakes were characterised by sensory (hedonic and intensity) and physicochemical (texture, colour, composition) analysis. SR of 25% showed the highest acceptance (SC_{25}). A SR of 50% negatively affected texture liking ($p<0.05$) and consequently OA ($p<.05$). Inulin and rebaudioside A cakes with 25% and 50% FR showed the highest acceptances ($SC_{30/25}$ and $SC_{30/50}$). Fat replacement (75%) impaired butter and sweet flavour ($p<0.01$), butter, sweet and caramel aroma ($p<0.05$) and consequently flavour and aroma acceptances ($p<0.05$) and OA ($P<0.01$). FR (0-75%) using PBRB increased the moisture ($P<0.05$) and crust lightness ($p<0.05$) of brownies but did not affect crumb hardness (N). Results indicate that PBRB replacing fat is feasible at 50% in sucrose-replaced (30%) Sponge cakes using inulin and rebaudioside A.

5.1 Introduction

Sponge cake, which is classified by its solid porous structure, is prepared using four main ingredients, eggs (27%), sugar (26%), flour (26%) and butter (19%) which are responsible for multiple functions in the batter matrix and during baking. The presence of fat and sugar in food products yields positive hedonic responses which may override metabolic responses such as satiety (Blundell & Macdiarmid, 1997). This contributes to dietary imbalances associated with overweight and obesity and consequently the prevalence of non-communicable diseases (National taskforce on Obesity, 2005). As cakes and biscuits contribute greatly to the dietary intake of sugar and fat in developed countries (IUNA) 2011) (Azaïs-braesco et al., 2017), it is necessary to focus on strategies to reduce these ingredients in cake products.

Before we consider sucrose and fat reduction strategies, it is important to understand the functions performed by these ingredients in cakes. Sucrose is the most common sugar used in baking (Bennion and Bamford, 2013) and is responsible for tenderisation of cakes by competing with gluten for available water during batter mixing, thus impeding on the formation of a strong gluten network (Wilderjans et al., 2013). During baking, sucrose increases the temperature of starch gelatinisation and protein denaturation by binding water and therefore limiting water availability to starch granules and protein (Ureta et al. 2016). In doing so sucrose affects the volume and bulk of the final product. The most commonly known function of sucrose is that it provides sweetness to cakes and also acts a flavour enhancer (Bennion and Bamford, 2013). Fat entraps air in the batter during the creaming step which provides a structure for leavening gases (Wilderjans et al. 2013). Thus fat also affects volume and bulk. Fat tenderises and adds lubricity to the texture of cakes by coating the protein and starch particles, thereby interfering with the protein matrix (Pyler, 1988). Fat also emulsifies

liquid in cake production which adds further moisture and softness to the product (Wade, 1988). Fat sources for cake formulation, such as butter contribute to flavour and can act as flavour enhancers (Lauterbach & Albrecht, 1994). Thus, as sugar and fat preform many key functions in cakes, it is difficult to reduce these ingredients without affecting the important physicochemical and sensory attributes associated with liking and overall acceptability.

Sucrose replacement (SR) as a strategy for sugar reduction in bakery products has been the focus of numerous studies (Ronda et al.,2004: Manisha et al., 2012; Gao et al., 2017; Martínez-Cervera et al., 2012). Fibres such as inulin are of particular interest for use as sugar replacers as they possess prebiotic properties (Shoaib et al., 2016). Furthermore, the incorporation of dietary fibre into foods is now considered as a principal prevention strategy against the risk of non-communicable disease (Stephen et al., 2017). Fibres such as inulin provide bulk properties (texture and volume) by binding water (Meyer et al., 2011) and therefore competing with gluten proteins for available water and delaying starch gelatinisation. Inulin contains 25-35% of the energy of digestible carbohydrates and its sweetness level is 10% that of sucrose (Shoaib et al., 2016). Cakes containing 30% sugar replacement with inulin were shown to be similar to control sugar cakes with regards to bubble size distribution and physicochemical properties in a study conducted by Rodríguez-garcía et al., (2014). As previously mentioned, inulin has a low sweetness value relative to that of sucrose, it is therefore necessary to combine inulin with intense sweeteners such as steviol glycosides. Approved for use in the EU in 2011, individual steviol glycosides, such as rebaudioside A are considered natural intense sweeteners (Scardigli, 2011). In a study conducted by Struck et al., (2016) a 30% SR in muffins with inulin and rebaudioside A produced cakes with a similar descriptive sensory profile to reference muffins.

The impact of inulin and rebaudioside A on the sensory acceptance of Sponge cake samples merits investigation. Therefore, the first objective of this study was to examine the effect of sucrose replacement (SR) (0, 25, 50 & 75%) using inulin and rebaudioside A on the sensory (hedonic) properties of Sponge cakes, in order to determine the levels of sucrose needed for sensory acceptance.

Fat replacement as a strategy for fat reduction in bakery products has also been the focus of numerous studies (Uruakpa and Fleisher, 2016; Rankim and Bingham, 2000; Rodríguez-García., 2012). Carbohydrate-based fat replacers also called fat mimetics, bind water and therefore contribute to texture and mouthfeel. In a study carried out on three commercial carbohydrate-based fat replacers, derived from pectin, gums and oat bran, moisture content increased with increasing levels of fat replacement in biscuits (Confortiv et al., 1997). Legumes such as beans are also considered carbohydrate-based fat replacers as they are a source of complex carbohydrates (20%). Legumes are low-glycaemic and high in protein and fibre and their consumption has been associated with a decreased risk of coronary heart disease (Bazzano & Ogden, 2001). In a study conducted on brownies, pureed black beans successfully replaced fat without affecting sensory acceptance at a level of 30% replacement (Uruakpa & Fleischer 2016). Furthermore, this study reported that a 90% substitution of fat with black beans still produced an acceptable product. In a study conducted on oatmeal cookies, white beans were successful in replacing fat up to a level of 25% without significantly affecting sensory properties (Rankin & Bingham, 2000). Cannellini beans have been used to successfully replace fat in brownies by up to 50% without affecting OA, texture and flavour properties (Szafranski et al., 2005). In the present study, pureed butter beans (PBRB) were used to replace fat in Sponge cake. The impact of PBRB on the sensory and physicochemical properties of Sponge cakes merits investigation. Therefore, the

second objective of this study was to examine the effect of fat replacement (FR) (0, 25, 50 & 75%) using PBRB on the sensory (hedonic & intensity) and physicochemical (texture, colour, compositional) properties of inulin and rebaudioside A Sponge cakes, in order to determine the levels of fat needed for sensory acceptance

5.2. Materials and Methods

5.2.1 Materials

Food ingredients used in this trial were; eggs (Upton brand, Ireland); caster sugar (99.9% sucrose, 0.3% moisture & 0.01% sodium, Tate & Lyle brand, UK); cream plain flour (82.7% carbohydrate, 2% of which were sugars, 11.7% protein, 3.4% fibre, 1.4% fat & 0.81% salt, Odlums brand, Ireland); creamery butter (81% total fat, 65.4% of which were saturated & 15.1% moisture, supermarket-own brand, Ireland); inulin (89% fibre & 8% sugar, Bioglan brand, Austrailia); rebaudioside A., (Bulk Powders brand, Ireland); butter beans (16.8% carbohydrate, 1.4% of which were sugars, 5.9% protein, 0.6% fats 0.1% of which were saturated, 5.2% fibre, 0.01% sodium & 0.03% salt, Suma brand, UK); baking powder (3 % sodium, Royal brand, US) and whole milk (4.7% carbohydrate, 4.7% of which were sugars, 3.5% fat, 2.3% of which were saturated, 3.4% protein & 0.1% salt, supermarket-own brand, Ireland). Food products were all purchased from a local supermarket unless stated otherwise and stored under refrigerated or cool, dry conditions where appropriate prior to sample preparation.

5.2.2 Sponge cake formulation for sucrose replacement

A standard Sponge cake recipe was utilised in this study (Table 5.1). Four Sponge cake formulations were manufactured containing four sugar replacement levels (0, 25, 50 & 75%). Before manufacture of Sponge cakes, a ranking test was used to determine the concentration of rebaudioside A necessary to replace the sweetness concentration of sucrose, ensuring iso-sweetness. Ranking tests were carried out twice using 21 assessors. Concentration adjustments for rebaudioside A were carried out according to the method of (Zahn et al., 2013), using similar concentrations of stevia (0.06-0.16g/l) and 24g/L sucrose. One-way ANOVA was used to compare the means of the data

obtained for each solution. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA). For each formulation (SC_0, SC_{25}, SC_{50} & SC_{75}), butter was blended in an electronic mixer (Kitchen Aid Professional mixer) and sugar was added gradually until soft and light in colour, at medium speed for 4 Mins. Eggs were added one at a time and beaten well between each addition at low speed for approx. 1 Min. Flour and baking powder were sifted in to the mixture and were mixed together at minimum speed for 2 Mins, before milk was added. Formulation was adapted for sucrose replacement where inulin and stevia were added to the mixture during the creaming stage in partial replacement of sucrose. Batter was poured into circular baking tins (9 inch) and baked for 30 min at 180° C in a Zanussi convection oven (C. Batassi, Conegliano, Italy). Following baking, cakes were left to set for 20 Mins in tins before being removed and placed on racks for cooling. Sponge cakes were stored in plastic containers for subsequent analysis and testing.

5.2.3 Sponge cake formulation for fat replacement

Four Sponge cake formulations were manufactured containing four fat replacement levels (0, 25, 50 & 75%) (Table 5.1). For each formulation; $SC_{30/0}$, $SC_{30/25}$, $SC_{30/50}$ & $SC_{30/75}$ (where the first digit denotes the sucrose replacement level and the second digit represents the fat replacement level) sponge cakes were formulated as previously described in (6.2.2) and adapted for fat replacement. Butter beans were drained and then pureed in a Stephan mixer (UMC-5 Stephan u. Sohner & Co, Hameln, Garmany) at 21 RPM for 5 Mins before being added in partial replacement of fat.

Table 5.1
Formulation (%) of different Sponge cake treatments

	%								
Samples [a]	Sucrose	Inulin	Reb A	Butter	Pureed butter beans	Flour	Milk	Baking powder	Eggs
SC_0	26.1	0	0	18.6	0	26.1	1.7	0.7	26.8
SC_{25}	19.55	6.49	0.02	18.6	0	26.1	1.7	0.7	26.8
SC_{50}	13.04	13.0	0.04	18.6	0	26.1	1.7	0.7	26.8
SC_{75}	6.51	19.49	0.06	18.6	0	26.1	1.7	0.7	26.8
$SC_{30/0}$	18.23	7.82	0.02	18.6	0	26.1	1.7	0.7	26.8
$SC_{30/25}$	18.23	7.82	0.02	13.96	4.7	26.1	1.7	0.7	26.8
$SC_{30/50}$	18.23	7.82	0.02	9.3	9.3	26.1	1.7	0.7	26.8
$SC_{30/75}$	18.23	7.82	0.02	4.7	13.96	26.1	1.7	0.7	26.8

[a] The first digit corresponds to the sucrose replacement level and the second digit corresponds to the fat replacement level

5.2.4 Sensory analysis

5.2.4.1 Sensory Acceptance testing (SAT)

Sensory acceptance testing (SAT) was carried out in the panel booths of the sensory science laboratory, at University College Cork according to international standards (ISO 11136:2014). Using untrained assessors (n = 25) who were familiar with the products being tested, SAT took place over six separate tasting. Samples (2 x 2 x 2 cm) were assigned a randomised three-digit code and sessions were carried out at room temperature under white light. Participants were instructed to use the water provided to cleanse their palates between tastings and used the following hedonic descriptors to rate their degree of liking of chocolate brownie samples; appearance, flavour, texture, colour and aroma liking. Assessors were asked to indicate their degree of liking for samples on a 9-point, numbers only hedonic scale. The terms 'extremely dislike' and 'extremely like' were used to anchor the far left and far right end of the scales respectively. Overall acceptability (OA) of samples was also determined using this scale.

5.2.4.2 Ranking descriptive analysis (RDA)

Ranking descriptive analysis (RDA) was also carried out in the panel booths of the sensory science laboratory, at University College Cork. A separate panel of assessors, (n = 21) all of whom had previous experience with descriptive analysis, were trained and participated in a separate RDA according to the method of Richter et al., (2010). RDA was only carried out for fat replacement trials. Thus, RDA sessions ran concurrently with SAT sessions during fat replacement and therefore took place over three weeks. Sensory descriptors were selected from panel discussion as the most appropriate and reflective of main variation in samples profiled. The consensus list of

intensity descriptors (Table 5.2) were measured on a 10cm continuous line scale. The terms ‘none’ and ‘extreme’ were used on the 0cm and 10cm anchor points on the scale respectively, unless stated otherwise in Table 5.2. All samples (2x2x2cm) were served coded in randomised order and presented simultaneously to assessors (Stone et al., 2012b). All samples were ranked on the same scale for each intensity descriptor.

Table 5.2

Consensus list of sensory descriptors and definitions selected by panel and used in ranking descriptive analysis of Sponge cake

Attributes	Anchor points on scale	Definition
Touch		
Springiness	None – extreme	Rate / speed by visual observation that the sponge cake returns to its original shape after the deforming force is removed.
Appearance		
Crust darkness	Light - dark	Degree of darkness ranging from light to dark brown.
Porous	None – extreme	Amount of bubbles and voids present in the inner mass of the sponge cake.
Aroma		
Sweet aroma	None – extreme	Fundamental smell sensation of which sucrose is typical.
Buttery aroma	None – extreme	Aromatics associated with butter produced from cow's milk.
Caramel aroma	None – extreme	Odour produced when caramelizing sugar without burning it.
Texture		
Moisture	Dry – moist	Wet texture in mouth.
Hardness	Soft – hard	The resistance of the cake to breaking upon pressure of the front teeth during biting.
Crumbly	None – extreme	Easily broken up in the mouth into a lot little pieces.
Dense	Light - heavy	A heavy texture in mouth.
Taste		
Sweet taste	None – extreme	Taste sensation associated with sucrose.
Buttery flavour	None – extreme	Flavour sensation associated with butter; creamy mouth-feel & buttery aroma

5.2.5 Physicochemical analysis

The following Sponge cakes were characterised by physicochemical analysis; SC_0, $SC_{30/0}$, $SC_{30/25}$, $SC_{30/50}$ and $SC_{30/75}$.

5.2.5.1 Texture Profile analysis (TPA)

Texture profile analysis (TPA) was used to measure the texture properties of Sponge cakes. TPA was conducted using a Texture analyser 16 TA-XT2I (Stable Micro Systems, Surrey, UK) and was carried out as described by Janjarasskul et al., (2016). Two Sponge Cake samples (30 x 30 mm) from the centre of each cake were used for TPA. Sponge Cake samples were sliced horizontally to a height of 30 mm. A 50% double compression test was carried out with a 35mm diameter flat-ended cylindrical probe (P/35), at a speed of 1mm/s with a 5 sec waiting time between the two cycles.

5.2.5.2 Colour measurement

Crust and crumb colour characteristics were assessed using a Minolta CR-200B Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan). Colour measurements were recorded on the CIE (L*a* b*) scale where L* represents lightness, a* signifies green - redness and b* represents blue - yellowness. Crust colour was measured at two separate points. Sponge cake samples were cut horizontally to remove the crust and crumb colour was measured directly at two separate points.

5.2.5.3 Moisture & fat

Moisture content was measured using the method of Bostian et al., (1985). Samples were homogenised for analysis using a Büchi Mixer B-400 (Büchi Labortechnik AG, Switzerland). Moisture content was determined using the CEM SMART system and fat was determined using the SMART Trac system (CEM GmbH, kamp-Lintfort, Germany). Two fibreglass pads were placed in the drying chamber of the CEM SMART system and the instrument was tared. Pads were removed and the homogenised samples (2-4g) were weighed accurately on the pads. Following this, one pad was placed over the sample, pressed together and placed back into the drying chamber to begin drying. The moisture (%) was displayed. Fat (%) was determined by wrapping the fibreglass pads with the sample in a sheet of Smart Trac film. Wrapped samples were placed in Smart Trac tube and positioned in the Smart Trac NMR unit. Percentage fat was displayed.

5.2.5.4 Protein

Protein content was determined using the Kjeldahl method which was carried out using the method of Jeddou et al., (2017). Before testing commenced the digestion block (Foss Tecator Digestor, Hillerød, Denmark) was pre-heated to 410˚C. Samples (0.5-2.0g) were weighed accurately into digestion tubes. Two “kjeltabs” were added to each sample in the fume hood followed by 15ml of sulphuric acid and 10ml hydrogen peroxide. Two controls containing no sample were prepared in the same way. Tubes were placed in the heated digestion block and left there for roughly 30-40 Mins until they became colourless. At this point the distillation unit was turned on and rinsed out by connecting a blank tube and receiver flask to the unit and pressing the steam button. Distilled water (50ml) was added to each digested sample in the fume hood after the

samples had cooled thoroughly. Samples were placed one by one into the distillation unit (Foss Kjeltec 2100, Hillerød, Denmark) along with a receiver flask containing 50mls of 4% Boric acid with indicator. After the distillation process was complete the contents of the receiver flask was titrated with 0.1N hydrochloric acid until the green colour reverted back to the original red colour. Nitrogen content was converted to protein content using the factor 6.25.

5.2.5.5 Ash

Ash content was determined using the muffle furnace. Homogenised samples (5g) were weighed into small silica dishes and placed into the muffle furnace. The samples were heated up to 600̊ C until only the inorganic material was left which was indicated by the light-white colour of the samples. The silica dishes containing the samples were then placed in a desiccator to cool and the dishes were weighed carefully. Ash (%) was calculated using the following equation;

$$\% \text{ Ash} = \frac{weight\ of\ Ash \times 100}{\text{weight of sample}}$$

5.2.5.6 Carbohydrate

Carbohydrate content was determined using the following calculation;

% Carbohydrate = 100 - (Moisture % + Fat % + Protein % + Ash %)

5.2.5.7 Dietary Fibre

Total dietary fibre was established according to AOAC 985.29 method. Sponge Cake samples went through sequential enzymatic digestion using a heat-stable α-amylase (95–100 °C, pH 6.0, 15 min), Subtilisin A (60 °C, pH 7.5, 30 min), and amyloglucosidase (60 °C, pH 4.5, 30 min) to remove digestible carbohydrates and protein. To establish total dietary fibre content, the enzyme digests were precipitated with four volumes of 95% (v/v) ethanol, filtered and, then, washed with 78% (v/v) ethanol, acetone before being dried in an oven at 110 °C.

5.2.5.8 Total sugars and Sucrose content

Total sugars and sucrose content of samples were determined by ion chromatography, using a method developed internally by an independent accredited laboratory based in Ireland.

5.2.6 Sponge Cake images

Photographs of the following Sponge Cake treatments; SC_0, $SC_{30/0}$, $SC_{30/25}$, $SC_{30/50}$ & $SC_{30/75}$, were taken one day after baking, in the photograph room of the Food Science Building, at University College Cork. Photographs were taken using a digital camera Nikon D5300 equipped with a lens Nikon DX AF-P NIKKON (18-55 mm, 1:3.5 - 5.6 G), Japan. Images were taken without flash with the following modes: macro, F6.3, 1/125, ISO 400. After images were taken, sharpness was increased to 100% and the saturation was adjusted for all images.

5.2.7 Statistical analysis

5.2.7.1 Sensory data

Three independent trials were carried out for all experimental treatments. Raw data obtained from sensory (hedonic & intensity) analysis was coded into Microsoft excel. The significance of sensory properties in discriminating between the Sponge cake treatments was analysed using ANOVA and Tukey's post-hoc test (SPSS statistics 20 software (IBM, Armonk, NY, USA). For sucrose replacement, the relationship between the set of Sponge cakes and the set of significant sensory variables was determined by partial least squares (PLS) regression using Unscrambler software (Unscrambler 10.3 CAMO software ASA, Trondheim, Norway). In the PLS regression only sensory properties that discriminated significantly between samples were used. The X-matrix was defined as the different sample treatments. The Y – matrix contained the significant sensory variables of the design. For fat replacement, the relationship between the set of sample treatments (X) and the set of sensory & physicochemical variables (Y) was examined by PLS regression. Again, only sensory and physicochemical properties that discriminated significantly between samples were used. Data were normalised during pre-processing, by taking the logarithm to achieve uniform precision over the whole range of variation. Data was also standardised by dividing each variable (sensory & physicochemical) by the corresponding standard deviation. To achieve significant results for the relationships determined in quantitative PLS, regression analysis, coefficients were analysed by jack-knifing which was based on custom cross-validation and stability plots (Martens and Martens, 1999). Statistical significance for the relationships analysed by PLS were defined as $P<0.05$-0.01 (significant), $P<0.01$-0.001 (highly significant) and $P<0.001$ (extremely significant).

5.2.7.2 Physicochemical data

Raw data obtained from physicochemical analysis was coded into Microsoft excel. As three independent trials were carried out for all experimental treatments, physicochemical data presented represents a mean of six values, unless stated otherwise. As two measurements for crust and crumb colour were taken for each individual sample, crust and crumb colour values represent a mean of twelve measurements (2 x 2 x 3). One-way ANOVA was used to compare the means of the data obtained from physicochemical analysis. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA). The relationship between samples and physicochemical properties was also examined by PLS regression as described in (5.2.7.1)

5.3. Results and Discussion

5.3.1 Rebaudioside A concentration adjustment for sucrose replacement

The sweetness rankings of six different concentrations of rebaudioside A and one standard solution of sucrose are presented in Table 5.3. The perceived sweetness of the solution containing 0.069 g of rebaudioside A was not different from the standard sucrose solution. Thus, a sucrose-to-rebaudiosideA-ratio of 1/350 was appropriate, meaning that the sweetness of 62.5 g sucrose (25% of the sugar in the formulation) was achieved by 0.17 g rebaudioside A.

Table 5.3

Iso-sweetness of Rebaudioside A in aqueous solutions

Sweetener	Concentration (g/L)[x]	Mean scores	Dilution factor[y]
Reb A	0.060	1.2[a]	400
Reb A	0.069	5.4[b]	350
Reb A	0.080	6.2[c]	300
Reb A	0.096	6.3[c]	250
Reb A	0.120	7.4[d]	200
Reb A	0.160	9.4[d]	150
Sucrose	24.0	5.6[b]	n/a

[abcd] mean values (± standard deviation) in the same column bearing different superscripts are significantly different, $P < 0.05$.

[xy] As described by (Zahn et al. 2013)

5.3.2 Sensory analysis

5.3.2.1 Relationship between sensory variables and Sponge cakes formulated with increasing levels of sugar replacement

The relationship between hedonic sensory variables (Y) and Sponge cakes prepared with increasing levels of SR (X) is visually represented by a Partial least squares regression plot (PLSR) shown in (Fig 5.1). Most of the variation is shown in Factor-1 where 33% of the data in X explains 56% of the Y data. The SC_0 sample which is situated in the outer circle of the lower right quadrant made a very significant contribution to Factor-1. As shown in the plot, this sample was correlated with all liking properties (aroma, appearance, colour, texture & flavour) and OA. The SC_{25} sample, which is positioned in the outer circle of the upper right quadrant was also associated with liking terms and OA. The SC_{75} sample which is situated in the outer circle of the upper left quadrant, was anti-correlated with all liking properties and OA. The SC_{50} sample, which is positioned in the outer circle of the bottom left quadrant, made a significant contribution to Factor-2 and was also anti-correlated with liking terms and OA. Liking properties were all correlated, as shown by their close proximity on the plot. Texture liking was highly correlated with OA.

Significance of estimated regression coefficients for the relationship between these two sets of variables can be seen in Table 5.4. Resembling results which are visually represented in the PLSR plot, SC_{75} was negatively correlated with aroma, colour, flavour liking, OA ($p<0.05$) and texture liking ($p<0.01$). SC_{50} was only negatively associated with texture liking ($p<0.01$) and consequently OA ($P<0.05$). The addition of inulin in orange cakes has been shown to negatively affect texture liking, but not aroma or flavour liking (Volpini-rapina et al., 2012). In a similar study on sucrose replacement, inulin and rebaudioside A muffins did not differ from the reference sample with regards

to aromatics, browning, cracks on crust or buttery and sweet flavour at a level of 30% SR. In the present study hedonic properties (aroma, appearance, colour and flavour) were not negatively affected by up to a level of 50% SR (SC_{50}). As a 25% SR (SC_{25}) had no negative impact on any hedonic properties (aroma, texture, appearance, colour and flavour) and because a 30% SR had been achieved using the same combination of replacers in muffins without negatively affecting sensory attributes (Zahn et al., 2013), a SR level of 30% was chosen for further experiments on fat replacement in the present study.

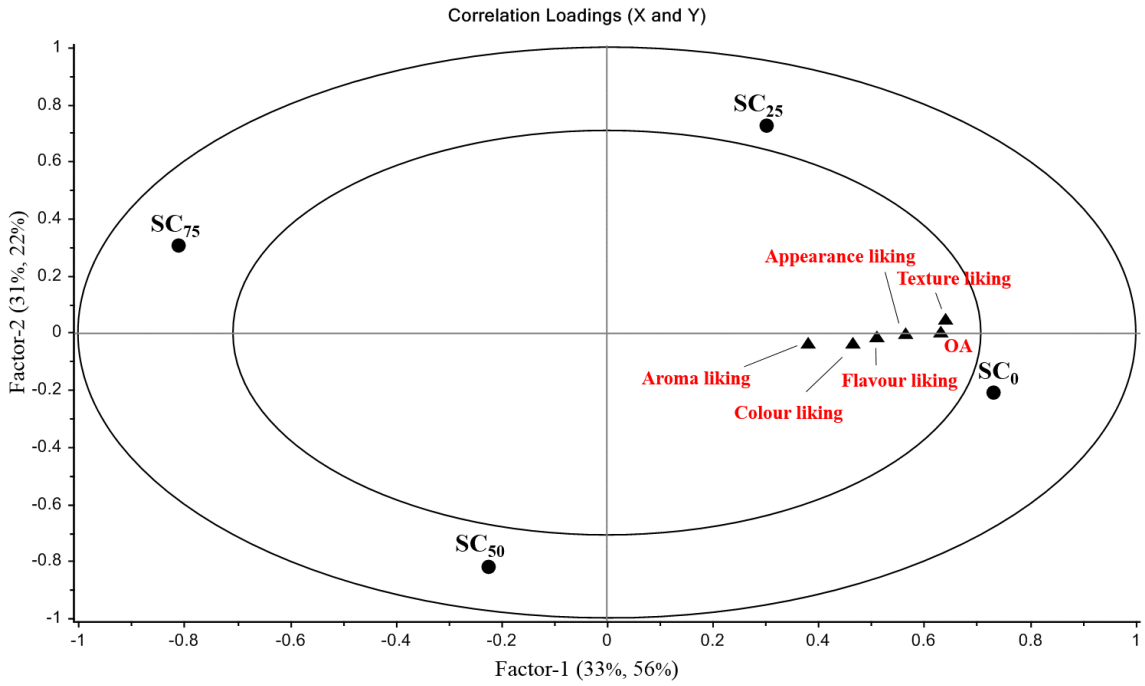


Fig 5.1 Partial least squares regression (PLSR) plot for the relationship between Sponge cakes formulated with increasing levels of sucrose replacement; 0, 25, 50 & 75%, (●) and hedonic sensory variables (▲).

Table 5.4

Significance of estimated regression coefficients (ANOVA values) for the relationship of hedonic sensory properties (Y) and Sponge cakes prepared with increasing levels of sucrose replacement (X).

	Hedonics					
	Aroma liking	Appearance liking	Colour liking	Texture liking	Flavour liking	Overall acceptability
SC_0	0.57	0.68	0.56	0.57	0.68	0.56
$SC_{25/0}$	0.55	0.84	0.56	0.57	0.76	0.66
$SC_{50/0}$	0.57	0.84	0.57	-0.04*	0.83	-0.03*
$SC_{75/0}$	-0.05	-0.03*	-0.04*	-0.01**	-0.03*	-0.02*

Significance of regression coefficients; (*) = $p \leq 0.05$, (**) = $p \leq 0.01$, (***) = $p \leq 0.001$.
(–) indicates whether the relationship is negatively correlated.

5.3.2.2 Relationship between sensory & physicochemical variables and reduced sugar Sponge cake formulated with increasing levels of fat replacement

The second part of this study involved the sequential replacement of fat in inulin and rebaudioside A Sponge cakes using PBRB. The relationship between sensory & physicochemical variables (Y) and inulin and rebaudioside A Sponge cakes, prepared with 0, 25, 50 and 75% FR (X) is visually represented by a PLSR plot in Fig 5.2. The following sensory terms were omitted from PLSR analysis because they did not significantly discriminate between samples; hedonic (appearance, colour, texture), intensity (porous, crumbly and dense). The following physicochemical properties were also omitted from PLSR analysis; texture (hardness, gumminess, springiness, cohesiveness), colour (crumb a* & b* components) and compositional (ash, carbohydrate, total sugars, sucrose content). The physicochemical characteristics of Sponge cakes ($SC_{30/0}$, $SC_{30/25}$, $SC_{30/50}$ & $SC_{30/75}$) are discussed in this section in terms of their correlation with sample treatments. Physicochemical properties will be discussed in detail in subsequent sections; 5.3.3 & 5.3.4. Most of the variation in the plot is shown in Factor-1, where 33% of the X data explains 51% of the data in Y.

The $SC_{30/0}$ sample, which is shown in the outer circle of the lower right quadrant makes a significant contribution to Factor-1. The $SC_{30/0}$ and $SC_{30/25}$ samples were positively correlated with hedonic properties (aroma, flavour liking and OA) which can also be seen on the right hand-side of the plot. The following sensory and physicochemical attributes were correlated with liking properties and OA; sweet, buttery and caramel aroma, sweet and butter flavour, crust and crumb darkness, perceived moisture and springiness and actual fat (%) content. Trends show that these sensory attributes were driving factors for liking and acceptability of samples. The $SC_{30/75}$ sample, which is positioned in the outer circle of the lower left quadrant was anti-correlated with these

driving factors and consequently hedonic properties (aroma, flavour liking & OA). $SC_{30/75}$ was correlated with the sensory and physicochemical variables situated on the left-hand side of the plot; perceived hardness, crust lightness, redness and yellowness, and actual moisture (%), protein (%) and fibre (%) content.

Significance of estimated regression coefficients for the relationship between Sponge cake treatments and sensory & physicochemical properties are displayed in Table 5.5. After custom cross validation of PLSR analysis, the inulin and rebaudioside A Sponge cakes containing 75% fat replacement with PBRB ($SC_{30/75}$) were the only samples negatively correlated with aroma, flavour liking ($p<0.05$) and consequently OA ($P<0.01$). The $SC_{30/75}$ samples were negatively correlated with intensity attributes that were associated with hedonic properties ($p<0.05$) in particular sweet taste and butter flavour ($p<0.01$). A reduction in perceived butter flavour was expected at this level of FR using PBRB, and similar results were reported by Laguna et al., (2014) in biscuits. A reduction in perceived sweetness was also expected at high levels of FR as the correlation between perceived sweetness and fat content in food products has been demonstrated by Drewnowski and Greenwood, (1983) and more recently by Biguzzi et al., (2012) who found that perceived sweetness declined with fat reduction in biscuits. Therefore, it is no surprise that perceived sweetness was negatively affected at a level of 75% FR ($SC_{30/75}$) in the present study. A decline in perceived aroma (butter, caramel, sweet) was also expected as butter aroma is stronger than the neutral aroma of butter beans. A reduction in perceived butter aroma may have had a carry-over effect on caramel & sweet aroma perception, due to the synergistic relationship between sugar and fat mentioned previously, in cake products. A decrease in perceived moisture was also expected at this level of FR (75%) as fats have a lubricating effect and produce a sensation of moistness in the mouth (Rios et al., 2014). Results obtained for perceived

moisture and actual moisture content however, were not correlated, with perceived moisture being associated with samples containing 0 or 25% FR and actual moisture content (%) being correlated with Sponge cakes containing high levels of FR ($SC_{30/75}$) ($P<0.05$). The correlation between Sponge cakes containing low levels of FR ($SC_{30/0}$, $SC_{30/25}$) and perceived moisture, highlights the importance of fat/butter content to the perception of moisture and lubricity in Sponge cake. Although $SC_{30/75}$ samples were perceived as dryer ($p<0.05$) and harder ($p<0.05$) than any other samples, no differences in texture liking was observed between samples. This finding suggests that texture differences did not negatively affect OA. $SC_{30/75}$ was also positively correlated with the L* colour component ($P<0.05$), indicating lighter appearances. Similar findings were reported by Confortiv et al., (1996) using carbohydrate-based fat replacers in biscuits. $SC_{30/75}$ samples were also correlated with the b* colour component ($p<0.05$) indicating more yellow appearances. Although colour changes were observed for the $SC_{30/75}$ as a result of FR, colour liking did not discriminate between any samples. Contradictory to our findings, in a previous study, colour liking of oatmeal cookies was negatively affected after a 50% replacement of fat for white beans (Rankin & Bingham, 2000).

Flavour and aroma liking were the only hedonic properties impaired ($p<0.05$) with fat replacement (0-75%) which suggests that flavour (sweet, butter) and aroma (sweet, butter, caramel) attributes were the primary driving factors for liking and OA.

PBRB were effective in the replacement of fat in inulin and rebaudioside Sponge cakes up to a level of 50% FR ($SC_{30/50}$). Similar findings were reported by Szafranski et al., (2005) for brownies formulated with pureed cannellini beans.

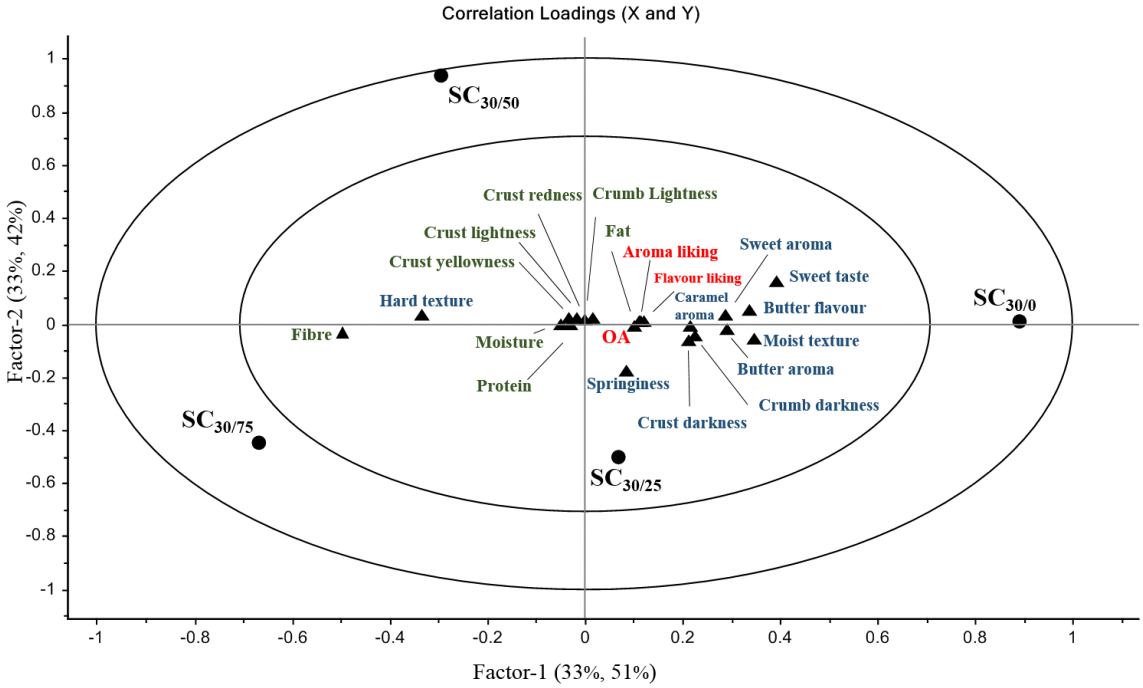


Fig 5.2. Partial least squares regression (PLSR) plot for the relationship between inulin and rebaudioside A Sponge cake (30%) prepared with increasing levels of fat replacement; 0, 25, 50 & 75%,(●) and sensory & physicochemical variables (▲). Hedonic (—) and intensity sensory terms (—) and physicochemical parameters (—)

Table 5.5
Significance of estimated regression coefficients (ANOVA values) for the relationship of sensory & physicochemical parameters (Y) and inulin and rebaudioside A Sponge cakes prepared with increasing levels of fat replacement (X).

	Hedonics			Attribute intensity										Physical properties				Proximate composition			
				Touch	Appearance		Aroma			Texture		Flavour		Colour				(%)			
Sponge cake sample	Aroma	Flavour	OA	Springi-ness	Crust darkness	Crumb darkness	Sweet	Butter	Caramel	Moist	Hard	Sweet	Butter	Crumb L*	Crust L*	Crust a*	Crust b*	Protein	Fat	Moisture	Fibre
$SC_{30/0}$	0.82	0.81	0.82	0.88	0.71	0.65	0.78	0.94	0.61	0.64	0.50	0.88	0.55	0.81	-0.95	0.99	0.92	-0.98	0.82	0.91	0.99
$SC_{30/25}$	0.84	0.96	0.93	0.89	0.75	0.86	0.98	0.95	0.58	0.56	0.81	1.00	0.81	0.68	0.92	0.99	0.96	0.99	0.79	0.88	0.79
$SC_{30/50}$	0.48	0.82	0.91	0.84	0.85	0.72	0.90	0.88	0.86	0.76	0.89	0.99	0.94	0.90	0.96	0.99	0.95	0.98	0.89	0.97	0.81
$SC_{30/75}$	-0.04*	-0.05*	-0.01**	-0.05*	-0.05*	-0.03*	-0.04*	-0.05*	-0.04*	-0.03*	0.05*	-0.01**	-0.01**	0.80	0.05*	1.00	0.04*	0.02*	-0.03*	0.05*	0.04*

Significance of regression coefficients; (*) = p≤0.05, (**) = p≤0.01, (***) = p≤0.001.
(–) indicates whether the relationship is negatively correlated.

5.3.3 Physical properties and images of Sponge cakes

Results from physical analysis on Sponge cake treatments (SC_0, $SC_{30/0}$, $SC_{30/25}$, $SC_{30/50}$ and $SC_{30/75}$) are displayed in Table 5.6. Cross section images of Sponge cakes are shown in Fig 5.3. SR (30%) using inulin and rebaudioside A, increased crumb hardness (N) ($p<0.05$). This was expected as sucrose plays a vital role in the tenderisation of baked goods and similar results have been reported by O' Brien et al., (2003) and Volpini-Rapina et al., (2012) with the addition of inulin in bread crumbs and orange cakes respectively. FR (0-75%) in inulin and rebaudioside Sponge cakes, using PBRB did not affect crumb hardness. As fats also plays a vital role in the tenderisation of dough, an increase in crumb hardness was expected at higher levels of replacement. Souza et al., (2018) reported an increase in crumb firmness of pound cakes with a 25% replacement of butter for green banana puree. However, in the present study and as previously mentioned results obtained from the physical analysis of texture did not correlate with sensory results and Sponge cakes containing 75% FR were perceived as harder.

SR (30%) in Sponge cake using inulin and rebaudioside A did not affect crust or crumb colour components. This finding was supported by images depicted in Fig 5.3. FR (0-75%) in inulin and rebaudioside A Sponge cake increased crust lightness ($p<0.05$). This finding was also visually supported by the images depicted in Fig 5.3. Crust yellowness (b* values) increased as FR increased in inulin and rebaudioside A Sponge cakes. Instrumental results obtained for crust colour, therefore correlated with sensory results. Contradictory to our findings, Confortiv et al., (1997) reported a decrease in crust yellowness with the substitution of fat for carbohydrate-based for replacers. An increase in crust yellowness observed with increasing levels of FR in the present study could be attributed to the yellow colour of butter beans.

Table 5.6

Physical properties of Sponge Cakes

		SC_0	**$SC_{30/0}$**	**$SC_{30/25}$**	**$SC_{30/50}$**	**$SC_{30/75}$**
TPA parameters	Hardness (N)	8.2 ± 0.55^a	15.1 ± 0.14^b	13.2 ± 0.44^b	14.1 ± 0.76^b	14.2 ± 1.52^b
	Gumminess (N)	4.8 ± 0.66^a	6.0 ± 0.78^b	5.8 ± 0.33^b	5.9 ± 0.72^b	6.4 ± 0.60^b
	Chewiness (N)	8.3 ± 0.58^a	3.9 ± 0.59^b	3.9 ± 0.82^b	3.8 ± 0.75^b	3.7 ± 0.55^b
	Springiness (mm)	0.7 ± 0.12^a	0.6 ± 0.03^a	0.7 ± 0.04^a	0.6 ± 0.07^a	0.7 ± 0.05^a
	Cohesiveness	0.6 ± 0.71^a	0.4 ± 0.04^a	0.4 ± 0.03^a	0.4 ± 0.04^a	0.4 ± 0.08^a
Crust colour	L*	39.8 ± 0.65^a	40.5 ± 0.41^a	41.0 ± 0.77^a	45.5 ± 0.40^b	49.8 ± 0.32^c
	a*	15.1 ± 0.25^a	15.8 ± 0.38^a	16.8 ± 0.88^{ab}	16.9 ± 0.60^b	17.5 ± 0.78^b
	b*	24.1 ± 0.61^a	24.8 ± 0.88^a	25.2 ± 0.66^{ab}	29.8 ± 0.20^b	34.1 ± 0.40^c
Crumb colour	L*	69.2 ± 0.44^a	70.8 ± 0.50^a	71.6 ± 0.31^{ab}	72.3 ± 0.45^b	70.7 ± 0.51^a
	a*	-3.1 ± 0.32^a	-2.8 ± 0.21^a	-2.7 ± 0.42^a	-2.8 ± 0.45^a	-2.7 ± 0.36^a
	b*	31.0 ± 0.24^a	29.8 ± 0.60^a	27.7 ± 0.80^a	27.2 ± 0.30^a	28.0 ± 0.54^a

^{abcd} mean values (± standard deviation) in the same row bearing different superscripts are significantly different, $p < 0.05$

5.3.4 Proximate composition of Sponge cakes

Results from the compositional analysis of Sponge cake treatments (SC_0, $SC_{30/0}$, $SC_{30/25}$, $SC_{30/50}$, and $SC_{30/75}$) are displayed in Table 5.7. A SR of 30% using inulin and rebaudioside A, did not affect moisture content (%). However, moisture content increased ($p<0.05$) as FR increased (0-75%) in inulin and rebaudioside A Sponge cakes. This may be due to the higher moisture content of butter beans (75%) compared to butter (15%). Similar results were reported by Confortiv et al. (1996) who found that carbohydrate-based fat replacers increased the moisture content of biscuits. As mentioned, sensory results obtained for perceived moisture did not correlate with instrumental results. Fat content (%) decreased significantly with each level of FR ($p<0.05$). As a 50% FR was permitted, using PBRB in inulin and rebaudioside A Sponge cakes, a 42% reduction in fat was achieved in the formulation. According to the standards set by the Food Safety Authority (2016) this percentage of fat reduction would permit the claim 'reduced fat'. Trends show that protein content increased with increasing levels of FR, however a significant increase in protein content was only observed after a 75% replacement of fat ($SC_{30/75}$) ($p<0.05$). Increase in protein content can be attributed to the protein content of PBRB (5.9%). Carbohydrate content decreased ($p<0.05$) after SR (30%) using inulin and rebaudioside A. Carbohydrate content remained constant (55%) in Sponge cakes containing increasing levels of FR (0-75%). Sucrose content and total sugars also decreased ($p<0.05$) after SR (30%), as expected. Although carbohydrate content was adequate in all samples containing increasing levels of FR, less than half of the carbohydrate was in the form of sucrose (21-28%) and total sugars (21-29%). A 30% SR in Sponge cakes equated to a 28% reduction in total sugars. SR (30%) using inulin and rebaudioside significantly increased fibre content ($p<0.05$) which can be attributed to the fibre present in inulin

(89%). Trends showed that fibre content increased with increasing levels of FR but only significantly so after a 50% FR with PBRB ($p<0.05$). The optimised reduced sugar and fat sample ($SC_{30/50}$) contained 2.8g/100g of fibre and 15% less energy content.

Table 5.7

Proximate composition (%) of Sponge Cakes

	SC_0	$SC_{30/0}$	$SC_{30/25}$	$SC_{30/50}$	$SC_{30/75}$
Moisture	21.6 ± 0.55^{a}	22.1 ± 0.01^{a}	27.5 ± 0.03^{b}	28.6 ± 0.42^{b}	32.4 ± 0.06^{c}
Fat	12.9 ± 0.41^{a}	12.6 ± 0.74^{a}	10.1 ± 0.26^{b}	7.5 ± 0.35^{c}	4.5 ± 0.33^{d}
Protein	6.1 ± 0.47^{a}	6.0 ± 0.75^{a}	6.7 ± 1.40^{ab}	6.8 ± 0.51^{ab}	7.5 ± 0.33^{b}
Ash	1.0 ± 0.35^{a}	1.3 ± 0.06^{a}	1.6 ± 0.63^{a}	1.3 ± 0.18^{a}	1.4 ± 0.05^{a}
Carbohydrate	58.4 ± 0.28^{a}	55.0 ± 0.60^{b}	54.2 ± 0.25^{b}	55.5 ± 0.10^{b}	54.2 ± 0.55^{b}
Sucrose	28.9 ± 0.66^{a}	20.4 ± 0.55^{b}	21.0 ± 0.55^{b}	21.0 ± 0.54^{b}	21.1 ± 0.41^{b}
Total sugars	29.8 ± 0.52^{a}	20.8 ± 0.41^{b}	21.4 ± 0.33^{b}	21.5 ± 0.58^{b}	21.4 ± 0.65^{b}
Dietary fibre	1.3 ± 0.57^{a}	2.0 ± 0.71^{b}	2.4 ± 0.22^{bc}	2.8 ± 0.46^{c}	3.3 ± 0.22^{cd}
Energy (Kcal/100g)	374	357	335	317	287

^{abcd} mean values (± standard deviation) in the same row bearing different superscripts are significantly different, $p < 0.05$.



Fig 5.3. Cross section images of Sponge cakes taken using a digital camera Nikon D5300. From left; SC_0, $SC_{30/0}$, $SC_{30/25}$, $SC_{30/50}$, and SC_{307}

5.4. Conclusions

A 50% FR with PBRB in sucrose-replaced (30%) Sponge cakes using inulin and rebaudioside A was feasible with no negative impact on sensory properties or acceptance. Although these modifications were found to affect the physicochemical properties of cakes, sensory acceptance was not negatively affected. As texture liking was the only hedonic property negatively impacted by a 50% SR in Sponge cakes perhaps the addition of emulsifiers or hydrocolloids in the formulation could improve texture and therefore permit higher levels of SR using these replacers. As FR using PBRB only negatively impaired flavour and aroma acceptances, the addition of flavourings could compensate for the reduction of butter/sweet flavour due to fat reduction and therefore could permit higher levels of fat replacement using this replacer. A simultaneous reduction in fat (42%) and sugar (28%) and an increase in fibre (2.8g/100g) was achieved. Overconsumption of these new developed products would be less likely due to increased satiety as a result of increased fibre content, which would contribute to a better caloric balance.

Chapter 6.

Impact of sugar size fraction and sucrose level on the sensory and physical properties of Shortbread biscuits

Abstract

The primary objective of this study was to investigate the impact of white sugar particle size on the sensory (hedonic & intensity) and physicochemical (dimensions, fracture properties, colour & moisture) properties of Shortbread biscuits, containing two levels of sucrose. A standard recipe was used for Shortbread biscuit formulation; flour (45%), butter (37%) and sucrose (18% & 9%). Three sugar sizes were examined including the Commercial sugar (CM) (102-378µm) and two of its sieved separates, Coarse (CS) (228-377µm) and Fine (FS) (124-179µm). Thus, six biscuit formulations were manufactured containing three sugar size fractions and two levels of sucrose (CM_{18}, CS_{18}, FS_{18}, CM_9, CS_9, & FS_9). The CS_{18} sample had the highest score for perceived sweetness ($p<0.05$) and had higher scores for flavour liking ($p<0.05$) and overall acceptability (OA) ($p<0.05$). Trends show that CS_9 also had higher scores for perceived sweetness. Sweetness correlated with butter flavour perception, with CS_{18} also having the highest score for perceived butter flavour ($p<0.05$). Biscuits containing the finer sugar fractions (FS_{18}, FS_9) had the largest width and length dimensions ($p<0.05$) and were darker in colour ($p<0.05$). Hardness (N) and moisture (%) did not discriminate between samples ($p>0.05$). Sugar particle size manipulation effects the physical and sensory properties of Shortbread biscuits and could be used as a new strategy to reduce sugar in biscuits.

6.1. Introduction

Biscuits are broadly recognised as cereal-based products with a low moisture content (1-5%) and are primarily classified into groupings based on their name, method of preparation and enrichment of ingredients such as fat and sugar (Manley, 2011b). Short dough biscuits are characterised by the presence of fat or sugar in the dough. Shortbread biscuits, an example of short dough biscuits typically contain high levels of fat (21-40%) and sugar (16-23%) which impede the formation of a strong gluten network. Hence, the dough is short (Manley et al., 2011). As short dough biscuits encompass a large variation of biscuits with an array of different ingredients, consumption of these products surpass the consumption of other biscuit types in developed countries (Manley et al., 2011).

Short dough biscuits and cakes, among other forms of sweet products, are major contributors to the intake of dietary sugars in Europe (Azaïs-braesco et al., 2017). In the US between 1977 and 2012, the consumption of added sugars from beverages declined in the latter decade, while the consumption of added sugars from food (sucrose, corn syrup, dextrose, fructose, glucose, high-fructose corn syrups) remained unchanged (Powell et al., 2017). Similar findings were reported in a survey conducted in Ireland where only 4% of participants admitted to drinking sugar rich drinks on a daily basis compared to 54% of participants admitting to eating sugar rich foods this regularly (Richardson et al., 2018a). Findings from the US and Ireland suggest a growing awareness of free sugar in beverages, but a lack of knowledge of the free sugar in sugar rich food products.

The over-consumption of sugars has been associated with various negative health implications. It has been established that intake of sugars is a determinant of body weight, with a clear association between higher intakes of sugars, body fat and long-

term weight gain in adults (Te Morenga et al., 2013). Excess weight in humans is a known risk factor for type-2 diabetes and it is estimated to be responsible for 90% of diabetes cases worldwide (Hossain et al.,2007) . The intake of dietary sugars is also the most important risk factor for dental caries (Moynihan & Kelly 2014). Therefore, the reduction of sugar in biscuits, amongst other products, could be a significant development in reducing the dietary intake of sugar and subsequently, the prevalence of these non-communicable diseases.

Sucrose is the most common sugar used in biscuit manufacture and provides sweetness and flavour enhancement to biscuit products (Manley, 2011c). Sucrose dissolves in the dough during baking and recrystallizes after, affecting biscuit spread and characteristics (Kulp et al., 1991). The process of sucrose dissolution and recrystallization, influences the textural properties of biscuits, for example biscuits high in sucrose possess a crunchy texture (Manley, 2011c). Sucrose competes with gluten for available water during baking, thereby impeding the formation of a very strong gluten network, resulting in a softer product texture compared to other dry cereal-based products (Kweon et al., 2009). Sucrose also increases the starch gelatinisation point to a higher temperature which allows the dough to rise more during baking (Spies & Hoseney 1982). Therefore, as a result of the numerous functional properties of sucrose, food researchers and industry professionals face many challenges when formulating sugar reduction/replacement strategies for bakery products.

The effect of sucrose reduction/replacement on biscuit properties has been extensively studied (Gallagher et al., 2003; Pareyt et al., 2009; Laguna et al., 2014). However, despite research to replace and/or reduce the sucrose content of biscuits, in a recent cross-sectional study conducted in the UK, it was reported that 74% of the commercially-available biscuits obtained a ‘red’ (high) label for sugar content per 100g

(Hashem et al., 2017). This could be attributed to high cost associated with bulk sugar replacers such as polyols (Zumbé et al., 2001) and oligosaccharides (inulin) (Sugar and Sweetener guide, 2013). These bulk sweeteners are typically combined with high intensity artificial sweeteners to replace sweetness due to sucrose reduction, which further increases cost. Additional problems arise when discussing the use of artificial sweeteners as controversy exists over their use in food products. In a recent US Mintel survey, it was found that 64% of respondents indicated they were concerned about the safety of "artificial" sweeteners (Gardner et al., 2012). This alone, justifies the need for a clean label approach when formulating sugar reduction/replacement strategies.

Due to the continued demand for biscuit products containing high levels of sugar, strategies such as sugar particle size manipulation, may allow for sugar reduction in these products. The impact of sugar particle size on the physical parameters of biscuits has been demonstrated previously (Kweon et al., 2009; Kissel et al., 1973). However, to date, following an extensive review of the scientific literature, the impact of sugar particle size on the sensory properties of short dough biscuits has not been investigated. In previous related work, the utilisation of small brown sugar particles (459-972µm) increased the perceived sweetness of chocolate brownies (Chapter 3) (Richardson et al., 2018b). The impact of sugar particle size, as a means of facilitating sucrose reduction in Shortbread biscuits merits investigation. Therefore, the objective of this study was to investigate the impact of three white sugar particle size fractions (including the commercial sugar) on the sensory (hedonic & intensity) and physical properties (dimensions, fracture properties, colour & moisture) of shortbread biscuits containing 18% and 9% sucrose.

6.2 Materials and Methods

6.2.1 Materials

Food ingredients used in this trial were; creamery butter (81% total fat, 65.4% of which were saturated, 2% salt, 15.1% moisture, supermarket own-brand, Ireland); cream plain flour (1.4% fat, 82.7% carbohydrate, 2% of which were sugars, 3.4% fibre, 11.7% protein and 0.81% salt, Odlums, Ireland); commercial white caster sugar (99.9% sucrose, 0.3% moisture & 0.01% sodium, Tate & Lyle brand, UK). Food products were all purchased from a local supermarket and stored under refrigerated or cool, dry conditions where appropriate prior to sample preparation

6.2.2 Preparation of caster sugar particle size fractions; grinding and sieving

Caster sugar was ground manually using rolling pins, to obtain adequate amounts of different sugar sizes and passed through a sequence of sieves of varying diameters (90, 125, 150, 180, 212 and 355 µm) placed on a mechanical device (Endeotts Octagon 200 London, England). Sugar was sieved (200g batches) for 10 Min at 5-mm amplitude and sieved fractions were measured. Two sugar fractions collected in different sieve apertures (355µm) (coarse) and (150µm) (fine) were selected for inclusion in the shortbread biscuit formulation.

6.2.2.1 Measurement of sugar particle size

Commercial caster sugar (unground and ground) was analysed using a light microscope (Olympus BX-61 Tokyo, Japan) and cellSens™ standard software (version 510_UMA_Database_cellsens19-Krishna-en_00). Particle images within each fraction were captured using a microscope digital camera lens (Olympus DP73 Tokyo, Japan). Particle size ranges and the average particle size of the commercial sugar and sugar

separates captured by different sieve apertures (90, 125, 150, 180, 212 and 355 µm) were determined by recording the 2D longest diameter of 100 particles per fraction in transparent light mode.

6.2.3 Shortbread biscuit formulation

A standard shortbread biscuit recipe containing butter (37%), flour (45%) and sucrose (18%) was utilised in this study. Six biscuit formulations were manufactured containing three sugar particle size fractions (commercial unground caster sugar (CM), coarse sugar (CS) and fine sugar (FS) and two sugar levels (18% and 9%)). For each formulation (CM_{18}, CM_9, CS_{18}, CS_9, FS_{18}, FS_9), butter was blended in an electronic mixer (Kitchen Aid Professional mixer) for 5 min at minimum speed in order to obtain a soft consistent cream. Sucrose was added slowly to the creamed butter and mixed at a low speed. Flour was sifted into the creamed butter/sucrose mixture at minimum speed 2 Min. The dough was pressed by hand to a thickness of 1cm and circular cutters were used (68mm) to cut biscuit shapes. Biscuits were baked for 20 Min at 180℃ in a Zanussi convection oven (C. Batassi, Conegliano, Italy). Following baking, biscuits were cooled for 1 hr prior to placement and storage in plastic containers for subsequent analysis and testing.

6.2.4 Biscuit dimensions

Biscuit height, width and length were measured (mm). Biscuit height was measured using a digital thickness gauge (FD50C, Käfer, Germany). Width, the biscuit dimension perpendicular to the direction of sheeting and length the biscuit dimension parallel to the direction of the sheeting, was measured using a ruler (30 cm).

6.2.5 Sensory analysis

6.2.5.1 Sensory Acceptance testing (SAT)

Sensory acceptance testing (SAT) was carried out in the panel booths of the sensory science laboratory, at University College Cork according to International Standards (ISO 2014). Using untrained assessors (n=25), who were familiar with the products being tested, SAT took place over three separate tasting sessions. Samples were assigned a randomised three-digit code, presented in duplicate and sessions were carried out at room temperature under white light. Participants were instructed to use the water provided to cleanse their palates between tastings and used the following hedonic descriptors to rate their degree of liking of biscuit samples; appearance, flavour, texture, colour and aroma. Assessors were asked to indicate their degree of liking for samples on a 9-point, numbers only hedonic scale. Overall acceptability (OA) of samples was also determined using this scale.

6.2.5.2 Ranking descriptive analysis (RDA)

Ranking descriptive analysis (RDA) was also carried out in the panel booths of the sensory science laboratory at University College Cork. A separate panel of assessors (n=21) who were regular consumers of biscuits and had received prior training in descriptive analysis were trained and participated in RDA according to the method of Richter et al., (2010). RDA sessions ran concurrently with SAT sessions and therefore, took place over three sessions. Sensory descriptors were selected from the panel discussion as the most appropriate and reflective of variation in samples profiled. The consensus list of intensity descriptors (Table 6.1) were measured on a 10 cm continuous line scale. The terms ‘none’ and ‘extreme’ were used on the 0cm and 10cm anchor points on the scale respectively (unless stated otherwise in Table 6.1). All samples were

ranked on the same scale for each intensity descriptor. Samples were served coded in randomised order and presented simultaneously to assessors (Stone et al., 2012).

Table 6.1

Consensus list of sensory descriptors and definitions selected by panel and used in ranking descriptive of Shortbread biscuits

Attributes	Anchor points	Definition
Appearance		
Colour intensity	Light - dark	Intensity of colour taking into account its hills and valleys.
Aroma		
Sweet aroma	None - extreme	Fundamental smell sensation of which sucrose is typical.
Buttery aroma	None - extreme	Aromatics associated with butter produced from cow's milk.
Caramel aroma	None - extreme	Odour produced when caramelizing sugar without burning it.
Texture		
Hardness	Soft - hard	Hardness: The resistance of the biscuit to breaking upon pressure of the front teeth during biting. Softness: Force required to bite through the biscuit after one bite between incisors.
Moisture	Dry - moist	Degree to which the sample feels wet in mouth.
Taste		
Sweet taste	None - extreme	Fundamental taste sensation associated with sucrose.
Buttery flavour	None - extreme	Taste sensation associated with butter; creamy mouth-feel & buttery aroma.
Toasted flavour	None - extreme	Toasty/malty flavour associated with biscuits.
Salty taste	None - extreme	Fundamental taste sensation associated with salt.
Astringent	None - extreme	Flavour of dryness, results in an immediate dry, chalky sensation in the mouth.

6.2.6. Determination of biscuit fracture properties

A 3-point bend test was used to measure the maximum force required to break biscuit samples using a Texture Analyser (16 TA-XT2I Stable Micro Systems, Surrey, UK). As described by Wehrle et al., (1999), biscuits were placed on two parallel fulcrums 50 mm apart. The third fulcrum was lowered at a speed of 5mm/s to apply the bending force.

6.2.7 Colour measurement

Colour was measured using a Minolta CR-200B Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan). Colour measurements were recorded on the CIE (L*a* b*) scale where L* represents lightness, a* signifies green - redness and b* represents blue - yellowness. Colour was measured directly from biscuit surfaces.

6.2.8 Moisture content

Moisture content was measured using the method of Bostian, et al., (1985). Samples were homogenised for analysis using a Büchi Mixer B-400 (Büchi Labortechnik AG, Switzerland). Moisture content was determined using the CEM SMART system (CEM GmbH, kamp-Lintfort, Germany). Two fibreglass pads were placed in the drying chamber of the CEM SMART system and the instrument was tared. Pads were removed and homogenised samples (2-4g) were placed on the pads. Following this, one pad was placed over the sample, pressed together and placed back into the drying chamber to begin drying. The moisture (%) was displayed.

6.2.9 Statistical analysis

6.2.9.1 Sensory data

Raw data obtained from sensory analysis was coded into Microsoft excel. One way ANOVA was used to compare the means of the data obtained from all analysis. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA). Using this method, the significance of sensory properties in discriminating between samples was determined. The relationship between the set of samples (6) and the set of significant sensory variables was determined by partial least squares (PLS) regression using Unscrambler software (Unscrambler 10.3 CAMO software ASA, Trondheim, Norway). For the PLS regression analysis, only sensory properties that discriminated significantly between samples were used. The X-matrix was defined as the different sample treatments. The Y-matrix contained the significant sensory variables of the design. Data was normalised during pre-processing of the data by taking the logarithm to achieve uniform precision over the whole range of variation. Data was also standardised by dividing each variable by the corresponding standard deviation. To achieve significant results for the relationships determined in quantitative PLS, regression analysis, coefficients were analysed by jack-knifing which was based on custom cross-validation and stability plots (Martens & Martens, 1999).

6.2.9.2 Physicochemical data

As three independent trials were carried out, physicochemical data presented represents a mean of 6 values ± standard deviation (3 trials x 2 measurements). One-way ANOVA was used to compare the means of the data obtained from all analysis. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA). Using this method, the significance of physicochemical properties in discriminating between samples was determined. The relationship between the set of samples (6) and the set of significant physicochemical properties was determined by (PLS) regression using Unscrambler software (Unscrambler 10.3 CAMO software ASA, Trondheim, Norway). For the PLS regression analysis, only physicochemical properties that discriminated significantly between samples were used. The X-matrix was defined as the different sample treatments. The Y-matrix contained the significant physicochemical variables of the design. Data was normalised during pre-processing of the data by taking the logarithm to achieve uniform precision over the whole range of variation. Data was also standardised by dividing each variable (sensory & physicochemical) by the corresponding standard deviation. To achieve significant results for the relationships determined in quantitative PLS, regression analysis, coefficients were analysed by jack-knifing which was based on custom cross-validation and stability plots (Martens & Martens, 1999).

6.3 Results and Discussion

6.3.1 Particle size distribution

Particle size distribution (PSD) of the commercial sugar, before and after grinding, is displayed in Fig 6.1. The large variation of sugar particle sizes within commercial sugar is evident from this plot. Before grinding, the majority of sugar (51%) was collected by the sieve with the second largest aperture (212μm). Sugar particles captured by this sieve and the sieve with the largest aperture (355μm) decreased after grinding. The presence of finer sugar particles increased after grinding as can be seen with the increase in particles captured in sieves with smaller apertures (180, 150, 125 & 90μm). A visual representation of the particle diameter distribution of the unground commercial sugar and six sugar separates can be seen in Fig 6.2. The commercial sugar had the widest particle size distribution as expected, with particles ranging from 102-378μm. This was a similar particle size range to caster sugar classified by the British sugar company (Speciality Crystalline White Sugar, 2015). After grinding and separation, particle size distribution within each fraction became smaller, in the range of 228-377μm for particles captured by the largest aperture (355μm) and 102-182μm for particles captured by the smallest aperture (90μm). Particle size ranges and average sizes for control sugar and each sugar-separate are shown in Table 6.2. Based on particle size ranges and average particle sizes of each sugar fraction, three fractions including the unground commercial sugar were chosen for shortbread biscuit formulation. Particles captured by the largest sieve aperture were chosen to represent the coarse sugar fraction (228-377μm). According to the British sugar company, this fraction of sugar can be classified as slightly coarser than caster sugar which typically has a range of particle sizes between 200-600μm (Speciality Crystalline White Sugar, 2015). The sugar fraction selected to represent the coarse sugar fraction in this study

however, had a narrower particle size range. Sugar particles captured by the third smallest aperture (150μm) were selected to represent the finer sugar fraction. This fraction was extremely different from the coarse sugar fraction with particles ranging from 124–179μm and an average particle size of 151μm. The British sugar company classifies this fraction as finer than typical caster sugar (Speciality Crystalline White Sugar, 2015).

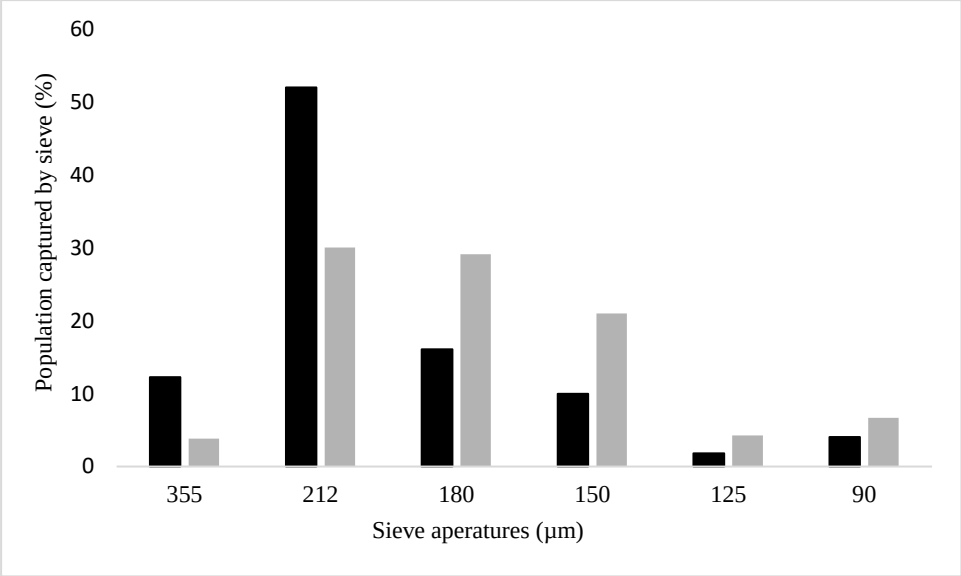


Fig 6.1 Particle size distribution of castor sugar (200g) before (■) and after (■) grinding

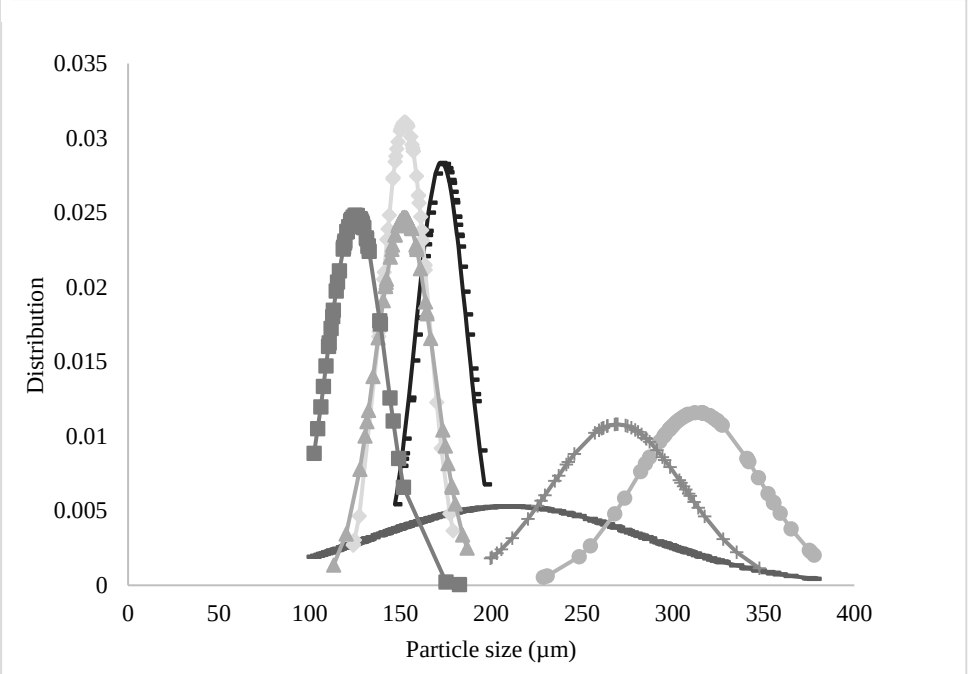


Fig 6.2. Particle diameter distribution of unground commercial castor sugar ( ) and sugar separates; 355 ( ), 212 ( ), 180 ( ), 150 ( ), 125 ( ) & 90µm ( )

Table 6.2

Particle size ranges and average particle size of commercial caster sugar and sieve separates captured by different sieve apertures (µm)

Sieve aperture (µm)	Particle size ranges (µm)	Average particle size (µm)
Commercial (CM)	102-378	210
355	228-377 (CS)	314
212	199-347	269
180	146-196	171
150	124-179 (FS)	152
125	113-186	150
90	102-182	125

6.3.2 Biscuit dimensions

Biscuit dimensions are presented in Table 6.3. The length and width of biscuits (CM_{18}, CS_{18} & FS_{18}) increased more during baking than CM_9, CS_9 & FS_9 ($p<0.05$). This was attributed to higher sucrose dissolution in samples containing more sucrose, which increased spread rates and diameter expansion. CM_{18}, CS_{18} & FS_{18} were also lower in height than CM_9, CS_9 & FS_9 ($p<0.05$) as a result of this. FS_{18} had the largest width (81mm) and length (81mm) ($p<0.05$). Similar results were presented by Kweon et al., (2009) who reported that cookies formulated with larger sugar particles were smaller in width (79.5mm) and length (79.5mm) than cookies prepared with finer sugar particles (83.4mm and 82.8mm width and length respectively) due to delayed sugar dissolution during baking.

Table 6.3

Dimensions of Shortbread Biscuits prepared with three different sugar size fractions and with two different levels of sucrose

Shortbread biscuit treatments	Biscuit geometry (mm)		
	Width	Length	Height
CM_{18}	75.0 ± 0.50^{a}	74 ± 0.41^{a}	8.5 ± 0.30^{a}
CM_9	68.5 ± 0.41^{b}	68.4 ± 0.55^{b}	10.0 ± 0.60^{b}
CS_{18}	74.0 ± 0.33^{a}	74 ± 0.45^{a}	8.4 ± 0.45^{b}
CS_9	68.0 ± 0.28^{b}	68.0 ± 0.55^{b}	10.0 ± 0.47^{b}
FS_{18}	81.0 ± 0.41^{c}	81.0 ± 0.45^{c}	7.2 ± 0.52^{a}
FS_9	70.0 ± 0.52^{b}	70.0 ± 0.61^{b}	9.0 ± 0.26^{b}

^{abc} mean values (± standard deviation) in the same column bearing different superscripts are significantly different, $P < 0.05$.

6.3.3 Sensory scores of biscuits containing three sugar particle sizes and two sucrose levels

6.3.3.1 Sugar size effect within sucrose levels on sensory properties

Sensory scores are presented in Table 6.5. F_{18} had the highest score ($p<0.05$) for perceived dark colour. Similar results were obtained for FS_9 ($p<0.05$). This may be due to finer sugar particles dissolving faster in the batter during baking, causing a faster onset of caramelisation. Early dissolution of sucrose also causes a faster onset of spreading which is responsible for increasing the diameter of the biscuits. An increase in diameter also increases the surface area of biscuits exposed to high temperatures during baking resulting in the more rapid and deeper browning of the biscuit surface. Consequently F_{18} and F_9 had significantly lower scores for colour liking ($p<0.05$).

In relation to aroma sensory attributes, such as sweet, buttery and caramel, no significant differences were observed between sample treatments. Irrespective of this, aroma liking did discriminate between samples and the CS_{18} sample had the highest score ($p<0.05$) while F_{18} had the lowest ($p<0.05$). For F_9, similar low scores were recorded for aroma liking ($p<0.05$).

The CS_{18} sample had the highest score for perceived sweetness ($p<0.05$). This finding was unexpected as it was initially hypothesised that biscuits containing the finest sugar fraction would be perceived as the sweetest samples, based on findings in Chapter 3 (Richardson et al., (2018b). The commercial soft brown sugar examined in chocolate brownies contained much larger sugar particles and had a wider particle size distribution (200-5181μm) (Richardson et al., 2018b) compared to the white sugar used in the present study (102-378μm). One similarity between the present and previous study on chocolate brownies was that samples perceived as the sweetest samples were both formulated with sugar fractions captured by the same sieve mesh size of 355μm.

Brown sugar captured by this sieve was the smallest sugar fraction investigated in the trial on chocolate brownies. As the commercial white sugar contained much smaller sugar particles, the sugar fraction chosen to represent the smallest white sugar fraction was much finer (124-179μm). This suggests that, rather than sugar particle size reduction increasing the perceived sweetness intensity of baked goods, it is more likely that an optimum sugar particle size exists for both white and brown sugar which contributes to an increased perception of sweet taste, and this may vary for different baked products such as biscuits and brownies. Trends showed that CS_9 also had higher scores for perceived sweetness than the CM_9 and F_9 samples, which further indicates that the utilisation of this sugar fraction may permit sugar reduction by increasing the perceived sweetness of samples.

CS_{18} had the highest score for perceived butter flavour ($p<0.05$). As butter content did not discriminate between samples, this result highlights the importance of perceived sweetness to butter flavour perception. No significant difference was reported for samples containing 9% sucrose in relation to perceived butter flavour.

Perceived toasted flavour was most pronounced in F_{18} ($p<0.05$) and F_9 ($p< 0.05$) samples. The toasted flavour associated with these samples may be due to the rapid caramelisation of the finer sugar fraction during baking. A limitation of this study is that the toasted aroma of samples was not measured. Previous work on fat replacement in biscuits showed a correlation between perceived toasted flavour and toasted odour of biscuit samples (Laguna et al., 2014). As discussed previously, samples containing the fine sugar fraction had significantly lower scores for aroma liking which may be due to samples having a more pronounced toasted aroma.

CS_{18} had the highest score for flavour liking ($p<0.05$). Flavour liking scores therefore, correlated with perceived sweetness and butter flavour scores. CS_9 also had higher

scores for flavour liking ($p<0.05$) compared to CM_9 and F_9 samples. Trends showed that CS_9 obtained higher scores for sweetness and butter flavour perception which may help to explain why this sample obtained higher scores for flavour liking.

No significant differences were observed between samples for perceived hard or moist texture. However, the CS_{18} had the highest score for texture liking ($p<0.05$). As this sample had the highest score for flavour liking, perhaps flavour liking of samples had a carry-over effect on texture liking of samples. The perception of flavour can change the perception of texture (Jeltema et al., 2015). Even though texture is very important to acceptability, texture awareness is low compared to flavour awareness even when a trained panel is used.

Similar to findings for flavour and texture liking, CS_{18} had significantly higher scores for OA ($p<0.05$). The CS_9 had the highest score for OA for all samples containing 9% sucrose, however results were not statistically significant.

Table 6.4

Sensory scores and physicochemical properties of Shortbread biscuits prepared with three different sugar size fractions and with two different levels of sucrose

Sensory and Physicochemical properties		Biscuit treatments					
		CM_{18}	CS_{18}	FS_{18}	CM_{9}	CS_{9}	FS_{9}
Attribute intensity	Dark colour	4.5 ± 1.14^{ax}	4.0 ± 1.00^{ax}	7.4 ± 1.00^{bx}	4.5 ± 1.00^{ax}	4.0 ± 1.00^{ax}	6.2 ± 1.00^{by}
	Sweet aroma	3.6 ± 1.20^{ax}	3.7 ± 1.20^{ax}	2.8 ± 1.20^{ax}	3.7 ± 1.12^{ax}	3.2 ± 1.20^{ax}	3.5 ± 1.40^{axx}
	Buttery aroma	3.9 ± 1.15^{ax}	4.3 ± 1.40^{ax}	3.3 ± 1.42^{ax}	4.3 ± 1.31^{ax}	3.8 ± 1.40^{ax}	4.0 ± 1.53^{ax}
	Caramel aroma	3.9 ± 1.73^{ax}	3.0 ± 1.43^{ax}	4.0 ± 1.40^{ax}	3.0 ± 1.13^{ax}	3.0 ± 1.40^{ax}	3.2 ± 1.50^{ax}
	Sweet taste	4.7 ± 1.20^{ax}	5.4 ± 1.00^{bx}	4.2 ± 1.40^{abx}	3.2 ± 1.00^{ay}	3.5 ± 1.03^{aby}	3.0 ± 1.00^{ay}
	Butter flavour	4.4 ± 1.20^{ax}	5.4 ± 1.00^{bx}	4.1 ± 1.30^{ax}	4.2 ± 1.00^{ax}	4.4 ± 1.40^{ay}	4.0 ± 1.40^{ax}
	Toasted flavour	5.0 ± 1.40^{ax}	5.0 ± 1.01^{ax}	6.4 ± 1.10^{bx}	4.7 ± 1.00^{ax}	4.0 ± 1.04^{by}	5.6 ± 1.10^{cy}
	Salty taste	2.5 ± 1.00^{ax}	2.6 ± 1.30^{ax}	2.8 ± 1.20^{abx}	4.0 ± 1.50^{ay}	3.5 ± 1.30^{by}	3.1 ± 1.20^{bx}
	Astringency	2.5 ± 1.50^{ax}	2.1 ± 1.84^{ax}	3.3 ± 1.55^{ax}	3.0 ± 1.51^{ax}	3.3 ± 1.53^{ax}	3.2 ± 1.50^{ax}
	Hard texture	5.2 ± 1.70^{ax}	5.2 ± 1.07^{ax}	5.5 ± 1.00^{ax}	5.0 ± 1.12^{ax}	5.6 ± 1.06^{ax}	5.0 ± 1.04^{ax}
	Moist texture	3.5 ± 1.50^{ax}	3.7 ± 1.00^{ax}	3.2 ± 1.12^{ax}	3.2 ± 1.40^{ax}	3.2 ± 1.08^{ax}	3.1 ± 1.14^{ax}
Hedonics/liking	Aroma	5.8 ± 1.34^{ax}	6.4 ± 1.42^{bx}	5.0 ± 1.54^{cx}	5.7 ± 1.21^{ax}	5.8 ± 1.12^{ay}	5.3 ± 1.31^{bx}
	Appearance	6.1 ± 1.32^{ax}	6.7 ± 1.23^{bx}	5.1 ± 1.70^{cx}	5.8 ± 1.40^{ax}	6.3 ± 1.45^{bx}	5.9 ± 1.40^{ay}
	Colour	6.0 ± 1.40^{ax}	6.7 ± 1.22^{abx}	4.7 ± 1.80^{cx}	6.0 ± 1.34^{ax}	6.5 ± 1.22^{abx}	5.4 ± 1.55^{cy}
	Texture	6.3 ± 1.40^{ax}	7.1 ± 1.10^{bx}	6.0 ± 1.41^{ax}	5.7 ± 1.55^{ax}	5.6 ± 1.43^{ay}	5.5 ± 1.43^{ax}
	Flavour	6.4 ± 1.60^{ax}	7.0 ± 1.24^{bx}	5.2 ± 1.90^{cx}	5.1 ± 1.65^{ay}	5.6 ± 1.45^{by}	5.0 ± 1.63^{ax}
	OA	6.3 ± 1.27^{ax}	7.0 ± 1.10^{bx}	5.2 ± 1.62^{cx}	5.2 ± 1.43^{ay}	5.4 ± 1.30^{aby}	5.1 ± 1.53^{ax}
Physicochemical	L*	49.5 ± 1.13^{ax}	50.4 ± 0.75^{ax}	41.3 ± 1.04^{bx}	58.5 ± 0.81^{ay}	62.3 ± 0.02^{ay}	45.7 ± 1.00^{by}
	A*	9.1 ± 0.84^{ax}	8.9 ± 0.75^{ax}	10.4 ± 0.26^{bx}	9.1 ± 0.81^{ax}	7.3 ± 0.84^{by}	10.2 ± 0.22^{cx}
	B*	33.0 ± 0.72^{ax}	33.1 ± 0.90^{ax}	24.8 ± 1.07^{bx}	34.3 ± 0.90^{ax}	35.4 ± 1.03^{ay}	32.2 ± 0.81^{by}
	Hardness (N)	9.9 ± 1.16^{ax}	8.3 ± 1.04^{ax}	9.1 ± 1.30^{ax}	8.4 ± 1.40^{ax}	11.2 ± 1.40^{ax}	11.0 ± 1.25^{ax}
	Moisture (%)	1.0 ± 0.10^{ax}	1.0 ± 0.22^{ax}	1.0 ± 0.37^{ax}	1.0 ± 0.13^{ax}	1.0 ± 0.10^{ax}	1.0 ± 0.21^{ax}

^{abc} For each sugar size group (CM, CS, FS), within individual sucrose levels (18%, 9%) mean values in the same row bearing different superscripts are significantly different $P < 0.05$. (Sugar size effect)
^{xyz} For each sucrose level (18%, 9%) within individual sugar size treatment groups (CM, CS, FS) mean values in the same row bearing different superscripts are significantly different $P < 0.05$. (Sucrose reduction effect)

6.3.3.2 Sucrose reduction effect within sugar particle size fractions

Sensory scores are displayed in Table 6.4. A 50% reduction in total sugars did not affect the following sensory properties of biscuits; sweet, buttery or caramel aroma, astringency, hard or moist texture. A significant decrease in dark colour perception ($p<0.05$) was observed after sucrose reduction for the biscuit samples containing the fine sugar fraction (F_9). This can be attributed to less sucrose present in the sample resulting in a slower rate of carmelisation.

With regards to perceived sweetness, all samples containing half of the original sucrose formulation (CM_9, CS_9, & F_9) were perceived as less sweet ($p<0.05$) than their full sugar counterparts. Sucrose reduction significantly decreased the perceived toasted flavour ($p<0.05$) of biscuits containing the coarse (CS_9) and fine (FS_9) sugar fractions. A 50% sucrose reduction increased the perception of saltiness ($p<0.05$) in samples containing the commercial (CM_9) and coarse (CS_9) sugar fractions. This may be due to the salty taste of butter (2% salt) being more pronounced in samples containing less sucrose.

A 50% sucrose reduction in the coarse sugar biscuits (CS_9) negatively affected aroma liking of samples ($p<0.05$). However, a 50% reduction of sucrose in biscuits containing the fine sugar fraction (F_9) improved appearance and colour liking of samples ($p<0.05$). Trends showed texture liking was negatively affected after a 50% reduction of sucrose in all biscuit treatments, however, only significantly so for the samples containing the coarse sugar fraction (CS_9) ($p<0.05$). Finally, similar to trends observed for perceived sweetness, flavour liking and OA scores were lower ($p<0.05$) after a 50% reduction of sucrose in sugar size treatments, with the exception of the biscuits containing the fine sugar fraction (F_9).

6.3.4 Physicochemical properties

Physicochemical properties of biscuits are presented in Table 6.4. Hardness, represented by maximum force at break in Newtons (N), did not significantly discriminate between biscuit samples. Sample hardness ranged from values between (8.3-11.2N). Previous trials have reported an increase in fracture strength of cookies and short dough biscuits with decreasing levels of sucrose (Laguna et al., 2014; Gallagher et al., 2003; Pareyt et al., 2009). Lower sucrose concentrations within the biscuit batter means less competition between the sucrose and gluten for available water. Therefore, a stronger gluten network is formed in samples with less sucrose. An increase in hardness was not observed after sucrose reduction of biscuits in the present study. Shortbread biscuits typically contain very high levels of fat, which may have lessened the importance of sucrose in tenderising of the final product. Results from instrumental analysis on texture correlate with sensory results as no difference in perceived texture hardness was observed by the sensory panel.

Shortbread biscuit moisture content was similar in all treatments (~1%) and was similar to previously reported moisture levels for biscuits (Manley et al., 2011)

The F_{18} sample had a significantly lower (L*) value ($p<0.05$) than any other sample, indicating a darker appearance. F_9 was also significantly darker than CM_9 and CS_9 ($p<0.05$). Instrumental colour results, therefore correlated with sensory results obtained for perceived colour. F_{18} had the highest (a*) value ($p<0.05$) indicating redness. The F_9 sample also had the highest (a*) value ($p<0.05$) out of all the samples containing 9% sucrose.

Colour lightness (L*) increased significantly ($p<0.05$) after sucrose was reduced by 50% in all biscuit treatments. In a study by Gallagher et al., (2003) sucrose substitution

with Raftilose decreased L* values of biscuits which means samples got darker after reduction of sucrose, this is due to the presence of reducing sugars in Raftilose which contributed to the effects of Maillard browning reactions. Perhaps the partial substitution of sucrose with a reducing sugar like fructose would contribute to a better instrumental colour profile of samples containing lower sucrose concentration

6.3.5 Relationship between biscuit treatments and sensory & physicochemical properties

The relationship between sensory & physicochemical variables (Y) and biscuit treatments (X) is visually represented by a PLSR plot in Fig 6.3. The following sensory terms were omitted from the PLSR analysis as they did not discriminate between samples; sweet, buttery & caramel aroma, astringency, hardness and perceived moisture. The following Physicochemical properties were also excluded from the PLSR analysis; actual hardness (N) and moisture content (%). Most of the variation is shown in Factor-1, where 63% of the data in X explains 75% of the data in Y. It is evident that the CS_{18} sample, which is situated in the outer circle of the upper right quadrant made a very significant contribution to the plot. The CS_{18} sample was the sample most positively associated with sweetness and butter flavour as seen by their close proximity in the PLS plot, and as a result was most correlated with flavour, texture, aroma liking and OA. Perceived butter flavour is situated in very close proximity with OA, which means that this attribute was also a driving factor for sensory acceptance. As butter content did not discriminate between samples, this finding demonstrates the importance of sugar content or perceived sweet taste to the perception of butter flavour and ultimately to liking and acceptability of samples. The CM_{18} sample, which is positioned in the inner circle of the upper right quadrant was also positively associated with butter flavour and sweetness intensity and therefore liking and OA. The F_{18} sample, which is positioned in the outer circle of the upper left quadrant also made a significant contribution to the plot. This sample was anti-correlated with butter flavour and sweetness and consequently liking properties and OA. Dark colour and toasted flavour were correlated and were the attributes most associated with F_{18}. This sample was associated with the (a*) colour component which means it had a red hue. The following

attributes were therefore, undesirable and contributed to the rejection of the F_{18} sample; redness, dark colour and toasted flavour. The F_9 sample, which is situated in the inner circle of the lower left quadrant was also anti-correlated with butter flavour and sweetness and consequently liking properties and OA, but not to the same extent as its full sugar counterpart. CM_{18} and CM_9 samples, which are located in the inner circle of the bottom left quadrant were the samples most associated the (L*) and (b*) colour parameters, which means these samples were lighter and more yellow in colour than any other samples. As a result, these samples were the most associated with colour and appearance liking.

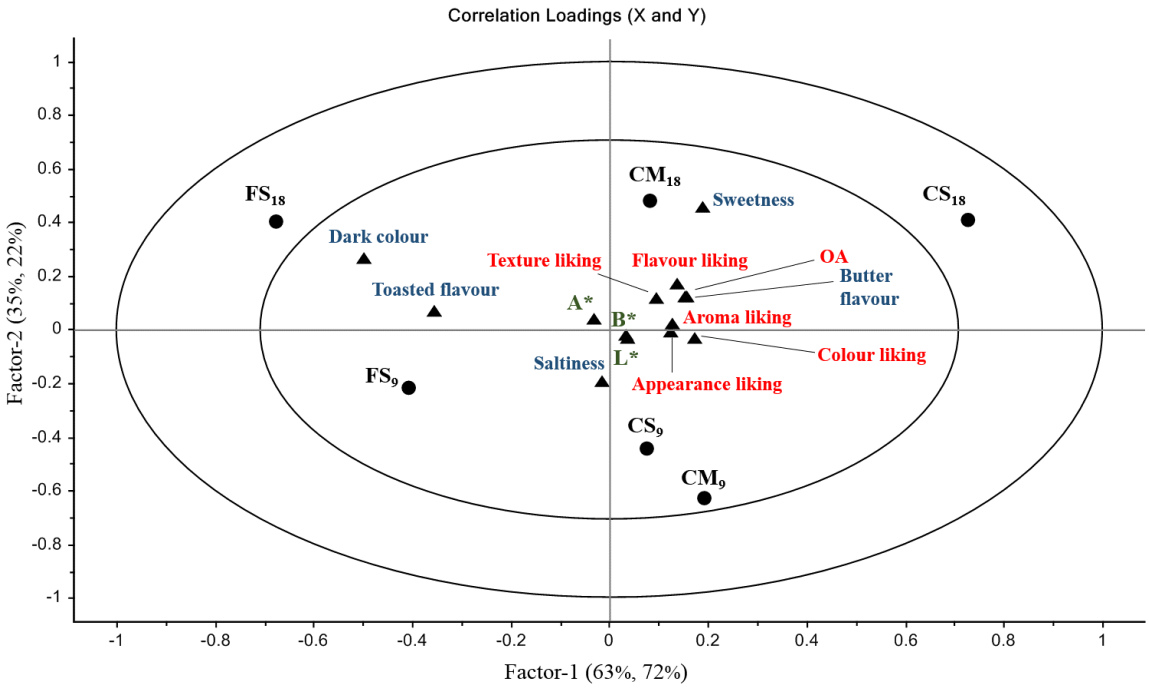


Fig 6.3. Partial least squares regression (PLSR) plot for the relationship between Biscuit treatments (●) and sensory & physicochemical variables (▲). Hedonic (—) and intensity (—) sensory terms and Physicochemical variables (—).

6.4. Conclusions

Sugar particles in the range of 228-377μm significantly increased the perceived intensity of sweetness in shortbread biscuits containing 18% sucrose. As sweetness intensity scores correlated directly with flavour liking scores, these findings promote the use of this sugar fraction in the formulation of low-sugar baked biscuits. The utilisation of a finer sugar fraction (124-179μm) in the preparation of Shortbread biscuits was unsuccessful as it contributed to a darker surface colour and more significant toasted flavour. However, as the utilisation of this sugar size promotes a dark surface colour, perhaps this fraction could be used in sugar replacement strategies to help maintain the desirable, brown surface colour that is typically lost as a result of sucrose reduction. Sugar particle size significantly affects biscuit dimensions and the sensory and instrumental colour properties of Shortbread biscuits. Additional work is necessary to demonstrate the impact of sugar particle size manipulation on the sensory and physical properties of other baked goods, using narrower, broader and various sugar particle size distribution ranges. Sugar particle size manipulation may represent a viable economic, technological approach to reduce sugar in baked goods and therefore promote the production of low-sugar, clean-label, and consumer acceptable, commercially available baked products.

Chapter 7

Consumer acceptability and stability of optimised sugar-and-fat-reduced cake products

Abstract

The primary objective of this study was to determine the consumer sensory acceptability (n=100) of optimised sugar and-fat reduced (OSFR) Sponge cake and Chocolate brownies, compared to experimental controls (EC) and commercially-available standards (CS). Physicochemical changes and microbial stability of cake products, were also assessed over time. For Sponge cakes, EC and OSFR samples had the highest scores for aroma liking ($p<0.05$). OSFR had the highest score for perceived hardness ($p<0.05$). Nonetheless, texture liking scores were similar for EC and OSFR samples ($p>0.05$). Perceived sweetness and overall acceptability (OA) did not discriminate between EC and OSFR ($p>0.05$) and OSFR were preferred to CS samples ($p<0.05$). A more rapid increase in firmness during storage was reported for OSFR samples. For Chocolate brownies, OSFR had the highest score for perceived hardness ($p<0.05$), but similar to results obtained for Sponge cake, this was not found to negatively affect texture liking ($p>0.05$). OSFR samples were preferred to CS samples and were similar to EC in relation to overall acceptability. Moisture content (%) and water activity were higher ($p<0.05$) in OSFR samples at each time point during storage. OSFR Sponge cake and Chocolate brownies were preferred to CS in the Irish market. Microbial stability of all products were similar. Further experiments are required to improve the texture and quality of OSFR samples for long term packaged storage.

7.1 Introduction

Consumer testing is a type of sensory acceptance test (SAT), carried out to validate new products at the end of development. A large number of panellists (n=100) are required for these tests as consumers are typically unfamiliar with the products being tested (Stone et al., 2012). In order to determine how experimental products will compare to commercial products, in relation to acceptability, a commercial standard (gold standard) is often used in a consumer acceptance test. As consumers are untrained, only hedonic sensory properties are typically measured. However, as it is critical to understand important factors that drive liking and acceptability, consumers are sometimes asked to rate important intensity sensory attributes. This approach was used by Li et al., (2014) and Rolon et al., (2017) to establish consumer preferences of coffee and ice creams respectively.

Cake-type products such as Sponge cake and Chocolate brownies contain excessive amounts of fat (15-30%) and sugar (30-45%) which makes these bakery products highly palatable. Therefore, the risk for passive over consumption of these products is high. This contributes to energy imbalances that are typically associated with overweight and obesity (National Taskforce on Obesity, 2005). Overweight and obesity are the most important risk factors for type 2 diabetes (Hossain et al., 2007) among other non-communicable diseases (National Taskforce on Obesity, 2005). As cakes and biscuits make a significant contribution to the dietary intake of both fat and sugar (IUNA) 2011), it is necessary to focus on ways to replace and reduce these ingredients in bakery products.

In our previous studies (Chapters 3, 4 & 5) optimised reduced sugar and fat (OSFR) cake products were established through sensory and physicochemical analysis. In Chapter 4, a simple and novel method was developed for fat reduction in chocolate

brownies, by reducing sugar particle size. This method was based on results obtained in Chapter 3 where it was found that small sugar particles (459-972µm) increased the soft and moist texture of Chocolate brownies. Given what is known about the function of fat in bakery products, it was hypothesised that brownies formulated with small sugar particles would be perceived as more moist and soft than those prepared with other sugar fractions and therefore permit higher levels of fat replacement (FR). Compared to other sugar sizes, the smallest sugar fraction permitted higher levels of FR (75%) using pureed black beans (PBLB), without negatively affecting overall acceptability. Sucrose was then replaced in PBLB Chocolate brownies using inulin and rebaudioside A. A sucrose replacement level (SR) of 25% was achieved without negatively affecting OA. Optimised samples contained significantly lower amounts of fat (9.9%) and sugar (33.6%). In Chapter 5, utilisation of pureed butter beans (PBRB) allowed for the partial replacement of fat (50%) in inulin and rebaudioside A Sponge cake (30%). Optimised Sponge cake samples contained significantly lower amounts of fat (7.5%) and sugar (21.5%).

The individual impact of fat and sugar replacement on the quality and sensory properties of cake products has been the focus of many studies (Schirmer et al., 2012; Zahn et al., 2013; Ates & Elmaci, 2018; Szafranski et al., 2005). The combined effect of fat and sugar replacement however, has been the focus of less research in the literature. In a study conducted by Rodríguez-garcía et al., (2014) a 50% fat replacement and a 30% sugar replacement both separately and combined using two types of inulin did not negatively affect overall acceptability of cakes. Polydextrose was used to replace both sugar (22%) and fat (25%) in a high ratio cake formulation in a study carried out by Kocer et al., (2007). In a recent study, sugar was replaced by 20-

40% and fat was replaced by 25% in pound cake using green banana puree (Souza et al., 2018).

From extensive review of the scientific literature, no consumer acceptability data exists whereby optimised sucrose and fat reduced cake products are compared to experimental controls and commercially available standards. Therefore, this was the main objective of the present study.

Sugar has many functions in cakes, during both the batter mixing and baking stages (Wilderjans et al., 2013). One important function of sugar is to prolong the shelf life of bakery goods by binding water and decreasing water activity (Nip, 2007). Baked products with a high water activity (<0.85) are susceptible to microbiological spoilage by bacteria and yeasts and moulds (Smith et al., 2004) whereas, the main factors that affect the shelf life of low moisture baked goods are the physicochemical changes that occur over time. According to Del Nobile et al., (2003) the physicochemical factors that cause deterioration in baked goods are staling and lipid oxidation. Staling of cakes is a result of moisture migration from crumb to crust and starch retrogradation (Luyts et al., (2013). Therefore, another objective of the present study was to determine the storage time of products using both microbiological and physicochemical analysis

7.2 Materials and Methods

7.2.1. Materials for Sponge cake preparation

Food ingredients used for the manufacture of Sponge cakes were; eggs (Upton brand, Ireland); caster sugar (99.9% sucrose, 0.3% moisture & 0.01% sodium, Tate & Lyle brand, UK); cream plain flour (82.7% carbohydrate, 2% of which were sugars, 11.7% protein, 3.4% fibre, 1.4% fat & 0.81% salt, Odlums brand, Ireland); creamery butter (81% total fat, 65.4% of which were saturated & 15.1% moisture, supermarket-own brand, Ireland); inulin (89% fibre & 8% sugar, Bioglan brand, Austrailia); rebaudioside A., (Bulk Powders brand, Ireland); butter beans (16.8% carbohydrate, 1.4% of which were sugars, 5.9% protein, 0.6% fats 0.1% of which were saturated, 5.2% fibre, 0.01% sodium & 0.03% salt, Suma brand, UK); baking powder (3 % sodium, Royal brand, US) and whole milk (4.7% carbohydrate, 4.7% of which were sugars, 3.5% fat, 2.3% of which were saturated, 3.4% protein & 0.1% salt, supermarket-own brand, Ireland). Food products were all purchased from a local supermarket unless stated otherwise and stored under refrigerated or cool, dry conditions where appropriate prior to sample preparation.

7.2.2 Materials for Chocolate brownie preparation

Food ingredients used in the formulation of Chocolate brownies were; light golden soft brown sugar (1.1% moisture, 98% sucrose, cane molasses & invert sugars, Siucra brand, UK); butter (81% total fats, 65.4% of which were saturated & 15.1% moisture, supermarket own-brand, Ireland); black beans (18% carbohydrate, 0.5% of which were sugars, 8.2% protein, 6.4% fibre, 0.8 % fat, 0.2% of which were saturated, 0.02% sodium, 0.05% salt & 73.2% moisture (after being drained and pureed), Suma brand, UK); inulin (89% fibre & 8% sugar, Bioglan brand, Austrailia); rebaudioside A (Bulk

powders brand, Ireland); cream plain flour (82.7% carbohydrate, 2% of which were sugars, 11.7% protein, 3.4% fibre, 1.4% fat, & 0.81% salt, Odlums, Ireland); eggs (Upton brand, Ireland); dark chocolate (55.8% carbohydrate, 97.2% of which sugars, 34.7% fats, 3.6% protein & 0.1% salt, Homecook wonder bar, Ireland). Food products were all purchased from a local supermarket unless stated otherwise and stored under refrigerated or cool, dry conditions where appropriate prior to sample preparation.

7.2.3 Product formulation

7.2.3.1 Cake treatments

The optimum parameters for the formulation of OSFR samples were determined by sensory and physicochemical analysis, as described in Chapters 4 & 5 and are shown in Table 7.1. For each product (Sponge cake (SC) and Chocolate brownies (CB)) an experimental control (EC) and commercial standard (CS) were also employed for analysis and are shown in Table 7.1. The commercial samples of cake products (gold standard) were purchased from Tesco, Ireland.

7.2.3.2 Sponge cake formulation

A standard Sponge cake recipe was utilised in this study and samples were prepared using methods described in Chapter 5. For each formulation (OSFR, SC/EC) butter was creamed in an electronic mixer and sugar was added gradually until soft and light in colour. Eggs were added one at a time and beaten well between each addition. Flour and baking powder were sifted in to the mixture and were mixed together before milk was added. For the optimum reduced sugar and fat sample (OSFR), inulin and rebaudioside A were added to the mixture in partial replacement of sucrose and pureed butter beans were added in partial replacement of butter. Batter was poured into circular

baking tins and baked for 30 Mins. Cakes were left to set for 20 Mins in the tin before being removed and placed on a rack for cooling. Sponge cakes were placed into plastic containers for storage prior to testing.

7.2.3.3 Chocolate brownie formulation

A standard Chocolate brownie recipe was utilised in this study and samples were prepared as described in Chapters 3 & 4. For each formulation (OSFR, CB/EC) dark chocolate and butter were melted in a heat stable bowl in a microwave oven. The melted mixture was stirred before sugar was added. Eggs were beaten in a separate bowl and added to the mixture. All of the ingredients were stirred until flour was sieved into the mixture. Mixture was stirred by hand until smooth. For the optimum reduced sugar and fat sample (OSFR), inulin and rebaudioside A were added to the mixture in partial replacement of sucrose and pureed black beans were added in partial replacement of butter. The batter was poured into tinfoil trays and batches were baked for 30 Mins. Batches of brownies were left to set for 30 Mins trays before being removed and cut into individual brownie pieces. Chocolate brownies were placed on a rack for cooling for one hour before being removed and placed into plastic containers for storage prior to testing

Table 7.1
Optimum parameters for the preparation of OSFR samples compared to EC and CS

	Sugar replacement level (%)	Fat replacement level (%)	Total sugars (%)	Sucrose (%)	Fat (%)
Products					
Sponge cake					
OSFR	30.0	50.0	21.5	21.0	7.5
SC/EC	0.0	0.0	29.2	28.9	12.0
SC/CS	N/A	N/A	32.4	----	10.4
Chocolate brownies					
OSFR	25.0	75.0	33.6	32.7	9.9
CB/EC	0	0	39.5	38.4	26.0
CB/CS	N/A	N/A	36.8	----	15.0

OSFR; Optimised sucrose and fat reduced sample, EC; Experimental control and CS; Commercial standard

7.2.4 Sensory analysis

Sensory analysis was carried out in the panel booths of the sensory science laboratory, at University College Cork according to international standards (ISO 11136:2014). Consumers were recruited based on their availability and preference for cake products. The study took place in one day and allocated time slots were given to accommodate for a large number of panellists (n=100). The following consumer hedonic descriptors were used and participants were asked to indicate their degree of liking on a 9-point, numbers only hedonic, scale; appearance, flavour, texture, colour and aroma. Overall acceptability (OA) of samples was also determined using this scale. Consumers were also asked to rate the intensity of important attributes, as described by Rolon et al., (2017). The following intensity descriptors were used for both cake products; sweet taste, butter flavour and hardness. Additionally, 'chocolate flavour' was measured for Chocolate brownie samples. Intensity descriptors were measured on a 10 cm continuous line scale. The terms 'none' and 'extreme' were used on the 0cm and 10cm anchor points on the scale respectively. All samples were ranked on the same scale for each intensity descriptor. The samples (2x2x2cm) were served, coded in randomised order and presented simultaneously to consumers, who were instructed to use the water provided to cleanse their palates between tastings (Stone et al.,2012b).

7.2.5 Microbial analysis

Microbiological analysis was carried out on EC and OSFR samples. The precise preparation dates for commercial standards employed for this trial, could not be established and therefore microbial analysis did not take place on these samples. Total plate count (TPC) and yeast and mould count (YM) were determined. Cake samples (10g) were weighed aseptically into a stomacher bag. A primary 10-fold dilution was performed by addition of sterile maximum recovery diluent (90ml) (MRD) (Oxoid, Basingstoke, UK). Samples were stomached (Steward Stomacher 400 Lab Blender, London, UK) for 3 min. Homogenates were 10-fold serially diluted using MRD. For the determination of TPC and YM, 1 ml of each appropriate dilution was inoculated on duplicated plates in the centre of compact dry-TPC and YM plates (Nissui Pharmaceutical, Co. Ltd., Japan). Following incubation at 37˚C for 48 hours for TPC and 20-25˚C for 4/5 days for YM, results were expressed as $\log_{10}$ colony-forming units (CFU) g-l cake. Each value represents the mean of 6 values (three independent trials x one sample x two measurements).

7.2.6 Physicochemical analysis

7.2.6.1 Texture analysis

Compression tests were used to measure the texture properties of cake samples. Compression tests were conducted using a Texture analyser 16 TA-XT2I (Stable Micro Systems, Surrey, UK) and were carried out as described Janjarasskul et al., (2016). Samples were cut into cubic specimens (30 x 30 x 30 mm^3). A 50% single compression test was carried out with a 35mm diameter flat-ended cylindrical probe (P/35), at a speed of 1mm/s. Hardness (N), the force necessary to attain a given deformation of samples was measured. Hardness (N) was measured during storage.

7.2.6.2 Moisture and water activity

Moisture content was measured using the method of Bostian, et al., (1985). Samples were homogenised for analysis using a Büchi Mixer B-400 (Büchi Labortechnik AG, Switzerland). Moisture content was determined using the CEM SMART system (CEM GmbH, kamp-Lintfort, Germany). Two fibreglass pads were placed in the drying chamber of the CEM SMART system and the instrument was tared. Pads were removed and homogenised samples (2-4g) were placed on the pads. Following this, one pad was placed over the sample, pressed together and placed back into the drying chamber to begin drying. The moisture (%) was displayed. Water activity (A_w) was determined using a_w meter, AquaLab Series 4 TE (LABCELL LTD., Hants, UK).

7.2.7 Statistical analysis

7.2.7.1 Sensory data

Raw data obtained from sensory analysis was coded into Microsoft excel. Data obtained from sensory analysis was presented as a mean of 100 values ± standard deviation. One-way ANOVA was used to compare the means of the data obtained. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA). Using the above method, the significance of sensory properties in discriminating between the samples was determined. The relationship between the set of samples and the set of significant sensory variables was determined by partial least squares (PLS) regression using Unscrambler software (Unscrambler 10.3 CAMO software ASA, Trondheim, Norway). In the PLS regression only sensory properties that discriminated significantly between samples were used. The X-matrix was defined as the different sample treatments. The

Y – matrix contained the significant sensory variables of the design. Sensory data was normalised during pre-processing of the data by taking the logarithm to achieve uniform precision over the whole range of variation.

7.2.7.2 Physicochemical data

As three independent trials were carried out, physicochemical data presented represents a mean of 6 values ± standard deviation (3 trials x 2 measurements). One-way ANOVA was used to compare the means of the data obtained from all analysis. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means using SPSS statistics 20 software (IBM, Armonk, NY, USA).

7.3 Results and Discussion

7.3.1 Consumer sensory scores for Sponge cake products

Scores obtained from sensory analysis on Sponge cakes are displayed in Table 7.2. SC/EC and OSFR samples had the highest scores for aroma liking ($p<0.05$). This may be due to consumers detecting a freshly baked aroma from these samples, which were prepared one day prior to testing. Previous studies have shown that odour is very important to consumer perception of fresh breads (Heenan et al., 2009). SC/EC had the highest score for appearance liking ($p<0.05$). Similarly, trends show that this sample also had the highest score for colour liking, however colour liking scores were not significantly different from the OSFR sample, but were significantly different from SC/CS ($p<0.05$). In relation to perceived hardness, OSFR samples had the highest score ($p<0.05$). Nonetheless, texture liking was the same for SC/EC and OSFR samples. Similar results were reported by (Souza et al., 2018) who found that texture liking scores were not affected by instrumental hardness of cakes. Scores obtained for SC/CS for texture liking however, were significantly lower than other samples ($p<0.05$). This may be due to other undesirable texture properties not measured in this study, such as dryness. Flavour liking scores discriminated between samples ($p<0.05$) with SC/EC having the highest score, followed by OSFR and SC/CS. Perceived butter flavour also discriminated between samples ($p<0.05$) with SC/EC having the highest score for this attribute, followed by OSFR and SC/CS ($p<0.05$). The low scores that were obtained for SC/CS for butter flavour may be due to this flavour being masked by emulsifiers, (mono- and di-glycerides of fatty acids) preservatives (potassium sorbate) and other flavourings that were present in this sample. Perceived butter flavour intensity therefore, correlated with flavour liking and similar results have previously been reported by Laguna et al., (2014).

The SC/CS had the highest score for sweet taste, ($p<0.05$) which was expected as this sample contained the highest amount of total sugars (32.4%). Actual sugar content is strongly related to perceived sugar content and sweetness intensity (Drewnowski & Schwartz, 1990). This sample, however, had the lowest score for OA ($p<0.05$). This could be due to the higher sweetness scores that were obtained for this sample. Previous research has shown that preference scores rise and then decrease with increasing levels of sucrose (Drewnowski and Greenwood, 1983). Lower OA scores obtained for this sample may also be due to lower butter flavour scores, discussed previously. OA did not discriminate between SC/EC and OSFR samples.

Table 7.2

Consumer sensory scores for Sponge cake

Sensory attributes	Products		
	SC/EC	OSFR	SC/CS
Hedonics			
Aroma liking	7.2 ± 1.42^{a}	7.0 ± 1.33^{a}	5.3 ± 1.90^{b}
Appearance liking	7.4 ± 1.09^{a}	6.8 ± 1.18^{b}	6.5 ± 1.96^{b}
Colour liking	7.6 ± 1.06^{a}	7.1 ± 1.13^{a}	6.0 ± 1.12^{b}
Texture liking	7.2 ± 1.19^{a}	6.9 ± 1.10^{a}	6.7 ± 1.08^{b}
Flavour liking	7.5 ± 1.16^{a}	6.8 ± 1.12^{b}	6.6 ± 1.75^{c}
OA	7.5 ± 1.20^{a}	7.1 ± 1.07^{a}	5.3 ± 1.04^{b}
Intensity			
Sweet taste	6.1 ± 1.30^{a}	5.6 ± 1.12^{a}	6.7 ± 1.43^{b}
Butter flavour	6.4 ± 1.40^{a}	5.5 ± 1.09^{b}	3.9 ± 1.69^{c}
Hard texture	3.7 ± 1.80^{a}	5.0 ± 1.33^{b}	3.9 ± 1.60^{c}

SC; Sponge cake, EC; Experimental control, OSFR; Optimised sucrose & fat reduced sampleand CS; Commercial standard.
^{abcde} mean values (± standard deviation) in the same row bearing different superscripts are significantly different, ($p<0.05$).

7.3.2 Relationship between sensory variables and Sponge cake treatments

The relationship between sensory variables (Y) and Sponge cake products (X) is visually represented by a PLSR plot in Fig 7.1. Most of the variation is shown in factor 1, where 50% of the data in X explains 60% of the data in Y. It is evident from the plot that SC/EC, which is positioned in the outer circle of the upper right quadrant of the plot was the product most associated with appearance, flavour, colour & texture liking and OA. Perceived butter flavour and flavour liking were highly correlated as seen by their close proximity to one another in the plot. Perceived butter flavour was therefore a driving factor for flavour liking and acceptability of samples and was most associated with SC/EC. The OSFR sample, situated in the outer circle of the lower right quadrant was also positively associated with all liking properties, in particular aroma liking and consequently OA. OSFR sample was the most associated with texture hardness, however, as mentioned texture hardness did not negatively affect liking or OA of samples. The SC/CS sample which is positioned in the outer circle of up the upper left quadrant, was very anti-correlated with perceived butter flavour, all liking properties and consequently OA. This sample was the sample most associated with perceived sweetness, which is situated in the inner circle of the upper left quadrant. 'Sweet taste' was anti-correlated with liking properties and OA which were situated on the right-hand side of the plot.

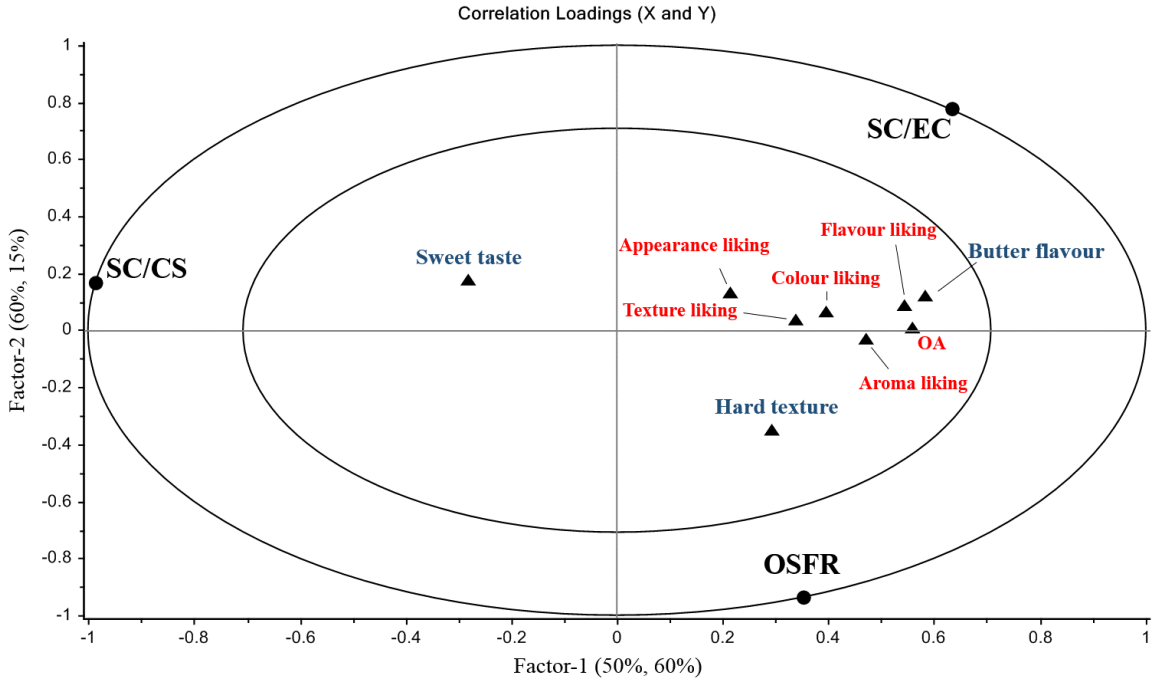


Fig 7.1 Partial least squares regression (PLSR) plot for the relationship between Sponge cake products (●) and sensory variables (▲). Hedonic sensory terms (▬) and intensity sensory terms (▬).

7.3.3 Consumer sensory scores for Chocolate brownie products

Scores obtained from sensory analysis on Chocolate brownies are displayed in Table 7.3. Similar to results obtained for Sponge cake, CB/EC and OSFR samples had the highest scores for appearance and colour liking ($p<0.05$). Similarly again, OSFR had the highest score for texture hardness ($p<0.05$) but this was not found to negatively affect texture liking. CB/CS samples had the lowest scores for texture liking ($p<0.05$). As previously mentioned, when discussing Sponge cake, this may be due to other undesirable textural properties being observed for this sample, such as texture dryness. Similar to findings for Sponge cake, perceived butter flavour discriminated significantly between all samples ($p<0.05$) with CB/EC obtaining the highest score, followed by OSFR and CB/CS. As mentioned previously, this may be due to butter flavour being masked in commercial samples containing flavourings and preservatives. CB/EC and CB/CS samples had the highest scores for sweetness ($p<0.05$). Similar to findings for Sponge cake, flavour liking and OA scores obtained for CB/CS were significantly lower than other samples ($p<0.05$). Hence, OSFR was preferred to the commercial standard and samples were similar to CB/EC in relation to OA.

Table 7.3

Sensory scores for Chocolate brownies

Sensory attributes	Products		
	CB/EC	OSFR	CB/CS
Hedonics			
Aroma liking	6.9 ± 1.61[a]	7.0 ± 0.92[a]	7.0 ± 1.31[a]
Appearance liking	7.0 ± 1.47[a]	7.1 ± 1.09[a]	5.9 ± 1.20[b]
Colour liking	7.1 ± 1.40[a]	7.1 ± 1.16[a]	6.5 ± 1.88[b]
Texture liking	7.2 ± 1.50[a]	6.9 ± 1.30[a]	6.1 ± 1.93[b]
Flavour liking	7.1 ± 1.66[a]	7.0 ± 1.09[a]	5.8 ± 1.20[b]
OA	7.2 ± 1.57[a]	7.1 ± 1.15[a]	5.8 ± 1.84[b]
Intensity			
Sweet taste	6.7 ± 1.40[a]	5.5 ± 1.55[b]	6.8 ± 1.80[a]
Butter flavour	6.3 ± 1.67[a]	5.6 ± 1.01[b]	4.8 ± 1.09[c]
Hard texture	3.7 ± 1.86[a]	5.4 ± 1.67[b]	3.6 ± 1.61[a]
Chocolate flavour	6.6 ± 1.63[a]	6.2 ± 1.30[a]	6.1 ± 1.07[a]

CB; Chocolate brownies, EC; Experimental control, OSFR; Optimised sucrose & fat reducedsample and CS; Commercial standard.

[abcde] mean values (± standard deviation) in the same row bearing different superscripts are significantly different, $P < 0.05$.

7.3.4 Relationship between sensory variables and Chocolate brownie treatments

The relationship between sensory variables (Y) and Chocolate brownie products (X) is visually represented by a PLSR plot in Fig 7.2. The following sensory terms were omitted from PLSR analysis as they did not significantly discriminate between samples; aroma liking and chocolate flavour. Most of the variation is shown is shown in factor-1, where 40% of the data in X explains 70% of the Y data. OSFR is positioned in the outer circle of the upper right quadrant and similar to results obtained for Sponge cake it was the sample most associated with percieved hardness. Regardless of this, OSFR was positively associated with liking properties and OA. CB/EC made a significant contribution to Factor-2 and is positioned in the outer circle of the bottom right quadrant. Similar to findings for Sponge cake, CB/EC was most associated with perceived butter flavour. Butter flavour was situated in very close proximity with texture & flavour liking and all other liking properties and OA. Similar to results obtained for Sponge cake, perceived butter flavour was a driving factor for overall acceptability of Chocolate brownie samples. The commercial standard was anti-correlated with liking properties, butter flavour and perceived hardness and was the sample most associated with sweet taste.

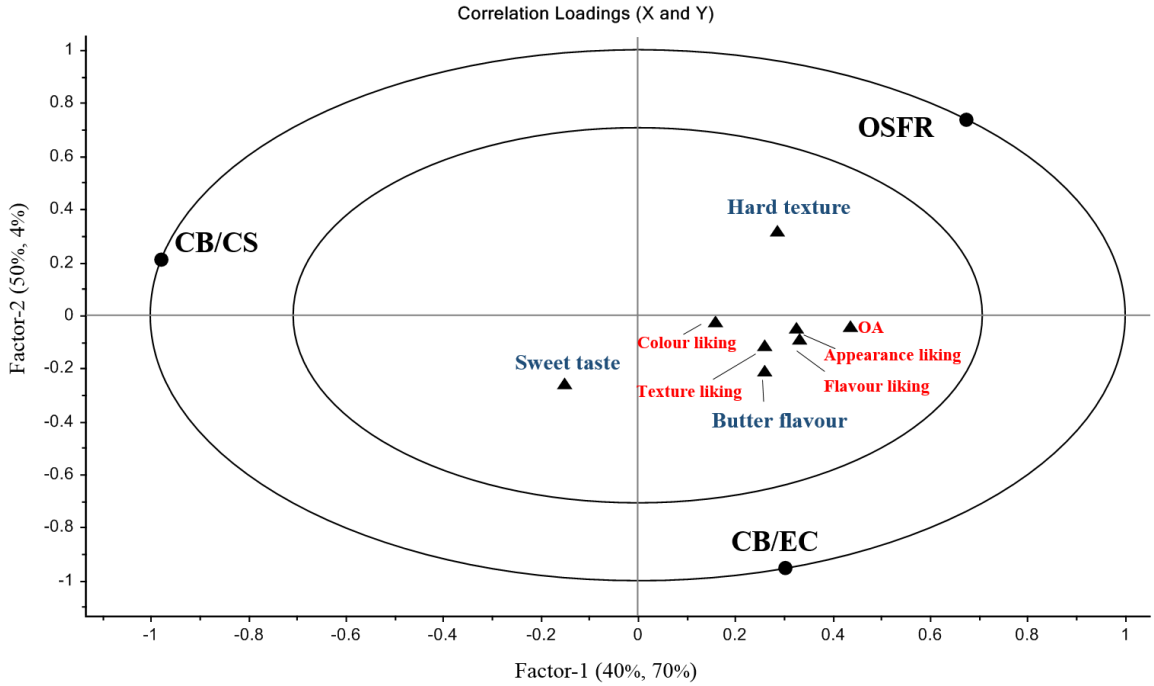


Fig 7.2 Partial least squares regression (PLSR) plot for the relationship between Chocolate brownie products (●) and sensory variables (▲). Hedonic sensory terms (▬) and intensity sensory terms (▬).

7.3.5 Microbial growth

7.3.5.1 Sponge cake

No differences were observed between SC/EC and OSFR in relation to YM growth over the storage of 4 days. At day ~1 the YM count was < 5cfu/g for both samples and remained constant at <10cfu/g for 3 days. At day ~ 4 the YM count for SC/EC and OSFR samples was 252cfu/g and 273cfu/g respectively. No visible mould growth was detected on either sample during this storage time.

Total plate counts (TPC) of SC/EC and OSFR samples over the storage of 4 days are displayed in Fig 7.3. TPC of SC/EC and OSFR samples on day ~ 1 of storage was <10cfu/g. On day ~ 2 the TPC of samples had increased to 1.4 x 10^4cfu/g and 1.8 x 10^5cfu/g for SC/EC and OSFR respectively. On day ~ 4 the TPC of samples had further increased to 1.0 x 10^6cfu/g and 1.4 x 10^6cfu/g. According to guidelines published by the FSAI, (2019) the TPC for bakery products is "satisfactory" below 10^4cfu/g, "border line" between 10^4–10^6cfu/g, and unsatisfactory at >10^6cfu/g. Therefore, SC/EC and OSFR samples were "border line" within two days of storage. Based on the graph it is evident that the OSFR and SC/EC samples became "unsatisfactory" on day 3 and day 4 of storage, respectively. OSFR samples have a slightly shorter storage stability of about one day.

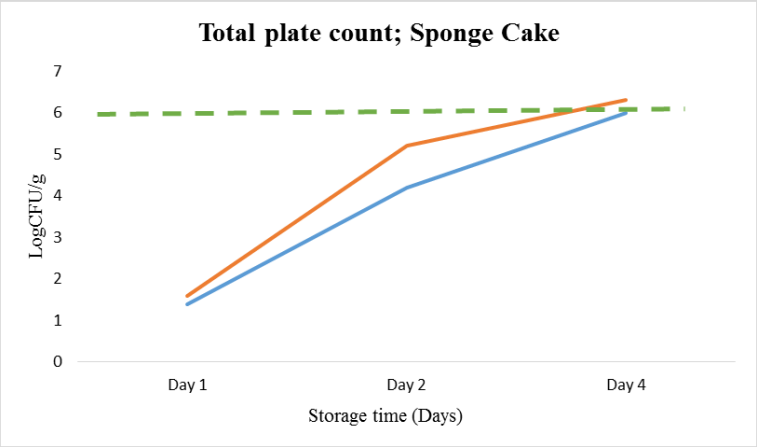


Fig 7.3 Total plate count (TPC) during ambient storage of the optimised reduced sugar and fat Sponge cake sample (—) and Experimental control (—)

7.3.5.2 Chocolate brownies

Similar to findings for Sponge cake, no differences were observed between CB/EC and OSFR in relation to YM growth over the storage of 5 days. YM count for both samples remained constant at <10cfu/g for 4 days. On day ~ 5 the YM count for CB/EC and OSFR was 190cfu/g and 212cfu/g respectively. No visible mould growth was detected on either sample during this storage time.

The TPC of both samples over storage of 5 days is displayed in Fig 7.4. On day ~ 5, the TPC of CB/EC and OSFR was 1.2 x 10^6 and 1.4^6 x 10^6 respectively. Both samples were therefore "unsatisfactory" at day ~ 5. Based on the graph it is evident that the OSFR and SC/EC samples became "unsatisfactory" on day 4 and day 5 of storage, respectively. OSFR samples have a slightly shorter storage stability of about one day.

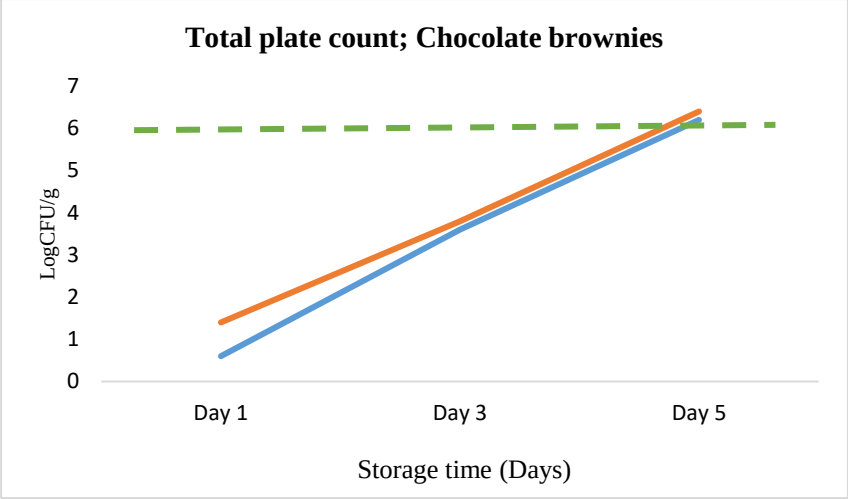


Fig 7.4. Total plate count (TPC) during ambient storage of the optimised reduced sugar and fat Chocolate brownie sample (–) and Experimental control (–).

7.3.6 Physicochemical changes during storage

7.3.6.1 Sponge cake

Physicochemical changes of Sponge cakes during storage are shown in Table 7.4. An increase ($p<0.05$) in hardness (N) was observed after ~ 4 days of storage for SC/EC, whereas an increase ($p<0.05$) in firmness was observed after only ~ 2 days of storage for OSFR. An increase in firmness during storage was expected and similar results were reported by Janjarasskul et al., (2016) in Sponge cake samples. SC/EC and OSFR were not different from each other in relation to firmness (N) on day ~ 1 of storage. However, differences were observed between samples on day ~ 2, with OSFR obtaining a higher ($p<0.05$) value for firmness. On day ~ 4 of storage, however, no differences were observed between samples. Starch retrogradation, which is the recrystallization of starch contributes to an increase in the firmness of bakery products over time (Seyhun et al., 2005). OSFR had a higher content of starch, as a result of the substitution of sugar and fat for inulin and pureed butter beans respectively. This may have contributed to the more rapid increase in hardness observed for this sample during storage.

OSFR had a higher moisture content ($p<0.05$) than SC/EC at each time point during storage. A higher moisture content was expected in OSFR samples because of the substitution of butter (15.1% moisture) for butter beans (75% moisture). Similar results have been reported in fat reduction studies using carbohydrate-based fat replacers (Confortiv et al., 1997). Moisture content of SC/EC decreased ($p<0.05$) after 2 days of storage, however on day 4 of storage the moisture content of the SC/EC sample had increased ($p<0.05$). Moisture content also decreased ($p<0.05$) after 2 days of storage for OSFR, but remained constant after 4 days of storage. It was expected that moisture content of the crumb for both samples would decrease over time as a result of moisture migration and that this process would affect firmness and contribute to staling. As the

moisture content for the crumb of both samples did decrease ($p<0.05$) after 2 days of storage, the process of moisture migration may have partially contributed to the increase in firmness and staling. This process however, was not fully responsible for an increase in firmness or staling as moisture content remained constant and increased for the SC/EC and OSFR samples respectively on the last day of storage. In a study conducted by Janjarasskul et al., (2016) no change in moisture content was observed in sponge cake samples over a storage period of 40 days.

Water activity of OSFR was higher ($p<0.05$) than SC/EC at each time point during storage. A higher water activity was expected for OSFR samples as sucrose binds water (Nip, 2007). A greater water activity observed for OSFR may also be due to an increase in moisture attributed to the substitution of butter (15.1%) for pureed butter beans (75%). The water activity of SC/EC remained constant at each time point and was between 0.82 and 0.84. The water activity of OSFR also remained constant between 0.89 and 0.91 throughout storage. Similar results were reported by (Janjarasskul et al., 2016). OSFR samples were more susceptible to microbial spoilage as a result of a higher water activity, hence these samples had a shorter shelf life. The higher water activity of these samples also meant there was more water available for starch molecules to bind water, recrystallize and contribute to sample firmness.

Table 7.4

Physicochemical changes of Sponge cake during storage

	Firmness (N)			Moisture (%)			Water activity A_w		
	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4
SC/EC	7.8 ± 0.80^{ax}	7.7 ± 0.68^{ax}	9.3 ± 0.55^{bx}	16.6 ± 0.44^{ax}	14.7 ± 0.52^{bx}	19.5 ± 0.33^{cx}	0.84 ± 0.29^{ax}	0.83 ± 0.51^{ax}	0.82 ± 0.25^{ax}
OSFR	7.6 ± 0.65^{ax}	8.7 ± 0.55^{by}	9.6 ± 0.56^{cx}	27.0 ± 0.61^{ay}	24.2 ± 0.62^{by}	24.5 ± 0.47^{by}	0.89 ± 0.33^{ay}	0.89 ± 0.52^{ay}	0.91 ± 0.36^{ay}

^{abc} mean values in the same row for each parameter (Firmness (N), Moisture (%) & water activity) bearing different superscripts are significantly different, $P < 0.05$.
^{xyz} mean values in the same column bearing different superscripts are significantly different, $p < 0.05$.

7.3.6.2 Chocolate brownies

Physicochemical changes of Chocolate brownies during storage are presented in Table 7.5. Similar to findings for Sponge cake, firmness (N) of CB/EC increased ($p<0.05$) after 5 days, whereas an increase in firmness ($p<0.05$) was observed after only 3 days of storage for OSFR. As previously mentioned, OSFR had a higher starch content, which may have contributed to a more rapid increase in hardness over time as a result of starch recrystallization.

Similar to findings for Sponge cake, the moisture content of OSFR was higher ($p<0.05$) than CB/EC at each time point. Moisture content (%) decreased ($p<0.05$) in CB/EC after ~ 3 days but increased on day ~ 5 of storage ($p<0.05$). For OSFR, moisture content increased ($p<0.05$) on day ~3 but decreased ($p<0.05$) on day ~ 5. No substantial evidence supporting moisture migration was observed, hence moisture migration may have only contributed slightly to an increase in firmness and staling of these samples. The increase in hardness over time was likely due to starch retrogradation.

Similar to findings for Sponge cake, the water activity of OSFR was higher ($p<0.05$) than CB/EC at each time point. The water activity of SC/EC (0.76-0.78) and OSFR (0.86-0.87) remained constant during storage. OSFR was more susceptible to microbial spoilage as a result of a higher water activity, hence this sample had a shorter microbial shelf life. Higher water activity also meant there was more water available in OSFR samples for starch molecules to bind water, recrystallise and contribute to firmness.

Table 7.5

Physicochemical changes of Chocolate brownies during storage

	Firmness (N)			Moisture (%)			Water activity A_w		
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5
CB/EC	31.6 ± 0.54^{ax}	31.5 ± 0.52^{ax}	35.5 ± 0.60^{bx}	13.6 ± 0.42^{ax}	11.8 ± 0.33^{bx}	17.5 ± 0.63^{cx}	0.78 ± 0.45^{ax}	0.77 ± 0.33^{ax}	0.76 ± 0.19^{ax}
OSFR	24.5 ± 0.36^{ay}	34.4 ± 0.54^{by}	38.9 ± 0.55^{cy}	21.9 ± 0.36^{ay}	24.2 ± 0.51^{by}	20.1 ± 0.54^{ay}	0.87 ± 0.21^{ay}	0.86 ± 0.42^{ay}	0.86 ± 0.44^{ay}

^{abc} mean values in the same row for each parameter (Firmness (N), Moisture (%) & water activity)bearing different superscripts are significantly different, $P < 0.05$.
^{xyz} mean values in the same column between treatments bearing different superscripts are significantly different, $p < 0.05$.

7.4 Conclusions

Results obtained for OSFR samples in relation to firmness over time may suggest a more rapid staling process for these samples. This is likely as a result of an initial high content of starch combined with a high water activity and moisture content. Perhaps the addition of hydrocolloids which have demonstrated excellent water retaining capabilities could improve the texture of OSFR samples and help to offset the staling process. Combined with the use of natural preservatives such as salt, hydrocolloids could be used to lower water activity and also improve microbial stability of optimised products. OSFR samples were preferred to commercially-available equivalent products available in the Irish market. Further experimentation to improve certain quality aspects of products such as texture and microbial stability of samples are necessary. Very positively, OSFR samples contained enough fibre to be considered a 'source of fibre'. Overconsumption of these new developed products would be less likely due to increased satiety as a result of increased fibre content, which would contribute to a better caloric balance.

Chapter 8.

General discussion, General conclusions and Future work

8.1 General Discussion

In the initial part of this study (Chapter 1), a review of the literature relating to sugar and fat reduction strategies in bakery products was carried out. The use of natural replacers, with particular attention to inulin and steviol glycosides as sugar replacers, and legumes as fat replacers was investigated. From extensive review of the literature, no work appeared to have been carried out on the simultaneous replacement of sugar and fat in bakery products using these natural replacers. Furthermore, few studies measured the impact of sugar and fat replacement on both the descriptive profile and sensory acceptance of these products. Thus, a thorough review of the scientific literature highlighted the need to measure the impact of a simultaneous replacement of sugar and fat in bakery products, using natural replacers, in order to determine the levels of fat and sugar needed for sensory acceptance.

A potential new approach for sugar reduction in bakery products, which involved the optimisation of the physical from of sugar was also highlighted. Optimising the physical form of salt (reducing salt particle size) has been used as an approach to reduce salt in crisps. From extensive review of the literature, no work had been carried out on the use of this strategy for sugar reduction in bakery products. Furthermore, no work had been carried out to measure the impact of sugar particle size on the descriptive profile and sensory properties of these products. Thus, this review highlighted the need for a novel approach to reduce sugar in bakery products by optimising its physical form.

The important factors which should be considered before formulating sugar and fat reduction strategies were also highlighted in this review, with particular attention to consumer attitudes towards the use of sugar replacers. Results from several surveys have highlighted a feeling of caution towards the use of artificial sweeteners and also a trend towards the consumption of clean-label products. From extensive review of the

literature, we found that no data had been collected on the attitudes, knowledge and consumption behaviour of Irish consumers on the intake of sugar and associated replacers. Thus, this was the main objective of the first experimental study carried out as part of this work.

In the initial survey (Chapter 2), consumers were asked to complete two questionnaires which were split into the following three sections; demographic questions, knowledge and belief-based questions and consumption-based questions. Following statistical analysis, it was determined that the majority of consumers (57%) were 'wary' of sugar replacers ($p<0.05$). The type of replacer, however, was important, with consumers being much more likely to purchase food and drinks containing natural sugar replacers than food and drinks containing artificial sugar replacers ($p<0.05$). Furthermore, the inclination to purchase foods and drinks containing artificial sugar replacers decreased with advancing age and education ($p<0.05$). Consumer attitudes towards artificial sugar replacers, justified the need for a clean label approach when formulating sugar reduction strategies. Furthermore, participants were much more likely to consume sugar rich foods on a daily basis (54.3%) then they were to consume sugar rich beverages (4.2%), which provided further basis for sugar reduction strategies in food products.

Following this survey and owing to the consumer demand for clean label food products, a new strategy of sugar reduction in bakery products was proposed in Chapter 3, by manipulating brown sugar particle size. After grinding and sieving commercial brown sugar (200-5181µm), five sugar treatments were identified for chocolate brownie formulation. Sensory analysis tests showed that samples containing the smallest brown sugar particle size fraction (459-972µm) were perceived as sweeter than any other samples ($p<0.05$). Based on these findings, sugar particle size reduction would permit sugar reduction as sweetness perception was increased in samples prepared with smaller

sugar particles. Furthermore, samples containing the smallest sugar fraction were perceived as significantly more moist ($p<0.05$) and soft ($p<0.05$) than samples prepared with any other sugar size fraction. Instrumental results for texture and moisture correlated with sensory results. As fat the perception of fat in foods is strongly related to texture and mouthfeel, this finding on sugar particle size, formed the basis of the work carried out in Chapter 4, where a simple and novel method was developed for fat reduction in chocolate brownies, by reducing sugar particle size. Compared to other sugar size fractions, the smallest sugar fraction (459-972µm) permitted a higher level of fat replacement (75%) using PBLB, in chocolate brownies without negatively affecting overall acceptability. As all sensory attributes (butter flavour, sweetness, and perceived moisture) were highly correlated with one another, it was difficult to determine exactly how smaller sugar particles permitted this level of fat replacement compared to the UC and L sugar fractions. As the utilisation of this sugar fraction had been shown to increase the perception of moisture in chocolate brownies in Chapter 3, perhaps this attribute was maintained enough in samples containing 75% FR to maintain the perception of other sensory attributes such as butter flavour and sweetness, associated with liking and OA. Thus, small sugar particles may create the illusion of a higher fat content by increasing the perceived moisture of reduced fat samples. Furthermore, utilisation of this fraction improved OA ($p<0.05$) of Chocolate brownies at a level of 25% fat replacement. Sucrose replacement (0-75%) using inulin and rebaudioside A, in PBLB brownies, did not negatively affect perceived sweetness of samples. Colour and texture properties of samples were negatively affected by a 50% sugar replacement which negatively affected hedonic properties ($p<0.05$) and OA ($p<0.05$). Hardness increased ($p<0.05$) as sugar replacement increased and the colour of samples became lighter ($p<0.05$) with increasing levels of sugar replacement. Results

indicate that inulin and rebaudioside A replacing sucrose is feasible at 25% in fat-replaced (75%) brownies prepared with small sugar particles, using PBLB. This equated to a 62.4% reduction in fat and a 15% reduction in total sugars. The substitution of fat for PBLB (75%) increased the dietary fibre ($p<0.05$) content of samples. Dietary fibre content increased ($p<0.05$) with each level of sucrose substitution for inulin and rebaudioside A. Dietary fibre was high enough in optimised samples (3g/100g) to permit the claim 'source of fibre'.

In Chapter 5, the impact of sugar replacement using inulin and rebaudioside on the sensory properties of Sponge cake was assessed. The level of sugar needed to maintain sensory acceptability was determined and following this elucidation, fat was replaced in inulin and rebaudioside A Sponge cake samples using PBRB. A 25% sugar replacement with inulin and rebaudioside A did not negatively affect liking properties or OA of samples. A 50% sugar replacement, however negatively ($p<0.05$) affected texture liking and OA. Similar findings on the impact of sucrose replacement on the texture properties of baked goods, using these replacers were reported in Chapter 4. Texture, colour and appearance liking did not discriminate between inulin and rebaudioside A (30%) Sponge cake samples containing increasing levels of fat replacement (0-75%) using PBRB. These fat replacers were successful in maintaining important sensory properties of Sponge cake, (butter flavour, sweetness, perceived moisture) up to a level of 50% fat replacement. Thus, liking and overall acceptability of samples did not discriminate between samples containing up to 50% fat replacement. A 75% fat replacement however negatively ($p<0.05$) affected important sensory properties and consequently; flavour, aroma liking and OA. Hardness (N) did not discriminate between inulin and rebaudioside A (30%) Sponge cakes containing increasing levels of fat replacement (0-75%) using PBRB. As fat replacement

increased, sample lightness increased ($p<0.05$), as determined by instrumental colour analysis. Results indicated that PBRB replacing fat is feasible at 50% in sucrose-replaced (30%) Sponge cakes using inulin and rebaudioside A. This equated to a simultaneous 42% reduction in fat and a 28% reduction in total sugars. Optimised samples contained more ($p<0.05$) dietary fibre.

In Chapter 6 of this thesis, using commercial white sugar, the impact of sugar particle size on the sensory and physicochemical properties of Shortbread biscuits was assessed. Sensory analysis tests showed that samples containing the coarse sugar fraction (228-377µm) were perceived as sweeter ($p<0.05$) than samples containing the fine or commercial sugar fractions in all samples containing 18% sucrose. Trends showed that samples containing this sugar size fraction were also perceived as the sweetest samples out of all the samples containing 9% sucrose. Sweetness intensity scores correlated directly with flavour liking scores which promotes the use of this sugar fraction (228-377µm) in the formulation of low-sugar Shortbread biscuits. Utilisation of a finer sugar fraction (124-179µm) increased biscuit spread and samples containing this sugar size had the widest diameter ($p<0.05$), which may be due to a faster rate of dissolution for smaller sugar particles. Biscuits containing the finest sugar fraction had a more pronounced toasted flavour ($p<0.05$) and darker surface colour ($p<0.05$). Findings from Chapters 3 and 6 suggest that there is an optimum sugar particle size for both white and brown sugar which contributes to an increased perception of sweet taste and that this may vary for different baked products such as biscuits and brownies.

Finally, methodologies reported in Chapters 3, 4 and 5 led to the successful development of optimised sugar and fat-reduced cake products. Consumer acceptability of these products (Chocolate brownies & Sponge cake) was determined and compared to experimental controls and products commercially available in the Irish market in

Chapter 7. Using physicochemical and microbiological analysis, the shelf-life of these products was determined. Optimised sugar and fat-reduced (OSFR) samples were preferred to commercially available equivalents present in the Irish market. Commercially available standards were perceived as sweeter ($p<0.05$) than other samples. Sweetness was anti-correlated with liking properties and OA, which suggests that commercially available standards of Sponge cake and Chocolate brownies are too sweet. Although OSFR samples were perceived as harder ($p<0.05$) than other samples, this did not affect overall acceptability, which did not discriminate between OSFR samples and experimental control samples. A rapid increase in sample firmness over time was observed for the OSFR samples. The rapid staling of these samples was likely as a result of an initial high content of starch combined with a higher water activity and higher moisture content. The microbiological numbers for OSFR samples and experimental control samples were similar, with OSFR samples having a slightly shorter storage stability of about one day. Further experiments to improve the texture and quality of OSFR products for acceptable packaged retail storage and display are necessary.

8.2 General Conclusions

- Irish consumers are much more likely to purchase foods and drinks containing natural sugar replacers rather than foods and drinks containing artificial replacers, which justifies the need for a clean-label approach when formulating sugar reduction strategies (Chapter 2).
- The difference between respondent's likelihood to consume sugar rich foods (54.3%) and sugar rich beverages (4.2%) on a daily basis suggests a growing awareness of the excess amount of free sugar in sugar sweetened beverages. These findings stress that more awareness needs to be highlighted of the excess amount of free-sugar also present in food products, perhaps through media/health promotion outlets or taxation (Chapter 2).
- Utilisation of small brown sugar particles (459-972µm) increases ($p<0.05$) the perception of sweetness in Chocolate brownies (Chapter 3).
- Application of small sugar particles (459-972µm) increases the perceived moisture ($p<0.05$) and soft texture ($p<0.05$) of Chocolate brownies (Chapter 3).
- Application of small sugar particles (459-972µm) permits a higher ($p<0.05$) level of fat replacement in Chocolate brownies (75%) than any other sugar size fractions using PBLB. Furthermore, samples containing small sugar particles with 25% FR showed the highest acceptances ($p<0.05$) (Chapter 4).
- Utilisation of inulin and rebaudioside A allowed for the replacement of sucrose by up to 25% in fat-replaced brownies. At 50% sugar replacement, texture properties and consequently texture liking are affected ($p<0.05$) (Chapter 4)
- Application of inulin and rebaudioside A for replacement of sucrose in fat-replaced Chocolate brownies was successful from a flavour perspective, as perceived sweetness and butter flavour did not significantly discriminate

between samples containing increasing levels of sucrose replacement (0-75%) (Chapter 4).

- Inulin and rebaudioside A replacing sucrose is feasible at 25% in fat-replaced (75%) brownies prepared with small sugar particles, using PBLB (Chapter 4).
- Utilisation of inulin and rebaudioside A for replacement of sucrose in Sponge cake negatively affects texture liking ($p<0.05$) at a level of 50% replacement. However, flavour liking was only negatively affected ($p<0.05$) at a 75% replacement level. This again, demonstrates the value of these sugar replacers in relation to maintaining flavour properties associated with liking (Chapter 5).
- Application of pureed butter beans for replacement of fat in sucrose-replaced Sponge cake did not affect important sensory properties such as butter flavour, sweetness, moist texture or OA, up to a level of 50% replacement. However flavour and aroma sensory properties were affected ($p<0.05$) at a level of 75% replacement (Chapter 5).
- Results indicate that PBRB replacing fat is feasible at 50% in sucrose-replaced (30%) Sponge cakes using inulin and rebaudioside A (Chapter 5)
- White sugar particles in the range of (228-377μm) increases the perceived sweetness ($p<0.05$), flavour liking ($p<0.05$) and OA ($p<0.05$) of shortbread biscuits (Chapter 6).
- Utilisation of a finer sugar fraction (124-179μm) in the preparation of Shortbread biscuits increases biscuits spread ($p<0.05$) and contributes to a darker surface colour ($p<0.05$) and more pronounced toasted flavour ($p<0.05$) (Chapter 6).

- Optimised sugar and fat-reduced (OSFR) cake products (Chocolate brownies and Sponge cake) were preferred to commercially available standards present in the Irish market (Chapter 7).
- OSFR samples have a slightly shorter storage stability of about one day (Chapter 7).

8.3 Future work

This thesis provides a background for future studies on sugar and fat reduction in bakery products. Some studies that could be carried out in future are as follows;

- Investigating the impact of sugar particle size on the sensory and physicochemical properties of other bakery products, using narrower, broader and various sugar particle size ranges.
- Utilisation of finer sugar particles in the formulation of low-sugar biscuits, to improve colour.
- Application of emulsifiers (polysorbates) or the addition of hydrocolloids (xanthan gum) (which have demonstrated excellent water retaining capabilities) to improve texture of sucrose-replaced cakes and thus, permit higher levels of sugar replacement using inulin and rebaudioside A.
- Addition of enhancers to improve flavour of fat-replaced cakes and thus, permit higher levels of fat replacement using PBRB/PBLB in Sponge cakes and Chocolate brownies respectively.
- Application of hydrocolloids to improve texture and delay staling of OSFR samples, in order to prolong the storage stability of these samples.

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