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DEPARTMENT OF FOOD AND NUTRITIONAL SCIENCES



Novel, clean label sweetening ingredients in reduced sucrose cake and biscuits

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Abstract

High levels of sucrose in food has been linked with an increase risk of developing obesity and type 2 diabetes. Therefore a reduced sugar intake is strongly recommended by health professionals. Sweet baked products contain high levels of sucrose. Sucrose was reduced by 30% in cake and biscuit formulations and a variety of potential sweetening alternatives were studied. The fundamental rheology of the control and reformulated raw cake batters and biscuit doughs were recorded. The microstructure of the batters and doughs were captured using light microscopy (LM), confocal laser scanning microscopy (CLSM) and cryo-scanning electron microscopy (cryo-SEM). At high frequencies (10Hz), batter containing polydextrose and apple pomace had a significantly greater complex modulus (G*), indicating a firmer batter. There was no significant change to the rheological properties of the biscuit doughs. LM revealed a reduction in sucrose yielded a batter with increased bubble size. Reformulated biscuit doughs remained similar to the control dough as seen by the LM. CLSM and cryo-SEM showed the reformulated batters and doughs were similar to the control however there was an increase in the cellulosic material in batters and dough containing fibrous material. The batters and doughs were baked and the resulting products' properties were examined.

The physical characteristics, texture profile, staling properties and microstructure of the reformulated cakes were examined. Sensory evaluation was also conducted. Oligofructose, whey permeate and polydextrose-containing cakes had similar characteristics to the control with regard to specific volume, colour, and texture. The addition of apple pomace resulted in cake with a decreased specific volume, harder crumb and a darker crumb and crust which received the lowest scores of the reformulated cakes in many attributes as ranked by the sensory panellists. The C-Cell revealed the 2D crumb structure did not significantly change. However CLSM revealed cakes containing apple pomace had an increase in cellulosic material. The reformulated cakes had a total sugar reduction of between 21.5-27.6%.

A biscuit formulation was reduced by 30% sucrose and a combination of bulking agents (polydextrose, maltodextrin, plant fibres) with extracts (yeast, apple) and natural flavourings were investigated as sucrose replacers. When compared to the control biscuits, the addition of citrus fibres and apple extract (CFA) resulted in biscuits with an increased hardness, moisture content and water activity and a decreased lightness (L^* value) and overall acceptability (as evaluated by the sensory panellists). Overall, biscuits containing cereal fibres and a natural flavouring received high sensory scores for colour, appearance, sweetness, aftertaste and overall acceptability. The polydextrose and a natural flavouring-containing biscuits achieved high scores for flavour, sweetness, aftertaste and overall acceptability compared to the control (over 5.5 on the hedonic scale). A total sucrose reduction of 14.7-28.6% was achieved compared to the control formulation.

The volatile profile, staling and sound (acoustic) properties of reduced sucrose biscuits enriched with citrus fibre and polydextrose were evaluated. There was a significant increase in the moisture content, water activity and fracturability in the reformulated biscuits and a significant decrease in the acoustic emissions and sensory scores as evaluated by the panellists. This indicates the high moisture content, water activity and fracturability negatively influenced the acoustic properties of the reformulated biscuits. Volatile profile analysis revealed a decrease in Maillard reaction-derived compounds and an increase in lipid oxidation-derived compounds. Sensory evaluation deemed the biscuits as acceptable.

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GENERAL INTRODUCTION

General introduction

Sugar is high in calories and is found in a wide variety of foods and beverages. Considering current negative press, consumer awareness around increasing risks associated with obesity (Stanhope, 2015), the demand for reduced sugar products has increased. However, as sugar has multiple functions in foods, its reduction can result in products with poor quality, and often with a reduced shelf life. This is particularly true for baked confectionary products. Sugary products such as cakes, biscuits and muffins are consumed throughout the world, and have a variety of flavours and shapes. They are often considered a 'reward' or a 'treat' and therefore must be of high quality.

Cakes are fat-in-water emulsions: air bubbles are found in the discontinuous phase, while the continuous phase is composed flour particles which are dispersed in the egg-sugar-water-fat mixture (Kocer et al., 2005). As well as providing sweetness to cake products, sucrose also has other important functions. It acts as a bulking agent in a cake batter, and promotes fat-crystal aggregates to form. These properties enhance air entrapment and help gas bubbles to remain stable while baking, creating a fine crumb. Sucrose also functions by increasing the denaturation temperature of egg albumen leading to a heat induced gel (Christ et al., 2005). The gelatinization temperature of the starch and the degree of gelatinization are increased by the addition of sucrose (Psimouli and Oreopoulou, 2012). The increase in temperature allows the gas bubbles in the batter matrix to expand fully, thus creating greater volume.

Sucrose improves the browning of cake crust as a result of the Maillard reaction and caramelisation. When heated, sucrose is converted to fructose and glucose which participate in Maillard reaction. Caramelisation occurs due to the thermal decomposition of the sucrose, which also creates a nutty flavour. Sucrose also helps form a crust, by crystallising on the surface of the cake thus improving moisture retention, increasing the shelf life the product.

Biscuits are characterised by a high fat/sucrose to flour ratio. Similar to cakes, sucrose improves the colour and sweetness of the biscuit formulation. Sucrose inhibits gluten development during dough mixing by competing with the flour for the recipe water. It

acts as a hardening agent by crystallising as the biscuit cools, creating the crispy product (Zhou and Hui, 2014).

Finding other ingredients that can mimic the functionality of sucrose and replace sucrose in reduced sucrose cake and biscuit formulations has been a focus of research in recent years. Many approaches examine the use of bulk sweeteners (polyols) and high intensity sweeteners. However recent consumer trends indicate a movement towards a 'clean label'. Although this term is not legally defined, it is generally accepted that the term indicates a product which **is** perceived as natural by the consumer. Clean label implies a food with a low number of ingredients, and ingredients with names that the consumer can understand (Skelke, 2018). Due to the increase in negative media coverage which surrounds polyols and high intensity sweeteners, they are not considered to be clean label, and therefore an alternative must be found.

A potential sucrose replacer must provide the same or similar properties as sucrose, while maintaining a clean label. This thesis explores, in detail, a variety of possible sucrose replacing ingredients in cake and biscuit formulations. Reduced sucrose cake batter and biscuit dough properties and baked cake and biscuit characteristics, including physical attributes, staling and texture profile, as well as flavour and sound (acoustic) changes which occur, will be examined. Sensory analysis will be used to evaluate the quality of the baked product with regard to appearance, texture flavour, and overall acceptability.

CHAPTER 1

Literature Review

State of the art in sucrose reduction: Alternative sucrose replacers and their application in bakery products.

1. State of the art in sucrose reduction: alternative sucrose replacers and their application in bakery products.

1.1 General introduction

Sweetening ingredients have multiple functions in cakes and other bakery products. They provide sweetness and affect structure, colour, flavour and texture of both the batter and the final baked product. Sucrose is one of the most common sweetening ingredients used in the baking industry. However, due to its high caloric content and link with diseases such as obesity and diabetes (Ronda et al., 2004), demand has increased for the use of low-calorie, low-sucrose sweetener alternatives. This presents a significant challenge for the food industry to provide products with reduced sucrose content while maintaining taste, structure and appearance. Baked goods are high in carbohydrates, primarily sugar and flour, therefore changing either component inevitably results in changes in taste and structure. It is important to maintain the eating quality of the products when creating novel foods. It is also important new formulations and alternative sweeteners do not suffer negatively from heating and are able to undergo browning in a similar manner to the traditional/ control products.

1.2 Sucrose: an introduction

Sucrose is the most common household and industry sweetener. Although it has a clean sweet taste, without any unpleasant aftertaste, reducing the intake of sucrose is important to decrease the risk of diabetes, obesity and to prevent dental cavities. It is estimated that over 225,000 people in Ireland live with diabetes, and up to 10% of Ireland's health budget is currently spent on treating the condition (Nolan, 2005). This number is predicted to increase to over 279,000 in 2030 (Shaw et al., 2010).

Sucrose is a disaccharide, composed of one molecule of glucose and one molecule of fructose, joined together by a α-1,2 glycosidic bond. The glycosyl units of sucrose are joined reducing end to reducing end, meaning the aldehyde group of D-glycosyl unit and the keto group of D-fructosyl unit are bound. Therefore sucrose does not have a reducing end and is not a reducing sugar (Whistler and BeMiller, 2008). As a result, sucrose does not participate in non-enzymatic browning.

It is extracted from sugar beet and sugar cane, and is found naturally in many foods such as fruit and vegetables. It is available in many grain sizes (1500µm-10µm) and colours (white, golden brown to dark brown).

Sucrose is important for sweetness. Sweetness is perceived and subjective. The chemical and physical composition of the food matrix, as well as the temperature, pH concentration and the other ingredients all affect the perceived sweetness of the compounds. Relative sweetness of a compound is compared with sucrose on a weight basis, weight-per-volume basis or molar equivalent, of which sucrose is one.

1.3 Cake: an introduction

Cakes are characterized by a sweet taste and a short tender texture. This short tender texture is a result of the fat coating the flour particles in the cake batter, which prevents the development of a gluten network, therefore creating a 'short' texture (Bennion and Bamford, 1997). The two categories of cakes are 'foam' and 'shortened'. Foam-style cakes are created from foaming eggs to form the structure and volume of the cake. A fat-liquid emulsion forms the structure of a shortened cake, created during the processing of the batter (Zhou and Hui, 2014). High sucrose inclusions (17- 20%) are the norm for most cakes recipes (Zhou and Hui, 2014).

1.3.1 Technological functions of sucrose in cake products

When the cake batter is created, the sucrose binds with water preventing full hydration of wheat proteins. This retards the development of a gluten network, and in a high sugar mixture, there is little water available to the gluten (Perego et al., 2007). Sucrose also functions by increasing the denaturation temperature of egg albumen. This prevents the proteins forming a heat induced gel, allowing the gas bubbles in the batter matrix to expand, thus creating greater volume (Christ et al., 2005). The gelatinization temperature of the starch and the degree of gelatinization are increased by the addition of sucrose (Psimouli and Oreopoulou, 2012). This is due to the sucrose binding with water and lowering the water activity of the dough or batter.

In cakes, sucrose has a tenderizing property by facilitating the incorporation air into the batter. When the cake is baked the air bubbles expand. If the batter is has a low

viscosity (i.e. does not contain sufficient sucrose), it will fail to retain the bubbles, resulting in a cake with a decreased volume. Due to the increase in gelatinization and coagulation temperatures as a result of the sucrose, (as discussed previously) the structure of the batter has a longer time to expand, resulting in an aerated crumb, and a baked product of good volume and texture (Moreira et al., 2011).

Although sucrose itself is a non reducing sugar, when it is heated, it is converted to fructose and glucose, both of which are reducing sugars (Struck et al,2014). This allows the sugars to react with the amino acid found in the flour and eggs and the Maillard reaction takes place. Sucrose also undergoes caramelisation, a non-enzymatic browning that occurs due to the thermal decomposition of the sucrose. A nutty flavour and a brown colour are produced. This property is unique to sucrose. The moisture retention of the baked product is also improved, as the browning reactions results in a crust being formed (Struck, 2014).

Sucrose is hygroscopic and is a humectant. Therefore, water migration from crumb to crust is minimized, and crumb moistness is maintained. Sucrose reduces water activity (a_w). This prevents the growth of some microbes, which is important for products such as baked goods that are stored at room temperature (Zhou and Hui, 2014).

1.4 Biscuits: an introduction

The word ‘biscuit’ originates from the Latin word ‘twice cooked’ where the biscuit was cooked at a low temperature to harden, and then crisped at a higher temperature. The popularity of biscuits is due to a number of reasons; they are a ready-to-eat snack, they are available in many varieties and they are affordable. The term ‘biscuit’ has two meanings. In the United States, biscuits are a small round bread. In Britain and Ireland, biscuits are defined as flat sweet cakes. Biscuits are a cereal based product, enriched with fat and sugar and baked to less than 5% moisture.

Misra and Timwari (2014) stated biscuits can be classified according to their dough characteristics. Doughs are defined as a soft, hard or fermented. The difference between hard or soft dough is mainly due to mixing conditions and the concentrations of fat and sugar. Soft dough usually contains higher levels of fat and sugar than hard

doughs. Biscuits can also be classified according to taste, and described as sweet, semi-sweet or salty. Sweet biscuits are produced from soft dough and have a high level of fat. Semi-sweet biscuits are commonly made form a hard-dough with a low fat and sugar content. Salt biscuits use a fermented or hard dough with a low concentrations of fat and sugar to create the cracker of puffed biscuit. It is common practise for the biscuit to be brushed or layered with fat.

1.4.1 Technological functions of sucrose in biscuit products

The sugar present in biscuits affects sweet flavour, dimensions, colour, hardness and surface finish. As it is hydroscopic, it also maintains moisture and helps prolong shelf life of the products.

The presence of sucrose is important for the overall structure of a biscuit or cookie. In moderate amounts, sucrose can act as a softening agent in cookies, due to the ability of sucrose to retain water (Mariotti and Alamprese, 2012). The amount of sucrose which dissolves in the aqueous phase of the dough determines the machinability of the dough. When sucrose is added to a mixture, it competes with the proteins in flour to bind with water. This prevents the gluten from hydrating (Manley, 2011), resulting in dough that has an increased viscosity.

Similar to cake batter, sucrose binds with the liquid in the mixture, increasing the gelatinization temperature of starch, and decreasing the level of gelatinization (Psimouli and Oreopoulou, 2012). This is a result of sucrose reducing the water activity of the dough, and forming bridges between the starch chains. The delay in gelatinization allows more time for gas bubbles to expand, thus increasing spread ratio of the biscuits (Moreira et al., 2011). The spread ratio is calculated based on the diameter and height of the biscuit. Gas bubbles expand in the dough, resulting in a decrease in density and increase in volume and spread ratio, giving the baked biscuit a more pleasant texture. Sucrose can act as a hardening agent by crystallising as the biscuit cools, thus making the product crisp (Misra and Timwari, 2014).

Browning is the formation of colour on the exterior of a baked product. The colour of the surface is an important attribute to the consumer to judge the “doneness” of a

product (Misra and Timwari, 2014). When sucrose is heated, it is degraded to fructose and glucose, which take part in the Maillard reaction to produce browning and flavour compounds. Compounds influencing biscuit flavour may also be produced by Maillard reactions. Sucrose also browns by caramelization.

As dough is baked, moisture is lost through evaporation, resulting in a moisture content of 1– 4%. As there is a high concentration of sucrose and low water content in the baked biscuit, the sucrose can crystalize on the surface of the biscuit. Water and heat is released during crystallisation and results in a crispy surface, which may sometimes lead to cracking (Misra and Timwari, 2014). Moisture migrates from the centre to the surface of the biscuit, as the moisture present on the surface evaporates into the environment (Saleem, 2005).

Currently, there is no perfect sucrose replacer. Commonly either nutritive or non-nutritive sweeteners are used to replace sucrose in industry.

1.5 Sweetening ingredients used in baking

Originally, low calorie/sugar–free goods were developed for diabetics or others with specific medical conditions. Today, these products are commonplace. Public awareness about the health risks of diets high in sugar have increased dramatically for the last number of years. While sugar does play an important biological role, excessive sugar intake has been linked with higher risk of diabetes and obesity (Stanhope, 2016). Governmental and professional groups have recommended sucrose to be reduced in certain food products (HSE, 2016); therefore the food industry is now seeking ways to reduce the sugar by substituting with other materials. However, most people enjoy the taste and texture of high sugar foods, and may not want to give them up. As sugar is present in such significant amounts in cakes and biscuits, altering the level used will greatly affect dough consistency and final product characteristics.

Efforts have made over the last number of years to explore alternative sweetening ingredients including bulk sweeteners and high intensity sweeteners as sucrose replacers (Di Monaco et al., 2018). Bulk sweeteners, such as polyols, are used at the same concentrations as sucrose and provide bulk and sweetness, but contain less

calories (2.4kcal/ g in comparison to sucrose which contains 4kcal/ g) (Mariotti and Lucisano, 2014). They are generally sweet, but have a lower intensity than sucrose, and can have a cooling effect. Polyols have good thickening and stabilizing properties (Livesey, 2003) and help retain moisture in foods. These include sorbitol, mannitol, maltitol, isomalt, xylitol and erythritol. However, it must be noted that concentrations of polyols greater than 10% must carry a warning on the label as in high concentrations polyols can have laxative effects.

High intensity sweeteners have an intense sweet taste, and therefore can be used in lower concentrations than sucrose, while having little or no caloric value. It must be noted the use of high intensity sweeteners is limited as they provide no functional effects, such as increasing bulk or browning, and can also degraded when heated (Mariotti and Lucisano, 2014). Examples of high intensity sweeteners are aspartame, sucralose, acesulfame-K and neotame. However, there has been recent media coverage around the dangers of artificial sweeteners and, although this lacks scientific evidence, it has resulted in an increase of consumer health concerns. Therefore the search for a naturally occurring sweetener began. Stevia is a natural sweetener, derived from the *stevia rebaudiana* plant. It is low in calories and up to 300 times as sweet as sucrose (Struck et al., 2016), however at high concentrations, the use of stevia can result in a bitter aftertaste (Gerwig et al., 2016).

Recent consumer trends indicate a movement towards ‘clean label’ processing. Although this term is not legally defined, it is generally accepted the term indicates a product which is minimally processed and perceived as natural by the consumer. Clean label implies a food with a low number of ingredients, and ingredients with names that the consumer can understand (Skelke, 2018). Polyols and high intensity artificial sweeteners are not considered to be ‘clean label’ ingredients, and therefore alternatives must be reviewed.

In the following sections, clean label alternative sweetening ingredients are discussed.

1.5.1 Dietary fibre

Many fibres have bulking properties and therefore may be used in bakery products potentially (with the addition of a natural flavouring) to mimic the bulking and thickening properties of sucrose.

Dietary fibre includes any nondigestible carbohydrate with a degree of polymierization (DP) of 3 or more, usually from plant sources (European Commission 2008). All dietary fibres have the energy value of 2kcal/ g, as adopted by the EU in 2008 (European Commission 2008; European Food Safety Authority, 2010). Fibre is classified as soluble and insoluble dietary fibre (Kale et al., 2011, Rodriguez et al., 2006). The ratio of soluble and insoluble is important for both functional and dietary properties. It is thought a ratio of 1:2 soluble/ insoluble for use as a food ingredient is most suitable (Figuerola et al., 2005). Cereal fibres have a higher proportion of insoluble fibres such as cellulose and hemicellulose, whereas fibre from fruit sources contains more soluble fibre such as pectin, mucilage and gums. Dietary fibres have multiple roles in human health and food functionality. There are associations with the reduction, prevention and treatment of some diseases including atherosclerosis, diverticulosis, colonic cancer and appendicitis (Foschia et al., 2013).

The presence of fibrous ingredients also confers functional properties in food formulations. They have the ability to increase viscosity, form gels, have high water holding capacity and swelling capacity (Elleuch et al., 2011). These properties relate to the structure of the fibre, and its ability to hold water through hydrogen bonds (Elleuch et al., 2011) However, the water holding capacity and swelling capacity can be reduced by grinding the dry fibres to a fine powder (Raghavendra et al., 2006).

It is recommended by physicians and nutritionists to consume 30g of fibre per day (Scientific Advisory Committee on Nutrition, 2015) and therefore there has been increased interest in the incorporation of fibre in regularly consumed food to increase the fibre consumption (Figuerola et al., 2005). Baked good are widely consumed worldwide, and therefore can be used as an efficient carrier for fibrous ingredients.

1.5.1.1 Fruit fibres and pomaces

Commonly, cereals are used as a source of fibre in functional food formulations, however fruit fibres (such as apple, mango, lemon and grapefruit (Sudha et al., 2015,

Figuerola et al., 2005, Masoodi et al., 2002) contain higher levels of total and soluble fibre (Figuerola et al., 2005, Borderias et al., 2005). This allows the fruit fibre to have a higher water and oil holding capacity while having a lower caloric content. Fruit fibres have also been reported to confer a pleasant, sometimes fruity flavour in novel bakery formations (Rupasinghe et al., 2009, Masoodi and Chauhan, 1998).

Studies have shown how the addition of fibre in baked goods (such as cake and biscuit) resulted in an increase in the viscosity of the batter, thus positively influencing the volume and texture of the final product (Lebesi and Tzia, 2011, Martinez-Cervera et al., 2011). Although the addition of fruit fibres significantly increases the total fibre content, the moisture, fat and ash are not significantly changed. For example, in one reported study, the addition of 24% apple fibre (7.6% DW basis) resulted in a 5.8 fold increase in total dietary fibre in muffins (Rupasinghe et al., 2008). The unchanged protein and ash content was similarly reported in a study with a mango fibre enriched muffin formulation. Muffins fortified with 25% mango pomace were found to have 6.7 times more total fibre than the control formulation (Sudha et al., 2015). The soluble fibres such as pectin, can also be used in reduced sucrose formations, for example fruit jam, while at the same time increasing the fibre content (Wang et al., 2013).

During the production of fruit juices, significant amounts of by-products are generated, which can cause problems with disposal. These have proven to be excellent sources of fibre (dos Santos et al., 2015, de Toledo et al., 2017) as well as a source of pectin (Rabetafika et al., 2014, Banerjee et al., 2017, Vendruscolo et al., 2008), which could present the opportunity of introducing these pomaces as a functional, nutritive sugar replacer.

Apple pomace

Apple is considered a good source of both soluble and insoluble fibres (O'Shea et al., 2012). Apple pomace is the solid material produced from apple juice extraction; approximately 25-30% of the whole fruit becomes pomace in commercial apple processing (Vendruscolo et al., 2008, Shalini and Gupta, 2010). Apple pomace ferments quickly, and has a high chemical oxygen demand and bio-chemical oxygen demand which causes environmental pollution (Kaushal *et al.*, 2002).

Apple fibres are a source of pectin, a soluble fibre which has a hypocholestrolemic effect (Theuwissen and Mensink, 2008). Apple fibres are also sources of bioactive compounds such as polyphenols, flavonoids and carotenes (Saura-Calixto, 2011, Will et al., 2006, Carle and Schieber, 2006). They also contain less phytic acid compared to cereal fibre, which reduces the bioavailability of some minerals such as zinc. Studies have shown that apple pomace contains 11.8-83.8g carbohydrates, 2.3-65.5g fibre, 3.6-15g sugar, 2.9-16.95g pectin, 0.53-3.5g ash (per 100g of DM) (Juskiewicz et al., 2015, Rabetafika et al., 2014, Kosmala et al., 2011, Mahawar et al., 2013, Albuquerque et al., 2006, Jin et al., 2006, Joshi and Sandhu, 1996, Villas-Bôas et al., 2002).

No studies have been found where apple pomace was used as a sweetening ingredient or as a sucrose replacer. However due to its high fibre content and pleasant fruity flavour, it is of interest to investigate its use as sweetening ingredient. The following studies demonstrate its function as a flour replacer in cakes and cake-like products.

Struck et al. (2016) replaced sucrose with pea, apple and wheat fibres coupled with reaudioside A in a muffin formulation. It was found as the fibre addition increased, there was a subsequent decrease in height, diameter and volume of the muffin. The decrease in height, diameter and volume was due to the decrease in starch gelatinization temperature influencing bubble formation in the batter. There was an increase in viscosity of the batter due to the increase water holding capacity (WHC) of the fibres compared to sucrose, which could negatively affect bubble formation causing a decrease in the previously mentioned parameters. There was an increase in a_w, bake loss, density and firmness as the sucrose concentration decreased. Firmness and density are significantly interrelated. A darker colour was recorded by both the instrumental and sensory evaluations as apple fibres increased.

Sudha et al. (2007a) incorporated finely ground apple pomace into cake in a study to boost the fibre levels of the cake. The wheat flour was replaced with apple pomace at 0% (control), 10% and 20% and 30% levels. The water absorption of the batter increased by approximately 10% as the apple pomace inclusion increased from 0% to 15%. The resistance to extension value also increased as the apple pomace concentration increased, creating a more viscous batter. The volume of the cake decreased from 850 cc to 620 cc following the increasing addition of apple pomace

from 0 to 30%. The density of the crumb increased due to the water binding properties of the apple pomace. There was also an increase in hardness of the cake with the higher presence of the pomace. Sensory evaluation concluded apple pomace at the level of 30% had a negative impact on crust and crumb colour. However a pleasant, fruity flavour was detected by panellists. The cakes containing 20% apple pomace were scored as highly acceptable. Nutritionally, the cakes containing apple pomace had similar fat, protein and moisture contents, but the total fibre content was significantly enhanced. The control cakes contained 0.47% total fibre (0.16% soluble fibre), whereas the cakes enriched with apple pomace contained up to 14.2% total fibre (5.8% soluble fibre).

Masoodi et al. (2002) enriched cakes with apple pomace flour in increasing concentrations (5%, 10% and 15%) in a variety of particle sizes (sieved through 30, 50 or 60 mesh). It was found as the apple pomace content increased, so did the batter viscosity. There was also a significant increase in batter viscosity as the flour particle was decreased. This is due to the increase in water absorption, producing a more viscous batter. These factors also resulted in a significant decrease in specific volume of the batter.

Rupasinghe et al. (2008) replaced wheat flour with dried apple skin pomace (ASP) at a number of different concentrations in a control muffin formulation. It was found the highest level (32% w/w) of replacement was not acceptable when rated by a taste panel, however muffins containing lower concentrations (8%, 16% and 24% w/w) showed no significant difference in overall acceptance when compared to the control. However it was remarked by the panel that the 24% ASP was difficult to masticate and swallow. As the concentration of apple skin powder increased in the muffins, so did the perceived sweetness. The overall acceptability of the muffins at the concentrations of 8%, 16% and 24% (w/w) were not significantly different from the control.

Citrus fibres

Citrus fruits include oranges, lemons, grapefruits and mandarins. These fruits are processed mainly for juice, canned to produce marmalades or to extract essential oils. Citrus fruits are a source of dietary fibres, with a high proportion of soluble fibre.

Soluble fibres have many functional properties including increasing viscosity and forming gels. Soluble fibres, containing ß-glucan can reduce risks of colon cancer and the absorption of glucose into the blood (Bingham et al. 2003). Citrus fruits are also a source of phytochemicals such as carotenoids and flavonoids (Nassar et al., 2008). Similar to apple pomace, citrus fibre has not been utilised as a sugar replacer, however it has been utilised to enhance the fibre content of biscuits.

Orange peel and pulp have been used to replace wheat flour (w/w) and increase the fibre content in a biscuit formulation in a study conducted by Nassar et al. (2008) . The addition of pulp and peel decreased spread ratio (length / thickness of the biscuit) and thickness of the biscuits. There was no significant difference between the control formulation and the biscuits containing 5% and 10% orange peel or pulp as ranked by the sensory panellists with regards to colour, texture, flavour and overall acceptability. However at higher concentrations (25% replacement), the reformulated biscuits were less favourable than the control biscuits. Total fibre increased as the inclusion level of orange peel and pulp increased from 2.7g/100g to approx. 15g/100g at 25% inclusion.

Larrea et al. (2005) added extruded orange pulp to replace wheat flour in cookies (5, 15 and 25% w/w replacement) to increase the fibre content. Cookies containing orange pulp had a significantly higher moisture content (1.1-3.6%), due to the water binding properties of the fibres in oranges. There was also a significant increase in hardness when orange pulp was added. At concentrations of up to the inclusion of 15% there was no significant changes to the diameter of the cookie, however at 25% inclusion, there was a decrease in the diameter from 50mm (control) to 48mm (25% replacement). There was also a significant decrease in specific volume when orange pulp was included at concentrations of 15 and 25%. A sensory panel ranked biscuits containing orange pulp with significantly higher scores with regards to appearance than the control. However, the reformulated biscuits containing 25% replacement scored lower on flavour, texture and general acceptance- there were no significant differences between the control and the biscuits containing 5 and 15% orange in these attributes.

1.5.1.2 Cereal fibres

Cereal fibres can include rice, corn, oat and wheat bran, and contain high concentrations of fibre, protein and other vitamins and minerals. The inclusion of high fibre ingredients in a food can influence the physiochemical properties of the matrix as highlighted by Nagi et al. (2012). Currently, no published material was found in which cereal fibres were used as a sweetening ingredients. However these fibres have been used to enhance the fibre content in baked products.

Sudha et al. (2007c) increased the fibre content of a biscuit formulation by replacing wheat flour with a number of cereal bran. The inclusion of oat bran significantly decreased the diameter of the biscuit at low concentrations (10 and 20% replacement w/w), and significantly increase diameter at high concentrations (40%). Spread ratio decrease with 10% inclusion and increased at 30% inclusion compared to the control. The hardness of the biscuits significantly increased with the addition of oat bran. In a sensory study conducted by Sudha et al. (2007c), panellist ranked biscuits containing oat bran similar to the control in regards to colour, texture, taste and mouth feel at low concentrations (10 and 20%). It was noted by the authors, taste and mouth feel of the biscuits were affected at 30% inclusion as there was an increase in dry mouth feel.

Nagi et al. (2012) added wheat bran, oat bran, corn bran and barley bran to a biscuit formulation to replace wheat flour (w/w). All fibres increased the moisture content (2.13-2.60%) of the biscuit, with the exception of oat bran which was the same as the control (2.00%).

Corn bran contains between 75-90% fibre (AACC, 2000, Burge and Duensing, 1989, Sosulski and Wu, 1988). Corn bran replaced wheat flour in a study conducted by Singh et al. (2012) in a cake formulation between 0-30%. All batters had a greater G' (storage modulus) than G" (loss modulus), indicating the batter had more elastic like properties. The elasticity decreased with the increase in bran replacement. The authors revealed this may have been due to the increase in water absorption of the fibres found in the corn bran. There was also a decrease in G". The baked cakes had a decrease in volume index when there was more than 20% flour replaced with corn bran. The L^* value increased and b^* values decreased in the crumb with the inclusion of over 25%. The a^* and b^* values of the crust was significantly decreased by 30% inclusion, most likely due to the yellow colour of the bran. Firmness of the cakes increased with the addition

of corn bran (773g-884g). The cake containing 0, 10 and 20% corn bran were scored by a sensory panel, however there was no significant differences between the control and the high fibre cakes.

1.5.2 Inulin/ Oligofructose

Fructans are a class of carbohydrate that describes any polysaccharide of fructose found in vegetables. Inulin and oligofructose belong to this class of carbohydrate and they also have prebiotic properties (Volpini-Rapina et al., 2012). As dietary fibre is classified as carbohydrate with a degree of polymerisation (DP) of 3 or more (European Commission 2008), both inulin and oligofructose as classed as dietary fibres; inulin has a DP of 3 to over 60, and oligofructose has a DP of 3 to 10. Inulin and oligofructose are classified dietary fibres and subcategorised as non-digestible oligosaccharides.

Both inulin and oligofructose are selectively fermented by the microflora of the gut. This selective fermentation promotes growth of beneficial bacteria, for example, bifidobacteria (Roberfroid, 2007, Roberfroid et al., 2010, Meyer and Stasse-Wolthuis, 2009), this allows inulin to be classed as a prebiotic ingredient. Bifidobacteria produces acetic and lactic acid in the gut and excretes substance which have antimicrobial properties, which can help protect the host from pathogens.

Currently there are no health or safety concerns about ingesting resistant oligosaccharides (Schaafsma and Slavin, 2015). The intake in the western diet of inulin intake ranges from 2-11g from dietary sources, mostly from wheat and onions (Apolinario et al., 2014, Shoaib et al., 2016, Khanvilkar and Arya, 2015). The EU and many countries including USA, Canada, Australia and Japan have classified inulin as food ingredients, rather than additives. Inulin has been confirmed as safe and therefore can be used without specific limitations (Coussement, 1999).

Inulin can be found in over 36,000 plants as a source of carbohydrate. Examples of plant sources include chicory, Jerusalem artichoke, onions garlic, honey, banana, tomato and rye (Collins et al., 2008; Yildiz, 2011; Khanvilkar & Arya, 2015). It is isolated most commonly from choicory in a process similar to the extraction of sugar from sugar beet. Inulin is water soluble, approximately 10% of concentration at room

temperature, and will produce a low-viscosity solution. It has approximately 10% the sweetness of sucrose, and therefore may only partially replace sucrose in a food product. Inulin is an excellent fat replacer, due to its ability to form gels (O'brien et al., 2003). The 3D microcystalline gel structure which forms when inulin is hydrated allowing inulin to mimic a creamy, fat-like texture in the mouth. It has proven to be a suitable fat replacer in spreads and other savoury products (Modzelewska-Kapitula and Klebukowska, 2009, Karaca et al., 2009, Laguna et al., 2014, Penksza et al., 2013, Tiwari et al., 2015). Inulin is effective in stabilising emulsion without negative impacts on texture. It has many functional benefits in food including bulking and gelling properties, providing a desirable mouth feel, it is highly soluble compared to other fibres, water binding capacity (Shoaib et al., 2016, Nair et al., 2010). It also has health benefits, as they are low in calories, non-cariogenic, they are suitable for diabetic formulation. Inulin can aid in decreasing levels of cholesterol in the blood and help the absorption of minerals such as calcium in the gut (Khanvilkar and Arya, 2015).

Oligofructose is formed by the partial enzymatic hydrolysis of inulin and then purification or by the transfructosylation of sucrose. It is also known as fruco-oligosaccharide (FOS) (Franck, 2002). Oligofructose is soluble up to 80% at room temperature in water, and has a greater sweetness than inulin, as it 35% concentration as sweet as sucrose (Schaafsma and Slavin, 2015). If oligofructose is used in products coupled with intense sweeteners, it masks an unpleasant aftertaste which can occur if the sweetener is used on their own (Schaafsma & Slavin, 2014). Oligofructose has similar properties to sucrose. It has good stability during processing in heat (Mensink et al., 2015). It also has humectant properties, and reduces a_w therefore confers microbiological stability to the product it is used in (Periche et al., 2016).

A study was conducted in which an inulin and oligofructose blend was added to an orange cake formulation to increase the prebiotic and fibre content of the cake (Volpini-Rapina et al., 2012) . The resulting cakes containing the prebiotic had a darker crust and deeper coloured dough. This is due to the Maillard reaction between the proteins and fructose in the oligofructose. The cakes were harder and less crumbly when compared to the control cakes (Volpini-Rapina et al., 2012). In another study cakes containing prebiotics had an increased level of stickiness, most likely due to the ability of inulin to uptake water while baking (Ronkart et al., 2009).

Handa et al. (2012) replaced the sugar in short dough cookies with oligofructose at levels of 40%, 60% and 80%, coupled with a low calorie sweetener. Due to the high hygroscopicity of oligofructose, there is less water available in a cookie dough for a gluten network to develop, therefore the cookies containing increased levels of oligofructose showed a decrease in height and an increased in diameter due to the high solubility of oligofructose when compared to sucrose. A decrease in cookie hardness was found, as the sugar was replaced. Hardness in a cookie is mostly caused by the recrystallization of the sucrose; this decrease in hardness is a result of the reduced sucrose content. When the cookies were stored over 7 months, it was found the control cookies hardened over time. The cookie containing the FOS decreased in hardness as oligofructose did not recrystallize. Sensory analysis revealed the control had the most appealing flavour, however it ranked lower in colour and texture when compared with the oligofructose-containing cookies. The FOS enriched cookies had a crisper texture and a golden colour, resulting in higher scores for these parameters.

Rossle et al. (2011) reduced sugar and fat in a quick bread formulation. The fat and sugar were replaced by inulin and FOS, and the effect on the baking properties of the quick breads were documented. It was found there was an increase in hardness of the crumb and crust, increase in volume as the concentration of FOS and inulin increased. As the inulin and oligofructose concentration increased, there was also a significant increase in the browning index of the crust, indicating a darker colour. Using an optimization tool, it was indicated the optimal design of the quick bread was a reduction of 10% fat and 10% sugar coupled with 10% oligofructose and 5.92% inulin, creating a quick bread with similar baking properties and textural attributes to the control.

1.6 Bulking agents

1.6.1 Polydextrose

Polydextrose is a bulking agent which is synthesised from glucose, sorbitol and citric acid, and provides a quarter of the energy provided by sucrose (1kcal/g). It has the average DP of 12 and it is classified as a dietary fibre. It is not hydrolysed in the small intestine, and is partially is fermented in the large intestine by the microbiota

(approximately 40%) (Holscher et al., 2015). It has been shown that polydextrose does not increase serum glucose values and does not cause insulin demand in diabetes, and is therefore suitable for diabetic food (Roberfroid and Slavin, 2000).

Polydextrose and sucrose share many similar physicochemical properties. Similar to sucrose, it increases the starch gelatinization temperature, but does not alter the denaturation temperature of the egg in a cake formulation. It is not sweet and has a neutral taste.

Polydextrose is an excellent thickening agent in bakery batters, with its addition resulting in an increase in the viscosity of batters and doughs (Schirmer et al., 2012). This increase in viscosity is sufficient to decrease the mobility of the air bubbles, providing a greater stability in batter. A high concentration of polydextrose in a cake batter can reduce the air-holding capacity of the batter, causing an increase in average bubble size and size variation (Kocer et al., 2007). The air bubble expansion rate is also slower in batter containing polydextrose. Polydextrose does not promote fat crystal aggregates; therefore air bubbles are less stable during baking (Schirmer et al., 2012).

Martinez-Cervera et al. (2012) replaced sucrose with polydextrose coupled with sucralose in a muffin formation (0%, 25%, 50%, 75%, 100% replacement). It was seen that total numbers of air cells within the muffin structure decreased as the concentration of sucrose also decreased. However, the muffins containing 25% less sucrose (and replacement with polydextrose) did not have a significant decreased number of air cells when compared with the control muffin. At 100% sucrose replacement, tiny bubbles which were seen in the uncooked batter were not seen in the baked muffin. This was result of the reduced viscosity of the batter allowing bubble buoyancy and a decrease in the ability of the batter to retain air while baking. The heights of the muffins were also affected by concentration of sucrose. The heights of control and 25% replacement products were significantly greater to higher concentrations (50%, 75% and 100% replacement). There was in significant increase in b^* (yellow hue) of the crumb as the concentration of polydextrose increased. The hardness and springiness of the 25% replacement formulation was less than the control, however not significantly less. The cohesiveness, springiness and resilience

properties all decreased as there was a reduction in sucrose. Chewiness of the muffins also decreased as the polydextrose concentration increased. Results from sensory studies indicated that levels of up to 50% replacement of sucrose were acceptable to the panellists with regards to colour, appearance, sweetness, flavour and overall acceptability.

Hicsasmaz et al. (2003) reduced the sucrose with polydextrose in a cake product at increasing concentrations (0%, 25%, 50%, 75% and 100%). It was shown that as the sucrose concentration decreased, there was an increase in the mean bubble size of the crumb. At 75% and complete replacement, the bubble sizes were similar to each other. However, even at total replacement, up to 90% of the bubbles had a similar range as the control, demonstrating the ability of polydextrose to mimic sucrose bubble size distribution. In formulations above 50% replacement, the reduced sucrose concentration were not able to maintain an even distribution of bubbles in the batter, as the lower sucrose content could not form a sufficient concentration of fat-crystal aggregates. It is important to note, there was a change in ratio of the shape and the size of the bubbles as less sucrose was present in the system. There is an increase in smaller, more spherical bubbles as the sucrose content decreases. The structure of the full replacement formulation is characterised by small spherical bubbles with almost total lack of interconnectivity. It was concluded the cell structural difference in the control and the cake formulation substituted with polydextrose must to due to insufficient expansion of the air bubbles. There was also an increase in Maillard reactions in the crumb, causing a darker colour and an increase in a value, and a decrease in L^* and b^* value of both the crumb and crust.

Ronda et al. (2005) completely replaced sucrose in a cake formulation with polydextrose and other bulking agents. It was found there was a decrease in lightness (L^* value) in the crumb- the crumb of the polydextrose cake had the lowest L^* values compared to the control and all the other bulking agents, however the crust had an increase in L^* value indicating a lighter colour cake when compared to the control formulation. There was a decrease in specific volume of the cake containing polydextrose compared to the control, a result of by the early gelatinization of starch and the denaturation of the proteins, reducing the time to allow bubble expansion. The firmness also increased in cakes containing polydextrose, particularly noticeable on

day four post-baking. Sensory evaluation concluded that the cakes containing polydextrose had the highest score for flavour intensity, but the lowest for sweetness compared to the cakes containing sugar alcohols.

Mieszkowska and Marzec (2016) replaced 20% wheat flour with equal weight of chickpea flour, as well as replacing 40 and 60% of the sucrose with polydextrose (w/w) in a cookie formulation. There was a significant decrease in brightness (L^* value) between the control and the reduced sucrose biscuits. This indicates a darker colour on the surface of the cookie. As sucrose is not a reducing sucrose, it only takes part in Maillard browning once it has been hydrolysed to glucose and fructose, whereas polydextrose is a glucose polymer, and therefore contains reducing side chain to participate in Maillard browning, producing a darker crust. Polydextrose also resulted in a harder biscuit and created the strongest acoustic emission, however these results were not significantly greater than the control. In the sensory analysis, panellists ranked the biscuits containing chickpea flour and 40% polydextrose as the highest overall, particularly in colour.

In a study conducted by Zoulias et al. (2002), polydextrose was used as a fat replacer (35%) in a biscuit formulation. There was a decrease in diameter of the biscuits and a significant increase in moisture, water activity of the biscuits with the addition of polydextrose as the fat replacer. This may have been a result of the increased water holding capacity of polydextrose. As fat is a tenderising agent, biscuits containing polydextrose (0% fat) showed an increase in hardness and brittleness compared to the control formulation (100% fat). Fat also improves to the flavour and overall eating of a biscuit, therefore, the sensory panellist ranked biscuits containing polydextrose significantly lower in flavour and general acceptance than the control formulation.

In a study conducted by Mancebo et al. (2018), 15% of flour weight of polydextrose and other soluble fibres (nutriose and inulin) were added to a biscuit formulation for fibre enhancement. There were no significant differences found between the control and polydextrose biscuit dough. Both polydextrose and the control doughs showed higher G' than G", indicating a more elastic than viscous characteristics of the dough. There was a decrease in thickness, and an increase in spread ratio and breaking strength of the biscuits containing polydextrose compared to the control. The presence

of polydextrose also significantly decreased the *L** value indicating a darker surface. The author concluded that this was not due to the Maillard browning, but more likely a result of the increase in spread, creating a thinner biscuit with a greater exposed surface area. There was also an increase in *a** value and decrease in *b** value, indicating an increase of redness and decrease in yellowness compared to the control. It was revealed the biscuits containing soluble fibre also had an uneven surface, due to the increase in air bubbles found at the surface of the biscuit, which may also be a result of the decrease in thickness.

1.6.2 Maltodextrin

Maltodextrin is spray dried into a soluble, hydroscopic powder which has a slightly sweet to neutral taste. It is digested in the body as a simple carbohydrate with a calorie content of 1kcal/g (Jones, 1996), and is converted to energy quickly (Prarikh et al., 2014). Maltodextrins are produced by the partial enzymatic hydrolysis of starch (usually manufactured from potato, tapioca, wheat or corn), which has a dextrose equivalent (DE) of less than 20. It interacts with amylose resulting in the formation of gels, therefore it can be used to replace fat in food matrices by thickening and stabilising matrices (Forker et al., 2012).

Maltodextrin has been used as a fat replacer in bakery products (Forker et al., 2012, Serin and Sayar, 2017, Sudha et al., 2007b). Studies have been undertaken where maltodextrin was used as a bulking agent in a reduced sucrose biscuit.

Savitha et al. (2008) replaced 30% sucrose with maltodextrin (0-40%) and sucralose and studied the rheology of the dough and quality attributes of the biscuits. It was found at the lowest replacement concentration (10%), there was an increase in lightness and breaking force while a decrease in spread factor compared to the control. In sensory evaluation, the biscuits containing 10% scored the lowest for texture and overall quality, while the biscuits containing 30% maltodextrin scored the most similar to the control of these parameters.

Fat was reduced by 35% and all of the sucrose in the formulation was removed in biscuits in a study conducted by Zoulias et al. (2002). The addition of polydextrose and maltodextrin resulted in a reduced diameter, and an increased moisture and water

activity. Polydextrose increased the hardness of the biscuit, while maltodextrin maintained a similar hardness to the control. The sensory panel scored the low-fat, sucrose free biscuits, significantly lower in flavour than the control.

1.7 Other sucrose replacers

1.7.1 Whey permeate

In cheese manufacture, the liquid whey and the solid curd are separated; the curd is then used to produce cheese. For every pound of cheese produced, there are nine pounds of whey. Whey contains 93% water and 0.6% protein, and almost 50% of the solids present in whole milk (Beucler et al., 2005). Globally, 115 to 160 million tons of whey are produced each year. Half of the whey used in food or to ferment ethanol, and the remaining is disposed (Guimarães, 2010).

Until recent years, whey was considered to be less important than the milk fat component in the milk. However whey is now recognised as an excellent source of essential amino acid and used commonly as a functional ingredient in food products. Whey proteins are concentrated by the use of ultra and microfiltration. Whey permeate (WP) is the substance that remains after filtration. Liquid WP consists of water (93%), with a smaller fractions of lactose (5%) and minerals (0.53%) (USDA, 2004). Spray dried WP comprises a minimum of 85% lactose, approximately 1.5% fat and maximum 7% minerals (Arla Foods Ingredients). Due to the increase in use and demand of whey protein there is an equal increase in quantity of its by-product. A viable use for its by-product whey permeate is being sought by food scientist and food industry. WP has high wettability and moderate solubility and is a free flowing bulking agent. It is perceived as sweeter than dried lactose powder, due to the flavour enhancing properties of the minerals found in WP.

WP has the potential to be applied as a functional ingredient, and can be used as a cheaper alternative to whey or lactose powders in bakery products, snack foods, dry mixes. Due to the high concentration of lactose, WP contributes to browning. It can also produce a caramelised flavour. The ability of lactose to crystallise on the crust of a baked product, such as cake or bread, which increase the moisture retention of the loaf (Burrington, 2005).

No documented studies have been found where whey permeate is used to replace sucrose in bakery formulations. However, given its high lactose content, and potential for browning, this ingredient presents new and novel opportunities for further studies in this area.

Jooyandeh et al. (2009) conducted a study in which feta cheese WP or fermented whey protein concentrate (FWPC) partially or fully replaced water (v/v) in bread at the following concentrations; 0%, 25%, 50%, 100%. When the samples containing WP were compared in a sensory evaluation with the control and bread containing FWPC, it was found the WP enriched bread received higher scores for crust and crumb colour. Bread supplemented with 25 and 50% WP had higher sensory scores than the control for appearance, colour, texture and overall acceptability. However the scores were not significantly different. The FWPC had a negative effect on the loaves in terms of texture and overall appearance. Even at 100% replacement of water with WP, there was no adverse effects seen for any of the attributes.

Al-Eid et al. (1999) replaced water with fermented and unfermented liquid milk permeates (MP) in a wheat bread formulation. Water was replaced at 0% (control) 25%, 50% and 100% concentration. It was seen the dough which contained 100% unfermented and 25% fermented MP had significantly higher water absorption than other dough. Replacing water with the MP (unfermented or fermented) significantly increased the gelatinisation temperature and viscosity of the dough when compared with the control. The increase in viscosity may be due to the presence of calcium and phosphate, influencing the starch pasting properties. It was found the breads containing 50% MP (unfermented or fermented) achieved highest loaf volume, significantly higher than the control. The presence of fermented MP resulted in a darker crust colour, and received significantly higher sensory scores for crust colour compared to the other treatments. Crumb texture was improved by the addition of MP, as rated by the sensory panel.

In another study, egg was completely replaced with whey protein phospholipid concentrate (WPPC) and delactosed permeate (DLP) in a cake formulation (Levin et al., 2016). No significant difference in yield was found between the control cake and

the cake containing WPPC and DLP (70:30), and WPPC. This was attributed to the high whey protein content (approx. 50%) of the WPPC, maintaining the WHC of the cake. There was a decrease in the volume and height of the cakes containing WPPC and DLP as the whey protein showed less foamability than egg proteins. There were no significant differences between the control and the reformulated cakes in regards $a_{w,}$ hardness, springiness, colour however a significant difference in cohesiveness was detected.

1.7.2 Fruit syrups

Fruit syrups are manufactured by extracting and concentrating the fruit juice. These syrups or extracts contain high levels of fructose and glucose, which are naturally found in high concentrations in fruits. Fruit syrups have been shown to have significant effects on colour, spread ratio and hardness of the biscuits (Majzoobi et al., 2016).

Majzoobi et al. (2016) replaced sucrose with date syrup and liquid date sugar in biscuits. Date syrup is extracted by applying pressure to the date fruit, filtrating and then concentrating the liquid. Date syrup contains a high concentration of sugar. However, the addition of date syrup increases the darkness and can decrease consumer acceptability. As the concentration of date syrup increased, there was a decrease in firmness of the dough. At high concentrations (above 80% sucrose replacement), there was a significant increase in moisture content of the baked biscuits compared to the control. The authors concluded the increase in moisture content was due to the difference in solubility of fructose, glucose and sucrose. Fructose and glucose in the syrup have a greater interaction with water in the dough, and therefore prevent a high level of evaporation during baking, leading to a biscuit with higher moisture content. Early starch gelatinisation absorbs water, therefore if there is a decrease in the concentration of sucrose, starch sets earlier resulting in an increase in the moisture content. Density increased with the addition of the date syrup. The authors hypothesised this increase in density was due to starch gelatinising earlier than the control. Biscuits containing date syrup was found to be harder than the control, as glucose and fructose have a lower crystallisation rate than sucrose. It was also purposed the decrease in hardness may be due to the increased moisture content. The colour of the biscuits were also significantly affected. The lightness (L^* value) of the biscuits decreased from 35.0 (control) to 30.3 (100% replacement with date syrup).

There was an increase in *a** value (green-red undertone) 12.6 (control) to 18.3 (100% replacement with date syrup) and a decrease in *b** value (blue-yellow undertone) 57.6 (control) to 35.33 (100% replacement with date syrup). The authors believed this change in colour was most likely the effect of Maillard browning. In regards to the sensory scores, panellists ranked texture, taste, colour and overall acceptability. At lower replacement levels (20% and 40%); there were no significant differences between the control and the reduced sucrose biscuits. However above 60% replacement, there was a decrease in sensory scores.

1.8 Conclusion

Sucrose has many functions in bakery products, with regard to sweetness, volume, shape, colour and overall sensory appeal. There are health risks associated with consuming large amounts of sucrose; however consumers still want to consume high quality baked goods. This review presents the most recently published research on an array of alternative sweetening ingredients in cakes and biscuits. Several studies have reported the successful formulation of the ingredients mentioned in this review as fibre enhancers or fat replacers; however they may have potential as sucrose replacing ingredients. These ingredients, e.g. maltodextrin, form weak gels, which can possibly replace the lost of bulk which is normally provided by sucrose. Other possible sucrose replacers, for example apple pomace, have a sweet or 'pleasant fruity taste' which may replace the sweetness of sucrose. These bulking ingredients, such as fruit or cereal fibres, which may not provide sweetness, could also be used in conjunction with natural flavourings or extracts. More research is necessary to fully explore the functionality of these ingredients in the production of high quality bakery products, which also are reduced in sucrose.

Objectives

The overall objective of this study was to investigate the effect of a number of clean label sweetening alternatives in reduced sucrose cake and biscuit formulations. In this thesis, a mixture of fibres, bulking agents, natural flavourings, natural extracts and by-products were used as sucrose replacers. Particular objectives were:

- To examine the properties of raw cake batters and biscuit doughs properties, using fundamental rheological measurements, and to visualise the microstructure of the batters and doughs using light microscopy, confocal laser scanning (CLSM) and cryo-scanning electron microscopy.

- To analyse the texture profile, staling kinetics, microstructure, composition and sensory properties of reduced sucrose cakes formulations enriched with apple pomace, whey permeate, oligofructose and polydextrose.

- To study the effects of the addition of cereal fibres and flavouring, maltodextrin and yeast extract, citrus fibres and apple extract, and polydextrose and flavouring as sucrose replacers in biscuit formulations, and to examine the physical characteristics, texture and staling profile and composition with regard to total sugar and total fibre of the reformulated biscuits.

- To investigate the texture, acoustic (sound) properties and volatile profile of reduced sucrose biscuit formulations.

CHAPTER 2

Fundamental rheology and microstructure of reduced sucrose cake batter and biscuit dough, with the inclusion of clean label alternative sweetening ingredients.

2. Fundamental rheology and microstructure of reduced sucrose cake batter and biscuit dough, with inclusions of clean label alternative sweetening ingredients.

Abstract

Sugar has multiple functions in cakes and biscuits. It provides sweetness and affects the structure, colour, flavour and texture of the batter and the baked product. However, due to its high caloric content and link with diseases such as obesity and diabetes, demand has increased for low-calorie, low-sucrose sweetener alternatives. An array of sweetening alternatives, including fibres, bulking agents and flavourings were used to replace sucrose in cake batters and biscuit doughs. Apple pomace, whey permeate, oligofructose and polydextrose were added to a reduced sucrose cake batter formulations. Reduced sucrose biscuit doughs were formulated using a combination of bulking agents (polydextrose, maltodextrin and plant fibres) with extracts (yeast, apple) and natural flavourings. The fundamental rheological properties and microstructural characteristics of the control and reformulated cake batter and biscuit dough were determined. A reduction of sucrose yielded cake batter with an increased storage modulus (G') and complex modulus (G*), indicating an increase in elasticity and firmness in the cake batter. In particular, cake batter containing polydextrose and apple pomace showed a significantly increased G' (3.45E+03Pa and 3.37E+03Pa respectively) and G* (3.94E+03Pa and 3.99E+03Pa respectively) compared to the control, at an oscillation of 10Hz ($P \leq 0.05$). There were no significant differences in the rheological properties of the biscuits doughs. With regard to the microstructure of the cake batter and biscuit dough, there was an increase in bubble size for both, and a wider range of bubble size when sucrose was reduced, as seen by light microscopy. Confocal laser scanning microscopy showed there was little change in the microstructure of the batter and dough. However, an increase in cellulose material was observed in samples containing apple pomace, oligofructose, polydextrose, citrus fibres and cereal fibres. Similar results were shown by scanning electron microscopy, in which fibrous material were seen in biscuit doughs containing citrus fibres and cereal fibres.

2.1 Introduction

Baked products are popular snack foods throughout the world; however they are high in fat, sugar and calories. Efforts have been made in recent years to decrease the calorie content of these foods by sugar reduction. Sugar plays a vital role in the functionality of cake batter and biscuit dough, influencing the characteristics of the baked cakes and biscuits. Reducing this component can result in products with an inferior quality. Sucrose is the most common sweetening ingredient used in the bakery industry. It contains 4kcal per gram and is produced from sugar cane or sugar beet. It is most commonly used in a crystallised form.

Cake batter is an oil-in-water emulsion consisting of two phases: a continuous aqueous phase containing the dry ingredients (including flour, sugar, and baking powder) and a dispersed oil phase. The oil phase is dispersed throughout the aqueous phase and does not mix. The mixing and baking of cake batter highly influences the interaction of these phases. The viscosity of the cake batter affects the final product with regard to volume and density. If the batter is too viscous, the bubbles cannot expand fully resulting in a decrease in volume (Zhou and Hui, 2014). Likewise, if the batter is not viscous enough, the bubbles cannot be trapped in the batter, will rise to the surface and escape, also resulting in a decreased volume. Changes to the batter during baking also influences the final structure of the cake with regards to cell formation and therefore affect the final texture of the cake (Schirmer et al., 2012). In cake formulations, sucrose promotes bubble stability (Hesso et al., 2015) and prevents full hydration of the gluten (Manley, 2011). Sucrose also competes with starch and gluten for water and increases the viscosity to the batter (Kweon et al., 2009).

Biscuit dough also contains two phases; oil/fat and the aqueous (hydrophilic) phase containing the sucrose solution. Air, flour particles, starch granules and proteins are dispersed in the hydrophilic phase. However it must be noted, as there is a low water content in biscuit dough, sucrose is present in both dissolved and crystalline form. The viscosity of biscuit dough significantly contributes to biscuit hardness and spread rate (Manley, 2011). When more water is present in the dough, more sucrose is dissolved during mixing, lowering initial dough viscosity, resulting in a biscuit that spreads faster during baking. A dough which has a high elasticity is not desirable in a biscuit

formulation, as it shrinks when baked (Sumnu and Sahin, 2008). The flour component, which absorbs large quantities of water, decreases the amount of water available to dissolve the sucrose in a biscuit formula. Thus, initial viscosity is higher and the biscuit spreads less during baking. Gluten present in flour interacts via hydrogen bonds, but no network is formed. Therefore the texture of the baked biscuit is attributable to starch gelatinization and supercooled sugar rather than a protein/starch structure (van der Sman and Renzetti, 2018).

The cake and biscuit making processes depend on the formulation and the processing conditions. Altering either of these can impact the quality of the dough and the final product. In this study, the rheology and microstructure of cake batters and biscuits doughs with reduced sucrose concentration were examined.

The rheological properties of a batter or dough give important information with regard to the quality of the raw ingredients, machinability of the dough/batter and the final texture of the baked product (Sumnu and Sahin, 2008). Mixing effects the rheological properties of the batter/dough; an increase in mixing time can result in the dough becoming softer, and can reduce the viscosity of the dough. In dynamic rheological tests, a strain is applied to a food sample, and the resulting stress is recorded. As a viscoelastic material is deformed, some of the energy is released in the form of heat, while other energy is remains stored in the food (Sahin, 2008). A stress sweep determines the linear viscoelastic region of the material, within which the dynamic test (frequency sweep) must be performed. A frequency sweep determines the effects of the ingredients in the batter/dough formulation. The temperature change during baking also impacts the batter and rheological properties. Temperature sweep demonstrates the change of the batter/dough during baking (Sahin, 2008). Changing the type or ratio of ingredients can significantly affect the rheological properties of the dough or batter. However, reducing sucrose without negatively impacting batter properties is possible. Schirmer et al. (2012) replaced sucrose with polydextrose in a cake batter and found the reformulated cake and batter had similar properties to the control formulation with regards to crumb grain and cake texture.

The rheology of a food can often be influenced by its microstructure. The microstructure of a batter and dough can determine the appearance, shelf life and

rheology of the batter and baked product. A number of methods can be employed to investigate the microstructure of product. These include confocal laser scanning microscopy (CLSM), light microscopy and cryo-scanning electron microscopy (SEM). Light microscopy highlights the different in the textures in the food, as well as the changes in structure as it undergoes processing. It is a highly versatile method, and materials can be stained to ensure the different structures are visible (Sánchez-Pardo et al., 2008). In another study, Kocer et al. (2007) reported that increasing polydextrose led to an increase in bubble size uniformity and decreased the average bubble area in a cake batter. The authors hypothesised this was due to a better incorporation of air bubbles into the batter. Rodriguez-Garcia et al. (2014) replaced sucrose with an oligofructose in cake batter. The microstructure was examined while the cake batter was heated (25-100˚C). It was found the bubbles in the control batter heated uniformly, whereas when the sucrose was replaced, bubbles coalescence and expanded quickly. Similar to light microscopy, in CLSM the microstructure is stained and individual components can be detected with dyes (fat, starch, protein, cellulosic materials). SEM imaging visualises the starch granules and protein matrix, as well as fibrous material in batters and dough. To date, no studies were found in which CLSM or cryo-SEM were used to examine the microstructure of reduced sucrose cake batter or biscuit doughs. This study aims to understand how ingredient changes contribute to differences in the rheological properties of cake batters and biscuit dough, as well as to create images to understand to the microstructure of the reformulated batters and dough in comparison to the control formulation.

2.2 Materials and methods

2.2.1 Ingredients

The cake formulations were prepared using the following ingredients: flour (Shackletons, Ireland), granulated sugar (purchased locally), water, margarine (purchased locally), sodium bicarbonate (baking powder, purchased locally). Beneco L91 (an oligofructose), apple pomace, whey permeate and polydextrose were all obtained from Irish ingredient companies.

The biscuit formulations were prepared using the following ingredients: flour (Shackletons, Ireland), granulated sugar (purchased locally), water, margarine (purchased locally), sodium bicarbonate (baking powder, purchased locally), salt. Cereal fibres, flavouring, polydextrose, flavouring, yeast extract, maltodextrin, citrus fibre and apple extract and polydextrose were all obtained from ingredient companies.

2.2.2 Cake batter preparation

A control cake batter formulation and five reduced sugar formulations were prepared as per Table 2-1, where 30% of the sugar was removed, and replaced by 5% of replacers (whey permeate, oligofructose, polydextrose and apple pomace) on a flour weight basis.

The cake batter was prepared in a Hobart PMFD158 (UK) mixer, using an 'all-in-one' method. Flour, sugar, egg, water margarine, and sugar replacer were placed into the mixing bowl and mixed at a low speed (speed 1) for 30 seconds using the K mixer attactment. The sides were scraped down using a spatula, and the speed was increased (to speed 2) for 2 minutes. Analysis was carried out immediately after batter was prepared. All batches were prepared in triplicate.

Table 2-1: Cake batter formulations, % of total cake batter weight.

	Control batter (%)	Reduced sugar batter (%)	Reformulated batter (%)
Flour	35.7	37.9	37.4
Fat	16.1	17.1	16.7
Egg	16.1	17.1	16.7
Water	12.4	13.2	13.0
Sugar	19.6	14.6	14.3
Sugar replacer	-	-	1.9

2.2.3 Biscuit dough preparation

The control biscuit dough formulation and 5 reformulated formulations were prepared as per Table 2-2. The inclusion levels of sucrose-replacing ingredients (cereal fibres and flavouring, maltodextrin and yeast extract, citrus fibres and apple extract, and polydextrose and flavouring) were based on the concentrations advised by the ingredient manufacturers.

Flour, sugar, margarine, salt, water and sugar replacer were placed into the Hobart PMFD158 (UK) mixer, and mixed at a low speed (speed 1) for 1 minute. The speed was increased (to speed 2) for 2 minutes. The dough was allowed to rest for 30 minutes at room temperature before analysis was carried out. All batches were prepared in triplicate.

Table 2-2: Biscuit dough formulations, based on 100g flour weight.

	Control	Reduced sugar (70)	Cereal fibres & flavouring (CFF)	Maltodextrin & yeast extract (MDY)	Citrus fibres & apple extract (CFA)	Polydextrose & flavouring (PXF)
Flour	100	100	100	100	100	100
Fat	35	35	35	35	35	35
Salt	0.7	0.7	0.7	0.7	0.7	0.7
Water	20	20	20	20	20	20
Sugar	35	24.5	24.5	24.5	24.5	24.5
Cereal fibre & flavouring	-	-	10.5	-	-	-
Yeast extract	-	-	-	0.04	-	-
Maltodextrin	-	-	-	8.2	-	-
Citrus fibres	-	-	-	-	2.9	-
Apple extract	-	-	-	-	14.3	-
Flavouring	-	-	-	-	-	0.3
Polydextrose	-	-	-	-	-	5

2.2.4 Batter and dough analysis

2.2.4.1 Fundamental cake batter and biscuit dough rheology

Batter and dough rheology was completed using a Physica MCR301 rheometer (Anton Paar, Sweden). Testing was performed within 30 minutes of batter and dough preparation. Approximately 2g of sample from each formulation was placed onto the base plate. The upper plate was brought to a gap of 1.025mm for the cake batter and 3.025mm for the biscuit dough, and the excess was trimmed. Damp cotton wool was placed around the sample to prevent drying out. The plate was then lowered to a test gap of 1mm for the batter and 3mm for the dough. Sample was covered by the Peltier hood. The batter and dough were allowed to rest for 10 mins to allow relaxation of residual stresses (Mancebo et al., 2016, Struck et al., 2015).

Amplitude sweep

Analyses were carried out between 10^{-3}–10^{2}% strain to determine linear viscoelastic region of the batter and dough samples. Measurements were carried out at 25˚C(Struck et al., 2015).

Frequency sweep

Experiments were carried out between a frequency of 0.1-10Hz (0.1% strain) in a viscoelastic linear region. Storage (G'), loss (G") and complex (G*) modulus versus frequency values were recorded. Ten measuring points were recorded. Measurements were carried out at 25˚C (Struck et al., 2015).

Temperature ramp test

A frequency of 1Hz and strain of 0.1% were kept constant while the temperature was changed continuously from 25 to 98˚C (increasing 2.4˚C/min). The G', G" and G* were measured as a function of the temperature (Struck et al., 2015).

2.2.4.2 Cake batter and biscuit dough microstructure

Light microscopy

Using a spatula, a thin layer of the batter or dough (from each formulation) was smeared onto a slide. The batter and dough was dyed by adding two drops of the iodine/KI mixture (0.1% w/v iodine in 0.2%, w/v KI). A cover slip was placed on top

and samples were examined at x10 or x20 objectives using an Olympus BX51 transmitted light microscope (O'Shea et al., 2013).

Confocal laser scanning microscopy

A spatula was used to spread a small piece of each of the batters and doughs to approximately 10mm diameter. Protein, starch and plant cell wall (cellulosic) material was visualised by adding a stain mixture containing Nile Blue, FITC and Fluorescent Brightener 28 in a ratio of 2:2:1 (O'Shea et al., 2013). The sample was examined with a Leica SP5 confocal scanning laser microscope (Leica Microsystems, Mannheim, Germany) using blue diode (405nm), 179 argon (488nm) and HeNe (633 nm) lasers. The 405, 488 and 633nm channels were pseudocoloured purple, green and red, respectively. The Nile Blue labelled protein as red and fat as green. Starch appeared as dark green with a black centre.

Cryo-scanning electron microscopy

Imaging was performed on a Hitachi SU 6600 field emission SEM (Hitachi UK, Maidenhead, Berkshire) and fitted with a Gatan Alto 2500 cryotransfer stage (Gatan UK, Abingdon, Oxon). Each cake batter and biscuit dough was prepared for Cryo-SEM by rapidly immersing into liquid nitrogen slush (-180°C). Following freezing, the frozen samples were immediately transferred using the vacuum transfer device into the cryo-preparation chamber using the vacuum transfer device. The sample was fractured using a chilled scalpel blade at -180°C in the chamber, which was maintained under a high vacuum of 10^{-4}Pa. The sample was then sputter coated in Au/Pd (60/40) using 300V, 10mA for 120s. The sample was then transferred under vacuum onto the cooled SEM stage and imaged at -140°C (O'Shea et al., 2013).

2.2.5 Statistical analysis

All baking trials and tests were replicated three times and mean values and standard deviations were calculated. Statistical significance was considered at ($P \leq 0.05$). One-way ANOVA was performed on data using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). Where ANOVA indicated that significant differences were present, a Tukey pairwise comparison of the means was conducted to identify where the sample difference occurred. Pearson Correlation Coefficients were used to determine correlations between results.

2.3 Results and discussion

2.3.1 Fundamental cake batter and biscuit dough rheology

As previously discussed, the rheological properties of cake batters and biscuit doughs are important, as they influence the machinability as well as the quality of the finished product. Cake batters and biscuit doughs display viscous and elastic properties, represented by the storage modulus (G') and the loss modulus (G") respectively. In the oscillatory test, strain is applied to the batter and dough, which are stressed and deformed under this strain. The complex modulus (G*) describes the stiffness or firmness of batter or dough.

2.3.1.1 Frequency sweep: Cake batter

As shown in Table 2-3, G' was greater than G" in the control batter, 70% sucrose batter and batter containing whey permeate at 0.1Hz and 10Hz, implying these batters showed more elastic rather than viscous properties at these frequencies. However, the addition of apple pomace, oligofructose and polydextrose resulted in a greater G" value, indicating a more viscous cake batter at both 0.1Hz and 1.1Hz compared to the control. All cake batters showed an increased G' and G" as the test frequency increase from 0.1Hz to 10Hz ($P \leq 001$) indicating shear-thickening behaviour. To date, no studies which were comparable to the current study were found.

With regard to the G', the apple pomace, oligofructose and polydextrose-containing batters showed a significant increase to the control formulation at 10Hz. The inclusion of polydextrose and oligofructose also significantly increased G" value at 10Hz ($P \leq 0.05$). The increase of G' and G" of the batters containing apple pomace, oligofructose and polydextrose ($P \leq 0.05$) may be a result of gel formation in the batters. Apple pomace contains high levels of pectin and sugars. Together, sugar and pectin are known to form gels (O'Shea et al., 2012).

Gels formed by pectin and sugars likely increased the viscosity and therefore increased the G'. The gel forming abilities of polydextrose and oligofructose can result in a creamy mouthfeel (Franck, 2002, Mancebo et al., 2018). The formation of weak gels in batters containing oligofructose and polydextrose increased G' and G" as the test frequency increased, seen in Figure 2-1 and Table 2-3**.** It should also be noted that

including new ingredients or changing the ratio of ingredients can alter the interactions between the proteins and polysaccharides, which may influence the gel structure formation. These interactions may also have had an effect on the batters containing apple pomace, oligofructose and polydextrose.

Table 2-3: Storage (G') and loss (G") modulus of the control and reformulated cake batters at increasing frequencies (0.1 – 10Hz). Values labelled with the same superscript in the same row are not significantly different (P≤ 0.05).

	Frequency (Hz)	0.1	1.1	10
Storage Modulus (G') (Pa)	Control	6.25E+02^{a}	1.12E+03^{ab}	1.87E+03^{b}
	70% Sucrose	9.06E+02^{a}	1.59E+03^{ab}	2.54E+03^{b}
	Apple Pomace	1.31E+03^{a}	2.16E+03^{a}	3.37E+03^{b}
	Whey Permeate	7.97E+02^{a}	1.45E+03^{ab}	2.40E+03^{b}
	Oligofructose	1.10E+03^{a}	1.99E+03^{a}	3.11E+03^{b}
	Polydextrose	1.09E+03^{a}	2.02E+03^{a}	3.45E+03^{b}
Loss Modulus (G") (Pa)	Control	2.84E+02^{a}	4.59E+02^{ab}	1.03E+03^{b}
	70% Sucrose	4.04E+02^{a}	6.17E+02^{ab}	1.31E+03^{b}
	Apple Pomace	5.46E+02^{a}	7.77E+02^{a}	1.55E+03^{b}
	Whey Permeate	3.53E+02^{a}	5.81E+02^{ab}	1.25E+03^{b}
	Oligofructose	5.11E+02^{a}	8.24E+02^{a}	1.61E+03^{b}
	Polydextrose	5.28E+02^{a}	8.73E+02^{a}	1.91E+03^{b}

Figure 2-1 shows the control formulation compared to the reduced sucrose formulation as frequency increased over time. As the frequency increased during the test, the G* significantly increased for all test samples all treatments (P≤0.001), indicating an increased firmness of the batter. The polydextrose and apple pomace containing batters had a significantly greater G* than the control formulation at 10Hz. Overall, the addition of polydextrose, apple pomace and oligofructose affected the rheological properties of the batter greatly, increasing the firmness of the batter. An increase in the firmness of the batter may lead to a decrease in specific volume of the baked cake. This can be seen in 2.9.1.1. Apple pomace- containing cakes had a significant decrease in specific volume compared to the control (P ≤ 0.05). While the cakes containing oligofructose and polydextrose also had an insignificant decrease in specific volume

compared to the control cake, highlighting the effect of batter firmness on the final volume of the baked cake.

As discussed above, this increase in G* may have had been a result of a formation of a gel, or also likely due to the increased fibre content present in these ingredients. Fibres have a greater affinity for water than gluten, therefore producing a firmer cake batter. This was reported by Sullivan et al. (2011) where the addition of type fibre in bread dough resulted in an increased G*.

Interestingly, the addition of whey permeate resulted in a batter which was most similar to the control formulation with regards to G', G" and G* moduli. This may have been a result of the high lactose concentration of the whey permeate, producing softer batter, similar to the control batter.

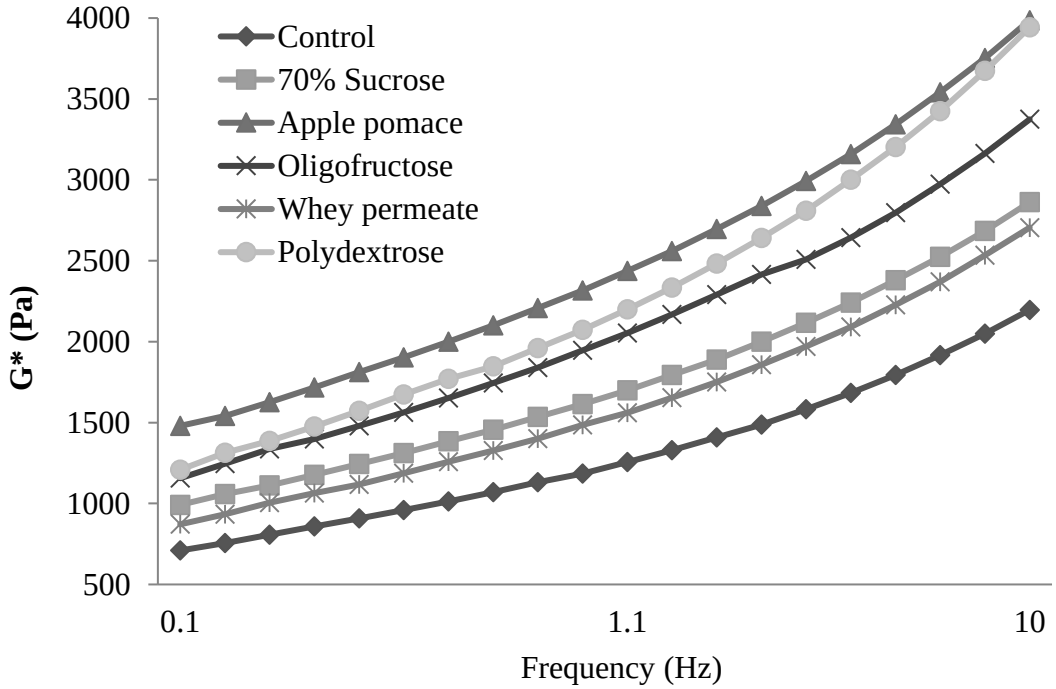


Figure 2-1: Complex modulus (G*) of control and reformulated cake batters containing 70% sucrose, apple pomace, oligofructose, whey permeate, polydextrose at increasing frequencies (0.1 – 10 Hz).

2.3.1.2 Temperature ramp test: Cake batter

Structural changes to the cake batter occur during the baking process, will affect the structure of the final cake. In the temperature ramp test, the temperature was increased

from room temperature (25ºC) to temperatures the batter is subjected to during baking (up to 98ºC). As sucrose is a softening agent, the control batter maintained the lowest G' from 25.2˚C to 75.4˚C. Figure 2-2, is an example of the change in G' properties of the test batters as the temperature increased.

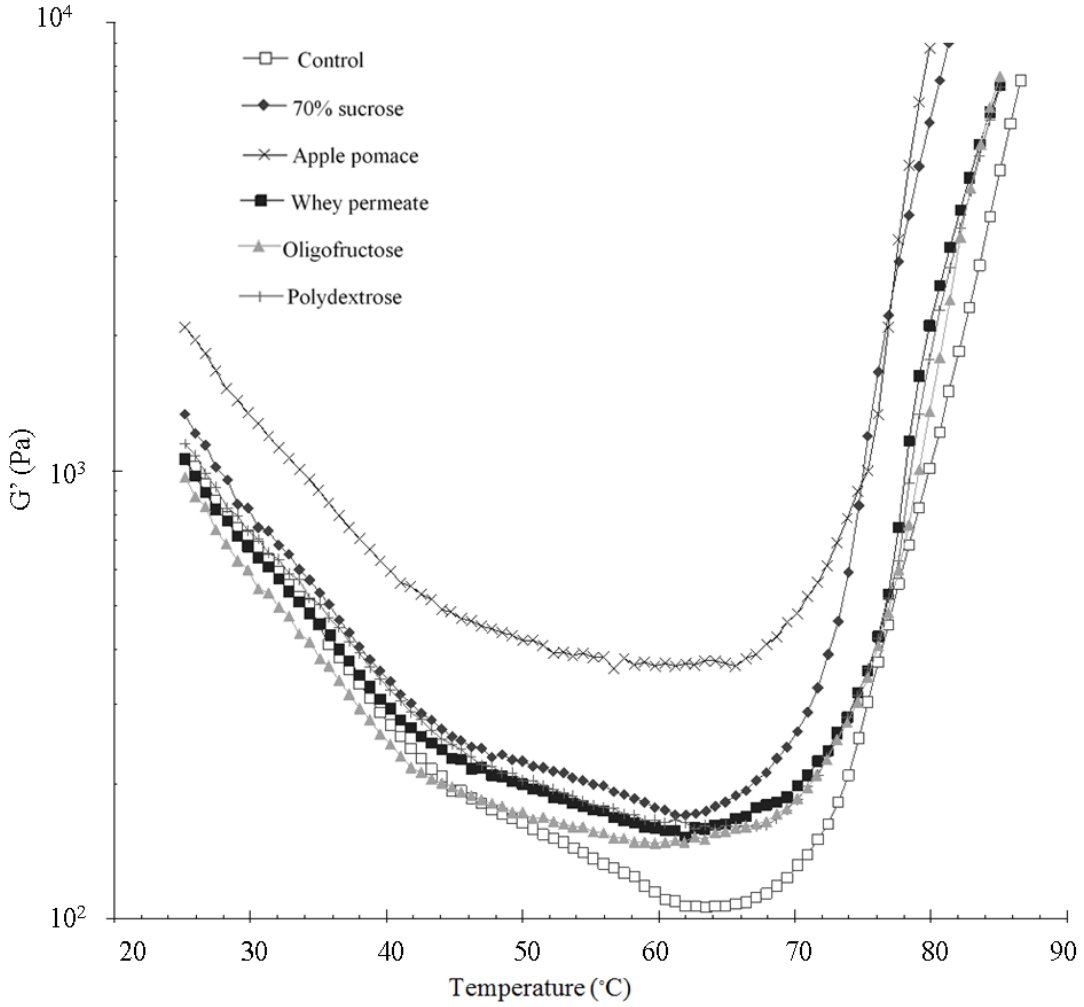


Figure 2-2: An sample temperature ramp test of control and reformulated cake batters containing 70% sucrose, apple pomace, oligofructose, whey permeate, polydextrose at increasing temperatures (20ºC to 90ºC).

As the temperature increased, the batter went through a number of changes. In the first stage, between 25ºC to approx. 55ºC, the fat begins to melt (Sumnu and Sahin, 2008). This resulted in a decrease in viscosity and therefore a decreased G' is seen in Figure 2-2. At 25ºC, the control formulation had the lowest G' value (940 Pa). The increase in G' of the reformulated batter highlights the ability of sucrose to soften the batter. The introduction of fibres, which bind to water creating a more viscous batter, also

have resulted in the increase of G' of batters containing polydextrose, oligofructose and apple pomace.

In the middle region of baking (55-70ºC), there is a slight increase in viscosity due to early gelatinisation of starch, resulting in an increase in G'(Sumnu and Sahin, 2008). At approximately 75˚C, the final stage of baking was reached. This resulted in a sharp increase in viscosity and G' (Sumnu and Sahin, 2008). At this high temperature, structural changes began to occur due to protein and starch forming gels resulting in the cake batter solidifying. The point at which these changes start to occur is known as the inflection point (Hesso et al., 2015).

The control formulation had the highest inflection point of all treatments (approx. 78˚C). The reduced sucrose batter and the apple pomace-containing batter appeared to have the lowest inflection points (approx. 72˚C), while batter containing oligofructose, whey permeate and polydextrose had a slightly greater inflection point (approx. 74˚C). Sucrose increases the temperature in which starch gelatinisation and egg protein denaturation occur, thus increasing the inflection point of the batter. A reduction in sucrose resulted in a decrease in the inflection point, as gelatinisation and denaturation of the protein occurs at a lower temperature compared to the control.

Polydextrose is known to increase the temperature gelatinisation of starch, therefore increasing the inflection point when compared to the 70% sucrose batter. However, as it does not increase the temperature of egg denaturation, a lower inflection point than the control formulation can be expected. Similarly, in other studies in which polydextrose was used as a sucrose replacer in cake type batters (Martinez-Cervera et al., 2012, Schirmer et al., 2012), an increase in both G' and G" was reported at a lower temperature than the control. Apple pomace has a high fibre content, which when heated, swells and increases viscosity. It is likely this high starch content resulted in a gel forming at a lower temperature than the control. It is also possible that the minor protein content of apple pomace also affected the setting temperature of the batter. The increase in G' continued until the final temperature (98˚C) was reached.

With regards to the inflection point of the batter, a slight decrease of between 4-6˚C was seen in the reduced sucrose samples compared to the control batter. It will be seen

in later in this thesis (section 3.4.1) that this decrease will not significantly affect the specific volume, density or cell structure of the reformulated baked cakes (with the exception of cakes containing apple pomace). This indicates that a 30% reduction of sucrose is achievable without significantly affecting the heating properties of the batter.

2.3.1.3 Frequency sweep: Biscuit dough

Although differences were measured between the control samples and the reformulated dough, the results were not found to be statistically significant with regards to storage modulus (G'), loss modulus (G") and complex modulus (G*) at any frequency. All doughs followed a similar trend of increased G' and G" as strain increased, displaying shear-thickening effects. All biscuit dough formulations had a greater G' than G" indicating the dough exhibited an elastic property. Results for the biscuit dough frequency tests are displayed on Figure 2-3 and Table 2-4.

The control dough had the lowest G' at 0.1 and 1.1Hz, and dough containing polydextrose and flavouring (PXF) had the lowest G' at 10Hz. The addition of sucrose is known to soften biscuit dough as it competes with gluten for the available water in a sample. An increased water content results in a reduced G' in biscuit dough (Maache-Rezzoug et al., 1998). Therefore, a reduction of sucrose in biscuit dough would increase the G', as seen in the current study.

Mancebo et al. (2018) observed the addition of polydextrose as a wheat flour replacer in cookie dough resulted in a decreased G' at 1Hz. The authors revealed this increase was likely related to the increase in WHC of the polydextrose. The addition of citrus fibre and apple syrup (CFA) resulted in the highest G' at 1.1 and 10Hz. Citrus fibres have a high concentration of dietary fibre, with a high proportion of soluble fibres, which have a high water holding and swelling capacity and form gels which increase viscosity. (Nassar et al., 2008). These properties of citrus fibres may have influenced the increase in G' in this study.

Table 2-4: Storage (G') and loss (G") modulus of the control and reformulated biscuit batters at increasing frequencies (0.1 – 10Hz). Values labelled with the same superscript in the same row are not significantly different (P≤ 0.05).

	Frequency (Hz)	0.1	1.1	10
Storage Modulus (G') (Pa)	Control	1.12E+05[a]	1.72E+05[a]	2.72E+05[a]
	70	1.26E+05[a]	1.84E+05[a]	2.73E+05[a]
	CFF	1.20E+05[a]	1.75E+05[a]	2.65E+05[a]
	PXF	1.16E+05[a]	1.73E+05[a]	2.59E+05[a]
	MDY	1.19E+05[a]	1.76E+05[a]	2.70E+05[a]
	CFA	1.18E+05[a]	1.87E+05[a]	2.99E+05[a]
Loss Modulus (G") (Pa)	Control	6.33E+04[a]	9.79E+04[a]	1.76E+05[a]
	70	6.01E+04[a]	9.24E+04[a]	1.63E+05[a]
	CFF	6.03E+04[a]	9.92E+04[a]	1.83E+05[a]
	PXF	5.89E+04[a]	9.25E+04[a]	1.59E+05[a]
	MDY	5.69E+04[a]	9.06E+04[a]	1.58E+05[a]
	CFA	6.15E+04[a]	9.52E+04[a]	1.70E+05[a]

70 = 70% sucrose, CFF = cereal fibre and flavouring, PXF = polydextrose and flavouring, MDY= maltodextrin and yeast, CFA = citrus fibres and apple extract.

The complex modulus (G*) is shown in Figure 2-3. The control formulation had the lowest G* value, indicating it was the softest dough, whereas the dough containing 70% sucrose had the highest G* value at 0.1Hz, and therefore was firmest dough. This further highlights the dough softening effects of sucrose and its importance in biscuit manufacturing.

Dough containing PXF was the least firm at 1.1 and 10Hz, whereas the addition of CFA to the dough resulted in the firmest dough at 1.1 and 10Hz. As discussed, the water holding capacity and gel formation of polydextrose and citrus fibres influence the biscuit dough firmness. The addition of apple extract may have also influenced the firmness of the dough. While, no previous studies were found in which apple extract was used as a sucrose replacer- a study using other sugar syrups (liquid glucose, invert

syrup and high fructose corn syrup) reported a reduction in viscosity of the biscuit doughs (Manohar and Rao, 1997). However the authors did not use additional fibres.

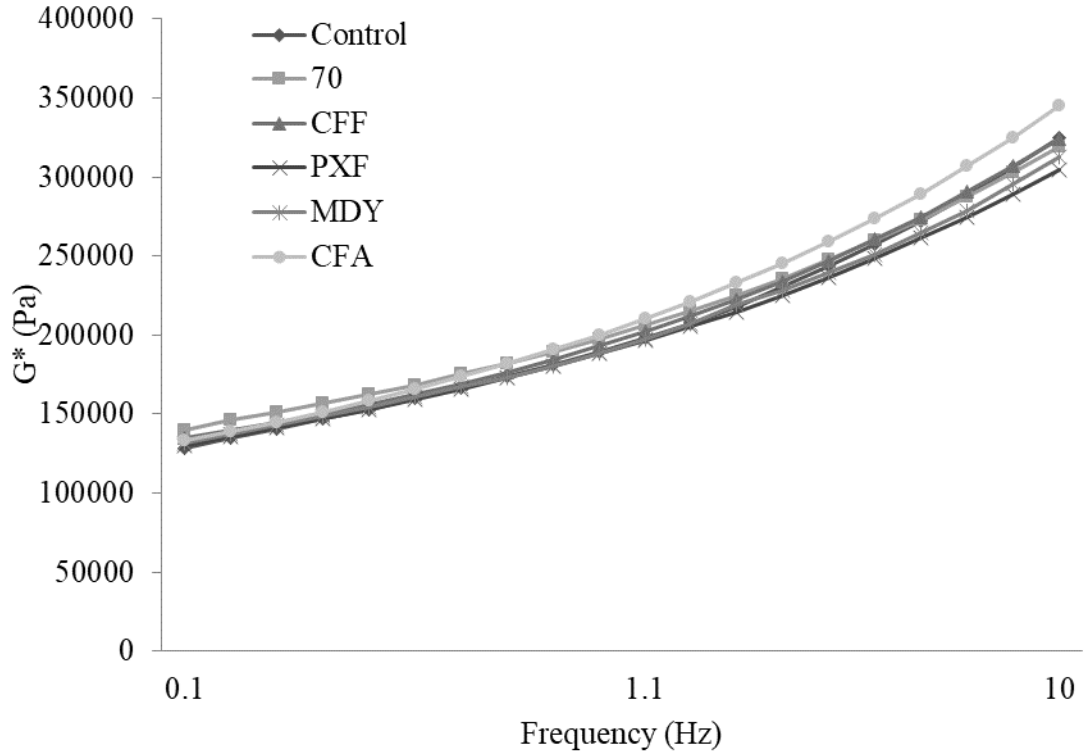


Figure 2-3: Complex modulus (G*) of biscuit doughs at increasing frequencies (0.1 – 10Hz), 70 = 70% sucrose, CFF = cereal fibre and flavouring, PXF = polydextrose and flavouring, MDY= maltodextrin and yeast, CFA = citrus fibres and apple extract.

Overall, there were no significant differences found between the control formulation and the reformulated biscuit doughs, which showed it was possible to reduce the sucrose content with adversely affecting the rheological properties of the dough. No other studies were found in which the rheological properties of reduced sucrose biscuit dough was investigated.

2.3.2 Cake batter and biscuit dough microstructure

2.3.2.1 Light microscopy

Light microscopy uses light and lenses to magnify images samples. Light microscopy images of cake batter are displayed in Figure 2-4 and biscuit dough displayed in Figure 2-5. The figures showed the reduction and replacement of sucrose had an effect on bubble size in the dough and batter.

The control batter formulation (A) showed small bubbles with a narrow bubble size distribution. When sucrose was removed, there was an increase in the number of larger bubbles and a broad size distribution of bubbles, which can be seen in Figure 2-4 & 2-5 (B-F). For example, in Figure 2-4 (B), bubbles ranged from approximately 20-150µm.

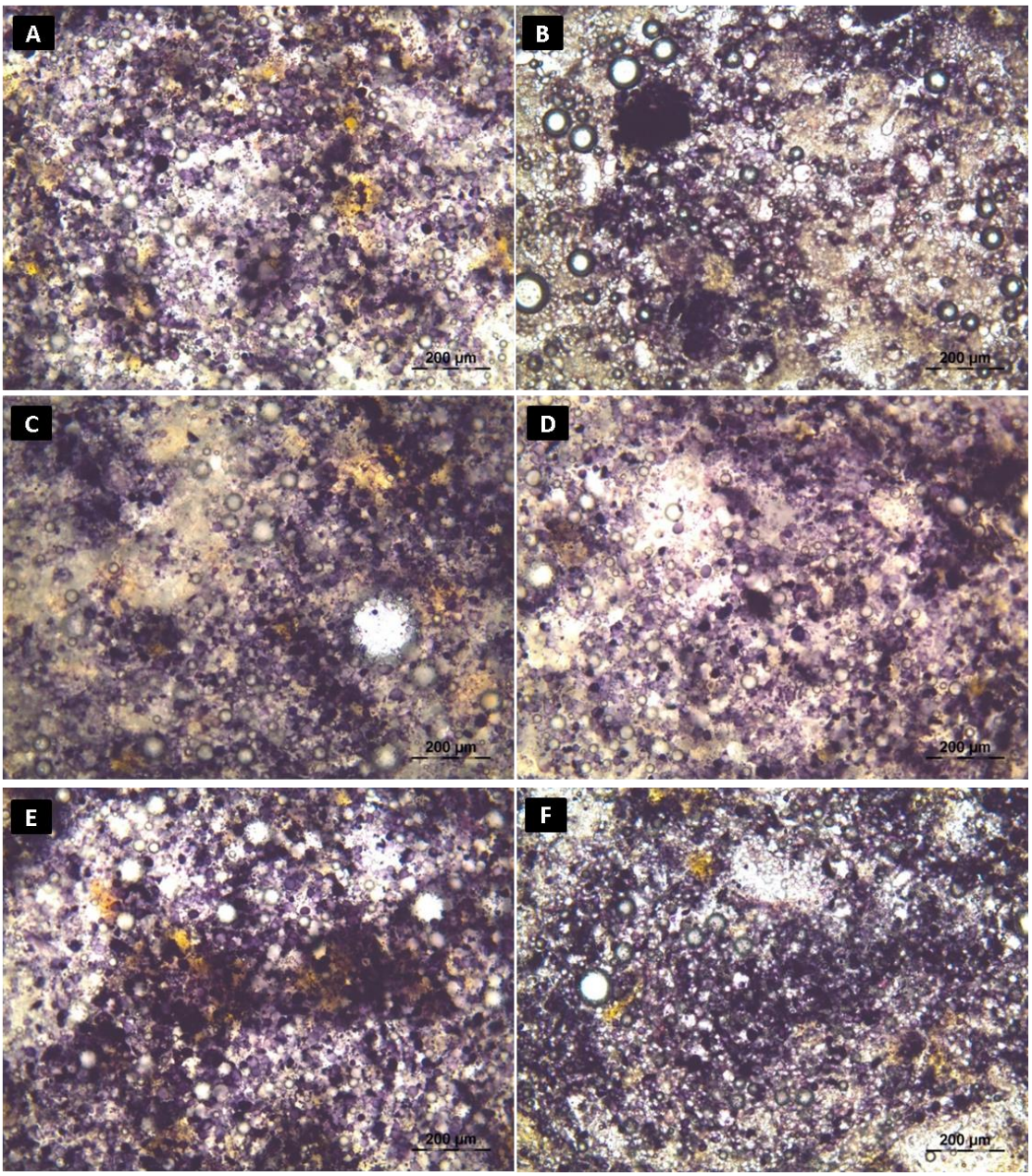


Figure 2-4: Light microscopy of cake batters; control (A), 70% sucrose (B), apple pomace (C), oligofructose (D), whey permeate (E), and polydextrose (F). Magnification=200µm.

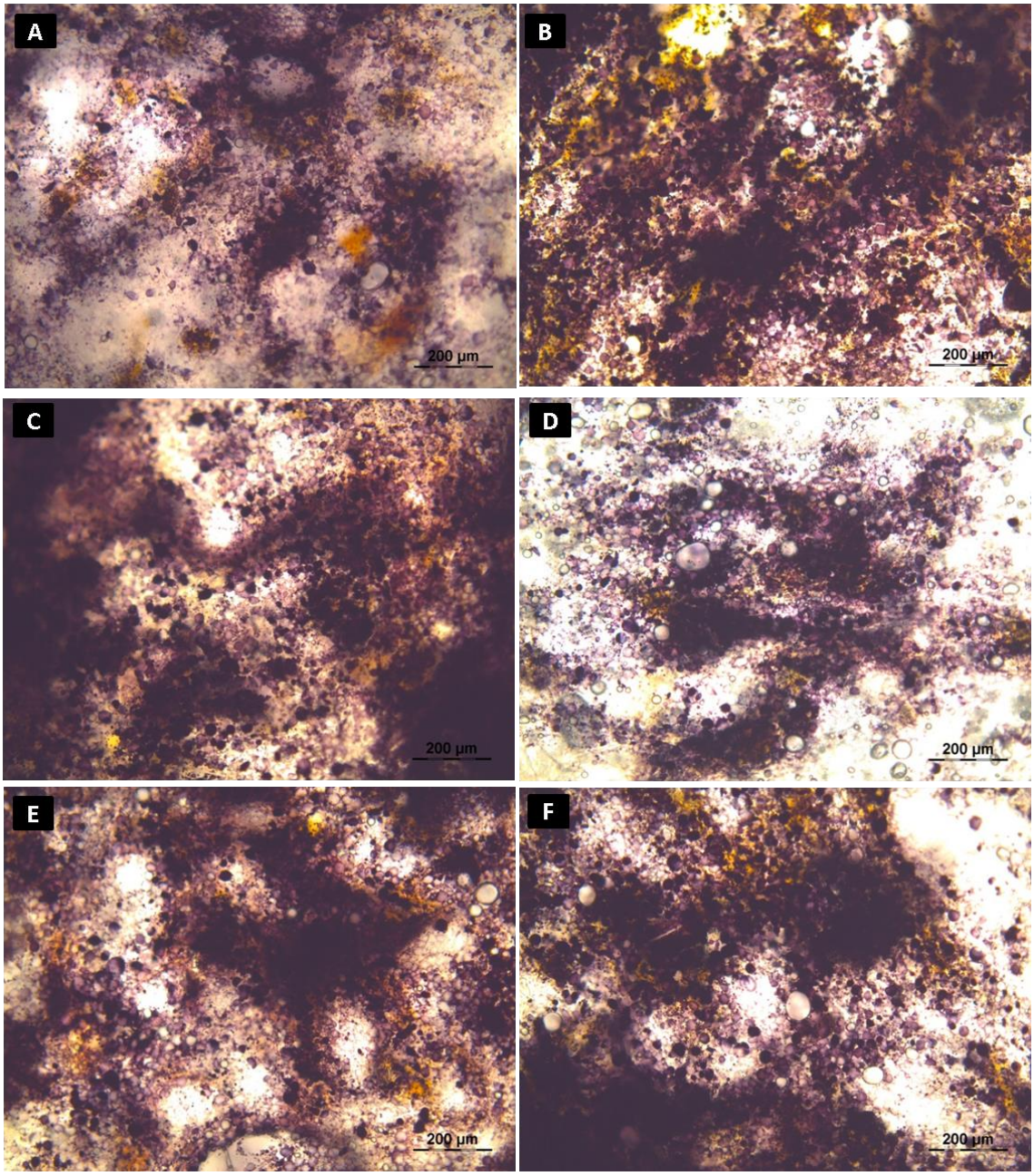


Figure 2-5: Light microscopy of biscuit doughs; control (A), 70% sucrose (B), CFF (C), PXF (D), MDY (E), and CCA (F). Magnification=200µm. 70 = 70% sucrose, CFF = cereal fibre and flavouring, PXF = polydextrose and flavouring, MDY= maltodextrin and yeast, CFA = citrus fibres and apple extract.

In the current study, the rheological testing showed that the control batter had the lowest viscosity compared to all batters. It is possible that the increase in bubble size, as seen in the reduced sucrose batters Figure 2-4 & 2-5 (B-F), is a result of an increase in viscosity which allowed the larger bubbles to be retained in the batter. This was also observed in a study conducted by Martinez-Cervera et al. (2012), where sucrose

was reduced by 50 and 100% and replaced with sucralose and polydextrose (w/w) in a cake batter. The authors reported a decrease in the number of larger bubbles in the reduced sucrose batters. They hypothesised the decreased sucrose content resulted in a less viscous batter which allowed the larger bubble to coalesce and escape, while the smaller bubbles were retained. Similar findings were found by Hanselmann and Windhab (1998), which showed a decrease in viscosity in a whey protein isolate and guar gum foam resulted in a decreased number of bubbles.

The microstructures of the biscuit doughs were similar, as shown by the light microscopy. All biscuit doughs were seen to contain a large concentration of small bubbles. As discussed in section 2.4.1, all biscuit doughs had a high G*, therefore it is unlikely large bubbles could be maintained in this matrix. There were not significant differences in the rheology of the control formulation and the reformulated doughs, therefore a similar bubble distribution was expected. Images of the dough microstructure were not examined in by any other author.

2.3.2.2 Confocal microscopy

Confocal laser scanning microscopy (CLSM) was carried out to investigate the effect of sugar reduction on the organisation and distribution of protein, fat, starch and structural polysaccharides in the samples. Images are shown in Figure 2-6 (cake batter) and Figure 2-7 (biscuit dough).

In the cake batter, proteins are represented as the red spheres, which were the largest component in the microstructure. In the biscuit doughs, however, protein presented itself as uneven small red shapes. The fat in the formulations can be seen as small bright green globules in Figure 2-6 and 2-7. The reduction in sucrose appeared to have little effect protein and fat organisation in both the cake batter and biscuit dough, as the structure and the organisation of the globules were maintained. The starch granules appear dark green with a near black centre. The dark centre of the starch granule is a result of the dye staining the exterior of the grain but not throughout. There was no visual change to the starch granules as sucrose was reduced.

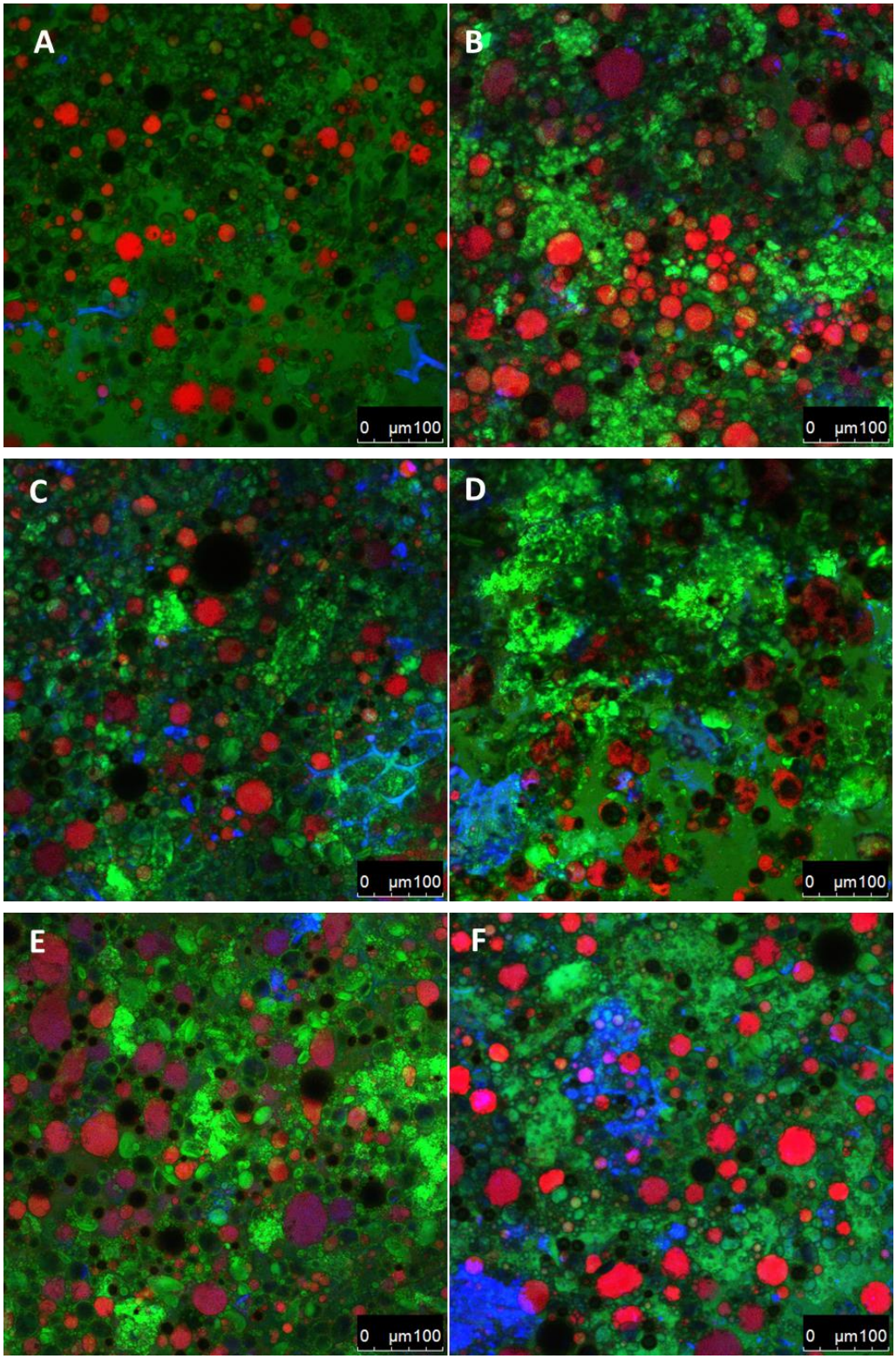


Figure 2-6: CLSM images of cake batters; control (A), 70% sucrose (B), apple pomace (C), oligofructose (D), whey permeate (E), and polydextrose (F). Protein=red, fat=bright green, starch=dark green with black centre, cellulosic material=blue. Magnification=100µm.

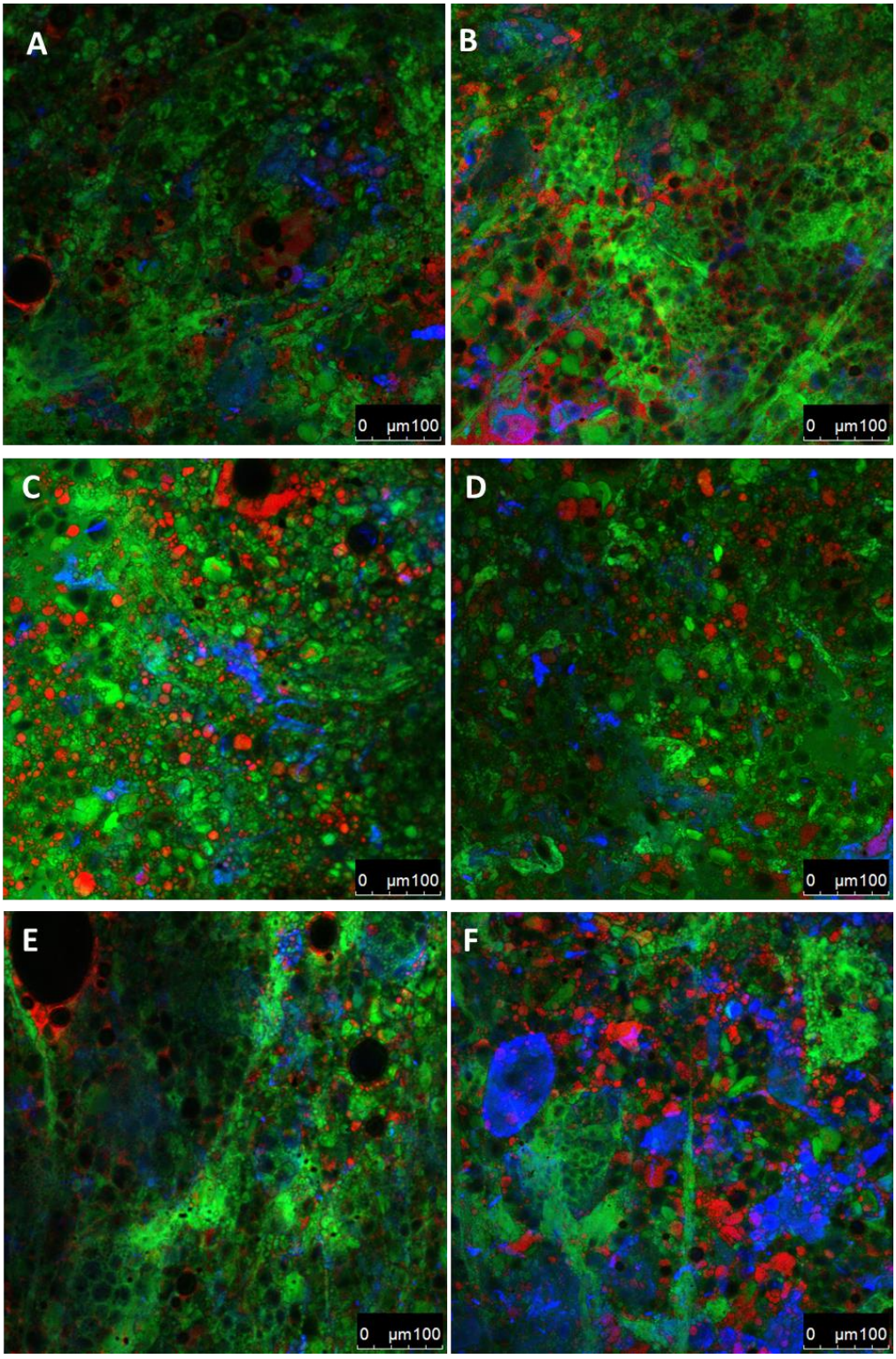


Figure 2-7: CLSM images of the biscuit doughs; control (A), 70% sucrose (B), CFF (cereal fibre and flavouring) (C), PXF (polydextrose and flavouring) (D), MDY (maltodextrin and yeast) (E), and CCA (citrus fibres and apple extract) (F). Protein=red, fat=bright green, starch=dark green with black centre, cellulosic material=blue. Magnification=100µm.

Cellulosic material appears as blue in the images. Apple pomace (Figure 2-7 C), oligofructose (Figure 2-7 D), polydextrose (Figure 2-7 F and Figure 2-8 D), cereal fibres (Figure 2-8 C) and citrus fibres (Figure 2-8 F) are all sources of varying ratios of soluble and insoluble fibres. The addition of fibrous ingredients was clearly seen in the microstructure by the increase in the blue colour (Figure 2-6 C, D and F, and Figure 2-7 C, D and F) indicating an increase in cellulosic material.

With the exception of the increase in cellulosic material, the overall structures of the batters and doughs appeared similar to the control formulation. As mentioned previously, the microstructure of the batter and dough influences the final shape and volume of the baked products. In sections 3.9.1 and 4.4.1 the effect of the fibre content of baked cakes and biscuits will be seen. Overall the batters and doughs containing higher levels of visible cellulosic material will have a smaller specific volume and spread ratio than the control cake and biscuit formulation respectively.

2.3.2.3 Cryo-scanning electron microscopy

Cryo-SEM examines the microstructure of the surface of a sample at low magnifications, displaying the micro components of batter and dough. Samples are frozen in liquid nitrogen, and fractured. Samples are then freeze-dried to prevent ice crystal formation. Figure 2-8 show the images of the cake batter and Figure 2-9 show the images of the biscuit dough. To date, no literature has been found where cryo-SEM was used to examine the effect of sucrose reduction in cake batter and biscuits doughs. Similar to the results from the confocal microscopy, there are many similarities between the control formulations and the reformulated batters and doughs.

The microscopy studies showed clear images of the microstructure of control and reformulated cake batter and biscuit dough. There were clear similarities between in control and reformulated samples of both batter and dough, with regard to the starch, protein and fat organisation within the microstructure. CLSM showed evidence of an increased concentration of cellulosic material in some samples. This was also visualized by the cryo-SEM imaging. Fibrous materials (FM) which may have resulted from the addition of the cereal and citrus fibres can be seen in the Figure 2-9 C and F.

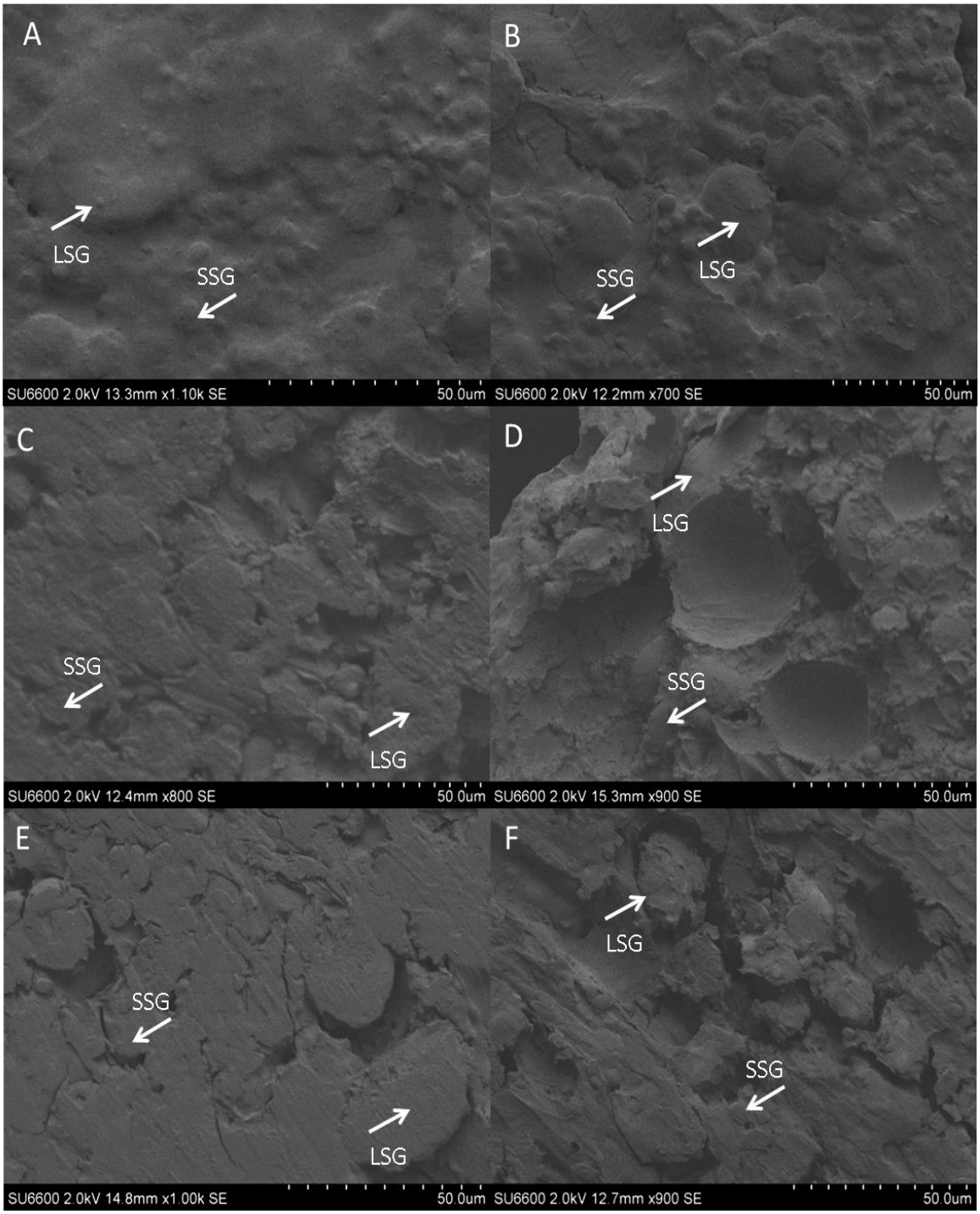


Figure 2-8: Cryo-SEM imaging of cake batters: control (A), 70% sucrose (B), apple pomace (C), oligofructose (D), whey permeate (E), and polydextrose (F). SSG: Small starch granule; LSG: Large starch granule; PM: Protein matrix. Magnification=50µm.

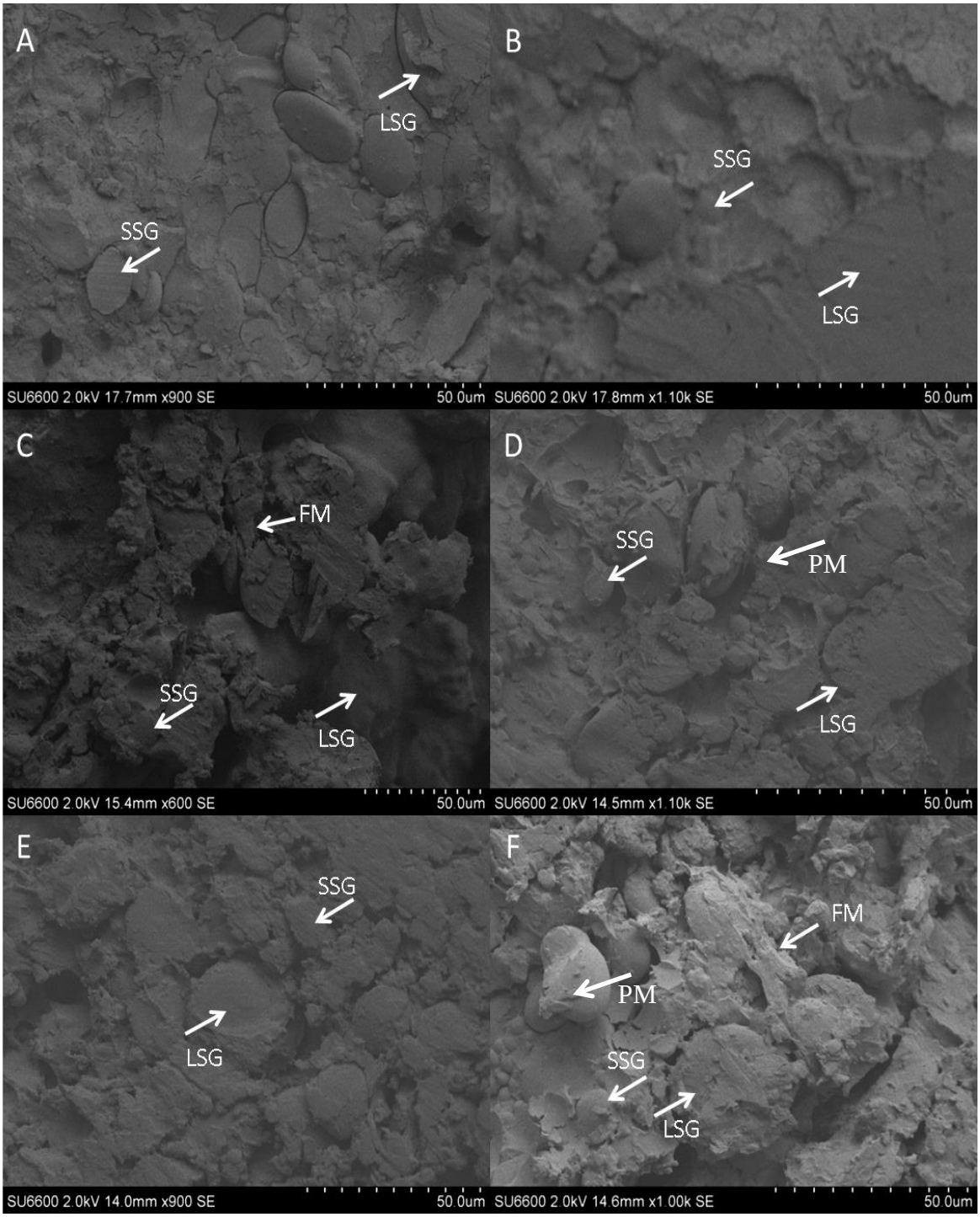


Figure 2-9: Cryo-SEM imaging of biscuit doughs; control (A), 70% sucrose (B), CFF (cereal fibre and flavouring) (C), PXF (polydextrose and flavouring) (D), MDY (maltodextrin and yeast) (E), and CCA (citrus fibres and apple extract) (F). SSG: Small starch granule; LSG: Large starch granule; FM: Fibrous material; PM: Protein matrix. Magnification=50µm.

The microstructure of the reformulated doughs and batters were overall similar to the control, demonstrating the reduction of sucrose was possible without greatly changing the microstructure.

Biscuit dough and cake batter contained a combination of large starch granules (LSG) and small starch granules (SSG) as seen in the micrographs (labelled in Figure 2-8 and Figure 2-9). The starch granules can be seen as a spherical SSG and larger, lentil-shaped granules which are embedded in the protein matrix (labelled in Figure 2-8 E and F and Figure 2-9 D and F). The protein network, which surrounds the starch granules, can be seen in the images and has previously been described in literature (Nandeesh et al., 2011). Starch and protein play an important role in developing the structure of biscuits and cakes, due to their interaction when baking.

2.4 Conclusion

As sucrose is such an important ingredient in bakery formulation, its reduction can have an impact on the batter/dough properties, thus influencing the final baked product. The G' and G" moduli of all cake batters increased significantly from 0.1Hz to 10Hz ($P \leq 0.001$). In particular, the inclusion of apple pomace, oligofructose and polydextrose resulted in a significant difference in G' and G" value at 10Hz ($P \leq 0.05$) during the frequency test. There were no significant differences in rheological properties between the control formulation and the reformulated biscuit doughs. Light microscopy revealed an increase in bubble size and distribution of bubbles in reduced sucrose formulation of batter and dough, likely due to the decrease in viscosity. CLSM showed the similarities of the control and the reformulated batter and dough with regards to the organisation of protein, fat and starch. However the addition of ingredients containing fibres increased the cellulosic material. Cryo-SEM showed the detailed surface of the microstructure, with small and large starch granules surrounded by a protein matrix. Fibrous material was seen in samples containing citrus and cereal fibres. It seems the addition of fibre, rather than the reduction of sucrose, had a greater effect on the microstructure, as seen by CLSM and cryo-SEM. Examining the rheology and microstructure of these cake batters and biscuit dough, allows a deeper understanding of the quality of the baked products.

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CHAPTER 3

Physical, textural and sensory characteristics of reduced sucrose cakes, incorporated with clean-label sugar-replacing alternative

3. Physical, textural and sensory characteristics of reduced sucrose cakes, incorporated with clean-label sugar-replacing alternative ingredients.

3.1 Abstract

High levels of sucrose in foods present a great risk of obesity and type 2 diabetes. Therefore a low sucrose intake is strongly recommended. Sweet baked products incorporate high levels of sucrose. Sucrose in the original cake formulation was reduced and replaced with apple pomace, whey permeate, oligofructose, polydextrose. An acceptable sucrose reduction of between 21-27% was achieved. Cakes containing apple pomace had the lowest specific volume (1.8cm^3/g) and highest crumb firmness (8.60N) ($P<0.05$). Apple pomace and whey permeate had a significantly decreased L* values of the crust ($P<0.05$). Moisture content of the cake crumb was increased significantly with the addition of oligofructose, whey permeate and polydextrose. All treatments resulted in a significant increase of the water activity of the cake crumb compared to the control ($P<0.05$). Crumb cell structure was maintained as shown by 2-D and confocal imaging. Sensory trials revealed the reformulated cakes were acceptable to panellists.

3.2 Introduction

There is an alarming increase in obesity and obesity related disease such as diabetes, heart disease and high blood pressure throughout the developing and developed world (Revels, Kumar & Assuli, 2017). Although risks of obesity can be increased due to genetic and environmental factors, the most common cause of obesity is an energy imbalance.

Sugar is high in calories and found in a wide variety of foods and beverages. Sugar reduction or removal in confectionary products is an important research objective for the food industry, considering negative press, consumer awareness around civilisation diseases and government strategies for sugar reduction in high sugar products (O'Sullivan, 2017). In Europe, some governments have implemented taxes and recommendations for food manufacturers and consumers for high sucrose products. For example, the Irish government introduced a sugar tax in 2018, which increased the price of beverages with a sugar content of greater than 5g/100ml of drink. In the UK, Public Health England has released an action plan to combat childhood obesity. This strategy aims to encourage the food industry to reduce the sucrose content of a variety of products by 20% by 2020. These initiatives are pressuring the food industry to produce reduced sucrose products, while consumers continue to demand high quality products.

Cakes are a high calorie food, containing a high sugar content. The quality of a cake is highly influenced by the quality and balance of ingredients. The primary ingredients of cake are; wheat flour, sugar, fat, egg and leavening agents. Due to the many functions of sucrose in cake products (Richardson, Tyuftin, Kilcawley, Gallagher, O'Sullivan & Kerry, 2018), the reduction of sucrose effects structure, colour, sweetness and shelf life of a cake.

Sucrose is hydroscopic and therefore binds to the water found in the cake batter. This results in an increase in the viscosity of the batter which is important as this helps to retain gas bubbles, increasing the final volume of the cake. As sugar binds with water, this prevents the full hydration of the gluten proteins (found in the flour), preventing the formation of a gluten network (Perego, Sordi, Guastalli & Converti, 2007).

Sucrose increases the temperature of starch gelatinisation and egg protein denaturation, allowing gas bubbles to expand before the formation of the gel (Christ, Takeuchi & Cunha, 2005; Psimouli & Oreopoulou, 2012).

Sucrose is important for the browning of the crust. Although sucrose is not a reducing sugar, when exposed to heat, it degrades to fructose and glucose. These monosaccharaides are reducing sugar and participate in Mallard browning. Sucrose also undergoes caramelization at high temperatures and produces a brown colour (Purlis, 2010).

The present study examines the use of clean label, novel sweetening ingredients (apple pomace, whey permeate, oligofructose, polydextrose) as possible sugar replacers.
Whey permeate is a by-product of cheese and whey protein manufacturing. When the curds and whey are separated in the cheese making process, the whey stream is filtered. Sprayed-dried whey permeate has a minimum of 85% lactose. Lactose is less sweet than sucrose, but it participates in Mallard browning and can crystallise on the surface of baked goods to form a crust and prevent the loss of moisture (Burrington, 2005). Due to the high lactose content, whey permeate is a possible sucrose replacer. However there no studies have been found where whey permeate was used in bakery products for sucrose reduction.

Apple pomace contains a high concentration of dietary fibre, manufactured as a by-product in the apple juice and cider industry. It has been used to enhance the fibre content of cakes and muffins (Masoodi, Sharma & Chauhan, 2002; Rupasinghe, Wang, Pitts & Astatkie, 2009; Struck, Gundel, Zahn & Rohm, 2016; Sudha, Baskaran & Leelavathi, 2007). It has been reported that the addition of apple pomace can increase the perceived sweetness of some baked goods (Rupasinghe et al., 2009).

Oligofructose is a dietary fibre, and formed by the partial enzymatic hydrolysis of inulin. In high concentrations (35%) it can be perceived as sweet as sucrose (Schaafsma & Slavin, 2015). Similar to sucrose, oligofructose has good stability at high temperature (Mensink, Frijlink, Maarschalk & Hinrichs, 2015), contributing to browning and demonstrates humectant properties. Partial replacement of sucrose in bakery products has been achieved in other studies (Handa, Goomer & Siddhu, 2012;

Rossle, Ktenioudaki & Gallagher, 2011; Volpini-Rapina, Sokei & Conti-Silva, 2012). The addition of oligofructose and inulin in cakes has been shown to increase the browning and hardness properties of the cakes (Volpini-Rapina et al., 2012).

Polydextrose is manufactured from glucose, sorbitol and citric acid. It provides 1kcal/g of energy and is a dietary fibre. It is not sweet; however it increases the gelatinization temperature of starch and thickens cake batters. Polydextrose browns when heated and has been used to replace sucrose in cakes and muffin in other studies (Hicsasmaz, Yazgan, Bozoglu & Katnas, 2003; Martinez-Cervera, Sanz, Salvador & Fiszman, 2012; Ronda, Gomez, Blanco & Caballero, 2005). A reduction of sucrose was shown to result in a decreased volume and L* value of the crumb and the sensory panellists deemed the cakes as acceptable.

The objective of the present study was to investigate the effects of reducing sucrose in a cake formulation and include natural sugar alternatives.

3.3 Materials and methods

3.3.1 Ingredients

The ingredients used to prepare the cake batter were: plain wheat flour (Shackleton, Ireland), granulated sugar (purchased locally), fresh whole egg (purchased locally), water, margarine (stork soft blend for cakes, purchased locally), sodium bicarbonate (Royal baking powder, purchased locally), Beneco L91 (an oligofructose), apple pomace, whey permeate, polydextrose.

3.3.2 Batter and cake preparation

Before the current trial, a preliminary baking trial was conducted to establish a maximum level of sugar reduction of the cake formulation and sugar replacing ingredients.

A control formulation and five reduced sugar formulations were prepared as per Table 3-1, where 30% of the sugar was removed, and replaced by 5% replacer flour weight basis.

The cake batter was prepared in a Hobart PMFD158 (UK) mixer. Flour, sugar, egg, water margarine, sodium bicarbonate and sugar replacer were placed into the mixing bowl and mixed at a low speed (speed 1) for 30 seconds. The sides were scraped down, and the speed was increased (to speed 2) for 2 minutes. 80g of cake batter was placed into a pup loaf tin (80mm x 60mm x 40mm) and baked in a conventional oven (Macpan MDBt8, Thiene, Italy) preheated at 180˚C for 45 minutes. Cakes were stored at room temperature until cool and then packed into polypropylene bags and stored overnight to be analysed. All batches were prepared in triplicate. From each batch two cakes were analysed.

Table 0-1: Cake batter formulations, % of total cake batter weight.

	Control cake (%)	Reduced sugar cake (%)	Reformulated cakes (%)
Flour	35.45	37.65	37.1
Fat	16	17	16.6
Egg	16	17	16.6
Water	12.35	13.15	12.9
Baking powder	0.7	0.75	0.7
Sugar	19.5	14.45	14.2
Sugar replacer	-	-	1.9

3.3.3 Cake analysis

3.3.3.1 Physical characteristics

Specific volume

The specific volume of each cake was determined as the ratio of the volume to its weight. Five whole cakes per batch per treatment were randomly chosen and used to calculate the specific volume using the TexVol instrument (BV-L370, Sweden). Samples were analysed on day of baking. The cake was placed into the volumeter vertically.

Crumb and crust colour

The *L**, *a** and *b** values of crust and crumb of the cakes were determined using a Chroma meter CR-410 (Konica Minolta, UK), which had been calibrated using a standardised white tile. Ten readings were recorded on the surface (on the top region of the cake), and ten readings on the crumb colour of the cake for each treatments on day 1 post-baking.

3.3.3.2 Crumb textural properties

Crumb texture

Two slices (1cm) were cut vertically from the centre of the cake. Texture profile analysis was carried out using a TAXT2i Texture Analyser. The test was performed

using each slice. Texture profile analysis was performed using a test speed of 1 mm/s with a strain of 40% of the slice height, a trigger a force of 0.049N was selected. The parameters obtained were hardness (first force peak), chewiness, springiness, and cohesiveness. Measurements were performed in duplicate on each test day (day 1, 3, and 7 post-baking).

Crumb moisture content

Crumb moisture was undertaken according to the AACC 44-15A method on days 1, 3 and 7 post-baking. The crust was removed from central two slices of the cake. The sample was cut into approximately $1cm^3$ cubes and allowed to dry overnight at ambient temperature. The weight of the sample was recorded before and after drying (moisture 1). The dried sample was ground for 30 seconds using NutriBullet 600 (Australia) and sieved with an Endecott test sieve (1680 microns) to ensure crumbs were a uniform size. Three grams of the crumbed cake was placed into the Brabender oven (Mollelec Ltd, UK) at 130˚C for 60min. The weights after second drying were recorded (moisture 2).

Moisture content was calculated by the following equations:

$$\frac{\text{Sample before drying-Sample after drying}}{\text{Sample before drying}} \times 100$$

Equation 0-1: Moisture 1

Moisture % as measured using the Brabender.

Equation 0-2: Moisture 2

$$(\frac{\text{Moisture 1 + Moisture 2)- (Moisture 1 x Moisture 2}}{100})$$

Equation 0-3: Total moisture (%)

Crumb water activity

A section of the central region of the two centre slices was crumbed and the water activity of the cakes was determined by using an Aqua Lab Lite (Decagon Devices, WA, USA). Samples were carried out in duplicate on each test day (day 1, day 3 and day 7 post-baking).

3.3.3.3 Crumb 2D imaging

C-Cell Imaging

The cake was sliced vertically in the centre, and a 1cm slice was cut from each half one day 1 post-baking. The sample was scanned by a C-Cell (Calibre Instruments Ltd, Warrington, UK) using the standard C-Cell software for data analysis. Slice area, height and width were measured, as well as crumb structure including; number of cells, number of holes, average cell diameter, wall thickness, distribution of cell diameters (coarse/fine clustering) and cell elongation characteristics. Measurements were performed in duplicate.

Confocal microscopy

Resin sections of the cake samples were placed on a microscope slide. One drop of 0.1% w/w ethanolic solution of fluorescein iso-thiocynate (FITC) was added to the surface of the sample. After 10 seconds, the FITC was drained off and replaced with one drop of 0.125% w/w aqueous solution of fluorescent brightener 28 (FB28), and one drop of Fast Green FCF (FG, 0.1% w/w aqueous solution) was added. Samples were rinsed with water. A coverslip was placed on top and the images were recorded in a Leica SP5 confocal scanning laser microscope (Leica Microsystems GmBH, Mannheim, Germany).

Readings were carried out at x20 and x63 oil immersion objective. Starch (green) were recorded at 488nm using an argon ion laser. Protein (bright red) was measured at 633nm using a helium-neon laser, and cellulosic materials (blue) were recorded at 405 nm using a blue diode laser.

3.3.3.4 Sensory evaluation

Sensory evaluation of the cakes were carried out by 20 semi-trained panellists on day 1 post-baking. Each panellist received half a slice of each cake (1cm thickness). The samples were coded by three digit random numbers and were served at room temperature in a random order. Water was supplied as a palate cleanser between each sample.

A 9 point hedonic scale was used (9=like extremely; 8=like very much; 7=like moderately; 6=like slightly; 5 =neither like nor dislike; 4=dislike slightly; 3=dislike

moderately; 2=dislike very much; 1=dislike extremely) to rate different parameters such as crust colour, overall appearance, texture while chewing, sweetness, aftertaste, overall acceptability.

3.3.3.5 Nutritional analysis

Sugar analysis

Sugar analysis was performed by ion chromatography (AM/C/1014). Total sugar (sum of individual sugars), mono (fructose, galactose and glucose) and disaccharides (lactose, maltose and sucrose) were measured. Samples were analysed in duplicate.

Fibre analysis

Total fibre analysis was performed using the AOAC 985.29 1986 method. Soluble and insoluble fibre was analysed using the AOAC 991.43, 1994 method. Samples were analysed in duplicate.

3.3.4 Statistical analysis

All tests were replicated three times and mean values and standard deviation were calculated Statistical significance was considered at ($P \leq 0.05$). One-way ANOVA was performed on data using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). Where ANOVA indicated significant differences were present, a Tukey pairwise comparison of the means was conducted to identify where the sample difference occurred. Coefficients were used to determine correlations between results.

3.4 Results and discussion

3.4.1 Physical characteristics

3.4.1.1 Specific volume

The volume of a cake is influenced by the incorporation of air into the batter, and the ability of the batter to entrap the gas. The presence of sugar has been found to have a significant impact on the volume of bakery products (Baeva et al., 2003, De La Hera et al., 2013, Khouryieh et al., 2005).

The apple pomace-containing cake had a lower specific volume than the control ($P \leq 0.05$) (2.05 ± 0.05g/ml). All other reformulated cakes had a lower (but not significant) specific volume (Figure 3-1). The presence of sugar affect bubble formation and expansion and therefore overall volume, as highlighted by this study.

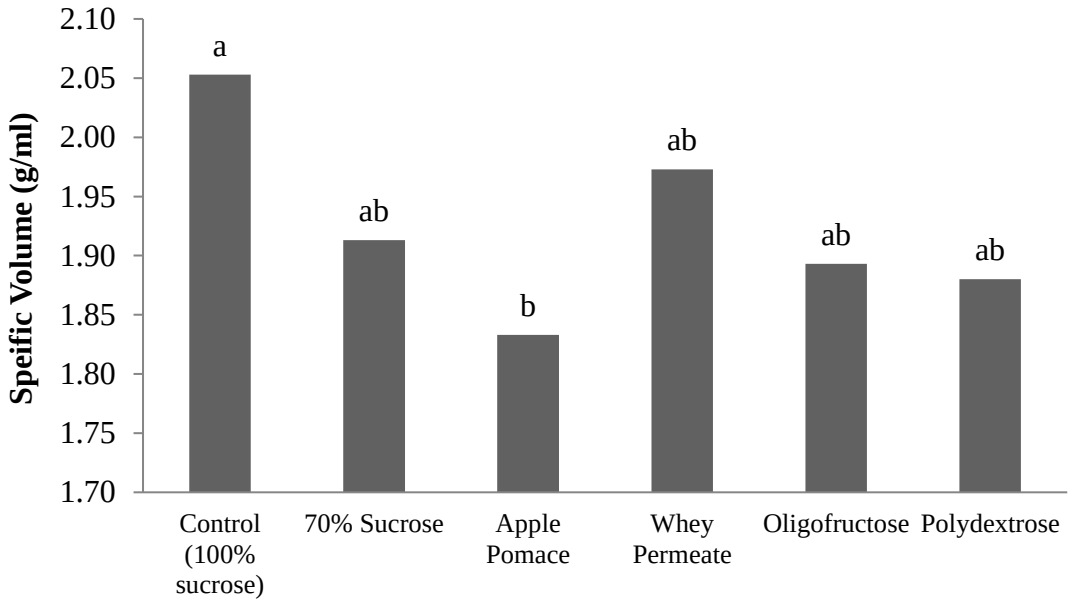


Figure 0-1: Specific volume (g/ml) of control and reformulated baked cakes. Values labelled with the same superscript are not significantly different ($P \leq 0.05$).

In a study conducted by Sudha et al. (2007a), involving fibre enrichment of cake using apple pomace, as the concentration of apple pomace increased there was a concurrent decrease in volume, from 850cm^3 (control) to 620cm^3 as apple pomace increased to 30%. Interestingly, in their trial studying muffin formulations, Rupasinghe et al. (2009) demonstrated how adding apple skin powder (ASP) (4-8%) initially resulted in

the volume of the muffins to increase, however higher concentrations (32%) of ASP resulted in a volume decrease. The authors concluded that this was due to the increase in water binding capacity of the fibres in apple pomace.

Masoodi et al. (2002) demonstrated how the particle size (30, 50, 60 mesh sieve) of the apple pomace effected on cake volume. There was a significant decrease in the volume of the cakes containing 15% inclusion levels, the volume decreased from 961.6 cm^3 (the volume of the control) to 886.6cm^3.

During baking, the volume of a cake can increase by 50-80% (Lostie et al., 2002). The presence of apple pomace has been shown to increase the water absorption of cake batter (Sudha et al., 2007a, Masoodi et al., 2002) due to the high fibre content, thus influencing the viscosity, elasticity, gluten network and subsequently the volume of the batter. When cake batter is heated during baking, the air bubbles begin to expand. The expansion of gas when exposed to heat is responsible for 10-15% of the air bubble inflation in the cake batter. The additional expansion of the bubble is due to the vaporisation of the batter components at the interface of the cell (Zhou and Hui, 2014). Lostie et al. (2002) concluded from their study concerning heat transfer in sponge cake batter, water vapour was the most predominant gas for air bubble expansion in a sponge cake. In the present study, was possible the fibres in the apple pomace bound to the water which usually evaporates from the batter. This could have prevented the build-up of vapour pressure in the air bubble; therefore the bubble could not expand fully, causing a decreased volume of the cake.

3.4.1.2 Crumb and crust colour

Crust and crumb colour of any baked product, especially cakes, is important for overall acceptability. Colour was measured on the Minolta scale, using three axes. *L** indicates the brightness of the sample, where a low number (0-50) indicates a dark coloured sample, and a high number (51-100) indicates a light coloured sample. The *a** value represents the red or the green undertone of a sample, where a positive value indicates more of a red colour, and a negative value indicates more of a green colour. The *b** value refers to the blueness or the yellowness of the sample, where a positive value represents a yellow hue, and a negative value represents a blue hue.

It can be seen in Figure 3-2, the addition of apple pomace and whey permeate resulted in a significant decrease (P≤0.05) in the *L** value of the crust compared to the control, indicating a darker external appearance.

A darker crust has been found in other studies, as well as a crumb colour change from a creamish yellow to a darker brown, following the inclusion of alternative sugar replacers. Studies have shown how the addition of apple skin powder decreased in the *L** value of the muffins, and adding fibres to breads increased in greyness and darkness in crumb colour (Rupasinghe et al., 2009). Likewise Struck et al. (2016) recorded a decrease in the *L** values of cakes containing 30-100% apple fibre.

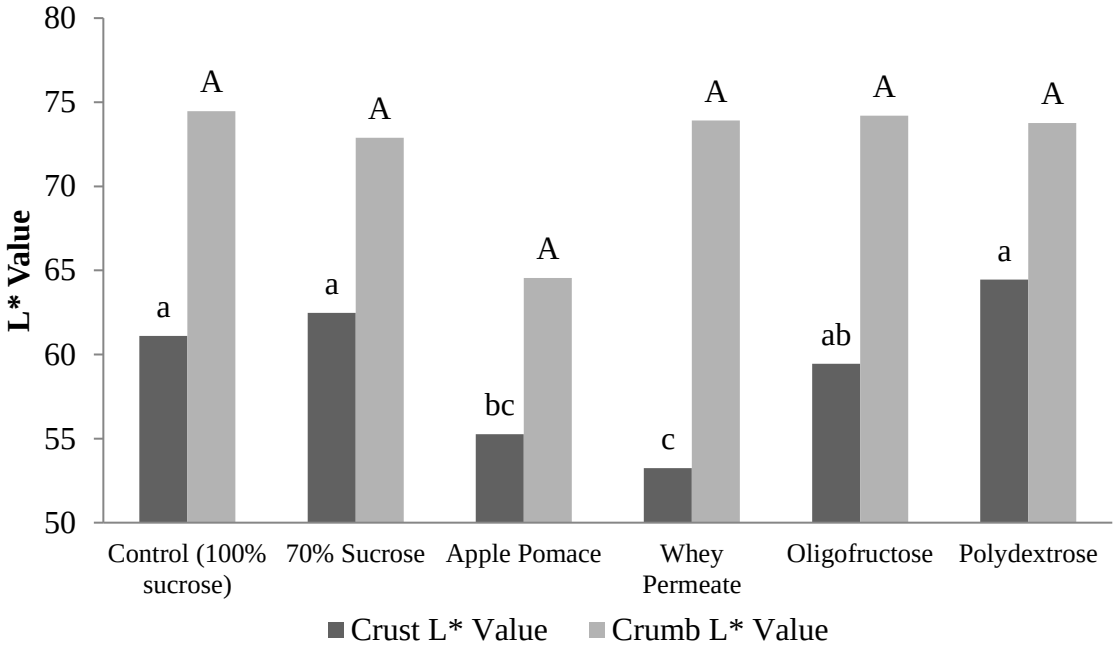


Figure 0-2: Crust and crumb lightness (*L**) values of the control and reformulated baked cakes. Values labelled with the same superscript are not significantly different (P≤ 0.05).

The crust and crumb undertones are represented by a* (red hue) and b* (blue hue) value. The addition of whey permeate in the formulation resulted in an increase in a* values (17.6 ±0.3) of the crust compared to the control cake (14.9±1.2), indicating an increase in a red undertone and a significant decrease in b* in both crumb compared to the control (37.6±1.7 and 39.7±0.9 respectively) (P < 0.05), possibly due to Mallard browning. The apple pomace inclusions significantly increased the in a* value of the

crumb, and decreased the b* value of the crust compared to the control (-0.1±0.2 & 3.4±0.2, 39.7±0.9 & 35, 9±0.5) ($P < 0.05$).

It was noted in other studies where bakery formulations contained inclusions of fruit and vegetable fibres (Rupasinghe et al., 2009), the lightness of the samples decreased, resulting in a darker crumb colour and increasing the greyness of the sample. It is also possible the difference in colour was due to ß-glucan content. Apples are a source of ß-glucan (Jozinovic et al., 2016), which is known to effect the structure and colour of a baked product (Foschia, Peressini, Sensidoni & Brennan, 2013). The apple pomace was produced from pulp and skins of apples; it was not surprising the pomace was a dark orange colour. The darker colour of the pomace coupled with the presence of ß-glucan could have resulted in the difference in colour of the apple pomace-containing sample and the control.

The whey permeate-containing cake had an increased *a** value and a decreased *L** value on the crust. As these colour differences were only found on the crust, this may give evidence the difference in colour is due to Maillard browning. Whey permeate contains high levels of lactose, which participates in Maillard browning (Al-Eid et al., 1999). Non-enzymatic browning has been seen to decrease *L** value in bakery products, and also to increase the red undertone (Rothschild et al., 2015), as also seen in this study.

3.4.2 Textural/ staling properties

Cakes are prone to staling, due to the airy structure of the product, which allows water to migrate from the crumb to the crust, and external environment. This leads to an increase in crumbliness and deterioration of the overall cake texture.

3.4.2.1 Crumb hardness

The hardness of a food is defined as the peak force of the first compression of the TPA profile. Hardness evaluates the ease of the first bite of a food product.

The results from this study are represented in Figure 3-3. Texture profile analysis was conducted on fresh samples (day 1 post-baking) and throughout storage (day 3 and 7 post-baking). All of the treatments showed a significant increase in crumb hardness

during storage (P≤0.05). Cakes containing apple pomace had a significantly harder crumb than the control formulation on day 1 (P≤0.05).

Soluble fibre (which are the fibres found in apple pomace and oligofructose) has been shown to impair textural characteristics and increase hardness of cakes and muffins. In other studies (Sudha et al., 2007a, Rupasinghe et al., 2009) the inclusion of apple fibres increased the firm texture of cakes and muffins. It was revealed that the formulations containing high concentrations of apple pomace did not rise well during baking and were more dense than control formulation. It was hypothesised by the authors this may had been related to the high water binging capacity of apple fibres, resulting in a firmer cake.

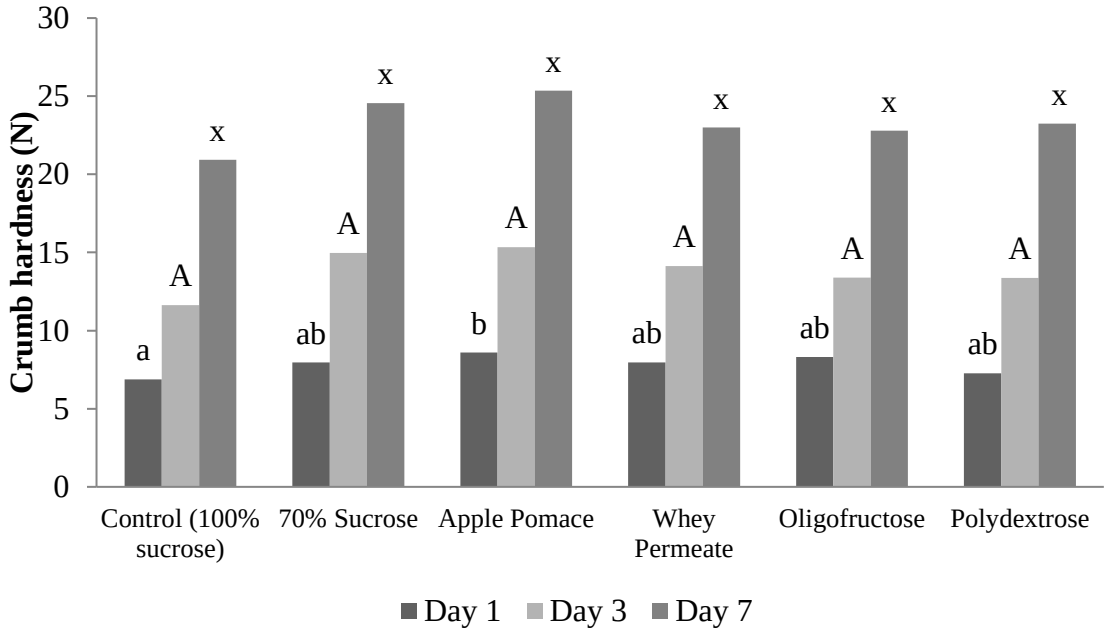


Figure 0-3: Crumb hardness (N) of the control and reformulated baked cakes day 1, 3 and 7 post-baking. Values labelled with the same superscript are not significantly different (P≤ 0.05).

The cake containing 70% sucrose had the largest increase in hardness from day 1 to day 3 compared to all other samples, and a similarly large increase from day 3 to day 7. Although the cake containing 70% sucrose was not significantly harder than the control formulation on day 1, it was not surprising to see a dramatic increase in hardness as days increased. Sugar is a humectant, which can reduce water migration

from crumb to crust. Therefore, a decrease in sucrose may result in an increase in water migration causing a harder crumb.

3.4.2.2 Crumb moisture content

Moisture content and crumb hardness have an inverse relationship. The moisture content of a food product influences textural attributes of the product. Sucrose is hygroscopic and a humecent, which binds to moisture, thus decreasing the rate of this migration. This highly influences the staling and therefore the shelf life of cake.
The results from this study are represented in Figure 3-4. Moisture content was measured using a two stage drying process (AACC 44-15A).

The cakes containing polydextrose had significantly greater moisture contents than the control formulation on each test day ($P \leq 0.05$). Cakes containing whey permeate and oligofructose had significantly greater moisture content on day 3 and 7. The cakes containing whey permeate, oligofructose and polydextrose had the greatest percentage moisture loss from day 1 to day 7 (6.30%, 6.02%, and 6.87% respectively). However at the end of the testing period, crumb moisture content of the cakes contain whey permeate, oligofructose and polydextrose were significantly higher than that of the control.

Jooyandeh et al. (2009) demonstrated how the use of whey permeate (WP) in bread resulted in a significant increase the moisture content of bread compared to the control. Water was replaced in the bread formulation with a solution containing 0-100% WP and water. There was an increase in crumb moisture content from 33.0 ± 0.93% (control) to 37.2 ± 0.57% (100% replacement). The authors hypothesised this increase in moisture was a result of the high water holding capacity of whey proteins (Jooyandeh et al., 2009). Although whey permeate contains a low percentage of whey proteins (maximum 3%), the concentration may still have increased the moisture content of the cake.

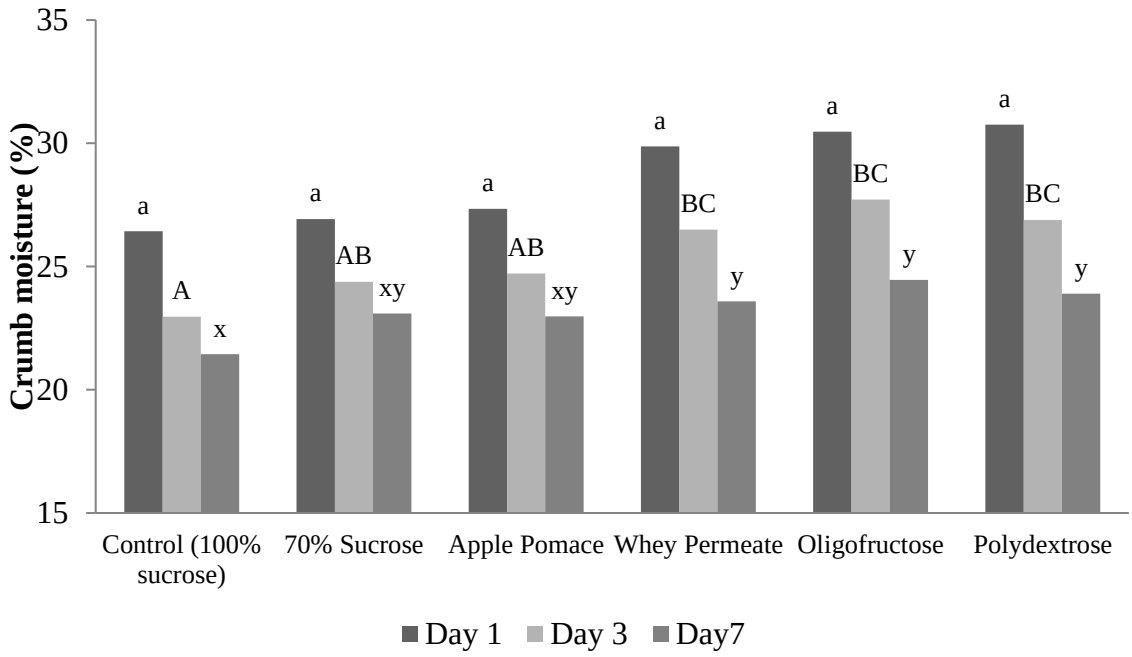


Figure 0-4: Crumb moisture content (%) of the control and reformulated baked cakes day 1, 3 and 7 post-baking. Values labelled with the same superscript are not significantly different ($P \leq 0.05$).

Praznik et al. (2002) observed a significant increase in the moisture content of bread with inclusions of 8 and 10% oligofructose compared to the control bread. This addition of 8 and 10% oligofructose resulted in the moisture content of the control to increase from 43.7 ± 0.2% (control) to 58.5 ± 0.3% and 57.3 ± 0.3% respectively. The significantly greater moisture content of cakes containing oligofructose and polydextrose may be due to the presence of soluble fibres in the ingredients, which have higher water holding capacities.

3.4.2.3 Crumb water activity

Water activity (a_w) is an important factor for the safety and textural attributes of a food. Water activity is influenced by water migration, which influences the texture of the food system. When the food has an a_w greater than the relative humidity of the environment (e.g. cake), moisture migrates from the internal structure to the surroundings, resulting in staling.

The water activity of each treatment was recorded on day 1, day 3 and day 7 post-baking using the Aqualab water activity meter. The results are represented in the Figure 3-5.

Overall, the control formulation had the lowest a_w, (0.891 ± 0.038). Sugar is hygroscopic and therefore this possibly explains why the control formulation had the lowest a_w when compared to the reduced sugar formulations. The a_w of the cakes containing 70% sucrose, oligofructose and polydextrose were significantly greater than the control formulation on all test days. Apple pomace, whey permeate-containing cakes had greater a_w on day 1 and day 3 post-baking (P≤0.05).

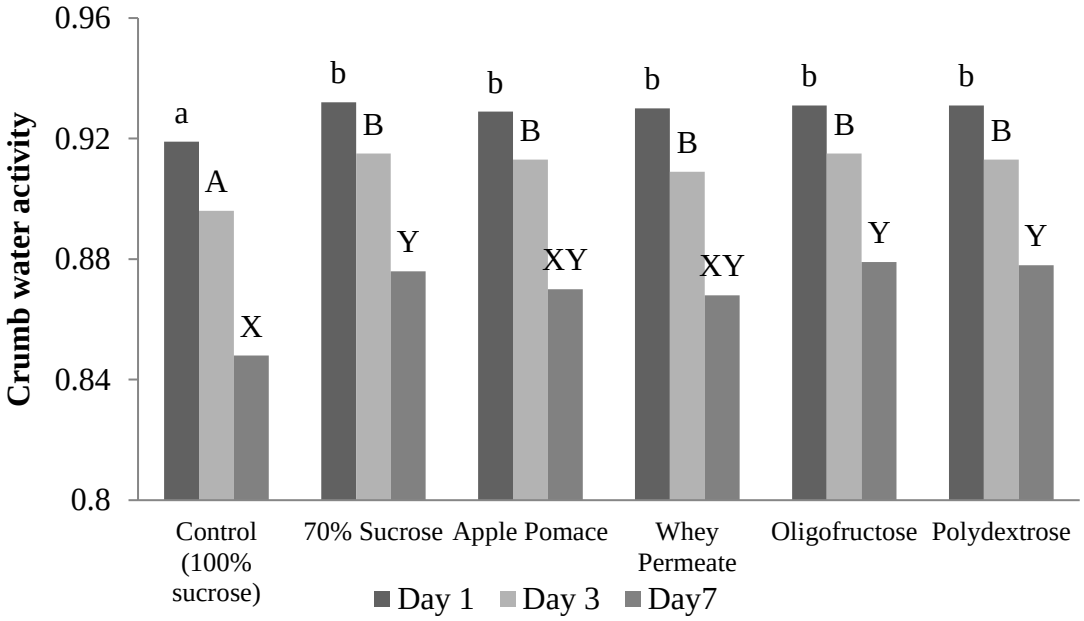


Figure 0-5: Crumb water activity of the control and reformulated baked cakes day 1, 3 and 7 post-baking. Values labelled with the same superscript (a-b) are not significantly different (P≤ 0.05).

The control cakes had the greatest decrease in a_w from day 1 to day 7 (0.919 ± 0.01 on day 1 to 0.849 ± 0.03), whereas the oligofructose containing cake maintained a high a_w from the first sample day to the last (0.931 ± 0.01 to 0.878 ± 0.03).

In a study conducted by Psimouli and Oreopoulou (2012) where sucrose was completely replaced in a cake formulation. The authors revealed the addition of polydextrose and oligofructose resulted in a significant increase in a_w. The high

concentration of fibres in polydextrose and oligofructose may have resulted in an increased a_w.

3.4.3 Crumb 2-D Imaging

3.4.3.1 C-Cell Imaging

Image analysis was used to examine the crumb grain of each formulation. The C-Cell is an image analysis system which evaluates the internal structure of a bakery product.

In this study, two central cake slices from each formulation were analysed. Most interesting results were related to the cell and hole within the slices. The area of cells and holes were calculated by the total number of pixels within a cell or hole, volume was based on the degree of shadow and area of a cell or hole in the crumb. A hole is defined as a cell which is greater than the value of three times one standard deviation.

Table 0-2: Crumb structure (number of cells and volume of holes) of the control and reformulated baked cakes. Values labelled with the same superscript (a-b and A-B) are not significantly different ($P \leq 0.05$).

Treatment	Number of Cells	Volume of holes
Control	1760.17 ± 61.82^{a}	32.79 ± 9.55^{A}
70% Sucrose	1670.75 ± 83.74^{a}	38.29 ± 9.37^{A}
Apple Pomace	1656.33 ± 104.81^{a}	41.245 ± 1.48^{A}
Whey Permeate	1667.42 ± 44.95^{a}	29.73 ± 4.59^{A}
Oligofructose	1735.67 ± 25.77^{a}	26.65 ± 11.52^{A}
Polydextrose	1724.25 ± 61.38^{a}	45.90 ± 7.53^{A}

The number of individual cells was recorded within the slice of each cake. From Table 3-2, it can be seen the control cake had the greatest number of cells. A higher number of cells can indicate a finer structure and a decrease in cell number can indicate a coarser crumb. There was no significant difference between the control formulation and the reformulated cakes. The C-Cell classifies large cells as ‘holes’. The volume of holes refers to the total number of pixels in each hole. Baked goods with a large amount of holes are not desirable as large gas pockets negatively impact the sensory and shelf life of the product. An increase in hole volume could indicate instability in

bubble growth during baking, resulting in the coalescence of smaller bubbles. However, no significant difference where found between the treatments and the control formulation was found in this study.

3.4.3.2 Confocal scanning laser microscopy

Confocal scanning laser microscopy (CLSM) was used to study the internal microstructure in the cake. Images were recorded using a Leica SP5 confocal scanning laser microscope. The images were read at three different wavelengths (405nm, 488nm and 633nm), with three types of lasers (argon, blue diode, helium neon).

Sugar has a major effect on the temperature of gelatinization of starch and also prevents the formation of a gluten network in cakes, therefore the reduction the sugar concentration should have resulted in a change in structure. Although there was a sugar reduction of over 20% in the reformulated cakes (Figure 3-6), there was no significant alteration in the microstructure of the formulation. Alternatively, the sugars and/or fibres found in the sugar replacers may have helped to maintain the structure of the cake (for example, polydextrose is known to increase the temperature of gelatinization in starch).

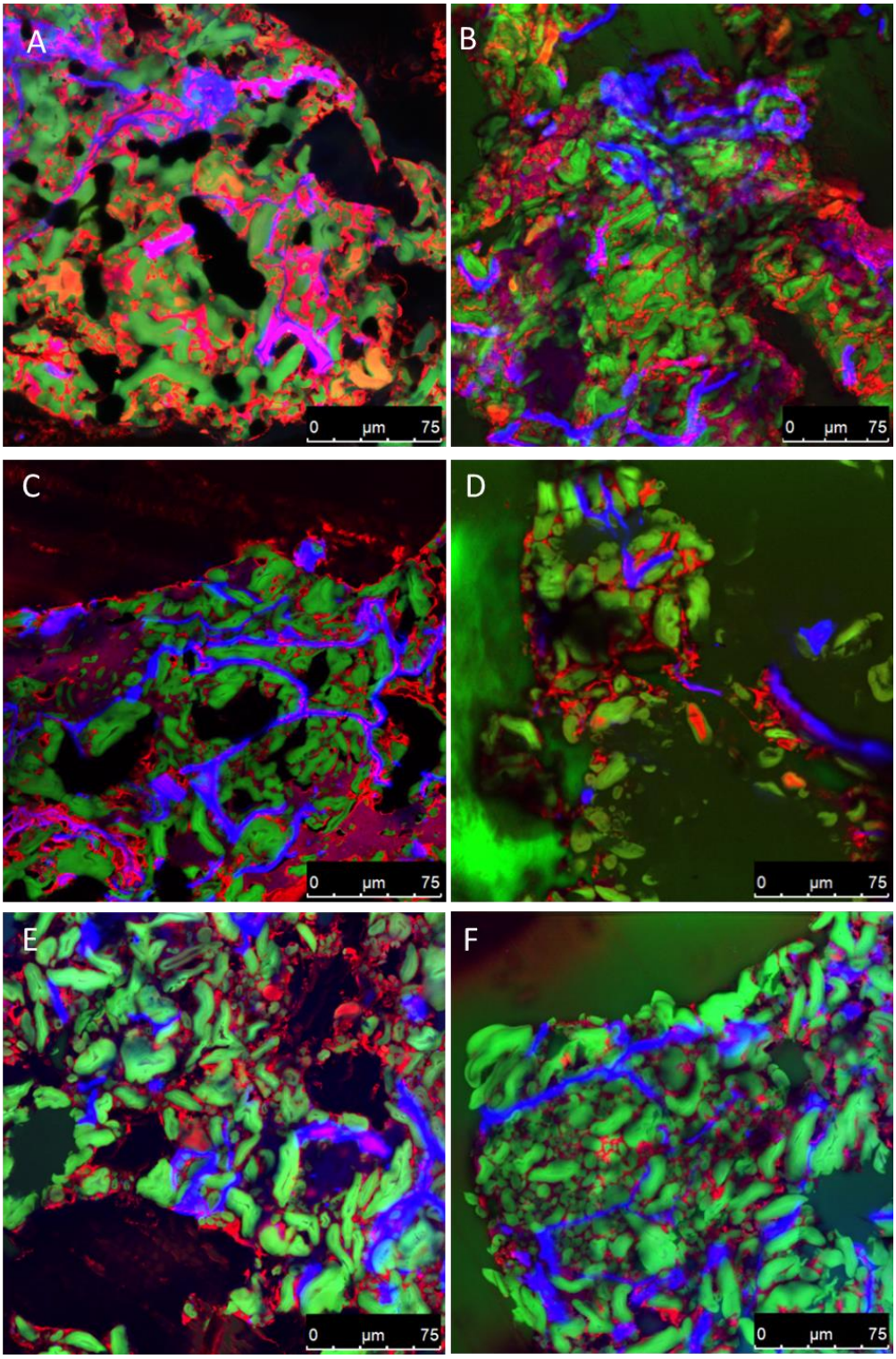


Figure 0-6: CLSM images of baked cake; control (A), 70% sucrose (B), apple pomace (C), oligofructose (D), whey permeate (E), and polydextrose (F). Protein=red, fat=bright green, starch=dark green with black centre, cellulosic material=blue. Magnification=75µm.

3.4.4 Sensory evaluation

Taste and texture are some of the most important attributes when developing a novel product. The panellists were asked to evaluate the cake formulation based on colour of crumb, overall appearance, texture while chewing, flavour, sweetness, aftertaste and overall appearance, rated on a hedonic scale (1 extremely dislike, 9 extremely like). The results are displayed on Figure 3-7.

Overall, the control formulation was favoured and received the highest preference scores in many of the attributes (texture while chewing, flavour, aftertaste, sweetness and overall acceptance). The apple pomace-containing cake was ranked lowest and classified as 'dislike slightly' ($P \leq 0.05$) (4.2). The panellists found the formulation containing apple pomace was the least favoured ($P \leq 0.05$) with regards to colour, texture while chewing, flavour, aftertaste and overall acceptance compared to the control ($P \leq 0.05$). Both apple pomace and oligofructose were significantly less favoured than the control in regards to texture while chewing ($P \leq 0.05$). Importantly, there was no significant difference in sweetness perceived between any treatment and the control ($P \leq 0.05$).

As previously discussed, the addition of apple pomace had a significant impact on many baking properties of the cake. Cakes containing apple pomace had a darker (reduced L^* value) crumb and crust and firmer a crumb. In contrast, other studies (Rupasinghe et al., 2009, Sudha et al., 2007a), cakes containing up to 20% AP were found to be acceptable to the panellists.

In another study, there was a significant decrease in scores for crumb colour in cakes containing apple pomace, as the crumb changed creamy to a darker brown colour according to a sensory evaluation conducted by Sudha et al. (2007a). The authors also found that the cake containing 20% apple pomace was still acceptable to the panel. However, apple pomace was not used as a sugar replacer in either of these studies, which may possibly explain how the authors were able to enrich the cake with apple pomace at such high concentrations without impacting the attributes of the cake negatively.

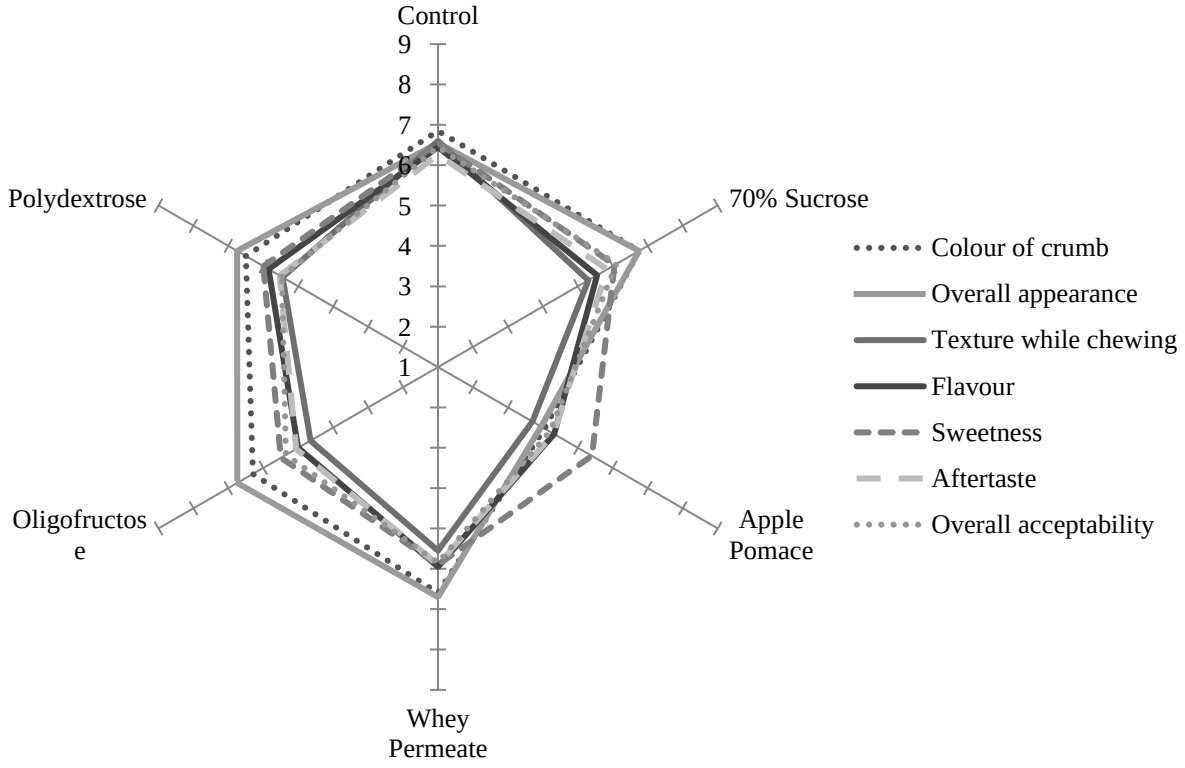


Figure 0-7: Sensory evaluation of baked cakes, as determined by the sensory panellists, scored on a 1–9 hedonic scale.

3.4.5 Nutritional analysis

3.4.5.1 Sugar analysis

Total sugar (sum of individual sugars), mono (fructose, galactose and glucose) and disaccharides (lactose, maltose and sucrose) were measured and results are displayed in Table 3-3.

The reformulated cakes had a total sugar reduction between 21.5% (whey permeate) and 27.6% (oligofructose). European regulation (European Commission, 2006) state for a food product to be classified as 'reduced' in a nutrient, there must be a minimum decrease of 30% however, a reduction of over 20% is a substantive decrease in sugar.

As expected, a notable increase in fructose was observed from the cake containing apple pomace. The cakes containing apple pomace also contained higher concentrations of glucose and maltose. Apples are a known source of fructose (Noro et al., 2006, Tavera-Quiroz et al., 2014) and glucose (Sato et al., 2010).

Table 0-3: Mono and disaccharide and total sugar composition (g sugar/ 100g of cake) of the control and reformulated cakes.

Treatment	Fructose	Galactose	Glucose	Lactose	Maltose	Sucrose	Total Sugars
Control	0.03	< 0.01	0.06	< 0.01	0.09	22.98	23.18
70% Sucrose	0.03	< 0.01	0.06	< 0.01	0.16	17.27	17.54
Apple pomace	0.60	< 0.01	0.26	< 0.01	0.24	16.64	17.76
Whey permeate	0.04	0.04	0.08	1.27	0.17	16.64	18.24
Oligofructose	0.08	< 0.01	0.07	< 0.01	0.16	16.49	16.91
Polydextrose	0.08	< 0.01	0.08	< 0.01	0.16	16.58	16.92

The cakes containing whey permeate contained the highest quantity of lactose and galactose compared to all the other reformulated cake. Whey permeate (as previously discussed) is primarily comprised of lactose (65-85%) (Burrington, 2005). Lactose is a disaccharide which consists of galactose and glucose. This explained the increase in lactose and galactose concentration of cakes containing whey permeate.

Cakes containing oligofructose and polydextrose had approximately 28% decrease in sucrose, and a decrease in total sugars by an approximately 27%. Oligofructose and polydextrose are comprised of predominantly fibres, which may have been the reason why there was less sucrose and therefore total sugars found in the reformulated cakes.

3.4.5.2 Fibre analysis

Soluble and insoluble fibre was conducted using the AOAC 991.43 1994 method and total fibre was determined by the AOAC 985.29 1986 method. Results are shown in Table 3-4.

The addition of apple pomace doubled the soluble fibre content of the reformulated cakes. This resulted in an over 60% increase of total fibres compared to the control. Other studies (Sudha et al., 2007a, de Toledo et al., 2017) revealed an increase in soluble and total fibre due to the addition of apple pomace. The soluble fibres increased by more than double in the polydextrose and oligofructose-containing cakes. The increase in concentration of soluble fibres in the oligofructose and polydextrose was as expected, as they are a known source of soluble fibre.

Table 0-4: Soluble, insoluble and total fibre composition (g fibre/ 100g of cake) of baked cakes.

Treatment	Soluble fibre (g/100 g)	Insoluble fibre (g/100 g)	Total Fibre (g/100 g)
Control	0.74	0.96	1.7
70% Sucrose	0.98	0.82	1.8
Apple pomace	1.48	1.22	2.7
Whey permeate	0.83	0.67	1.5
Oligofructose	1.6	<0.5	1.6
Polydextrose	1.6	<0.5	1.6

3.5 Conclusion

A sugar reduction of between 21-27% was achieved in the cake formulations. The use of polydextrose, whey permeate and oligofructose as sweetening ingredients produced a cakes with similar physiochemical properties, crumb hardness, moisture and a_w as the control cake. Crumb cell structure was maintained for all cakes: there was no significant difference between the control formulation and the reduced sugar cakes as shown by 2-D. It is therefore possible to reduce the sucrose concentration in a cake formulation without dramatically altering the taste, staling profile and overall structure of the cake. Cakes containing apple pomace had the lowest specific volume, highest crumb firmness and scored the lowest in overall acceptability by the sensory panellists. Overall this study showed sucrose can be reduced in a cake formulation and replaced with a range of sugar replacing ingredients without significant changes to the physiological, sensory or staling properties.

CHAPTER 4

The physiochemical effects of sucrose substitution with clean label sweetening ingredients in biscuits.

4. The physiochemical effects of sucrose substitution with clean label sweetening ingredients in biscuits.

4.1 Abstract

Sugar in a biscuit formulation was reduced and replaced with clean label alternative sweetening ingredients. Reduced sucrose biscuits were formulated using a combination of bulking agents (polydextrose, maltodextrin and plant fibres) with extracts (yeast, apple) and flavourings. A sugar reduction of between 14 and 29% was achieved. Biscuits containing cereal fibres and flavouring (CCF) had the greatest spread ratio (P≤0,05), which is a desirable quality of a biscuit. The addition CFF and citrus fibre with apple extract (CFA) had a significant impact on colour, which resulted in a decrease in brightness (*L** value) (P≤0.01). Water activity significantly increased in biscuits containing 70% sucrose and CFA (P≤0.05) on all test days (P≤0.05). The inclusion of CFA also increased the moisture content and hardness compared to the control formulation (P≤0.05). With regards to sensory analysis, the reformulated biscuits were acceptable to the panellists.

4.2 Introduction

Obesity is a worldwide issue and a number of programmes and policies have been implemented by several governments to combat this issue. In Ireland 60% of the population is considered overweight or obese (Safefood, 2011). In response, the Irish government introduced a sugar tax in 2018 on drinks containing 5g of sugar/100ml.

Bakery products, including biscuits, cakes and muffins are calorific due to their high fat and sugar content. Therefore, manufacturers are focussing on reducing calories, sugar or fat while maintaining a high quality and stable shelf life. The approach which was examined in this study was to reduce the sucrose content of a short dough biscuit formulation and replace it with clean label sucrose replacers (cereal fibre, citrus fibres, apple extract, polydextrose, maltodextrin, yeast extract and two natural flavourings).

Sucrose has multiple functions in biscuits, and reducing it can result in a product of inferior quality. Sucrose is the main sweetening ingredient utilised in the biscuit industry and its presence affects sweet flavour, dimensions, colour, hardness and surface finish. Sucrose can inhibit gluten development during dough mixing by competing with the flour for the water in the recipe. It can act as a hardening agent by crystallising as the biscuit cools, thus making the product crisp (Zhou and Hui, 2014). However, in moderate amounts, sucrose can act as a softening agent in biscuits, due to its ability to retain water. Sucrose is hydrolysed to glucose and fructose (reducing sugars) during baking and biscuit colour is affected via Maillard browning reactions between these reducing sugars and amino acids. Compounds which influence the flavour of the biscuit may also be produced by Maillard reactions.

Fibres have many functional properties in a biscuit formulation, including increasing the viscosity in the dough, forming gels and increasing the water and oil holding capacities. Citrus fibres are a by-product of the juice and oil industry and provide a high content of soluble and insoluble fibres. They can also be a source of phytochemicals which are beneficial to human health. The addition of orange fibres in biscuit formulations at low concentrations (less than 10-15%) can increase the total fibre content without negatively affecting baked biscuits (Nassar et al., 2008, Larrea et al., 2005).

Cereal fibres including rice, corn, oat and wheat bran, contain high concentrations of fibre, protein and other vitamins and minerals. Oat bran, corn bran, barley have been added to increase the fibre content in bakery products (Sudha et al., 2007c, Nagi et al., 2012, Singh et al., 2012). At low concentrations (up to 20%), the biscuits were acceptable to the sensory panellists. As the fibre content increased, the firmness/hardness of the baked products also increased. Polydextrose is synthesised from glucose, sorbitol and citric acid and is classified as a dietary fibre. Similar to sucrose, it increases the temperature of gelatinization of starch and is a thickening agent. Polydextrose has been added to a biscuit formulation in a study conducted by Mancebo et al. (2018) and the resulting biscuits were found to have an increased spread ratio and hardness compared to the control formulation. The colour of the biscuit was significantly impacted, as there was a decrease in L^* and a^* value, and a decrease in b^* value, as polydextrose undergoes Maillard browning.

Maltodextrin is a bulking agent and forms gels, allowing it to increase the viscosity. Savitha et al. (2008) replaced sugar with maltodextrin in a biscuit formulation, and found at the lowest concentration (10%), there was an effect on the lightness, hardness and sensory scores of reformulated biscuits compared to the control.

Fruit syrups naturally contain high concentrations of the sugars found in fruit, namely fructose and glucose. No studies were found where apple syrup was used to reduce the sugar content in biscuits or other bakery products; however date syrup has been investigated. Majzoobi et al. (2016) replaced sugar with date syrup in biscuits and found there was a decrease in lightness and consumer acceptability at high concentrations (over 60% replacement).

Biscuits may be flavoured in three ways; by introducing the flavour to the dough or batter before baking, by dusting or spraying the flavour after baking or by flavouring a non–baked portion such as fillings which are applied at a later stage (Manley, 2011). Vanilla (or synthetic vanillin) is a common flavour in baked goods. It may be used on its own as a characterising flavour, or to enhance other flavours (Zhou and Hui, 2014). In this study two natural flavourings and a yeast extract were selected to mask the reduction of sweetness in the reformulated biscuit.

The objective of this study was to reduce sucrose in biscuits and replace it with clean label sweetening alternatives, whilst minimising any negative baking and sensory impacts.

4.3 Materials and methods

4.3.1 Ingredients

Biscuits were prepared using the following ingredients: wheat flour (Shackletons, Ireland), granulated sugar (purchased locally), water, margarine (purchased locally), sodium bicarbonate (baking powder, purchased locally), salt cereal fibres, flavouring, polydextrose, flavouring, yeast extract, maltodextrin, citrus fibre and apple extract.

4.3.2 Dough and biscuit preparation

Following preliminary trials, it was established that 30% sucrose reduction was viable for further investigation. The control formulation and 5 reformulated formulations were prepared as per Table 4-1. The inclusion levels of sucrose replacing ingredients were based on the concentrations advised by the ingredient manufacturers.

Flour, sugar, margarine, salt, sodium bicarbonate, water and sugar replacer were placed into the Hobart PMFD158 (UK) mixer, and mixed at a low speed (speed 1) for 1 minute. The sides were scraped down using a spatula, and the speed was increased (to speed 2) for 2 minutes. The dough was allowed to rest for 30 minutes and sheeted using a pastry dough sheeter (Rondo Doge Econom; RONDO Burgdorf AG, Switzerland) to a thickness of 4mm and cut to shape (diameter of 50 mm). Biscuits were baked for 24 mins at 180˚C in a deck oven (MacPan, Thienne, Italy), allowed to cool at room temperature and placed in sealed polyethylene bags for storage. The biscuits were stored overnight to be analysed at room temperature. All batches were prepared in triplicate.

Table 4-1: Biscuit dough formulations, based on 100g flour weight.

	Control	Reduced sugar biscuits (70)	Cereal fibres & flavouring (CFF)	Maltodextrin & yeast extract (MDY)	Citrus fibres & apple extract (CFA)	Polydextrose & flavouring (PXF)
Flour	100	100	100	100	100	100
Fat	35	35	35	35	35	35
Salt	0.7	0.7	0.7	0.7	0.7	0.7
Sodium bicarbonate	0.5	0.5	0.5	0.5	0.5	0.5
Water	20	20	20	20	20	20
Sugar	35	24.5	24.5	24.5	24.5	24.5
Cereal fibre & flavouring	-	-	10.5	-	-	-
Yeast extract	-	-	-	0.04	-	-
Maltodextrin	-	-	-	8.2	-	-
Citrus fibres	-	-	-	-	2.9	-
Apple extract	-	-	-	-	14.3	-
Flavouring	-	-	-	-	-	0.3
Polydextrose	-	-	-	-	-	5

4.3.3 Biscuit analysis

4.3.3.1 Physical characteristics

Biscuit dimensions

The weight of each biscuit per batch was recorded before and after baking to calculate cook loss. Biscuit width, length, thickness were measured using a Vernier calipers. Spread ratio, volume and density were calculated for each sample using the following equations. All measurements were performed on 20 biscuits on day 1 post-baking.

$$\frac{\text{Length}}{\text{Height}}$$

Equation 4-1: Spread ratio

$$\text{Lenght x Width x Height}$$

Equation 4-2: Volume

$$\frac{\text{Mass}}{\text{Volume}}$$

Equation 4-3: Density

Biscuit colour

Biscuit colour was carried out on the surface of the biscuits using a Minolta Lab colorimeter (CR-400/410, Konica, Minolta, Ireland) on day 1 post-baking. After the colorimeter had been standardized using a white tile, the *L**, *a** and *b** values of 10 biscuits were recorded.

4.3.3.2 Textural properties

Dough and biscuit texture

The texture profile of the biscuit dough was measured using a Texture Analyzer (TA-XT2*i*; Stable Microsystems, Surrey, UK), using the Exponent software version 5.0.6.0. Dough texture was analysed using a 20-mm cylindrical Perspex probe and a 40% compression rate at a test speed of 1mm/s. The hardness of the baked biscuit was measured with a three-point bending rig (HDP⁄3PB). The sample was placed on top of the base with adjustable supports, set 40mm apart. The blade moved downwards at a rate of 2mm/ s, to break the biscuit. The maximum force required to break the sample

was recorded as the hardness of a sample, and the distance at the point of break represented the fracturability of the biscuit. Ten samples for each treatment were recorded on each test day (day 1, 7, 28 and 59 post-baking).

Moisture content

The moisture content of the biscuits was measured on day 1, 7, 28 and 59 post-baking. The samples were ground for 30 second using a NutriBullet 600 (Australia) and sieved with an Endecott test sieve (1680 microns). Three grams of the sample was weighted and dried in the drying oven at 130˚C for 60 mins. Samples were carried out in duplicate. The moisture content was calculated using the following equation:

$$\frac{\text{Sample before drying- Samples after drying}}{\text{Sample before drying}} \times 100$$

Equation 4-4: Moisture content (%)

Water activity

Approx. 2 g of ground sample was placed in the AquaLab meter (Decagon Devices Inc., Pullman, WA, USA), and a_w was recorded. Five measurements were taken for each sample on days 1, 7, 28 and 59 post-baking.

4.3.3.3 Sensory evaluation

Sensory analysis was evaluated one day post-baking with 28 semi-trained panellists. Each panellist received half a biscuit. The samples were coded by three digit random numbers and were served at room temperature in a random order. Water was supplied as a palate cleanser between each sample.

A 9 point hedonic scale was used (9=like extremely; 8=like very much; 7=like moderately; 6=like slightly; 5 =neither like nor dislike; 4=dislike slightly; 3=dislike moderately; 2=dislike very much; 1=dislike extremely) to rate different parameters which included biscuit colour, overall appearance, texture while chewing, sweetness, aftertaste and overall acceptability.

4.3.3.4 Nutritional analysis

Sugar analysis

Sugar analysis was performed by ion chromatography (AM/C/1014). Total sugar (sum of individual sugars), mono (fructose, galactose and glucose) and disaccharides (lactose, maltose and sucrose) were measured. Samples were analysed in triplicate.

Fibre analysis

Total fibre analysis was performed using the AOAC 985.29 1986 method. Soluble and insoluble fibre was analysed using the AOAC 991.43 1994 method. Samples were analysed in duplicate.

4.3.4 Statistical analysis

All tests were replicated three times and mean values and standard deviation were calculated Statistical significance was considered at ($P \leq 0.05$). One-way ANOVA was performed on data using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). Where ANOVA indicated significant differences were present, a Tukey pairwise comparison of the means was conducted to identify where the sample difference occurred. Coefficients were used to determine correlations between results.

4.4 Results and discussion

4.4.1 Physical characteristics

4.4.1.1 Biscuit dimensions

There were no significant differences found between the control biscuits and the reformulated biscuits with regards to length, width, height, volume or density (data not shown).

Spread ratio

The spread ratio is calculated from diameter and height. A high spread ratio is considered a desirable attitude as this implies a large diameter and a lower height. Results are shown on Figure 4-1.

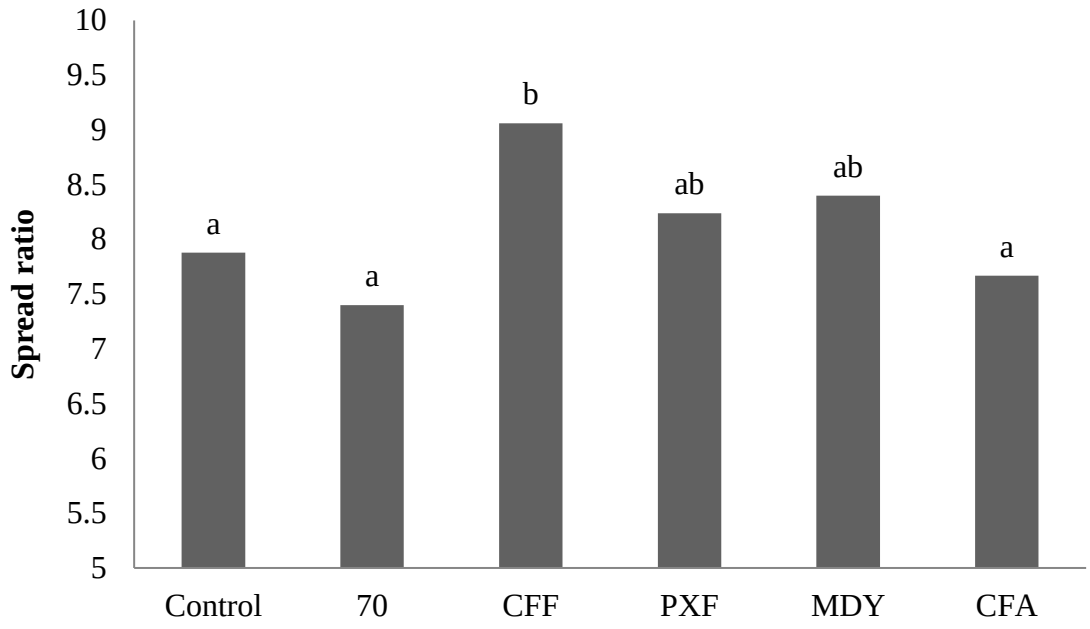


Figure 4-1: Spread ratio of the control and reformulated biscuits containing cereal fibres and flavouring (CFF), maltodextrin and yeast extract (MDY), polydextrose and flavouring (PXF), citrus fibres and apple extract (CFA). Values labelled with the same superscript are not significantly different ($P \leq 0.05$).

Biscuits containing CFF had a significantly higher spread ratio than the control formulation (9.06 and 7.88 respectively). In a study conducted by Sudha et al. (2007c), involving enriching biscuits with cereal fibres (wheat, rice, oat and barley), the authors

demonstrated an increase in fibre resulted in a decrease in spread ratio. A similar trend was seen by Larrea et al. (2005) when citrus fibres were added to biscuits.

4.4.1.2 Biscuit colour

Colour is an important attribute for a consumer as it indicates the 'doneness' of the biscuit. Colour was measured on the Hunter scale, using three axes. *L** indicates the brightness of the sample, where a low number (0-50) indicates a dark coloured sample, and a high number (51-100) indicates a light coloured sample. The *a** value represents the red or the green undertone of a sample, where a positive value indicate more of a red colour, and a negative value indicate more of a green colour. The *b** value refers to the blueness or the yellowness of the sample, where a positive value represents a yellow hue, and a negative value represents a blue hue. The addition of CFF and CFA resulted in a significant decrease in *L** value (70.02 ± 1.12 and 68.09 ± 0.75 respectively) (P≤0.01) compared to the control biscuit (75.33 ± 0.93) (Figure 4-2).

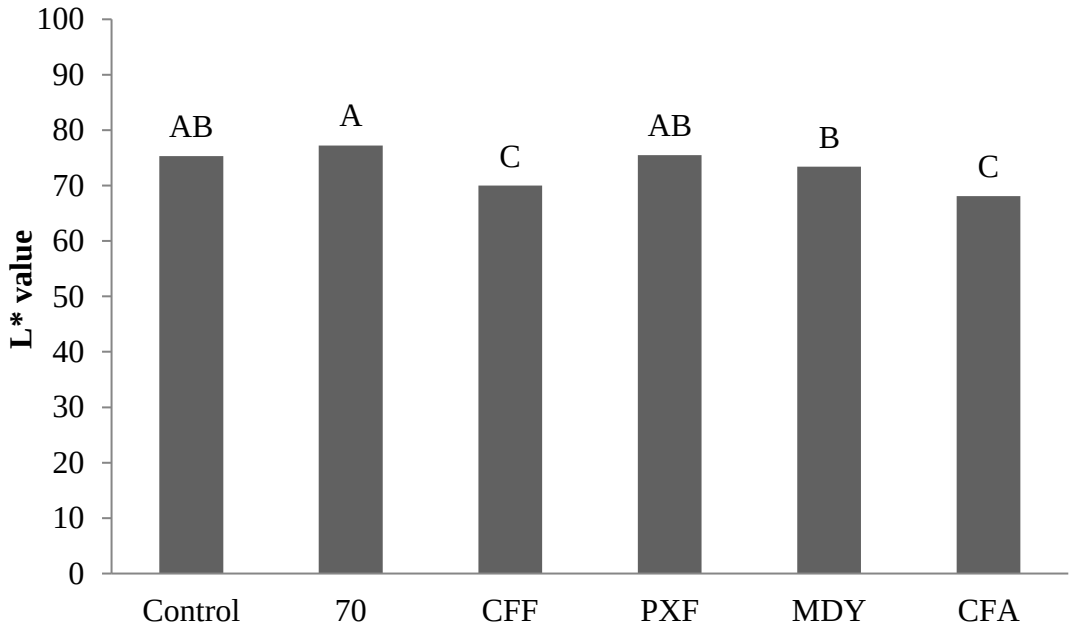


Figure 4-2: Lightness (*L**) values of the control and reformulated biscuits containing cereal fibres and flavouring (CFF), maltodextrin and yeast extract (MDY), polydextrose and flavouring (PXF), citrus fibres and apple extract (CFA). Values labelled with the same superscript are not significantly different (P≤ 0.05).

The biscuits that contained 70% sugar had the greatest *L** value (77.25 ± 1.10), indicating a lighter colour, however this result was not significant (P≤0,05). It was not surprising the 70% sucrose biscuit had lighter colour. Sucrose role in browning (Maillard and caramelization) and therefore a reduction in sucrose will reduce the level of browning. The addition of CFF and CFA resulted in an increase in browning, compared to the 70% sucrose (P≤0,05).

Spread ratio of biscuits containing CFF could have played a role in the decrease in *L** value. An increase in spread ratio creates an increased surface area, and therefore a thinner biscuit, resulting in a darker exterior surface.

Fruit syrups can have a significant impact on the colour of the biscuits. Majzoobi et al. (2016) demonstrated when date syrup was added to a biscuit formulation there was a significant decrease in *L** value compared to the control. The author noted this increase in darkness may have been due to the increase in Maillard browning. Fruit syrups contain a high concentration of fructose and glucose, which are reducing sugars and brown more than sucrose when in contact with proteins in a formulation.

Colour hues are the undertone of the biscuits. The *a** value represents the red to green undertone and the *b** value represent the yellow-blue undertone. Results are displayed in Figure 4-3. The biscuit formulation containing CFA also had a significantly higher *a**value and *b** value (P≤0.01). Biscuits containing CFF had a significant increase in *a**value (5.19 ± 0.52) compared to the control (2.01± 0.19) (P≤0.01). This indicates an increase in yellowness and redness. Majzoobi et al. (2016) replaced sucrose with date syrup in a biscuit formulation, and found there was an increase in *a** value, which the authors believed was a result of Maillard browning.

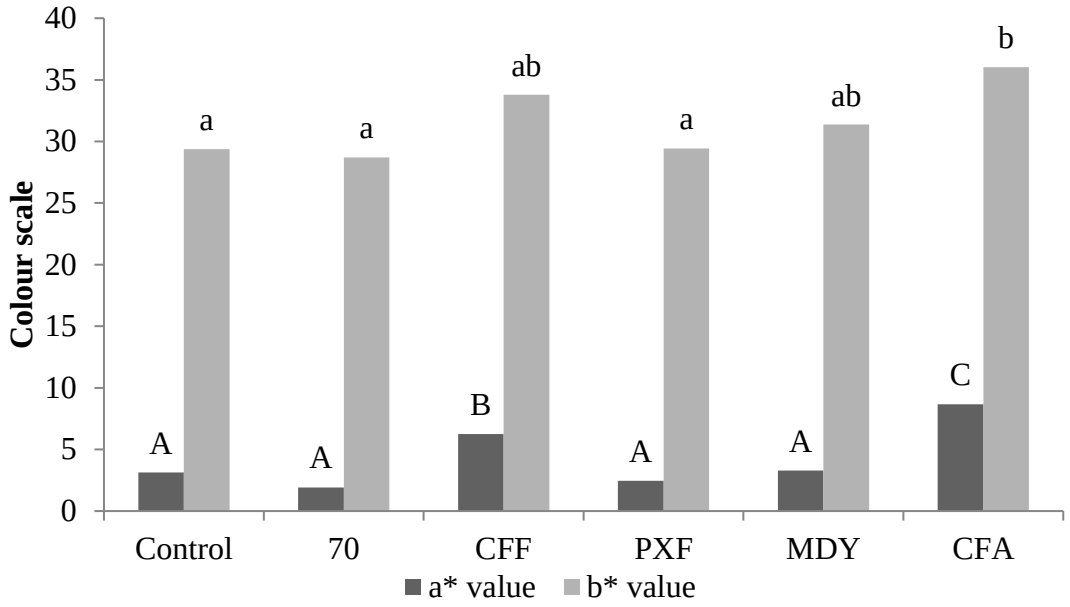


Figure 4-3: Colour hue (*a** and *b** value) of control and reformulated biscuits containing cereal fibres and flavouring (CFF), maltodextrin and yeast extract (MDY), polydextrose and flavouring (PXF), citrus fibres and apple extract (CFA). Values labelled with the same superscript are not significantly different (P≤ 0.05).

4.4.2 Textural properties

4.4.2.1 Dough and biscuit texture

The texture profile of the reduced sucrose doughs were not significantly different from the control formulation (data not shown). Similarly, when the rheological properties of the control and the reformulated biscuit doughs was examined in Chapter 2, there were also no significant differences found.

Hardness was defined as the peak force for the first bite of the first compression of a TPA profile. Consumers perceive biscuits to have a crunchy texture; therefore hardness is an important attribute.

Biscuits containing CFA was significantly harder than the control on all test day (Table 4-2, P≤0.05). The addition of CFF and MDY created a less hard biscuit than the control; however this was not significantly different to the control. There was a significant increase in the hardness of biscuits containing CFA between day 1 and 7. All other treatment did not change significantly through the testing period, indicating

the biscuits were stable with regard to hardness. There was an inverse relationship between spread ratio and hardness on day 1 (r^2=-0.63), biscuits with a large spread ratio were also less hard than the control biscuits, for example biscuits containing CFF, PXF and MDY had a lower breaking force. This was most likely due to the decrease in thickness of the biscuit as it spread when baking. Water activity also had a strong correlation with the hardness of the biscuits (r^2=0.79). Biscuits with a high a_w also tended to be harder than the control although this was not significantly different to the control.

Similar results were found in a study conducted by Larrea et al. (2005), who replaced wheat flour with extruded orange pulp in biscuits. The authors demonstrated how the addition of citrus fibres increased the hardness of the biscuits significantly, even at the lowest inclusion concentration (5%). In a study using date syrup to replace sugar in biscuits, it was found there was a decrease in hardness in the biscuits. The authors indicated this may have been due to the increase in the moisture content of the biscuits and the lower crystallisation rate of fructose and glucose compared to sucrose (Majzoobi et al., 2016). However in the current study apple extract was used, which resulted in a harder biscuit.

Table 4-2: Hardness (N) of baked biscuits day 1, 7, 28 and 59 post-baking. Values labelled with the same superscript are not significantly different (P≤ 0.05).

	Day 1	Day 7	Day 28	Day 59
Control	33.55^{ac}	32.98^{ac}	34.43^{ac}	30.42^{ac}
70	36.59^{abc}	41.74^{bc}	38.07^{abc}	35.43^{abc}
CFF	25.71^{a}	25.96^{a}	25.28^{a}	25.84^{a}
PXF	27.35^{ac}	26.22^{ac}	26.23^{ac}	26.17^{ac}
MDY	36.66^{abc}	27.43^{ac}	26.89^{ac}	28.35^{ac}
CFA	49.47^{d}	69.32^{d}	65.53^{d}	72.80^{d}

70 = 70% sucrose, CFF= cereal fibres and flavouring, MDY = maltodextrin and yeast extract, PXF = polydextrose and flavouring, CFA = citrus fibres and apple extract.

4.4.2.2 Moisture content

The moisture content was recorded on day 1, 7, 28 and 59 post-baking. The results are of the study are shown in on Table 4-3. Biscuits undergo hydrolytic and oxidative rancidity at low moisture contents, and therefore moisture content is important for the shelf life of a biscuit. It has been seen over time (1.5 to 4 months), biscuits show an increased moisture content (Jan et al., 2017, Nagi et al., 2012, Kumar et al., 2014, Singh et al., 2000) This is a result of the hydroscopic properties of biscuits, which are influenced by the environmental factors such as temperature and relative humidity.

The control formulation had the lowest moisture content on day 1 and day 7 (3.83 and 4.11%), while biscuits containing CFF had the lowest moisture content on day 28 and 59 (4.61 and 4.22%). The biscuits containing CFA had significantly higher moisture content than the control formulation on day 1 and day 7 post-baking (7.22 and 7.17% respectively). All of the formulations, with the exception of the biscuits containing CFA, had an increased moisture content between day 1 and 28, and a decrease from day 28 to day 59.

Table 4-3: Moisture content of baked biscuits day 1, 7, 28 and 59 post-baking. Values labelled with the same superscript are not significantly different ($P \leq 0.05$).

	Day 1	Day 7	Day 28	Day 59
Control	3.83^{b}	4.11^{ab}	4.66^{abde}	4.39^{ab}
70	5.89^{abcde}	6.39^{acde}	6.61^{acde}	6.05^{abcde}
CFF	4.33^{ab}	4.28^{ab}	4.61^{abe}	4.22^{ab}
PXF	5.11^{abcde}	5.34^{abcde}	5.5^{abcde}	5.11^{abcde}
MDY	4.39^{ab}	5.67^{abcde}	5.78^{abcde}	5.12^{abcde}
CFA	7.22^{c}	7.17^{cd}	$6.95c^{de}$	6.45^{acde}

70 = 70% sucrose, CFF= cereal fibres and flavouring, MDY = maltodextrin and yeast extract, PXF = polydextrose and flavouring, CFA = citrus fibres and apple extract.

Fruit syrups have been shown to increase the moisture content in reduced sugar biscuits. Majzoobi et al. (2016) observed the significant increase in moisture content of a biscuit formulation in which date syrup replaced sucrose. There was an increase

in moisture content from 3.85% ± 0.04 (control) to 4.44% ± 0.08 (100% date syrup replacement). The author revealed the increase in moisture was likely a result of the increased solubility of fructose and glucose compared to sucrose. Fructose and glucose have a greater interaction with moisture in the biscuit, and therefore moisture in the dough is prevented from evaporation during baking, resulting in an increased moisture content. Larrea et al. (2005) demonstrated the addition of orange fibres also increased the moisture content in cookie. The author hypothesised this increase in moisture was a result of the water binding properties of the orange fibres.

4.4.2.3 Water activity

Water activity (a_w) is an important attribute as it contributes to the safety and overall quality of the food. In a food with a low a_w moisture is absorbed from the surrounding into a food. In biscuits, this results in the biscuits becoming softer. The a_w of each treatment did not significantly change over the testing days, indicating a shelf stable product ($P \leq 0.05$). The control biscuit maintained the lowest a_w throughout storage, while the biscuits containing CFA had the highest a_w. As sucrose reduces a_w in a food, it is not surprising the control had the lowest a_w. Biscuits containing CFA and the reduced sucrose biscuits had significantly higher a_w on all test day ($P \leq 0.05$).

Table 4-4: Water activity of baked biscuits day 1, 7, 28 and 59 post-baking. Values labelled with the same superscript are not significantly different ($P \leq 0.05$).

	Day 1	Day 7	Day 28	Day 59
Control	0.287^{a}	0.282^{a}	0.288^{a}	0.284^{a}
70	0.458^{bcdf}	0.441^{bcdf}	0.455^{bcdef}	0.444^{bcdef}
CFF	0.293^{a}	0.284^{a}	0.307^{ae}	0.307^{ae}
PXF	0.341^{ade}	0.342^{acde}	0.355^{ade}	0.357^{acde}
MDY	0.342^{ade}	0.374^{abcdef}	0.395^{abcde}	0.387^{abcdef}
CFA	0.523^{f}	0.501^{bcf}	0.493^{bf}	0.494^{bcf}

70 = 70% sucrose, CFF= cereal fibres and flavouring, MDY = maltodextrin and yeast extract, PXF = polydextrose and flavouring, CFA = citrus fibres and apple extract.

The significant increase in biscuits containing CFA may be a result of the increased fibre content. Citrus fibres are high in soluble fibres, and therefore may bind to more water. This may have resulted in a higher a_w Similarly, fructose and glucose found in fruit syrups have been found to help maintain moisture (Majzoobi et al., 2016), and therefore increase in these monosaccharaides may result in an increase a_w.

Biscuits containing 70% sucrose also had significantly greater a_w than the control. This demonstrates the important of the inclusion of sucrose to maintain a low a_w, which is needed to ensure shelf stability.

4.4.3 Sensory evaluation

Sensory analysis examines some of the most important attributes for new foods including taste, appearance and texture. This study was conducted using 28 un-trained panellists to evaluate colour, appearance, texture, sweetness, aftertaste and overall acceptability. These attributes were measured using a hedonic scale (1 extremely dislike; 9 extremely like).

With regard to colour, biscuit containing 70% sucrose and biscuits containing PXF were significantly less preferred to the control (Figure 4-4, $P \leq 0.01$). These biscuits had higher L^* values than the control formulation (75.3), indicating a lighter colour. The biscuits containing CFF had a low L^* value (70.0), but received the highest preference colour score (7.0) of all treatments, indicating the sensory panellists preferred a darker colour biscuit. However, it is important to note the darkest biscuit, which had the lowest L^* value (CFA), received a lower colour preference score compared to the control. This indicates the panellists had a minimum L^* value which they found acceptable. Biscuits containing 70% sucrose and PXF also had significantly lower appearance scores than the control biscuit ($P \leq 0.05$).

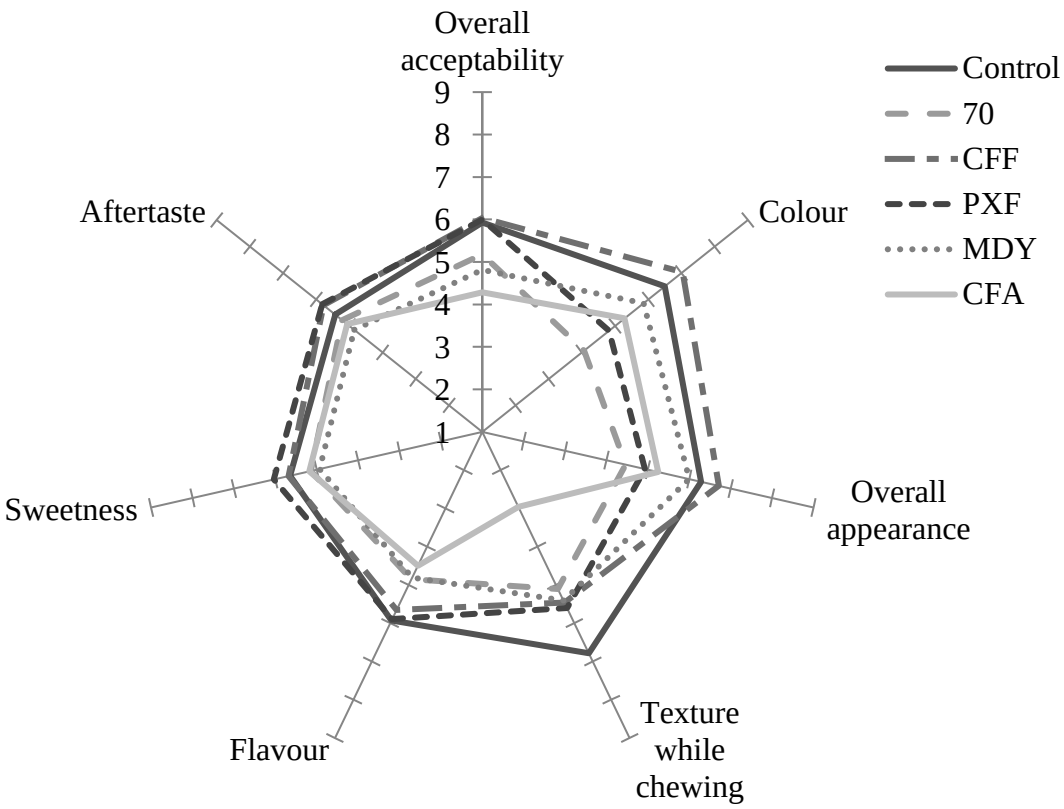


Figure 4-4: Sensory evaluation of baked biscuits, as determined by the sensory panellists, scored on a 1–9 hedonic scale. 70 = 70% sucrose, CFF= cereal fibres and flavouring, MDY = maltodextrin and yeast extract, PXF = polydextrose and flavouring, CFA = citrus fibres and apple extract.

The control formulation had the highest score for texture while chewing (6.8) which was significantly higher than biscuits containing CFF, MDY and CFA and the reduced sucrose biscuit. This highlights the importance of sucrose in for texture of a biscuit. Interestingly, the instrumental analysis which measured the hardness of the biscuit only distinguished a significant difference between the control formulation and the biscuits containing CFA. This demonstrates the importance of carrying out a sensory analysis tests of texture evaluation.

Biscuits containing CFA scored significantly lower with regard to overall acceptability (4.3) compared to the control (5.9). There were no significant differences found between the control and the treatments with regard to flavour, sweetness or aftertaste.

Biscuits containing PXF received the highest scores with regard to flavour and sweetness, while both biscuits containing PXF and CFF received the highest scores for aftertaste and overall acceptability. Biscuits containing CFF also received the highest score for colour and overall appearance.

Singh et al. (2000) added corn bran to a cake formulation to replace wheat flour and found there was no significant differences found between the control and the cakes containing 10 and 20% corn bran with regards to their sensory properties (appearance, colour, texture, taste and overall acceptability). Likewise Sudha et al. (2007c) enriched a biscuit with oat bran and found no significant differences as ranked by the sensory panel at concentrations of 10 or 20% with regards to colour, texture, taste and mouth feel. In other studies (Nassar et al., 2008, Larrea et al., 2005) it was found citrus fibres in biscuits did not significantly impact the sensory scores at low concentrations (less than 25%). Overall it appears at low concentrations (less than 20-25%) addition of fibre, biscuits are still deemed as acceptable to a sensory panel. However, Similar results were documented by Majzoobi et al. (2016) as there were no significant differences found by the panellist in biscuits in which sucrose was replaced with date syrup at 20 and 40%. Zoulias et al. (2002) replaced fat in biscuits with polydextrose, it was found there was a significant decrease in flavour and general acceptance as scored by a sensory panel.

4.4.4 Nutritional analysis

4.4.4.1 Sugar analysis

The sugar concentration of the biscuits was analysed using ion chromatography (AM/C/1014). Total sugar and mono and disaccharides were measured and are shown in Table 4-5.

There was a total sugar reduction of between 28.6% (PXF) and 14.7% (CFA). For a food product to be classified as ‘reduced in sugar’, there must be a reduction of at least 30% (European Commission, 2006). Although the biscuits cannot be labelled as ‘reduced’, a reduction of more than 15% without many negative alterations to the biscuit is a substantial decrease.

The biscuits containing CFA showed an increase in fructose and glucose. These biscuits contained an apple extract, and apples contain high levels of fructose and glucose (Noro et al., 2006, Tavera-Quiroz et al., 2014, Sato et al., 2010). Therefore this increase is expected.

Table 4-5: Total sugar composition (g sugar/ 100g of biscuit) of baked biscuits.

Treatment	Fructose	Galactose	Glucose	Lactose	Maltose	Sucrose	Total sugar
Control	0.06	<0.01	0.06	<0.01	0.17	22.85	23.1
70	0.04	<0.01	0.04	<0.01	0.34	16.21	16.6
CFF	0.09	<0.01	0.53	<0.01	0.7	15.63	17
PXF	0.08	<0.01	0.05	<0.01	0.28	16.08	16.5
MDY	0.04	<0.01	0.11	<0.01	2.79	15.7	18.6
CFA	2.66	0.04	1.95	<0.01	0.32	14.71	19.7

70 = 70% sucrose, CFF= cereal fibres and flavouring, MDY = maltodextrin and yeast extract, PXF = polydextrose and flavouring, CFA = citrus fibres and apple extract.

4.4.4.2 Fibre analysis

The fibre content of the baked biscuits were analysed using the AOAC 991.43 1994 (soluble and insoluble fibre) and AOAC 985.29 1986 (total fibre) methods.

Table 4-6: Total fibre composition (g fibre/ 100g of biscuit) of baked biscuits.

Treatment	Soluble fibre	Insoluble fibre	Total fibre
Control	2.9	<0.5	2.9
70	2.5	<0.5	2.5
CFF	2.7	0.5	3.2
PXF	2.3	<0.5	2.3
MDY	2.3	<0.5	2.3
CFA	2	1.6	3.6

70 = 70% sucrose, CFF= cereal fibres and flavouring, MDY = maltodextrin and yeast extract, PXF = polydextrose and flavouring, CFA = citrus fibres and apple extract.

The European Commission (2006) states for a food product to be a ‘source of fibre’, there must be at least 3g of fibre per 100g. As shown in Table 4-6, biscuits containing

CFF and CFA contained more than 3g of fibre (3.2g and 3.6g respectively) due to the increase in cereal and citrus fibres. However, although these samples can be classified as a 'source of fibre', these products have a high calorie and fat content, and therefore this claim should not be used as it may mislead the consumer.

4.5 Conclusion

The sucrose content of the reformulated biscuits were reduced by up to 29%, and the fibre content was increased by 10-24%. There were no significant differences found between the reduced sugar biscuits and the control with regard to dough properties, volume, density and biscuits dimensions. The spread ratio of biscuits containing CFF significantly increased. Biscuits containing CFF, PXF and MDY had similar textural properties, such as a_w, hardness, moisture, to the control on all test days. All treatment received similar sensory scores as the control in regards to flavour, aftertaste and sweetness. This study demonstrates the potential use of fibres and natural flavouring (particularly CFF and PXF) to create a reduced sucrose biscuit which maintains overall high sensory scores and a similar staling profile as a control biscuit formulation.

CHAPTER 5

The impact of sucrose reduction on the acoustic (sound) properties, volatile profile, and sensory attributes of a biscuit formulation.

5. The impact of sucrose reduction on the acoustic (sound) properties, volatile profile and sensory attributes of a biscuit formulation.

5.1 Abstract

Although sucrose has many functions in baked products, it is linked to diseases such as diabetes and obesity and therefore, a reduction in the consumption in sucrose is recommended by health professionals. In this study, sucrose was reduced by 40% in a biscuit formulation. The effectiveness of polydextrose (PDX) and citrus fibres (CF) as clean label sucrose replacers was studied. The texture, acoustic, sensory and aromatic properties of the biscuits were examined on day 1, 7 and 28 post-baking. There was a significant increase in the moisture content of the reduced sucrose biscuits and biscuits containing CF and PDX ($P \leq 0.001$). The hardness of the biscuit formulations was not significantly affected following the reduction in sucrose. However, the control formulation had the lowest fracturability compared to the biscuits containing 60% sucrose on all test days, CF on day 1 and 7, and PDX on day 1 post-baking ($P \leq 0.05$). The acoustic (sound) emissions of the reformulated biscuits were significantly less than the control on all test days ($P \leq 0.05$). The reformulated biscuits contained significantly less volatile compounds associated with Maillard browning, while the volatile compounds associated with lipid oxidation increased. Sensory panellists scored all formulations as acceptable.

5.2 Introduction

Due to the increase in risk of obesity and diabetes, the Irish government introduced a sugar tax on high sugar drinks in 2018. Pressure is now being placed on food manufacturers by consumers to reduce sucrose in food products. However, sucrose has multiple functions in foods and therefore its reduction results in significant changes to the texture, appearance, taste and staling properties of the food. This is particularly true in baked goods, including cakes, biscuits, muffins and brownies (Richardson et al., 2018).

Sucrose is a common sweetener used in biscuit production, and it influences the shape, texture, appearance and flavour of the product. The presence of sucrose reduces the development of a gluten network, allowing biscuits when baked to have a 'shortness' which gives a crumbly texture. Sucrose crystallizes on the surface of the biscuit when baked to produce a crisp exterior creating a desirable texture (Zhou and Hui, 2014). Although sucrose itself does not partake in Maillard browning, its simple sugars, glucose and fructose (reducing sugars), react with amino acids which result in browning during baking.

When dough is baked, there are changes not only to the textural and physicochemical properties, but also to the organoleptic aspects of a biscuit. Baking induces browning (Maillard reaction and caramelization) and lipid peroxidation (Ameur et al., 2008) which produces a mixture of flavour compounds. These compounds are most commonly produced on the surface of the product, due to exposure to the high oven temperatures. Flavour compounds produce an aroma, which is a key attribute to the quality of a baked product. Solid-phase microextraction (SPME) can be used for volatile aroma analysis. Which has been employed to investigate the effect of lipid oxidation (Purcaro et al., 2008, Mildner-Szkudlarz et al., 2009) and the addition of antioxidants (Pasqualone et al., 2014) in biscuits. However, no studies were found which examined the effect of sugar reduction on flavour compounds.

The texture of a food product, such as a biscuit, is evaluated on the mechanical (hardness, fracturability) and acoustic (crispy and crunchy) properties during biting, which contributes to the overall sensory experience. For a food to be considered

'crunchy' or 'crispy' it must be difficult to deform, but will fracture and break under pressure to create a sound. Acoustic emission is the process of sound waves forming as well as a signal registration (Lewicki et al., 2009, Duizer, 2001). The acoustic vibrations are received by the eardrum through the air and the soft tissue, and interrupted as the sound of the biscuit breaking/ deforming (Błońska et al., 2014). When pressure is applied to a sample, microfractures occur, which release acoustic energy. This gives a helpful indication of the texture of the food. Crunchy foods often have several fracture events, compared to crispy foods, which have fewer fractures.

Sound is the result of the food product quickly deforming; if the food deforms slowly it does not create an acoustic emission. Although the acoustic energy of a sample is only a small part of the energy which is required to break a product, it affects the sensory perception of crispy and crunchy (Luyten et al., 2005). The consumer has a perceived expectation of the acoustic properties, particularly in the case of a biscuit. If the sound does not meet the consumer's expectation, the food product may be perceived as inferior. Changing the ingredients of a formulation has been reported to alter the acoustic properties of a food.

While no studies were found which investigated the effects of sucrose reduction on the acoustic properties of a food product, the effects of addition of apple pomace to extruded snacks was examined (O'Shea et al., 2012). It was found that the addition of apple pomace resulted in a decrease in the acoustic emission, indicating a less crunchy product. The author hypothesised that this was due to the increased concentration of soluble fibres such as pectin. In another study, fat was replaced with inulin in a biscuit formulation, the acoustic energy which was required to break the biscuit significantly increased (Błońska et al., 2014). The author reported this increase in acoustic energy was a result of the flour components' exposure to water which produced a crunchier product. Crispy and crunchy sounds are created from dry foods with low water activity. Foods with a low activity absorb moisture from the environment over time; they can become less crunchy or crispy.

Sucrose has many functions in a biscuit formulation as discussed above, as well as sweetening the product. To replace some of these other functions of sucrose, bulking agents such as polydextrose, cellulose, inulin, maltodextrin and plant fibres have been

used in baked products (Alonso and Setser, 1994, Hicsasmaz et al., 2003, Kocer et al., 2007, Devereux et al., 2003, Rose et al., 2010, Ronda et al., 2005, Gómez et al., 2010). In this study, the potential of using fibres as sucrose replacers was examined as they show excellent bulking properties (Lundberg et al., 2014).

Citrus fruits are currently an underused source of fibre and are a by-product of the juice and oil industry. Although the use of citrus fibres as a sucrose replacer has not been studied in literature, they are rich in soluble and insoluble fibre and therefore increase the viscosity of a batter or dough. The addition of orange fibre as a flour replacer in biscuit formulations at low concentrations (less than 10-15%) has been shown to increase the total fibre content without negatively affecting baked biscuits (Nassar et al., 2008, Larrea et al., 2005). Polydextrose is classified as a dietary fibre, and is manufactured from glucose, sorbitol and citric acid. It has similar properties to sucrose, with regard to its ability to thicken batters, and to increase the temperature of gelatinization of starch. Polydextrose has shown the potential to be used as a sucrose replacer; in a cake formulation, polydextrose was used to completely replace sucrose (Ronda et al., 2005). An increase in the brightness (L^* value) was seen in the crust of cakes, containing polydextrose, indicating a lighter colour compared to the control. A decrease in specific volume was also noted due to the early gelatinization of starch and the denaturation of the proteins, reducing the time to allow bubble expansion. However, the sensory panellists deemed the cakes to be acceptable.

This study aimed to investigate the effect of sugar reduction on the instrumental volatile profile and overall sensory quality of a biscuit formulation. Clean label ingredients (citrus fibres and polydextrose) were used to replace the sucrose.

5.3 Materials and methods

5.3.1 Ingredients

Four biscuit formulations were prepared using wheat flour (Shackleton, Ireland), granulated sugar (purchased locally), water, margarine (Stork, purchased locally), sodium bicarbonate (Royal baking powder, purchased locally), salt, polydextrose (Kerry Group, Ireland) and citrus fibre (Herbafood, Germany).

5.3.2 Biscuit preparation

The control and the reformulated biscuits were prepared as per Table 5-1 and using the method from chapter 4, Section 4.3.2.

Table 5-1: Biscuit formulations, based on 100g flour weight.

	Control	Reduced sugar biscuits (60% sucrose)	Citrus fibres (CF)	Polydextrose (PDX)
Flour	100	100	100	100
Fat	35	35	35	35
Salt	0.7	0.7	0.7	0.7
Sodium bicarbonate	0.5	0.5	0.5	0.5
Water	20	20	20	20
Sugar	35	21	21	21
Citrus fibres	-	-	6	-
Polydextrose	-	-	-	6

5.3.3 Biscuit analysis

5.3.3.1 Physical characteristics

Biscuit colour

A minolta Lab colorimeter (CR-400/410, Konica, Minolta, Ireland) was calibrated using a white tile. The brightness (*L**), the green-red undertone (*a**) and the blue-yellow undertone (*b**) of 10 biscuits were recorded on day 1 post-baking.

5.3.3.2 Textural properties

Moisture content

Biscuits were ground in a NutriBullet 600 (Australia) for 30 seconds, and sieved with an Endecott test sieve (1680 microns). Samples were weighed and 3g were dried at 130˚C for 60 mins in a drying oven. The tests were carried out in duplicate on day 1, 7 and 28 post-baking. The moisture content was calculated using the following equation:

$$\frac{\text{Sample before drying-Sample after drying}}{\text{Sample before drying}} \times 100$$

Equation 5-1: Moisture content (%)

Water activity

Water activity (a_w) was recorded using an AquaLab meter (Decagon Devices Inc., Pullman, WA, USA) using approximately 2g of ground sample. Five measurements were taken for each formulation on day 1, 7 and 28 post-baking.

Biscuit texture

Biscuit hardness was recorded using Texture Analyzer (TA-XT2*i*; Stable Microsystems, Surrey, UK), equipped with the Exponent software (version 5.0.6.0) with a three-point bend rig (HDP⁄3PB) attachment. The sample was placed on top of the base with adjustable supports, set 40mm apart. The blade move was set to downwards at a rate of 2 mm/s, to break the biscuit. The maximum force required to break the sample was recorded as the hardness, and the distance at the point of break represented the fracturability of the biscuit. Hardness and fracturability of 10 biscuits were measured on day 1, 7 and 28 post-baking.

Acoustic properties

Acoustic emissions and mechanical properties of the biscuits were measured simultaneously as they were fractured on the three point bend rig. A combination of the Texture Analyser equipped with acoustic envelope detector was used to record the acoustic properties of the biscuit. Before the test began, the microphone was calibrated using an Acoustic Calibrator Type 4231 (94dB and 114dB SPL-1000Hz).

The test was performed in a quiet laboratory and at room temperature. A gain amplifier was used at level 1, set to signal and filter out any surrounding noise from the laboratory. The microphone (Brual & Kjaer 4188-A-021) was held in a stand 20 mm away from the sample. The Texture Exponent software recorded 500 points per second for acoustic emissions. Acoustic energy and linear distance of each sample were measured. Testing was performed using ten biscuits from each formulation on day 1, 7 and 28 post-baking.

5.3.3.3 Volatile analysis-head space solid phase microextraction

Sample preparation

3g of sample was added to an amber 20ml screw capped SPME vial and equilibrated to 60°C for 5mins with pulsed agitation of 5sec at 350rpm. The samples were analysed in triplicate. Sample introduction was accomplished using a Gerstal MultiPurpose Sampler (MPS) (Garvey, et al., 2019).

GCMS Method

Samples were prepared as per Garvey, et al. (2019). A single 50/30um Carboxen™/ divinylbenzene/ polydimethylsiloxane (DVB/CAR/PDMS) fibre was used. The SPME fibre was exposed to the headspace above the samples for 60min at depth of 21mm at 60°C. The fibre was retracted and injected into the GC inlet and desorbed for 3min at 250°C. Injections were made on a Shimadzu 2010 Plus GC with an Agilent DB-624 UI (60m x0.32mm x1.80µm) column using a split/splitless injector in splitless mode with a merlin microseal. The temperature of the column oven was set at 40°C, held for 5min, increased at 5°C/min to 230°C then increased at 15°C/min to 260°C, yielding at total GC run time of 65min. The carrier gas was helium held at a constant pressure of 43.8psi. The detector was a Shimadzu TQ8030 mass spectrometer detector, ran in single quad mode. The ion source temperature was 220°C and the interface temperature was set at 260°C. The MS mode was electronic ionization (70v) with the mass range scanned between 35 and 250amu. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, a commercial flavour and fragrance library and an in-house library created in GCMS Solutions software (Shimadzu, Japan) with target and qualifier ions and linear retention indices for each compound. Spectral deconvolution was also performed to confirm identification of compounds using AMDIS. An auto-tune of the GCMS was carried out prior to the

analysis to ensure optimal GCMS performance. A set of external standards was ran at the start and end of the sample set and abundances were compared to known amounts to ensure that both the SPME extraction and MS detection was performing within specification.

5.3.3.4 Sensory evaluation

Sensory acceptance testing was conducted using 31 untrained assessors (Stone et al., 2012b, Stone et al., 2012a) on day 1 post-baking. Panellists received one biscuit from each treatment at room temperature, labelled with a random 3 digit code. Water was supplied as a palate cleanser between each sample.

A 9 point hedonic scale was used (9=like extremely; 8=like very much; 7=like moderately; 6=like slightly; 5 =neither like nor dislike; 4=dislike slightly; 3=dislike moderately; 2=dislike very much; 1=dislike extremely) to rate different parameters which included overall acceptability, colour, overall appearance, texture while chewing, sweetness and aftertaste.

5.3.4 Statistical analysis

All tests were replicated three times and mean values and standard deviations were calculated. Statistical significance was considered at ($P \leq 0.05$). One-way ANOVA was performed on data using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). Where ANOVA indicated that significant differences were present, a Tukey pairwise comparison of the means was conducted to identify where the sample difference occurred. Pearson's correlation coefficients were used to determine correlations between results.

5.4 Results and discussion

5.4.1 Physical characteristics

5.4.1.1 Biscuit colour

The exterior colour of a biscuit in an important attribute for consumer acceptability as it shows the 'doneness' of the product. A reduction of sucrose resulted in biscuits with an increased *L** value indicating a lighter colour however results were not significant (data not shown). The colour undertone (*a** value and *b** value) results are shown on Figure 5-1.

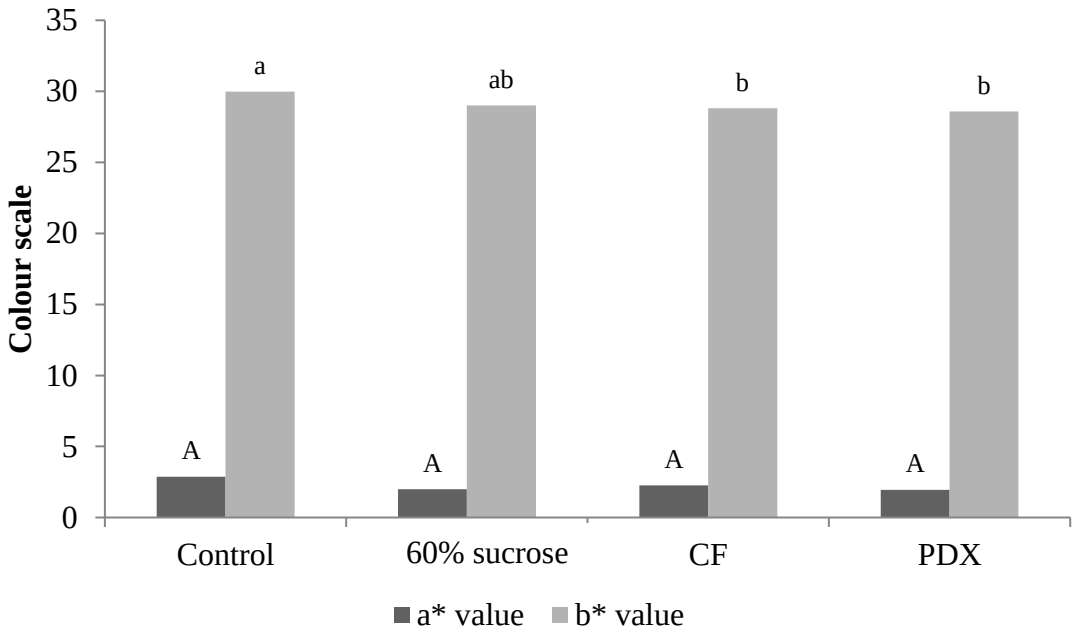


Figure 5-1: Colour hue (*a** and *b** value) of the control and reformulated biscuits containing 60% sucrose, citrus fibres (CF) and polydextrose (PDX). Values labelled with the same superscript are not significantly different (P≤ 0.05).

There were no significant differences found between the control and the reformulated biscuits with regard to *a** value. The addition of CF and PDX had a significant impact on the *b** value of the biscuit (28.81 ± 0.86, 28.58 ± 0.39 respectively) (P≤0.05). The *b** value refers to the blue and yellow undertones of the sample, where a positive value represents a yellow undertone, and a negative value represents a blue undertone. The biscuits containing CF and PDX showed a significant decrease in *b** value indicating a decrease in the yellow undertone. However, while these results were significantly

different to the control formulation, the differences were small enough that they are unlikely to be seen by the human eye.

5.4.2 Textural properties

5.4.2.1 Moisture content

As the biscuit stales, it absorbs water from the environment and therefore the moisture content of the biscuit typically increases (Jan et al., 2017, Nagi et al., 2012, Kumar et al., 2014, Singh et al., 2000). The moisture content is important with regard to the pleasant taste of the biscuit. Fats which are exposed to a high moisture content can undergo oxidative and hydrolytic oxidation resulting in an off taste and odour of the biscuit (Giannou et al., 2014). Moisture results are displayed on Figure 5-2. The moisture content of the biscuit samples were recorded on day 1, 7 and 28 post-baking.

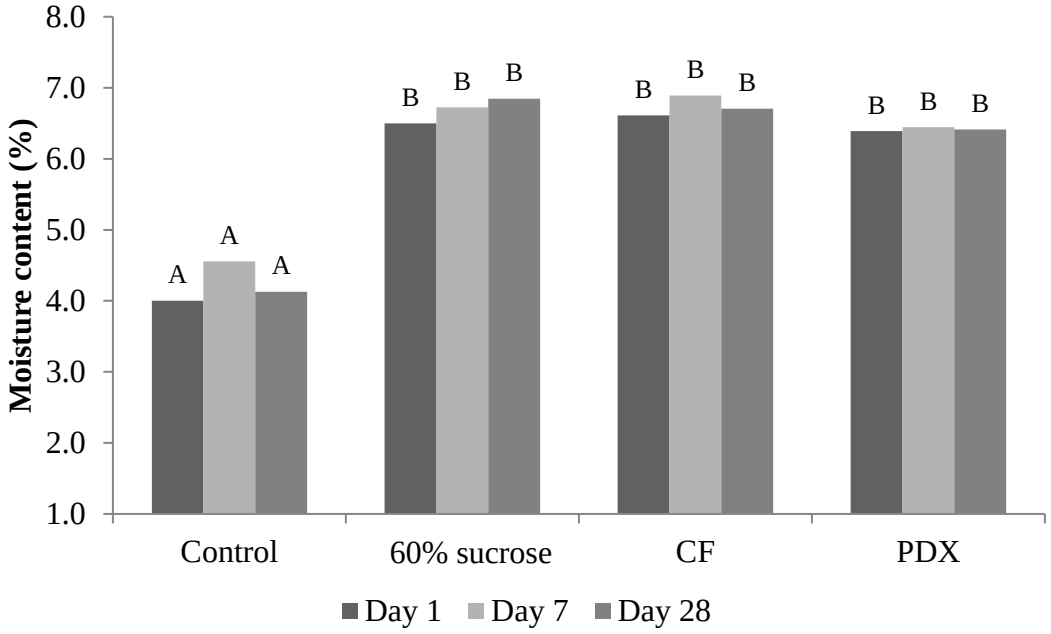


Figure 5-2: Moisture content of the control and reformulated biscuits containing 60% sucrose, citrus fibres (CF) and polydextrose (PDX) day 1, 7 and 28 post-baking. Values labelled with the same superscript are not significantly different ($P \leq 0.05$).

In this study, all reformulated biscuits had a significantly higher moisture content than the control formulation on all test days ($P \leq 0.001$). Within each formulation no significant increase was seen over the 28 day test period. Biscuits most commonly have a moisture content of less than 4% (Giannou et al., 2014). The control formulation had the lowest moisture content on all days (4.00%, 4.56% and 4.13%

respectively). All reformulated biscuits maintained higher moisture content (greater than 6%) due to the reduction in sucrose as seen in Figure 5-2. The addition of orange pulp fibres was seen to significantly increase the moisture content of the biscuit formulation by 0.25-2.64% of sample, due to the high water binding properties of the orange fibres (Larrea et al., 2005).

In chapter 4 Section 4.4.3.2, a reduction of 30% sucrose did not result in a significant increase in moisture content in the reformulated biscuits when compared to the control formulation. From these studies, it can be concluded that a sucrose reduction of greater than 30% but less than 40% is possible without adverse effects to the moisture content of a biscuit formulation.

5.4.2.2 Water activity

Water activity (a_w) is a key factor in the safety of a food product. Generally a low a_w prevents the growth of a number of pathogenic bacteria. The a_w of a food also indicates if the product is likely to absorb or lose water throughout its shelf life. All biscuit formulations followed a similar trend with regard to a_w. All treatments showed an increase (though not significant) throughout storage, as moisture from the environment is absorbed by the biscuits. The results are shown in Figure 5-3.

The control formulation had the lowest a_w throughout the testing period (0.191, 0.249 and 0.301), while biscuits containing 60% sucrose continuously had the greatest a_w value on day 1 post-baking and throughout storage (0.391, 0.397 and 0.453). All treatments had significantly higher a_w than the control formulation on day 1 post-baking ($P \leq 0.05$). Biscuits containing 60% sucrose had significantly higher a_w values than the control on day 7 and 28 ($P \leq 0.05$) highlighting the importance of sucrose in reducing the a_w in food products.

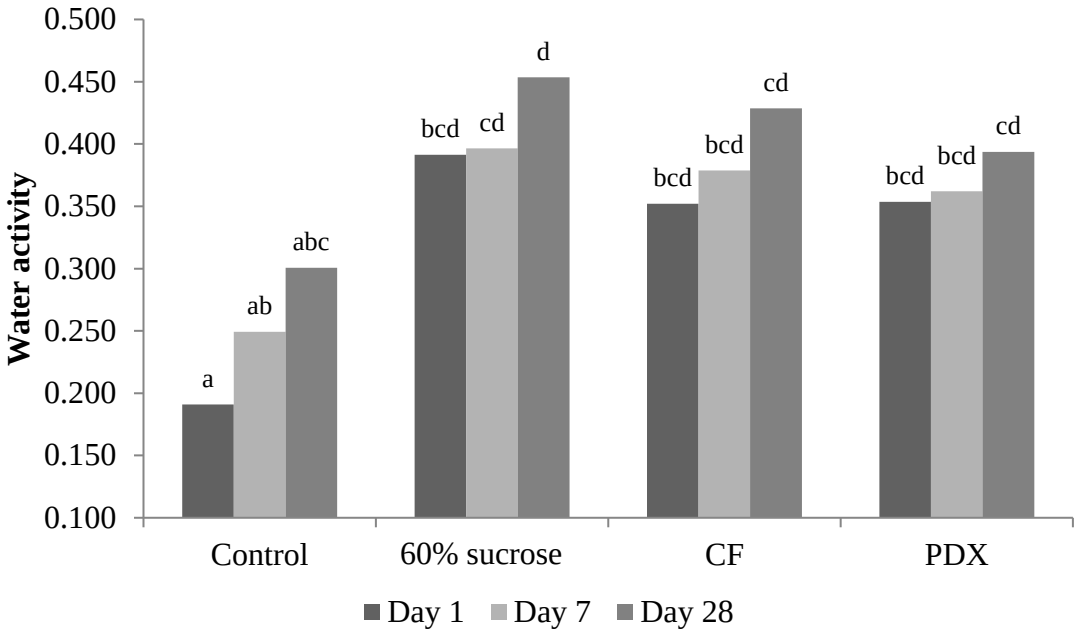


Figure 5-3: Water activity of the control and reformulated biscuits containing 60% sucrose, citrus fibres (CF) and polydextrose (PDX) day 1, 7 and 28 post-baking. Values labelled with the same superscript are not significantly different ($P \leq 0.05$).

5.4.2.3 Biscuit hardness

The hardness property of a biscuit, as measured by a Texture Analyser, is the maximum force required to break the biscuit. Hardness is an important quality as it contributes to the texture of the food. As consumers have a preconceived expectation of biscuits, the hardness helps the quality of the biscuit to be established.

In this study, there were no significant differences between the reformulated biscuits and the control (Figure 5-4). Similar results were observed in chapter 4, Section 4.4.3.1, where no significant differences were found between the control biscuit formulation and the reduced sucrose biscuit or biscuits containing polydextrose over all test days. This showed a reduction of up to 40% sucrose was possible without affecting the hardness of the biscuits.

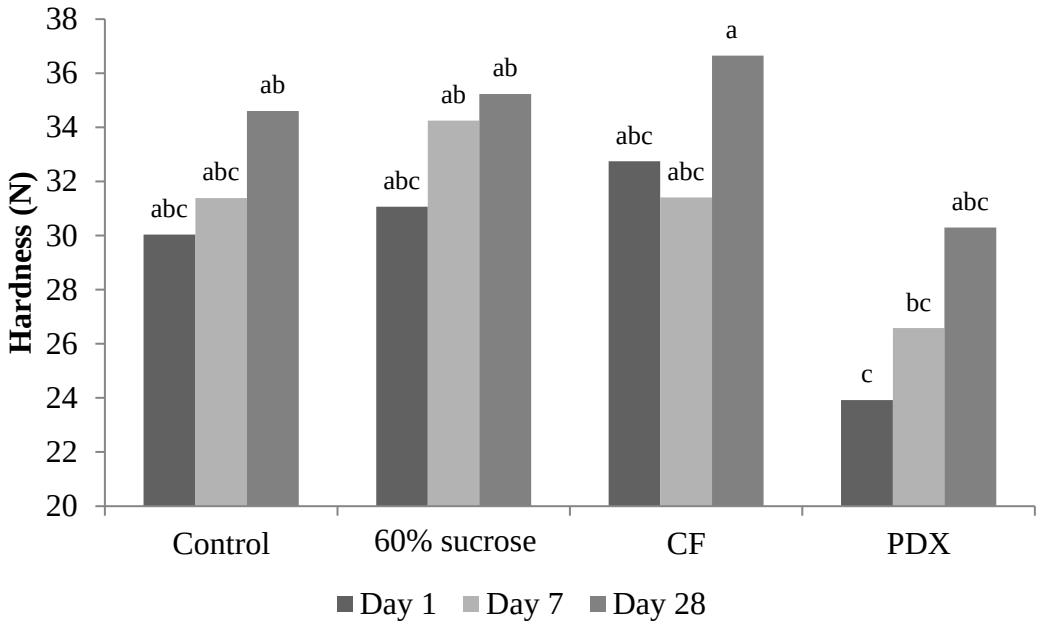


Figure 5-4: Hardness (N) the control and reformulated biscuits containing 60% sucrose, citrus fibres (CF) and polydextrose (PDX) day 1, 7 and 28 post-baking. Values labelled with the same superscript are not significantly different (P≤ 0.05).

5.4.2.4 Biscuit fracturability

The fracturability or brittleness of a food sample is defined as the tendency to fracture, crack or shatter under the influence of force. Fracturability includes the crispiness or crunchiness of a food and therefore 'dry' products such as snacks or biscuits exhibit this property. Dry and brittle products do not deform when force is applied as it snaps quickly, to produce a sound.

In the present study, sucrose reduction significantly affected the fracturability of the biscuit samples. Overall, the reduction in sucrose resulted in biscuits with higher fracturability than the control indicating a less "brittle" texture. As sucrose controls the hydration of starch and protein molecules, baked products with high sucrose content are more brittle/ less fracturable. Therefore a reduction in sucrose can result in an increase in the fracturability of a food product. Results are shown in Figure 5-5.

All reformulated biscuits had a significant increase in fracturability on day 1 post-baking (P≤0.05). Biscuits containing 60% sucrose and CF showed a significant increase in fracturability compared to the control sample on day 7 (P≤0.05). The biscuit samples containing 60% sucrose also had a significantly higher fracturability

value than the control on day 28 (P≤0.05). The control formulation had the lowest fracturability for all test days (0.56mm, 0.63mm, 0.66mm). There was no significant change in the fracturability of the treatments between test days. Mancebo et al. (2016) replaced flour (15%) with polydextrose to increase the fibre content of a cookie formulation. While the authors reported a significant increase in hardness, there was no significant differences were observed with regard to the fracturability compared to the control.

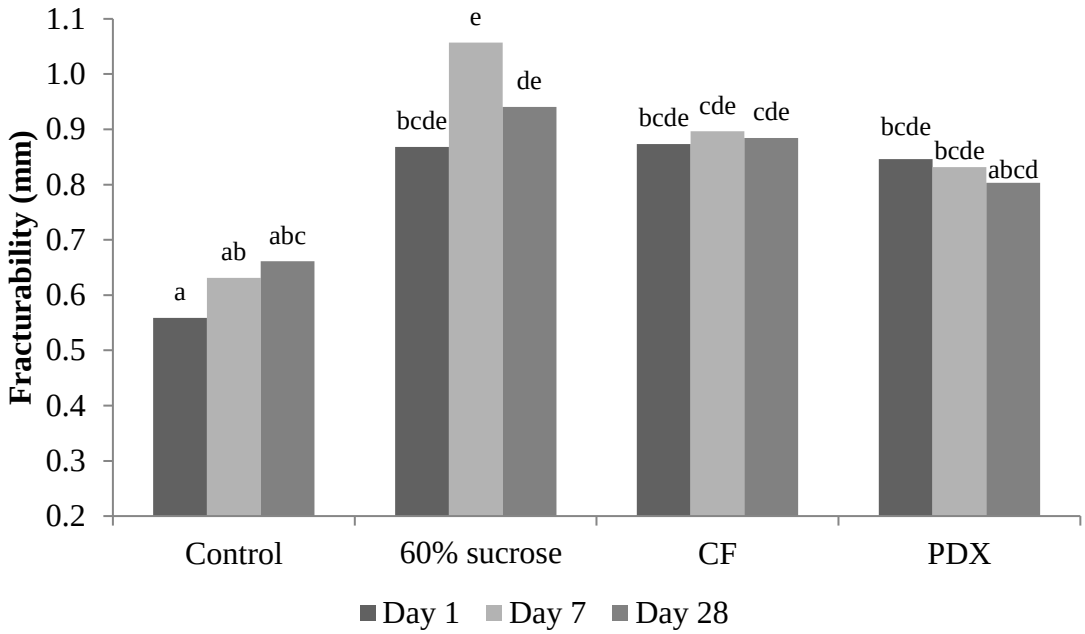


Figure 5-5: Fracturability (mm) of the control and reformulated biscuits containing 60% sucrose, citrus fibres (CF) and polydextrose (PDX) day 1, 7 and 28 post-baking. Values labelled with the same superscript are not significantly different (P≤ 0.05).

The fracturability of the biscuits were highly correlated with the moisture content (r^2=0.889) and water activity (r^2=0.876). This is likely a result of a combination of the decrease in the sucrose content and the increase in the fibre content, as both contribute to the moisture content and water activity of a biscuit (this will be explained further in detail as the thesis progresses). As fracturability measurement relies on foods to be 'dry', an increase in moisture and water activity would result in a less fracturable sample.

5.4.2.5 Acoustic properties

Acoustic emission

As the sample breaks, energy is released in the form of sound energy. The acoustic emission is the peak of sound produced when the sample deforms. As sucrose cools on the surface of the biscuit, it crystalizes forming a hardened casing, resulting in a crispy surface. This property of sucrose is highlighted in this study, as the control formulation had a significantly greater acoustic emission than all reformulated biscuits on all test days ($P \leq 0.01$). The reduction of sucrose in the reformulations created a less crunchy biscuit compared to the control. Results are shown in Figure 5-6.

There was a decrease in acoustic emission as the biscuit staled. Dry crispy foods tend to absorb moisture as staling occurs, resulting in a more flexible structure. There were no significant differences found between the reformulated biscuits throughout the test period, however there was a significant difference found between the control formulation on day 1 and day 28.

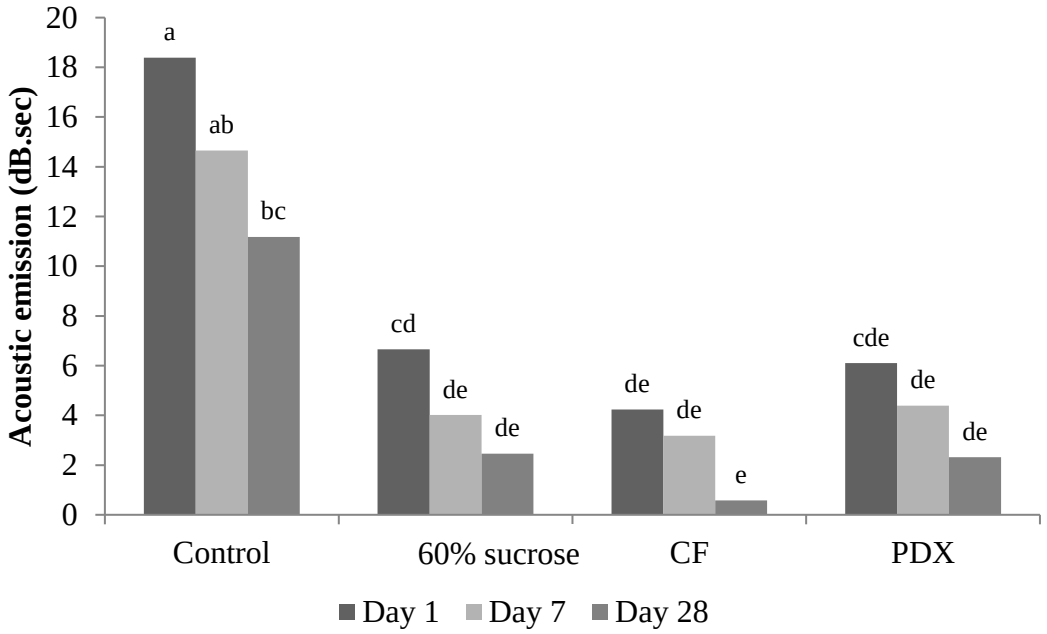


Figure 5-6: Acoustic emission (dB.sec) of the control and reformulated biscuits containing 60% sucrose, citrus fibres (CF) and polydextrose (PDX) day 1, 7 and 28 post-baking. Values labelled with the same superscript are not significantly different ($P \leq 0.05$).

The decrease in the acoustic emission of the reformulated samples may have resulted from the addition of fibres. The addition of soluble fruit fibres (apple pomace) have been shown to decrease the acoustic emission of an extruded snack compared to the control (O'Shea et al., 2012). It was hypothesised by the authors the soluble fibres absorbed more water in the matrix, preventing vaporisation during baking. This reduced the expansion of air bubbles, creating a less crunchy snack.

In the current study all reformulated biscuits had a significantly greater moisture content and water activity than the control. Moisture content and water activity were seen to have an inverse relationship with the acoustic emission (r^2=-0.873 and -0.763 respectively). The reformulated biscuits, with a greater water concentration, were more flexible before snapping, therefore creating a lower acoustic emission. The relationship between the available water, the texture and acoustic properties has also reported in other studies (Roudaut et al., 1998, Marzec et al., 2007).

There was also a strong negative correlation between the acoustic emission and fracturability (r^2=-0.864) indicating samples with a higher fracturability produced a lower acoustic emission. As mentioned previously, the crystallisation of sucrose on the surface of the biscuits contribute to the crispy/ crunchy texture, resulting in the production of sound when snapped. A reduction of sucrose resulted in less crystals forming on the surface of the biscuit thus creating a lower acoustic emission.

Linear distance

The linear distance is an indication of the crispness of a sample. When there are many fracture events, displayed by a jagged line during the tests on the Texture Analyser, the product is perceived as crispy or crunchy. Crunchy samples display clear defined force points, with a longer distance between them. In contrast, a crispy sample shows many smaller fluctuations close together. Results, shown in Figure 5-7, highlight that the linear distance was significantly affected by the reduction of sucrose. Figure 5-8 show an example of a crunchy and crispy graph of the control formulation and biscuits containing CF. Within each formulation, there was no significant change found in the linear distance of the test formulations over the 28 day testing period.

The reduction in sucrose was seen to significantly decrease the linear distance compared to the control formulation on all test days (P≤0.05). This indicated that the reformulated biscuits had a crispier texture, compared to the control biscuits, which had a crunchier texture. Crunchy foods often have several fracture events, compared to crispy foods, which have fewer fractures. The linear distance recordings of the samples were highly influenced by the moisture content (r^2= -0.873) water activity (r^2= -0.763), and fracturability of the biscuit (r^2= -0.726). As discussed previously, fracturability, moisture content and water activity have a strong correlation with each other.

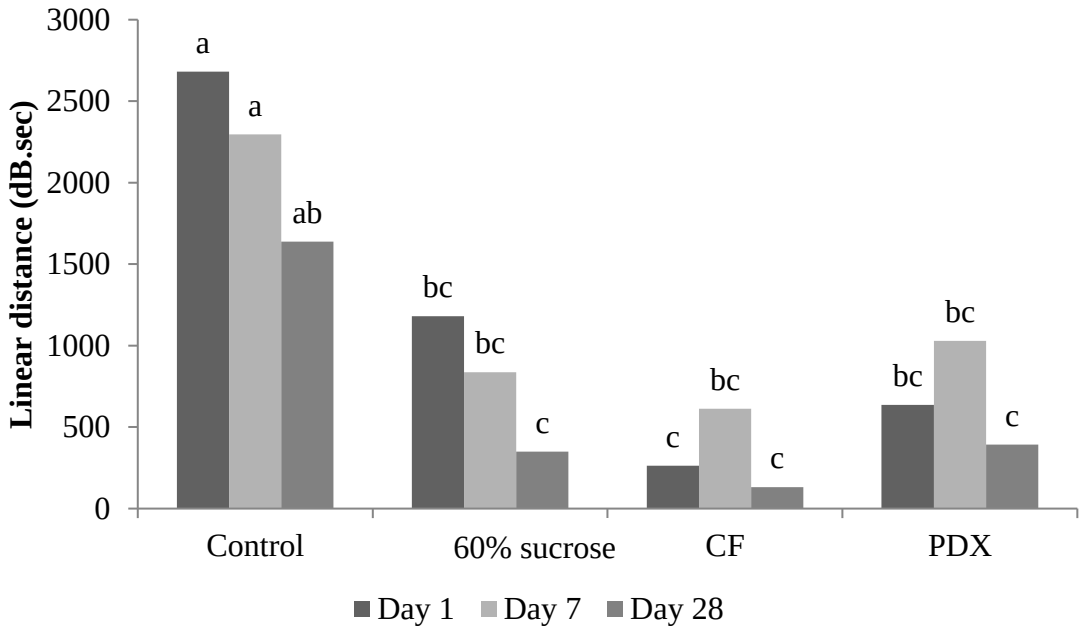


Figure 5-7: Linear distance (dB.sec) of the control and reformulated biscuits containing 60% sucrose, citrus fibres (CF) and polydextrose (PDX) day 1, 7 and 28 post-baking. Values labelled with the same superscript are not significantly different (P≤ 0.05).

The addition of fruit fibres has previously been seen to decrease the linear distance in an extruded snack to produce a 'crispier' product (O'Shea et al., 2012). Similar results were seen in the current study. This may have been due to an increase in product density. When sucrose is reduced, early starch gelatinisation occurs, preventing the full expansion of air bubbles. Fibres bind to water in the mixture, which prevents vaporisation of the moisture, therefore decreasing bubble expansion. This possibly led

to a decrease in the size of air pockets in the baked reformulated biscuits. The control formulation had complete air pocket expansion, leading to an aerated structure. When pressure was applied in this test, the control biscuits had multiple microfractures, which produced multiple fracture events, thus increasing the linear distance compared to the reformulated biscuits. To date, no other study was found which examines the effect of sucrose reduction on the acoustic properties (acoustic emission and linear distance) of a biscuit formulation.

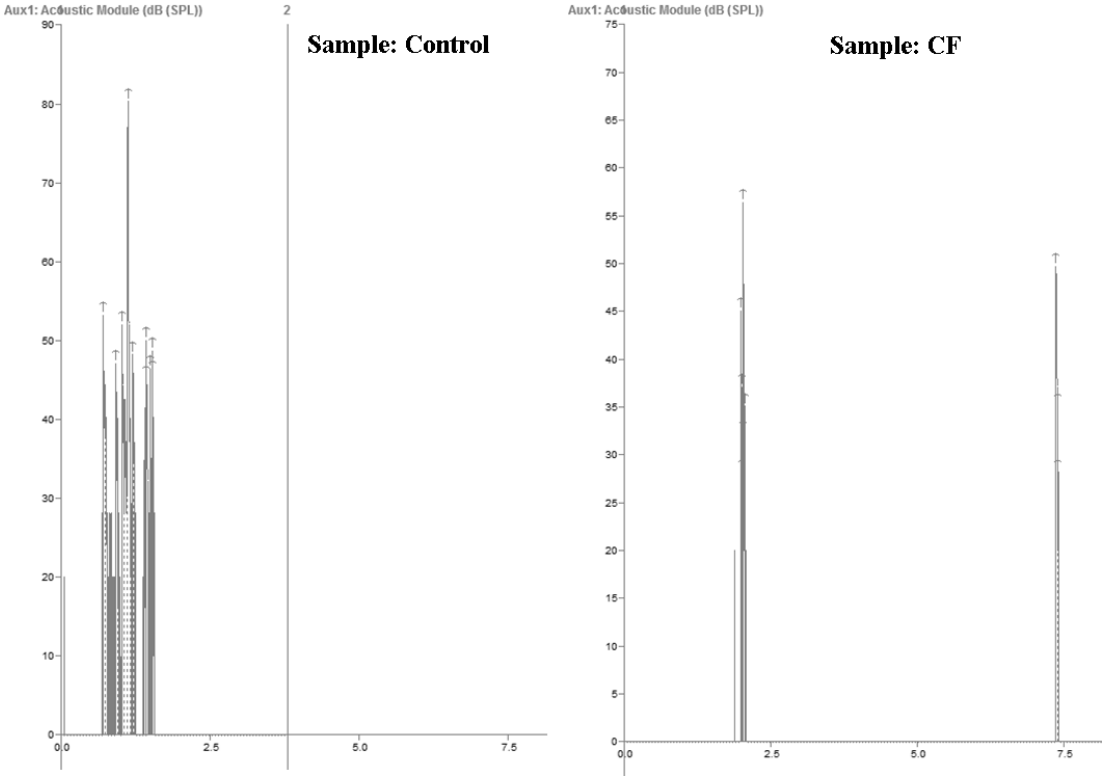


Figure 5-8: An example of a crunchy and crispy graph, control and citrus fibres (CF) formulations respectively.

5.4.3 Volatile analysis- head space solid phase microextraction

The volatile profile of the control and reformulated biscuits were analysed using the SPME method. Odour and flavour compounds were identified and the descriptors associated with each compound are shown in Table 5-2. Figure 5-9 and 5-10 show the principal component analysis (PCA) biplot.

In total, 79 volatile compounds were identified, consisting of 28 aldehydes, 9 ketones, 9 furans, 6 alcohols, 5 pyrazines, 5 terpenes, 4 acids, 3 lactones, 3, hydrocarbons, 2

benzenes, 2 pyranones, 2 phenols and 1 ester. It can be seen from Table 5-2, many of the compounds identified from the biscuit samples were derived from the Maillard reaction, lipid oxidation and fermentation. While a minor group of compounds were a result of caramelization, the thermal degradation of glucose or from plant and carotenoid sources. Overall, the reduction in sucrose had a significant effect on the volatile profile of the biscuits samples. The abundance as compounds formed from the Maillard reaction decreased, while the compounds associated with lipid oxidation increased in the reformulated biscuits compared to the control. Biscuit formulations containing CF showed an increase in the volatile compounds derived from plant sources, such as terpenes.

Table 5-2 shows a wide array of compounds formed as a result of Maillard browning. The most abundant of these compounds were the pyranones. Maltol and hydroxy-dihydromaltol are both described of producing a caramelised odour (Pico et al., 2015, Maire et al., 2013), which is important for the flavour profile of biscuits. However, there was a decrease in these compounds in the reformulated biscuits. Sucrose plays an important role in browning, and the production of Maillard reaction-derived compounds as seen above.

Table 5-2: Volatile compounds of control and reformulated biscuits of containing 60% sucrose (60), citrus fibres (CF) and polydextrose (PDX).

Compound	Control	60	PDX	CF	Odour Descriptor	Source
Aldehydes						
Acetaldehyde	3.62E+04	4.68E+04	5.84E+04	6.08E+04	Pungent, fresh, aldehydic, refreshing, green [1]	MR, F
2-Propenal (acrolein)	1.41E+04	1.28E+04	1.34E+04	1.53E+04	Cooking grease [7]	MR, LO, F
Propanal	3.35E+04	3.50E+04	3.15E+04	3.33E+04	Malthy [7]	LO
2-Methylpropanal	1.37E+04	1.15E+04	9.55E+03	8.57E+03	Fresh, sweet mint, floral , malty [1,5]	MR, F
n-Butanal (Butyraldehyde)	1.35E+04	1.44E+04	1.29E+04	1.48E+04	Pungent, cocoa, musty, green, malty, bready [8]	
3-Methylbutanal	8.15E+04	7.60E+04	6.33E+04	7.40E+04	Ehtereal, aldehydic, choclate, peach, fatty, malty [1,3]	MR, F
Pentanal	2.81E+05	2.89E+05	2.70E+05	3.01E+05	Pungent , fremented, bready, fruity, nutty, berry [5,8]	LO
(E)-2-Pentenal	4.27E+04	4.00E+04	4.44E+04	4.88E+04	Pungent, green, fruity, apple, orange, tomato [8]	LO
Hexanal	1.18E+06	1.12E+06	1.17E+06	1.21E+06	Green, fatty, leafy, fruity, aldehydic , sweaty, grass [1]	LO
2-Hexenal	4.44E+04	4.04E+04	4.21E+04	4.61E+04	Green, fatty [7]	LO
Heptanal	2.46E+05	2.12E+05	2.83E+05	2.49E+05	Fresh green, sweet, herbal, wine-like, ozone [1]	LO
2-Heptenal isomer	1.90E+05	1.67E+05	1.82E+05	2.06E+05		LO

Benzaldehyde	5.74E+05	2.04E+05	6.05E+05	5.14E+05	Strong, sharp, sweet, bitter, almond, cherry [1]	MR, F, LO
Octanal	1.68E+05	1.20E+05	1.59E+05	1.34E+05	Aldehydic, waxy, citrus, orange green peel [1]	LO
2,4-Heptadienal isomer	1.27E+05	1.59E+05	1.48E+05	1.58E+05	Fatty, green, oily, aldehydic, vegetable, cinnamon [8]	LO
2,4-Heptadienal isomer	2.23E+05	2.16E+05	2.42E+05	3.10E+05	Fatty, green, oily, aldehydic, vegetable, cinnamon [8]	LO
Benzeneacetaldehyde	3.77E+05	2.43E+05	2.53E+05	2.68E+05	Honey, floral [5]	F
Nonanal	1.38E+06	1.15E+06	1.20E+06	1.06E+06	Aldehydic, waxy, rose, orris, orange peel, verbena , green, fatty [1,4]	LO
2,6-Nonadienal isomer	2.91E+04	3.04E+04	3.58E+04	3.65E+04	Cumcumber-like [3,7]	LO
(2E)-Nonenal	1.42E+05	1.29E+05	1.56E+05	1.61E+05	Green, tallow, cumcumber [3,4]	LO, MR
Decanal	9.43E+04	3.42E+04	7.15E+04	7.70E+04	Sweet, waxy, orange peel, citrus, floral [1]	LO
2,6-Nonadienal isomer	3.90E+04	3.71E+04	4.32E+04	4.11E+04	Cucumber-like [3,7]	LO
(2E)-Decenal	7.28E+04	4.92E+04	6.90E+04	5.87E+04	Waxy, fatty, earthy, coriander, green, mushroom, aldehydic [8]	LO
2,4-DecadienaL	9.94E+04	1.03E+05	1.08E+05	1.56E+05	Fatty, waxy [3]	LO
2,4-Decadienal	5.63E+05	6.50E+05	6.76E+05	9.67E+05	Fatty, waxy [7]	LO
2-Undecenal	4.67E+04	2.90E+04	4.10E+04	3.49E+04	Fresh fruity, citrus, orange peel [1]	LO
Ketones						
2-Propanone (acetone)	1.26E+05	1.53E+05	1.57E+05	1.73E+05	Ether, grape , sweetish, aromatic [7]	F
2,3-Butanedione (diacetyl)	1.58E+05	2.10E+05	2.34E+05	2.66E+05	Strong, butter, sweet, creamy, pungent, caramel, buttery [1,4]	MR, F
2-Butanone	4.92E+04	7.01E+04	7.42E+04	8.58E+04	Acetone-like, sharp, sweet [5,7]	MR, F
1-Penten-3-one	1.74E+04	1.40E+04	1.36E+04	1.44E+04	Pungent, peppery, mustard, garlic, onion [8]	LO
1-hydroxy-2-propanone	3.81E+06	5.01E+06	6.45E+06	6.39E+06	Sweet, burnt, pugent, caramel, ether [5, 7]	MR, C
Acetoin (3-hydroxy-2-butanone)	1.17E+05	1.67E+05	2.10E+05	2.41E+05	Buttery , butterscotch, yoghurt, cream [4,7]	MR, F
1-Hydroxy-2-butanone	2.64E+04	2.83E+04	3.51E+04	3.78E+04	Sweet, coffee, musty, grain, malty, butterscotch [8]	MR
2-Heptanone	1.12E+05	2.64E+05	3.08E+05	3.66E+05	Fruity [5]	LO
2-Octanone	5.09E+04	3.86E+04	4.82E+04	4.56E+04	Cheesy, musty [6]	LO
3,5-Octadien-2-one isomer	8.37E+04	9.04E+04	1.09E+05	9.68E+04		LO
3,5-Octadien-2-one isomer	4.20E+04	4.22E+04	5.07E+04	4.54E+04		LO
Furans						
2-Ethylfuran	5.61E+04	4.80E+04	4.47E+04	5.38E+04	Musty, earthy [5]	MR
Dihydro-2-methyl-3(2H)-furanone	2.35E+04	1.55E+04	1.36E+04	1.42E+04	Sweet, solvent, bready, buttery, nutty [8]	MR
Furfural	4.32E+05	2.71E+05	2.18E+05	5.60E+05	Almond, bread, woody, caramellic [2,4]	MR, F, LO
2-n-Butyl furan	1.56E+04	1.28E+04	1.44E+04	1.73E+04	Wine, sweet [5]	MR
2-Furanmethanol (furfuryl alcohol)	5.05E+05	3.24E+05	3.24E+05	3.99E+05	Alcoholic, chemical, musty, sweet, caramel, bread, coffee [1]	MR, LO
2-Acetylfuran	3.72E+04	2.70E+04	2.50E+04	3.29E+04	Smoky, roasty [7]	MR
2-Pentylfuran	7.20E+05	6.09E+05	7.30E+05	8.25E+05	Fruity, green, earthy, beany, vegetable, metallic [1]	MR, LO, F
2(5H)-Furanone	5.74E+05	5.20E+05	6.05E+05	5.13E+05	Buttery [8]	MR
5-Hydroxymethylfurfural	1.32E+05	0.00E+00	0.00E+00	9.80E+04	Fatty, musty, waxy, caramel [1]	MR, C
Alcohols						
Ethanol	5.60E+03	5.54E+03	7.80E+03	1.44E+04	Ethanol [2]	F
1-methoxy- 2-propanol	1.33E+05	1.27E+05	8.43E+04	6.47E+04	Wine, malty [5]	

1-Penten-3-ol	8.85E+04	1.03E+05	9.30E+04	1.25E+05	Buttery, milky [5]	LO
1-Pentanol	9.87E+04	7.87E+04	1.00E+05	1.34E+05	Fusel oil, sweet balsam [1]	LO
Hexanol	2.20E+04	4.95E+04	6.08E+04	4.54E+04	Ethereal, fusel oil, fruity, alcoholic, sweet, green [1]	LO
1-Octen-3-ol	1.79E+05	1.10E+05	1.24E+05	1.68E+05	Mushroom , fruity, buttery [2,4]	LO
Pyrazines						
Pyrazine	2.66E+04	2.99E+04	3.06E+04	3.16E+04	Roasted [7]	MR, LO
2-Methylpyrazine	2.65E+05	1.95E+05	1.60E+05	2.31E+05	Roasted, burnt, sweet [7]	MR, LO
2,5-Dimethylpyrazine	2.53E+05	1.65E+05	1.46E+05	1.85E+05	Chocolate, earthy [6]	MR, LO
2,3-Dimethylpyrazine	3.91E+04	2.29E+04	1.57E+04	2.43E+04	Nutty, coffee, peanut butter, walnut, caramel, leather [1]	MR
2,3,5-Trimethylpyrazine	6.04E+04	3.35E+04	3.40E+04	4.26E+04	Potato-like, earthy [7]	MR
Terpenes						
a-Pinene	1.09E+05	1.58E+05	1.87E+05	1.86E+05	Pine [5]	P
Sabinene	2.41E+04	3.06E+04	3.45E+04	3.30E+04	Citrus, spicy [5]	P
D-Limonene	9.43E+04	1.03E+05	1.31E+05	1.27E+05	Citrus, [5]	P
o-Cymene	4.14E+04	4.90E+04	5.02E+04	6.29E+04	Sweet [5]	P
Vanillin	3.02E+04	2.18E+04	1.85E+04	2.38E+04	Sweet vanilla, creamy, chocolate [1]	P
Acids						
Acetic acid	8.49E+05	3.95E+05	5.46E+05	7.89E+05	Sharp, pungent, sour, vinegar[1]	MR, C, F
Isovaleric acid (3-methyl butanoic acid)	9.88E+04	1.39E+05	1.30E+05	1.27E+05	Sweaty, rancid [2,6]	MR, F, LO
Valeric acid	1.81E+05	7.17E+04	1.01E+05	1.57E+05	Sweaty [7]	
Nonanoic acid	1.36E+04	1.02E+04	1.76E+04	2.21E+04	Waxy, dirty, cheese, cultured dairy [1]	LO
Lactones						
Butyrolactone	4.48E+05	4.13E+05	5.06E+05	4.77E+05	Burnt, sweet [4]	MR, F
γ-Hexalactone	5.89E+04	5.57E+04	5.96E+04	5.61E+04	Herbal, coconut, sweet, coumarinic, tabacco [8]	MR
γ-Nonalactone	4.96E+04	4.18E+04	4.97E+04	4.43E+04	Coconut-like [3]	MR
Hydrocarbons						
2-Acetylthiazole	6.47E+04	4.93E+04	4.79E+04	4.21E+04	Nutty, popcorn, peanut, roasted peanut, hazelnut [8]	MR
2-Acetyl-1H-pyrroline	1.00E+05	5.38E+04	5.63E+04	5.62E+04	Corn chip, roasty, crust-like, sweet, cereal, popcorn-like, bread [9]	MR
2-Pyrrolidinone	2.41E+04	1.53E+04	1.95E+04	1.79E+04		MR
Benzenes						
Toluene	8.46E+04	8.04E+04	1.01E+05	1.02E+05	Benzene-like [5]	LO, Car, GluT
Xylene isomer*	7.29E+04	6.90E+04	6.74E+04	6.44E+04	Sweet [8]	
Pyranones						
Maltol	1.10E+06	6.07E+05	8.68E+05	9.13E+05	Sweet, caramel, cotton candy, jam, fruity, baked bread [1]	MR
Hydroxy-dihydromaltol	1.74E+06	3.00E+05	4.58E+05	6.61E+05	Caramelised[9]	MR
Phenols						
p-Cresol	3.95E+05	1.19E+06	1.45E+06	7.62E+05	Phenolic, narcissus, animal. Mimosa [8]	Car, F
4-Vinyl guaiacol	2.67E+05	1.57E+05	1.51E+05	1.64E+05	Clove , amber, cedar, [4,6]	MR
Esters						

Phenethyl acetate	8.25E+04	9.32E+04	1.03E+05	1.03E+05	Rose [7]	F

LO	Lipid oxidation
MR	Maillard Reaction
C	Carmelization
Car	Cartenoids
GluT	Thermal degradation of Glucose
F	Fermentation (includes Strecker reaction products)
P	Plant dervied

[1] Maire et al. (2013)
[2] Jensen et al. (2011)
[3] Cho and Peterson (2010)
[4] Pico et al. (2017b)
[5] Pico et al. (2017a)

Figure 5-8 and 5-9 show the PCA of the volatile compounds, which revealed the compounds which are associated with each sample. The samples were separated based on their relative volatile compound concentration. It can be seen in Figure 5-9, the reformulated biscuits were negative on the PC1, whereas the control sample was positive on the PC1. The difference between positive and negative PC1 accounts for a difference of 50%. The reformulated samples were separated mainly on PC2, which accounts for 31% of the overall variance.

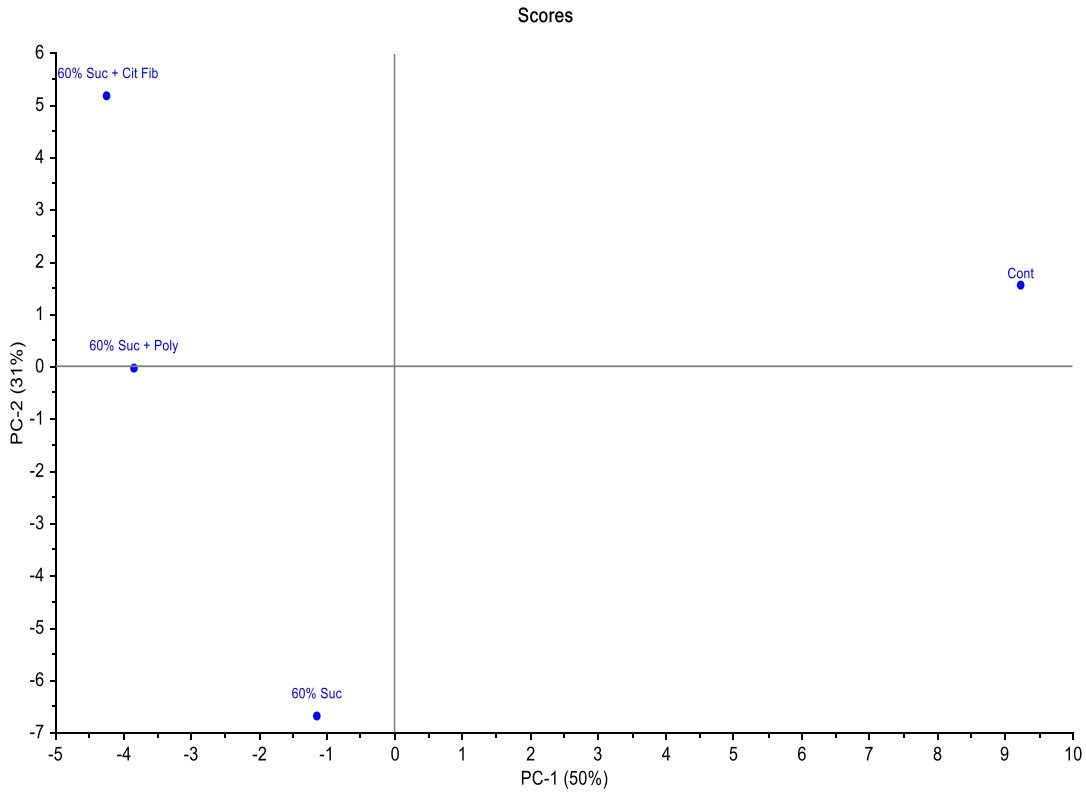


Figure 5-9: Principal Component Analysis Biplot of the volatile profiles of the control (Cont) and reformulated biscuits containing 60% sucrose (60% Suc), citrus fibres (60% Suc + Cit Fib) and polydextrose (60% Suc + Poly).

The compounds associated with each sample are displayed on Figure 5-10. Compounds created by the Maillard reactions are associated with the control formulation. These compounds have been shown to have antioxidant potential (Bailey and Um, 1992, Whitfield and Mottram, 1992), and therefore this may reduce the formation of volatile compounds from lipid oxidation. It that appeared the lipid oxidation compounds were associated with the reduced sucrose samples. Bakery products contain high concentrations of unsaturated fat (from margarine). Unsaturated fats are at risk of undergoing lipid oxidation due to the presence of oxygen during mixing and exposure to high temperatures while baking. The reformulated samples contained an increased concentration of lipid oxidation-derived samples compared to the control, highlighting the importance of the antioxidant effect of the Maillard reaction, and compounds associated with it.

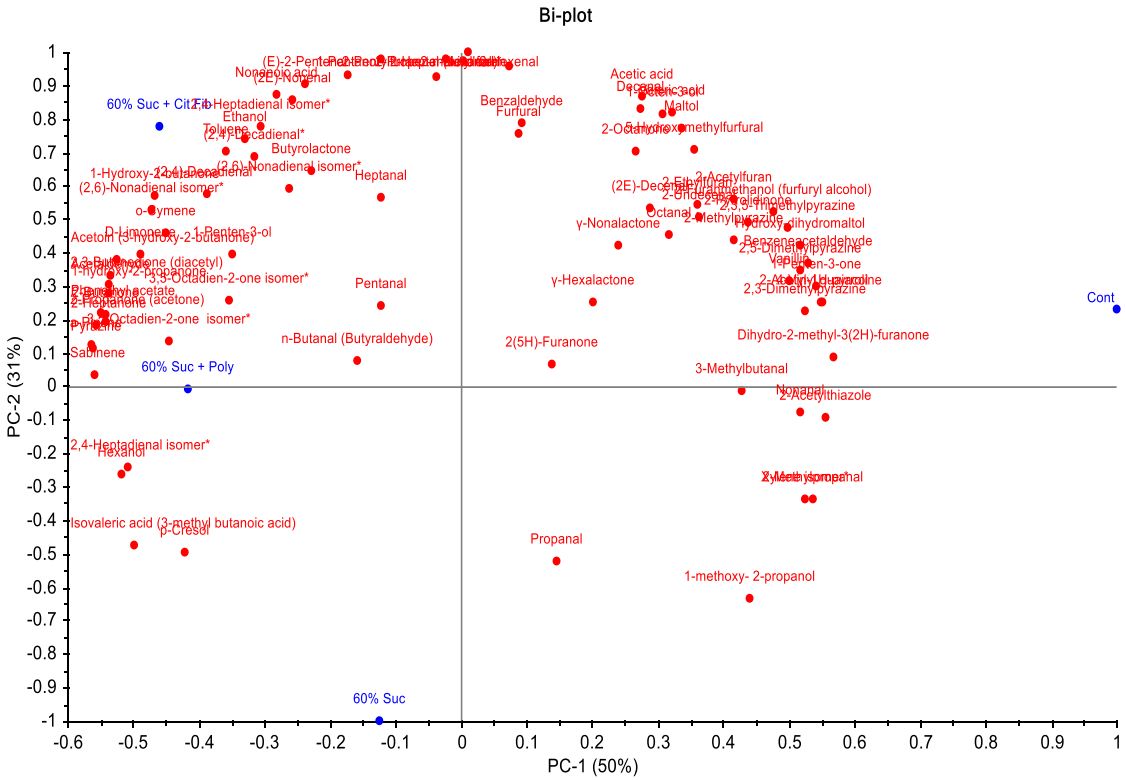


Figure 5-10: Principal Component Analysis Biplot of the volatile profiles of the control (Cont) and reformulated biscuits containing 60% sucrose (60% Suc), citrus fibres (60% Suc + Cit Fib) and polydextrose (60% Suc + Poly).

When comparing the reformulated biscuits, biscuits containing CF and PDX showed a greater affiliation with compounds associated with lipid oxidation, than the biscuits containing 60% sucrose. It was seen in Section 5.4.1, the addition of CF and PDX resulted in a biscuit with a decreased *b** value. This reduction in the yellow undertone may be attributed to a decreased level of Maillard browning. As stated above, Maillard browning produces antioxidants which prevent lipid oxidation. Hence it would be expected that CF and PDX biscuits have a higher proportion of compounds associated with lipid oxidation than those containing only 60% sucrose.

The texture of the samples may also have an effect on the release of volatile compound (De Roos, 2003). This was observed in a snack product enriched with brewer's spelt grain (Ktenioudaki et al., 2013). The authors hypothesised this was a result of the change in texture of the snack products, altering the release of volatile compound to the air. In the current study, there were significant differences found with regard to

fracturability and acoustic properties. The volatile profile of reduced sucrose biscuit has not been studied previously.

5.4.4 Sensory evaluation

Multiple quality attributes including appearance, eating quality and overall acceptability of the control and reformulated biscuits were evaluated by 31 un-trained assessors and results are shown on Figure 5-11. These attributes were measured using a hedonic scale (1= extremely dislike; 9= extremely like).

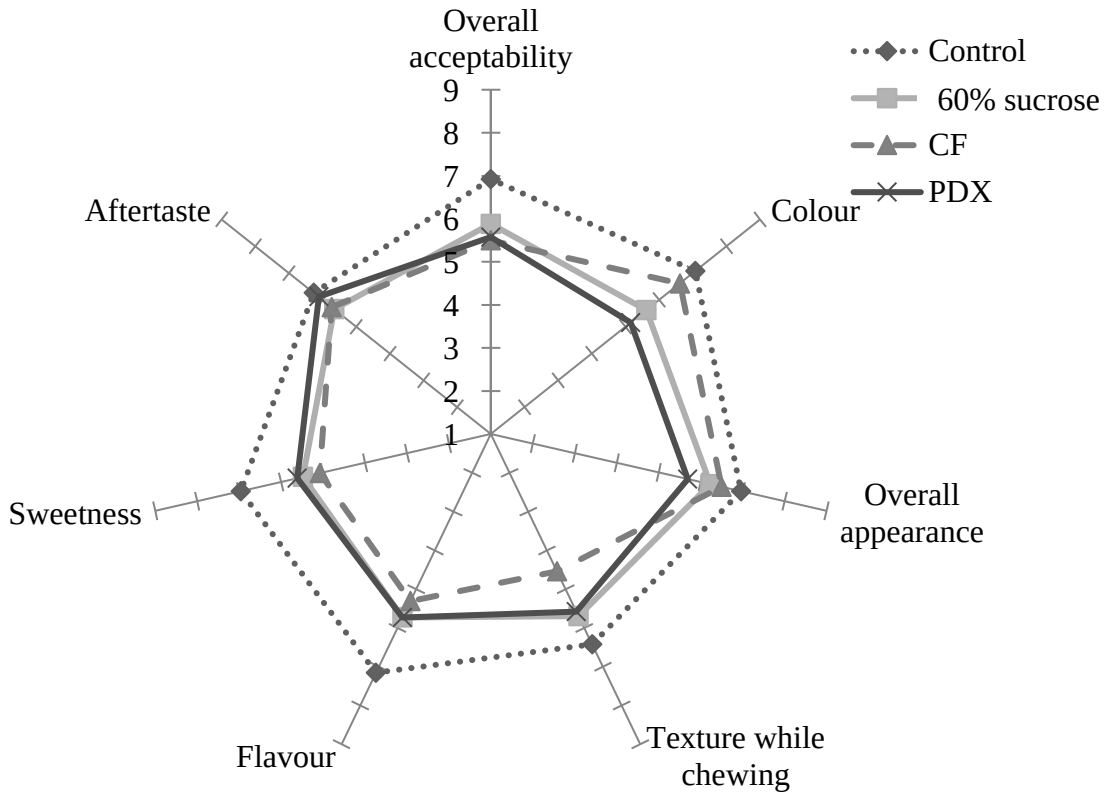


Figure 5-11: Sensory evaluation of the control and reformulated biscuits containing 60% sucrose, citrus fibres (CF) and polydextrose (PDX), as determined by the sensory panellists, scored on a 1–9 hedonic scale.

The control formulation ranked the highest across all parameters. Significant differences were found between the control and the reformulated biscuit with regard to overall acceptability ($P \leq 0.001$), flavour and sweetness ($P \leq 0.01$). Biscuits containing CF received significantly lower scores with regards to texture while

chewing (P≤0.001). There also were significant differences found between the biscuits containing 60% sucrose and the control with regards to colour (P≤0.001), overall appearance and aftertaste (P≤0.05). The addition of polydextrose as a sucrose replacing ingredient had a significant negative impact on colour and overall appearance compared to the control (P≤0.001). Overall, this study demonstrates the importance of sucrose in the acceptability of a biscuit formulation.

As discussed in Section 5.4.1., there were no significant differences found between the biscuits containing CF and the control with regards to the *L** values (data not shown). It is possible that the brightness of the biscuit played a role in the acceptability of the biscuit, with regards to colour and overall appearance in the sensory evaluation. The addition of CF resulted in a significant decrease in the 'texture while chewing' parameter as scored by the panellists. This implies that the sensory panellists recognised a difference between the reformulated biscuits, although instrumentally, there were no significant differences, which highlights the importance of sensory evaluation for food product reformulation and innovation.

5.5 Conclusion

The sucrose concentration in biscuit formulations was reduced by 40% and polydextrose (PDX) and citrus fibres (CF) were added. The moisture content significantly increased in the reformulated biscuits on all test days. There was significant increase in the water activity in all treatments on day 1 post-baking and in biscuits containing 60% sucrose on day 7 and 28. Fracturability was significantly affected by sucrose reduction, as seen on day 1 by all treatments, and biscuits containing 60% sucrose and CF on day 7 (P≤0.05). There were significant decrease in the acoustic properties (acoustic emission and linear distance) of the reformulated biscuits compared to the control on test days (P≤0.05). Flavour analysis revealed a decrease in volatile compounds associated with Maillard browning and an increase in volatiles associated with lipid oxidation in the reformulated biscuit compared to the control. Assessors ranked the reformulated biscuits as less preferred in all attributes. However, overall, the biscuits were deemed to be acceptable.

GENERAL CONCLUSION

General conclusion

Sucrose has many important functions in food products, and its reduction results in foods with inferior quality. This is particularly true in baked products, such as cakes and biscuits. A high sucrose diet has also been linked to increased risk of obesity and obesity related diseases such as type 2 diabetes and heart disease. In 2018, the Irish government implemented a sugar tax, which is pressuring food manufacturers to reduce the sucrose content of their foods. Therefore a solution must be found. There is a consumer trend towards 'clean label', a term that indicates a product which is minimally processed and perceived as natural by the consumer. Many of the current sucrose replacing ingredients such as polyols and high intensity artificial sweeteners are not considered to be 'clean label' ingredients, and therefore there is a need for alternatives to be studied.

This thesis has studied the potential of a number of possible sucrose-replacing ingredients in cake and biscuit formulations, examining the batter and dough properties and the baking quality and structure of the final products. Understanding the batter and dough rheological properties is an important factor for explaining their behaviour while baking, and also how these properties contributed to the quality parameters of the final product.

Alternative sweetening ingredients (apple pomace, whey permeate, oligofructose and polydextrose) were added to a reduced sucrose cake formulation. Several factors which are important for the overall quality of a cake, such as colour, structure and texture, as well as nutritional composition, were analysed. With the exception of apple pomace, which produced harder cake, with a lower specific volume than the control, all of the ingredients were shown to be viable sucrose replacers.

The application of whey permeate, oligofructose and polydextrose maintained a similar specific volume and comparable texture to the control. When these ingredients were added to the cake formulation, a higher moisture content than the control was obtained indicating a moister cake. Chapter 2 discussed how, the whey permeate-containing batter had similar viscoelastic properties as the control batter, most likely a result of the high lactose content. This produced a cake with a similar specific

volume and texture to the control, as seen in chapter 3. Lactose is known to crystallise on the surface of baked products to form a crust, thus possibly preventing moisture loss as the cake staled. As the presence of polydextrose increases the gelatinisation temperature of starch in a batter or dough when baked, bubbles are allowed to expand fully in the batter before the gel is set, producing a volume similar to the control. Both polydextrose and oligofructose are soluble fibres. Soluble fibres have a high water holding capacity and therefore an increase in the moisture content of these cakes was expected.

The crumb cell structure, as measured by the C-Cell was unaffected by the sucrose reduction. The high cell number and low hole volume of the reformulated cake crumb, which indicated a finer crumb, remained similar to the control. The microstructure of the cake was also examined using CSLM imaging. This revealed the addition of fibres (apple pomace, polydextrose and oligofructose) increased the concentration of cellulosic material in the baked cake, however, the fat, protein and starch organisation remained similar to the control. It is also important to note the addition of polydextrose and oligofructose as sucrose replacers in cake resulted in an approx. decrease of 28% in total sugar content, while cakes containing whey permeate had the decrease of 21.5% in total sugar.

In relation to sensory aspects, the reformulated cakes received lower scores in all parameters as scored by the sensory panellists compared to the control, with the exception of overall appearance. Cakes containing 70% sucrose, whey permeate, oligofructose and polydextrose scored higher than the control with regard to overall appearance. With the addition of a clean label anti-staling ingredient such as ascorbic acid, the texture properties of the cakes could be improved, potentially increasing the sensory scores.

Fibres, bulking agents and natural flavouring (cereal fibres and flavouring, maltodextrin and yeast extract, citrus fibres and apple extract, and polydextrose and flavouring) were added to a reduced sucrose biscuit formulation. The fibres and maltodextrin acted as a bulking agent in the formulation, while the natural flavourings added sweetness. A number of these combinations (cereal fibres and flavouring, maltodextrin and yeast extract and polydextrose and flavouring) resulted in biscuits

with similar properties to the control with regard to hardness and the sensory properties (as ranked by the panellists). In particular, biscuits containing cereal fibres and flavouring had a greater spread ratio than the control (a desirable trait in biscuits), and received higher scores than the control in overall appearance, sweetness and overall acceptability as scored by the panellists, while decreasing the total sugar content of the biscuit by over 25%. These biscuits also had moisture content and water activity similar to the control. Polydextrose and flavouring-containing biscuits also received high sensory scores, had the greatest decrease in total sugar content of all reformulated biscuits (28.5%), and maintained a similar texture profile to the control.

It is interesting the note the biscuits containing 'sweet' natural flavouring (cereal fibres and flavouring and polydextrose and flavouring) received higher preference scores for taste related attributes (flavour, sweetness and aftertaste) than the control biscuits. Although these biscuits were ranked lower for texture while chewing, they received higher scores for acceptability than the control formulation as scored by the sensory panellists. This demonstrates the effect taste has on the overall acceptability of a food product. Overall, biscuits containing cereal fibres and flavouring, and polydextrose and flavouring showed the most potential as sucrose replacers in biscuit formulations.

An additional study was carried out in which polydextrose and citrus fibres were added to a reduced biscuit formulation. There were significant differences found between the control and the reformulated biscuits when examining the acoustic properties and volatile profile.

There was an increase in the moisture content of the reformulated biscuits. This increased moisture content was attributed to the increased water holding capacity of the soluble fibres (citrus fibres and polydextrose). The acoustic emission and linear distance also significantly decreased as a result of sucrose reduction, indicating a less crunchy biscuit. It was hypothesised that this was partly due to the decrease of crystallised sucrose on the surface of the reformulated biscuits, and also a result of the increased moisture content. The increased water holding capacity of the fibres may have prevented water vaporisation during baking. This reduced the expansion of air bubbles, creating a less crunchy biscuit.

The volatile profile revealed the reduction in sucrose yielded biscuits with a higher concentration of compounds associated with lipid oxidation while there was a decrease in compounds formed from the Maillard reaction. Maillard reaction-derived compounds have been shown to have antioxidant potential, which prevent lipid oxidation. As there was a reduction in compounds associated with the Maillard reaction in the reformulated biscuits, there was an increase in the concentration of compounds derived from lipid oxidation. Although there were significant differences found between the control and the reformulated biscuits, these ingredients did show potential as sucrose replacers. The reformulated biscuits had similar colour and hardness to the control. Perhaps with the addition of natural flavouring, the sweetness and other flavour attributes could be improved.

This thesis highlights that through the careful choice of appropriate ingredients and the execution of comprehensive baking trials is possible to reduce the sucrose concentration in sweet baked products while maintaining the product quality. The addition of clean label flavourings, bulking agents and fibres can uphold the taste, texture and appearance of these products, while contributing to a shelf-life similar to the control.

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Appendix

Publication and awards.

Oral presentations

Milner, L., Kerry, J. P., O'Sullivan, M., & Gallagher, E. (2018), Using cake as a model system to investigate the effects of sugar-replacing ingredients. *7th European Sensory Science Society Annual Symposium, Dublin, Ireland.* (May).

Milner, L., Kerry, J. P., O'Sullivan, M., & Gallagher, E. (2018), Fundamental dough properties and baked characteristics of novel products with reduced sugar. *32nd EFFoST International Conference, Nantes, France.* (November).

Poster presentation

Milner, L., Kerry, J., O'Sullivan, M., & Gallagher, E. (2017), Textural, nutritional and sensory analysis of reduced-sugar cake products. *46th Annual Food Science and Technology Conference, Dublin, Ireland.* (December).

Milner, L., Kerry, J. P., O'Sullivan, M., & Gallagher, E. (2018), Fundamental rheology and instrumental texture of reduced-sugar baked products. *Cereal Grains Conference, London, England.* (October).

Awards

Travel scholarship awarded:
Milner, L., Kerry, J., O'Sullivan, M., & Gallagher, E. (2017), Characterising the ingredient interactions and optimal processing parameter of novel baked products with reduced sugar.
Sao Paulo School of Advanced Sciences on Reverse Engineering of Processed Foods, UNICAMP, Sao Paulo, Brazil. (October).