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**Physicochemical and sensory quality of muffin made with  
Stevianna<sup>®</sup> or inulin as sucrose replacer**

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A thesis  
submitted in partial fulfilment  
of the requirements for the Degree of  
Doctor of Philosophy in Food Science

at  
Lincoln University  
by  
Jingrong Gao

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Lincoln University  
2018

## Declaration

Some aspects of this thesis have been published and accepted for publication (*copies of the published and submitted papers are attached at the back of the thesis*) or presented at conferences.

## Publications

- Gao, J., Brennan, M. A., Mason, S. L., & Brennan, C. S. (2016). Effect of sugar replacement with Stevianna® and inulin on the texture and predictive glycaemic response of muffins. *International Journal of Food Science & Technology*, 51(9), 1979-1987.  
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## Presentations

- Stevia and Inulin for Partial Sucrose Replacement in Bakery Products and Contribution to Human Nutrition. A poster presentation at the International Journal of Food Science and Technology 50th Celebration conference at Lincoln University, New Zealand in 2015.

- Stevia and Inulin for Partial Sucrose Replacement in muffins and Contribution to Human Nutrition. An oral presentation at the Postgraduate Conference 2015 at Lincoln University, New Zealand.
- Effects of sugar substitution with “Stevianna” on the sensory characteristics of muffins and the potential of vanilla and/or cocoa to mask aftertaste. A poster presentation at the 2nd Asian Sensory and Consumer Research Symposium at Shanghai, China in 2016.
- Effect of Using Stevianna® and Inulin as a Sucrose Replacer on Quality and *In Vitro* Digestibility of RVA Gels from Muffin Batter Formulas. A poster presentation at the 1st Asia-Pacific ICC Grains Conference at Xiamen, China in 2017.
- Physical Characteristics of Muffin Batter made with Stevianna® or Inulin as Sucrose Replacer. An oral presentation at the 67th AGSA Conference Program Australian Grain Science Association Conference at Christchurch, New Zealand in 2017.

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Philosophy in Food Science.

Physicochemical and sensory quality of muffin made with Stevianna® or inulin as sucrose replacer

by

Jingrong Gao

Bakery products are a popular food which have sugar and starch as the main ingredients. However, high sugar levels can lead to chronic diseases such as weight gain leading to diabetes. Consumers have become increasingly aware of the link between diet and health, and consequently food manufacturers have used functional sugar ingredients to replace sugar and that can satisfy requirements of consumers. This study evaluated the impact of Stevianna® and inulin as partial or complete sugar replacers in muffin products.

Stevianna® is a mixture of rebaudioside-A and erythritol, which can be used as a natural sweetener and may prove to be an effective and acceptable replacement to sucrose in baked systems. Additionally, Stevianna® is also a nutritive sweetener in food, as it lends a minimal calorie intake to the user. Inulin is a term applied to a heterogeneous blend of fructose polymers widely distributed in nature as plant storage carbohydrates. It has a neutral taste, is colourless, and thereby only minimally influences the organoleptic characteristics of product. Furthermore, inulin provides nutritional benefits, which results in better health and attenuation of the risk of many diseases.

The purpose of this research was to determine the physicochemical effect of replacing the Stevianna® or inulin for sucrose at various levels (0, 25, 50, 75 and 100 %) in muffins. Two levels of Stevianna® (50 and 100 %) with cocoa powder and/or vanilla containing muffin were used in sensory analysis compared with a control sample. Analytical testing of air cell, height, density, volume, texture, moisture, colour, pasting properties and differential scanning calorimetry (DSC) was conducted on the different muffin formulations in addition to potential glycaemic impact and a sensory evaluation.

Texture analysis showed the partly reduced sucrose muffins were more tender than the 100 % reduced sucrose muffin ( $p < 0.05$ ) by sugar replacers. Muffin batters containing low levels of inulin showed a lower viscosity compared to the high level of inulin within batter, which indicates that sugar replacement with 100 % inulin has a significant effect on the batter viscosity. DSC results indicated that as the replacement level of sucrose with inulin increased, the starch gelatinisation temperatures also increased.

A sensory descriptive panel found that muffins prepared with 100 % Stevianna® exhibited significantly harder texture, poorer acceptance, and a drier mouthfeel compared against a reference muffin control. Optimal results were obtained with 50 % Stevianna® sample, which was consistent with physical quality characteristics in terms of colour, volume, density and texture. Additionally, cocoa powder and vanilla were included to mask the stevia bitterness to aftertaste.

The potential glycaemic impact was evaluated by *in vitro* carbohydrate digestibility analysis to mimic starch digestion in human intestine system. The results illustrated that the inclusion of inulin or Stevianna® have the potential to inhibit the glycaemic response.

**Keywords:** muffin, inulin, Stevianna®, moisture content, height, density, volume, texture, viscosity, differential scanning calorimetry, air cell, scanning electron microscopy, sensory evaluation, total starch, *in vitro* starch digestion.

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## Abbreviations

ADI: Acceptable Daily Intake

AUC: Area Under the Curve

BI: Browning Index

DF: Dietary Fibre

DP: Degree of Polymerisation

DSC: Differential Scanning Calorimetry

GGE: Glycaemic Glucose Equivalence

GI: Glycaemic Index

MC: Moisture Content

PCA: Principal Component Analysis

RDS: Rapidly Digestible Starch

RVA: Rapid Visco Analyzer

SDS: Slowly Digestible Starch

SEM: Scanning Electron Microscopy

T<sub>onset</sub>: Onset Temperature

T<sub>peak</sub>: Peak Temperature

T<sub>endset</sub>: End Temperature

V<sub>s</sub>: Volume of the Rapeseed Required

V<sub>t</sub>: Total Volume

WHO: World Health Organisation

$\Delta E^*$ : total colour difference

$\Delta H$ : the change of enthalpy



# Chapter 1

## General Introduction

Consumers have been changing their behaviour with regards to the purchase and use of foods as well as eating habits. As a consequence of consumers requiring fast, convenient, and easy to transport food products, ready-to-eat convenient foods have gained popularity, especially considering that they are frequently available as small products intended for mobile consumption. Typical bakery products in this category include bagels, breadsticks, biscuits, donuts or muffins which are now available worldwide (Zahn et al., 2010).

Muffins are high-calorie baked products which are highly appreciated by consumers due to their taste and soft texture. Sugar is a principal ingredient in sweet muffins, not only in terms of contributing to the sweetness of the product but also for rheological and textural roles. However, high sugar levels are undesirable for human health.

In the WHO European Region in the early 2000s the prevalence of obesity and overweight had risen three-fold since the 1980s (Branca et al., 2007). The prevalence of obesity has increased dramatically with suggestions that in US the prevalence of obesity was stable over the period 1960-1980 but increased significantly in the 1980's and the 1990's (Branca et al., 2007). In particular, the rates for childhood obesity are even more alarming. Over one-third of children ages 12 to 19 years old are overweight (Ogden et al., 2008) and the prevalence of childhood obesity has increased three-fold from 1980 to 2000 according to the centre of Disease Control Health data Division of Adolescent and School Health. Consumption of sugar-sweetened foods can significantly influence the glycaemic index of diet (Bhupathiraju et al., 2014). Additionally, over-intake of high glycaemic food can result in higher postprandial glucose and insulin levels and potentially lead to a number of metabolic and hormonal changes that stimulate hunger levels and promote fat deposition (O'Keefe and Bell, 2007). High

sucrose diets have adverse effects on body weight and are associated with other medical complications, such as type-2 diabetes, high blood cholesterol, coronary heart disease and cancer (Röbke et al., 2011).

For this reason, sweet bakery products may be suitable candidates for calorie reduction by utilizing sucrose substitutes. However due to the complex structural functionality of sucrose in baked products, obtaining good quality low-sucrose products is a difficult task. Therefore, current trends have seen consumers tending to seek foods that are not only healthy and nutritious, but also appetising and flavourful. This represents a considerable challenge for the cereal food industry where consumers are interested in consuming sugar reduced, or sugar free, products based on health reasons, and yet wish to have the sweet flavour from a hedonic view point.

Inulin, derived from chicory root, is a mixture of oligomer and polymer chains with a variable number of fructose molecules, joined by  $\beta$  (2-1) bonds and usually including a glucose molecule at the end of the chain (Bayarri et al., 2011). The degree of polymerisation (DP) of chicory fructans varies from 2 to 60 (average DP = 12). It is moderately water soluble and can form a short, spreadable gel network (Zahn et al., 2010). Inulin has been reported as a potential ingredient to imitate the functional and sensorial properties of sugar and fat, while at the same time providing high-quality baked products with considerably fewer calories (Röbke et al., 2011). Inulin is not only a dietary fibre but also a prebiotic, which is linked to a variety of beneficial physiological effects such as improving bowel habits, increasing calcium absorption, lowering serum lipids, a positive effect on the feeling of satiety, and stimulating the immune system (Meyer et al., 2011). Also, the successful incorporation of inulin in cookie formulations was reported by Laguna et al. (2013), and the use of inulin as prebiotic, fat-replacing ingredient in bread-making was reported by Peressini and Sensidoni (2009) and Poinot et al. (2010). Therefore, this kind of ingredient has shown considerable promise to be used in sugar reduced cereal products.

Stevianna® sweetener has been produced and marketed by STEVIANNA International New Zealand Limited (SINZ) for the consumer market. This sweetener is a mixture of rebaudioside-A and erythritol formulated specifically for a consumer to replace sucrose in applications at a 1:1 ratio (SINZ).

Rebaudioside-A is one compound within the stevia plant that provides approximately 300 times the sweetness than that of sucrose (Lin and Lee, 2005). Clinical studies provide further evidence that purified rebaudioside-A has no effect on either blood pressure or glucose homeostasis, thus providing a minimal calorie intake to the user. A strategy that used rebaudioside-A for partial sucrose replacement in muffins has been considered as an alternative to artificial sweeteners in bakery products (Zahn et al., 2013).

Erythritol is a 4-carbon sugar alcohol or polyol with about 60 to 80 % of the sweetness of sucrose (Goossens and Roper, 1994) and is intended for use in bakery products principally as a low-calorie sweetener. Erythritol has been used as the bulking agent for sugar replacement in chiffon cake (Lin et al., 2003) or Danish cookies (Lin et al., 2010), showing good stability during baking, and giving desirable physical quality characteristics with up to 50 % sucrose replacement. Additionally, Akesowan (2009) observed that no significant differences in chiffon cake firmness when 50 % sucrose was replaced by a sucralose-erythritol mixture.

Depending on the plant, *Stevia rebaudiana* leaves contain a complex mixture of sweet diterpene glycosides, including stevioside, steviolbiosides, rebaudiosides (Reb A, B, C, D, E and F) and dulcoside A. Stevioside and rebaudioside-A are the predominant steviol glycosides found in *Stevia rebaudiana*. Stevioside constitutes 5-15 % of the dried leaves of stevia, and is also the major compound responsible for the sweetness (Kulthe et al., 2014). However, steviosides are considered to have adverse taste characteristics, such as bitterness and a black liquorice aftertaste, compared with rebaudioside-A (Prakash et al., 2008). Although rebaudioside-A is perceived as less bitter, it is ranked second in bitterness to stevioside (Schiffman et al., 1995). Therefore, cocoa powder was used to provide a bitterness suppressing effect when combined with Stevianna® in muffin systems in this project. The research carried out in Chapter 5 (Gao et al., 2017) found that cocoa powder seemed to be effective in suppressing the bitterness of muffin.

Stevia has been used for many years throughout the world as a sweetener in many different food systems (Goyal and Goyal, 2010), but it was not an approved food sweetener in the United States up

until 2008 (Food and Drug Administration (FDA 2008) 2008). Stevia was approved for use as a sweetener by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (Anton et al., 2010), and has also recently received GRAS approval from the Food and Drug Administration. Furthermore, Barriocanal et al. (2008) reported steviol glycosides does not have negative side effects in safety studies.

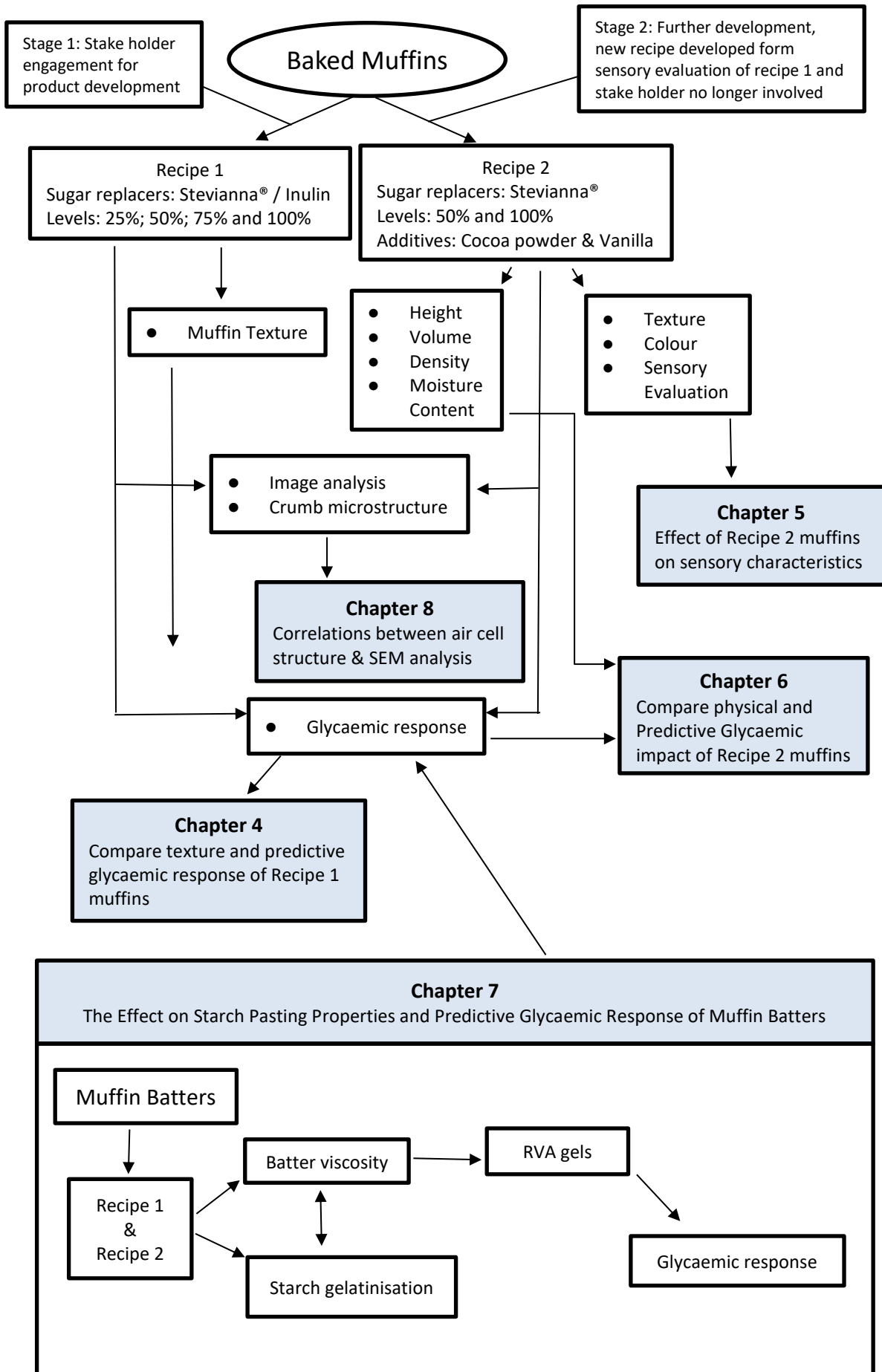
The aim of this research was to determine the physicochemical and sensory effects of using a sugar replacer for sucrose in a muffin. Different levels (0, 25, 50, 75 and 100 %) of sugar replacers (inulin and Stevianna®) were used in two kinds of muffin recipes. Analytical testing on height, volume, texture, air cells, moisture, colour and rapid visco analyzer, differential scanning calorimetry, scanning electron microscopy was conducted on the two recipes as well as total starch, *in vitro* starch digestion and sensory evaluation. With these tests, we set out to determine what replacement level if any may be acceptable both from a physicochemical and sensory perspective. This study contributed to the innovation of bakery products which are not only nutrient-rich but are also acceptable to the consumer, and will consequently increase market requirement for these bakery products.

## Objectives of Research

The objectives of the study were to:

1. Develop and bake model bakery product (muffin) and evaluate their physical-chemical properties against a reference product to determine the role Stevianna® or inulin in contributing to product quality and glycaemic response. This is discussed in Chapter 4.
2. Use physical-chemical analysis to determine the role Stevianna® or inulin combinations the structure of a broader range of commonly consumed baked muffin product evaluating product characteristics, such as appearance, texture profile and colour. Reported in Chapter 5, 6 and 8.
3. Evaluate the effect of Stevianna® on the glycaemic impact of sugar containing baked muffin products, compared to ones with Stevianna® or inulin, using *in vitro* carbohydrate digestibility analysis to mimic starch digestion in the human intestine system. Reported in Chapter 4 & 6.
4. To evaluate the consumer preference for Stevianna® or inulin containing cereal food products against a control reference sample. Furthermore, to evaluate the effects of adding cocoa powder and/or vanilla, as a type of mask ingredient, on the sensory evaluation. Reported in Chapter 5.

**Figure 1. 1 Schematic illustration of the thesis outline.**



## Chapter 2

### Literature Review

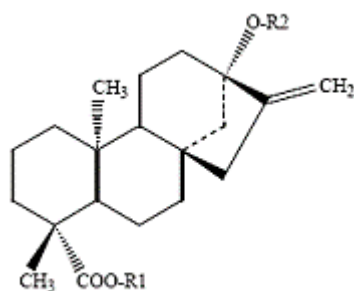
#### 2.1 The history of Stevia Plant and its Sweetening Compounds Properties

*Stevia (Stevia rebaudiana)* is a small, herbaceous, semibushy, perennial shrub of the Asteraceae family, and is native to South America and Central America (North to Mexico, Paraguay and Brazil). Native Indians of the Guarani Tribe appear to have used the leaves of this herb as a sweetener since pre-Columbian times. It is also referred to as sweet leaf or sugar leaf and is a genus of about 150 species of herbs and shrubs. It grows well in the sandy soil of elevated land and may grow to a height of 80 cm when it is fully mature. In 1887, a South American natural scientist named Antonio Bertoni first discovered it. Bertoni named the “new” variety of the stevia genus in honour of a Paraguayan chemist named Rebaudi, who became the first to extract the sweet constituent of plant (Panpatil 2008). *Stevia rebaudiana* Bertoni is cultivated in continental China, Taiwan, Thailand, Korea, and Malaysia in Israel, Ukraine, UK, Philippines, Canada, Hawaii, California and all over South America (Erkucuk et al., 2009).

All diterpene glycosides isolated from *Stevia rebaudiana* leaves have steviol and differ in the content of carbohydrate residues (Erkucuk et al., 2009). The quantification of steviol glycosides has determined eight glycosides which are stevioside, steviolbioside, rebaudiosides A, B, C, D, E and dulcoside A (Goyal and Goyal, 2010). The major diterpene glycosides are stevioside (5-18 %), rebaudioside-A (2-4 %). These two types of low calorie natural substances have been rated as possessing about 300 times the relative sweetness intensity of 0.4 % (w/v) sucrose (Erkucuk et al., 2009). However, Rebaudioside-A is sweeter and less bitter than stevioside. Due to its sweetness and pleasant flavour, rebaudioside-A has the largest potential for replacing sucrose in beverages and baked goods (Carakostas et al., 2008).

Steviol is the aglycone of all the principle and secondary sweetener components (Panpatil, 2008). Figure 2.1 illustrates the chemical structure of major sweet glycosides. The constituents attached to the base structure of steviol are glucose, rhamnose, and xylose sugar moieties at R1 or R2 (Table 2.1).

All the other isolated diterpenoid glycosides possess an entkaurene diterpene steviol skeleton but differ in the residues of carbohydrate at position C-13 and C-19 (Gasmalla et al., 2014). For instance, rebaudioside-A contains one glucose molecule attached at R-group one and three glucose molecules at R-group two which is unique to other steviol glycosides. A complete diagram of each structure can be found in Appendix A the main steviol glycosides and their respective R-groups.



**Figure 2. 1 Chemical structure of major sweet glycosides (Gasmalla et al., 2014).**



**Table 2. 1 Steviol Glycosides and Their Respective R-Groups (Gasmalla et al., 2014).**

| Compound Name  | R1  | R2   |
|----------------|---|--|
| Stevioside     | $\beta$ -Glc                                  | $\beta$ -Glc- $\beta$ -Glc(2 $\rightarrow$ 1)  |
| Rebaudioside-A | $\beta$ -Glc                                  | $\beta$ -Glc- $\beta$ -Glc(2 $\rightarrow$ 1)<br> <br>$\beta$ -Glc(3 $\rightarrow$ 1)  |
| Rebaudioside B | H   | $\beta$ -Glc- $\beta$ -Glc(2 $\rightarrow$ 1)<br> <br>$\beta$ -Glc(3 $\rightarrow$ 1)  |
| Rebaudioside C | $\beta$ -Glc                                  | $\beta$ -Glc- $\alpha$ -Rha(2 $\rightarrow$ 1)<br> <br>$\beta$ -Glc(3 $\rightarrow$ 1) |
| Rebaudioside D | $\beta$ -Glc- $\beta$ -Glc(2 $\rightarrow$ 1) | $\beta$ -Glc- $\beta$ -Glc(2 $\rightarrow$ 1)<br> <br>$\beta$ -Glc(3 $\rightarrow$ 1)  |
| Rebaudioside E | $\beta$ -Glc- $\beta$ -Glc(2 $\rightarrow$ 1) | $\beta$ -Glc- $\beta$ -Glc(2 $\rightarrow$ 1)  |
| Steviolbioside | H   | $\beta$ -Glc- $\beta$ -Glc(2 $\rightarrow$ 1)  |
| Dulcoside A    | $\beta$ -Glc                                  | $\beta$ -Glc- $\alpha$ -Rha (2 $\rightarrow$ 1)  |

Stevia leaf is about 5 cm long and 2 cm wide and leaf arrangement is crosswise, facing each other (Figure 2.2). Environmental factors like soil, irrigation methods, sunlight, air purity, cleanliness, farming practices, processing and storage affect stevia quality. Quality of stevia should be compared on the basis of aroma, taste, appearance and sweetness (Panpatil, 2008). Processed forms of stevia are high-intensity sweeteners ranging from 50 to 300 times sweeter than sugar, with low water solubility and high melting points (Kaushik et al., 2010). These high intensity sweeteners are also highly stable at broad pH and temperature ranges in solution. For example, steviol glycosides are stable between a pH of 2-10. However, a significant decrease in steviol glycoside concentration at 80 °C was found only at highly acidic condition (pH 1) in solution and at 140 °C as a solid (Kroyer, 1999). Rebaudioside-A has been shown to be stable to sunlight exposure as well as in stability studies performed over a four-month period (Kaushik et al., 2010). Kroyer (2010) studied the interaction of rebaudioside-A in binary aqueous solutions with other non-nutritive sweeteners including saccharin, sodium cyclamate, aspartame,

acesulfame potassium and neohesperidin dihydrochalone. This experiment indicated there was no interaction between the individual sweeteners at 80 °C up to four hours or incubated for four months at room temperature.



**Figure 2. 2 Stevia leaves (leaf).**

## **2.2 Human Safety of Rebaudioside-A**

A dietary exposure assessment estimated that for the majority of consumers, the acceptable daily intake (ADI) (0-4 steviol equivalents) was not exceeded when steviol glycosides were added to the range of foods requested. From an extended review by Geuns (2010), an ADI value between 0 and 10 mg steviol equivalents/kg BW can be suggested. The joint FAO / WHO expert committee of food additives (JECFA 2006) recommends a final ADI of 0-4 mg steviol equivalents/kg BW (safety factor 100x). FSANZ (Food Safety Australia and New Zealand) fixed a value of 0-4 mg steviol equivalents. However, in December 2008, the FDA (USA) accepted the GRAS status of rebaudioside-A and, in 2009, for the mixture of steviol glycosides. In September 2009, the French authorities approved rebaudioside-A (>97 % purity) as a food additive, and in January 2010 rebaudioside-A was also authorised as a table top sweetener. FSANZ has also concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day.

Rebaudioside-A has been used for years throughout the world as a natural non-nutritive sweetening alternative to sucrose and other nutritive variants (Goyal and Goyal, 2010). It has very low toxicity, which reported the LD<sub>50</sub> is 8000 mg/kg (Tolstikova et al., 2009). In an acute toxicity study of rebaudioside-A in male Wistar rats, after being administered a single dose of 2000 mg/kg of body weight, no significant changes were seen in any signs of toxicity for sixteen hours post dosing (Williams and Burdock, 2009). Therefore, the toxicity studies of the rebaudioside-A showed no clinical signs of toxicity or carcinogenic changes were found, and safe for human consumption.

Rebaudioside-A appears not to be absorbed or digested in the small intestine due to its high molecular weight (967.013 g/mol), In digestive studies using human volunteers receiving dosages of 750 mg/day, stevioside (molecular weight is 804.9 g/mol) was not detected in faecal matter of any subject (Chatsudthipong and Muanprasat, 2009). However, rebaudioside-A is metabolized by human microflora in the large intestine (Tolstikova et al., 2009). Additionally, “steviol glucuronide excreted in urine and free steviol in faeces account for 62% and 5.2% of the total dose of stevioside administered respectively” after 72 hours of ingestion, according to a study performed in 2008 (Chatsudthipong and Muanprasat, 2009). This confirmed that bacteria in the large intestine can convert stevioside into steviol.

A reproductive toxicity study conducted on hamsters to see the effect of daily ingestion of Rebaudioside-A and its effects on two subsequent generations showed no significant difference in the average growth of the first generation of hamsters in the groups receiving 500, 1000 and 2500 mg/kg bw of rebaudioside-A (Carakostas et al., 2008). There was no reported effect on mating performance or fertility, and no deformities were seen in the foetuses.

There are some reported health benefits to supplementing a diet with rebaudioside-A. The toxicological studies revealed that test subjects were given a diet supplemented with 1000 mg/day of rebaudioside-A for sixteen consecutive weeks, which found no statistical effect on blood pressure or blood lipids in the patients. This test result indicated that simply supplementing a diet with

rebaudioside-A would have no negative or positive effects on glucose homeostasis (Carakostas et al., 2008).

## **2.3 Nutritional Qualities of Stevia and Erythritol**

### **2.3.1 Stevia**

Studies have revealed that stevia has been used since ancient times for various purposes throughout the world. *Stevia rebaudiana* Bertoni has attracted economic and scientific interests in recent times due to the sweetness and the supposed by therapeutic properties of its leaf (Lemus-Mondaca et al., 2012). Japan was the first country to utilize *Stevia rebaudiana* extracts as a low-calorie sweetener. Subsequently, stevia products have been introduced in other Asian countries and more recently in Australia and New Zealand.

Unlike sugar, stevia is not fermented by the microflora of the dental plaque. It is also metabolized more slowly, allowing blood sugar levels to remain more stable over time than sugar. Therefore, stevia has been applied as a substitute for sucrose, and has been recommended as a treatment against various diseases such as diabetes, dental caries, obesity, and stomach infections (Yadav and Guleria, 2012). Additional secondary components were found by Lemus-Mondaca et al. (2012), who reported the presence of the anti-nutritional factor oxalic acid (2295.0 and 0.010 mg/100 g, dry weight basis), which can inhibit the bioavailability of calcium, iron and other nutrients.

Stevia glycosides possess valuable biological properties, regular consumption of these compounds decreases the content of sugar, radionuclides, and cholesterol in the blood (Atteh et al., 2008), improves cell regeneration and blood coagulation, suppresses neoplastic growth and strengthens blood vessels (Lemus-Mondaca et al., 2012). Similarly, stevioside has now been identified as an insulinotropic, glucogonostatic, and anti-hyperglycaemic with its ability to lower blood pressure (Yadav and Guleria, 2012). It has been reported to maintain blood glucose level by increasing glucose utilization, because it enhances the effect of stevioside on insulin secretion. Secondly, stevioside has been found to down-regulate the expression of phosphoenol pyruvate carboxylase (PEPCK) enzyme, a

regulatory enzyme of gluconeogenesis. As a result, it reduces glucose production by down-regulating the process of gluconeogenesis (Yadav and Guleria, 2012).

Shivanna et al. (2013) have highlighted the functional prospect of stevia leaf powder, which contains a number of beneficial components including fibre, protein, carbohydrates, rutin, vitamin C and A. Lemus-Mondaca et al. (2012) have reported that, stevia leaves contain a wide variety of amino acids, including tyrosine and cysteine. In addition to these, stevia powder also contains a number of phytochemicals some of which include  $\beta$ -carotene, riboflavin, thiamine, and various terpenes and flavonoids as well as high concentrations of tannins.

As proposed by Lemus-Mondaca et al. (2012), the composition and health-promoting contents present in the leaves of the *Stevia rebaudiana* plant have the potential to be extracted and incorporated in to foods as a functional food ingredient. Consequently, stevia offers therapeutic benefits such as anti-inflammatory, anti-tumour, anti-diarrhoeal, hyperglycaemic, anti-hypertensive, and diuretic and immunomodulatory effects (Lemus-Mondaca et al., 2012). Table 2.2 shows the benefits of rebaudioside-A in food products.

**Table 2. 2 Benefits of Rebaudioside-A in Food Products.**

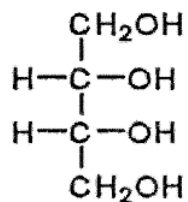
|                            | Carbonated Soft Drinks<br>(Canned soft drinks)   | Dairy Products<br>(Yogurt Ice Cream)                                       | Seasoning,<br>Sauces and<br>Canned Foods   | Baked Goods<br>(Biscuits,<br>Cakes)  |
|----------------------------|--|--|--|--|
| Rebaudioside-A<br>Benefits | Reduces calories,<br>making them suitable<br>for people with low<br>sugar diet;<br><br>Sugar-free labelling;<br>Sweet taste at low<br>concentrations;<br><br>Synergistic with sugar;<br>pH 3-9 stable; Dissolves<br>easily | Non-fermentation<br>properties extend<br>shelf-life;<br><br>Non-cariogenic | Improves<br>taste;<br><br>Shortens<br>salting time<br>and prevents<br>dewatering;<br><br>Extends shelf<br>life | Do not cause<br>Maillard<br>reaction;<br><br>No browning<br>effect;<br><br>Use of<br>bulking<br>agents would<br>be necessary |

Sourced from ingredient supplies website: <http://www.sunwininternational.com/>

### 2.3.2 Erythritol

Erythritol ((2R,3S)-Butan-1,2,3,4-tetrol) belongs to the family of sugar alcohols also known as polyols, which are formed due to hydrolysis processes of the aldehyde or ketone group in various carbohydrates (Billaux et al., 1991). Figure 2.3 illustrates the chemical structure of erythritol. Polyols are naturally abundant in fruits and vegetables, like grapes and mushrooms as well as in fermented foods like soy sauce (Moon et al., 2010). The most valuable properties of these sugar alcohols are their sweetness and low calorie content combined with being noncariogenic (Billaux et al., 1991). Within the sugar alcohols, erythritol plays a somehow extraordinary part. It consists of only four carbon atoms and has therefore the smallest molecular weight of all sugar alcohols, which is associated with slightly different physical and chemical properties. Erythritol has a sweetness of 60 to 80 % than of sucrose (O'Donnell and Kearsley, 2012). It is a carbohydrate in which the carbonyl group has been produced from wheat or corn starch by enzymatic hydrolysis yielding glucose (Goossens and Roper, 1994). As a more important feature, the dissolved erythritol exhibits a strong cooling effect due to its high negative

heat of solution, which is also a non-hygroscopic substance (Park et al., 2005). This sugar alcohol characterizes with a high stability in temperature and acid or alkaline environments as well as does not take part in Maillard-type browning reactions (O'Donnell and Kearsley 2012).



**Figure 2. 3 Chemical structure of erythritol (Bernt et al., 1996).**

Unlike other polyhydric alcohols, it is the only polyol that is non-calorie, providing no energy to the body. Most (60 to 90 %) ingested erythritol is rapidly absorbed via the small intestine in humans due to its small molecular structure (O'Donnell and Kearsley, 2012). *In vitro* studies have demonstrated that erythritol is not metabolized systemically and is excreted and recovered in urine (Bornet, 1994). Therefore, when erythritol is taken as an acute oral-tolerance test it does not raise plasma glucose or insulin levels in healthy subjects. This makes it a particularly useful sweetener for people who suffer from diabetes, and wishing to reduce their post-prandial blood sugar levels (O'Donnell and Kearsley, 2012). Furthermore, erythritol is also a free radical scavenger with the ability to exercise its anti-oxidant activity while circulating the body before it is excreted into the urine (Moon et al., 2010). Erythritol is currently used as a low-calorie, tooth-friendly, bulk sweetener, which provides volume, texture as well as microbiological stability in such products as tooth-friendly chewing gums, candy products, ice creams and beverages (O'Donnell and Kearsley 2012).

Erythritol has been used since 1990 in Japan as a new natural sweetener to become present on the market (Boesten et al., 2015). Erythritol has been reported that is less sweet than sucrose (Goossens and Roper, 1994). The range of applications for erythritol is still growing. It may be found on its own, or in combination with other polyols in foods, cosmetics, and pharmaceuticals.

To date, the use of erythritol in foods has been approved in more than 60 countries, including Europe, the USA, Japan, Canada, Mexico, Brazil, Argentina, Turkey, Russia, China, India, Australia and New Zealand (Boesten et al., 2015). Within the food sector, erythritol is mainly utilized as sweetener to balance the finished product with regard to its sensory characteristics, such as flavour, colour, and texture. Therefore, erythritol can be used to produce no-sugar added, reduced-sugar, or sugar-free alternatives. Erythritol as sugar replacement can be found as tabletop sweetener, in beverages, chewing gum, chocolate, candies, and in bakery products (Moon et al., 2010). Due to its mild sweetness, it allows a volume-for-volume replacement of sugar.

Estimating total polyol intake from dietary surveys of motivated groups, such as persons with diabetes, led to average daily intakes of 4 g polyol/day based on a 7- day UK survey (Ministry of Agriculture, Fisheries and Food, 1990) and 5 g polyol/day based on a 2-day Finnish survey (Virtanen et al., 1988). Additionally, a recent 3-day food intake survey also conducted by the U.S. Department of Agriculture and on an independent chewing gum survey, and assuming that erythritol will be used in all intended applications with no other polyols, a “maximum possible” erythritol intake was also estimated (Fleming, 1995). Based on the available survey data, it can be concluded that the 90th percentile user of erythritol would be expected to have a daily erythritol intake of no more than 4 g/day (67 mg/kg body wt) with a more likely intake estimate of approximately 1 g/day (17 mg/kg body wt/day) for mean users.

## **2.4 Physicochemical Properties of Inulin**

Inulin is a natural dietary fibre found in onions (1-5 % on a fresh weight basis), garlic (4-12 %), bananas (0.2 %), leeks, wheat, asparagus, Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intybus*) root (Meyer et al., 2011). Some 36,000 plants from a wide variety of genera contain inulin as a storage carbohydrate. Chicory is the most commonly source used to produce inulin by means of an extraction process followed by purification and crystallization stages in the industry (Giri et al., 2014). Chicory root is equal to 15-20 % inulin as a storage carbohydrate, consequentially, it can be considered as concentrated source of inulin. Chicory inulin contains up to 10% mono and disaccharides, mainly

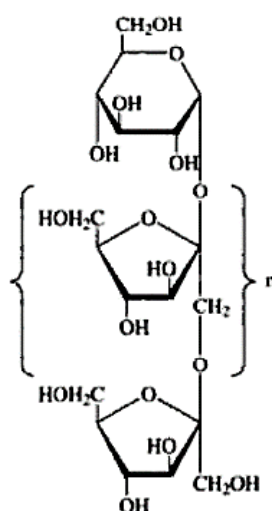


sucrose and fructose, and an oligosaccharide content of approximately 30 % (Nair et al., 2010). Inulin content of some of the sources is presented in Table 2.3.

**Table 2. 3 Inulin content of few food sources (Nair et al., 2010).**

| Source  | Inulin (g/100g) |
|---|-----------------|
| Raw onion bulb ( <i>Allium cepa</i> )                     | 1.1-7.5         |
| Jerusalem artichoke tuber ( <i>Helianthus tuberosus</i> ) | 16.0-20.0       |
| Chicory root ( <i>Cichorium intybus</i> )                 | 35.7-47.6       |
| Asparagus raw ( <i>Asparagus officinalis</i> )            | 2.0-3.0         |
| Garlic ( <i>Allium sativum</i> )                          | 9.0-16.0        |
| Wheat (flour baked) ( <i>Triticum sp.</i> )               | 1.0-3.8         |

Inulin is a polydisperse mixture of molecules all with same basic chemical structure, which can be symbolized as GF<sub>n</sub>, where G is the glucosyl moiety, F is the fructosyl moiety and n is the number of fructosyl moiety linked by β(2,1) linkages (Figure 2.4). A glucose molecule typically resides at the end of each fructose chain and is linked by an α(1,2) bond, as in sucrose (Nair et al., 2010). The degree of polymerization (DP) of inulin typically ranges from 2 to 60 (Giri et al., 2014).



**Figure 2. 4 Structure of Inulin, n = 2-60 (Nair et al., 2010).**

Native or medium chain length inulin has a DP ranging from 3 to 60 monosaccharide units with an average of about 10; its partial enzymatic hydrolysis product is called oligofructose (OF) that is a short-chain inulin with a DP ranging from 2 to 8 with an average of about 4. Long-chain inulin with average DP of about 23 and a DP ranging from 10 to 60 can be produced from native inulin by applying specific separation techniques (Meyer et al., 2011).

The physico-chemical properties of inulin are connected to the degree of polymerisation. The short-chain fraction, oligofructose, is much more soluble and sweeter and it can be used for partial sucrose replacement (Tárrega et al., 2011). Furthermore, short-chain inulin can contribute to improve mouthfeel because its properties are closely related to those of other sugars. Long-chain inulin, however, is less soluble and more viscous than the native inulin (Meyer et al., 2011), and can be used to add rheological and textural properties to fat-free products to give a smooth creamy texture and taste (Tárrega et al., 2011). Inulin has been found to improve the stability of foams and emulsions and can it can be used to replace other stabilizers in food (Nair et al., 2010). Other physico-chemical properties that are influenced by DP include the melting and glass transition temperature (Meyer et al., 2011). In addition, native chicory inulin has a water-binding capacity (1:1.5) for gel formation and the subsequent gel strength (de Gennaro et al., 2000).

Inulin forms a particle gel network when thoroughly mixed with water or another aqueous liquid, leads to a white creamy structure with a short spreadable nature. This property can easily be incorporated into foods to replace fat by up to 100 %. The gel formed comprises a tridimensional network of insoluble sub-micron crystalline inulin particles in water (de Gennaro et al., 2000). Inulin also has the interaction with other food components such as starch or hydrocolloids (Meyer et al., 2011). Apart from the above properties, inulin is one of the categories of non-digestible carbohydrates. Since inulin is resistant to digestion in the upper gastrointestinal tract and is essentially intact until the large intestine, where it is fermented by colonic bacteria causing an increase in faecal biomass, production of short chain fatty acids and decrease in cecocolonic pH (Roberfroid, 2005).

## 2.5 Human Safety of Inulin

According to the American Association of Cereal Chemists International (AACCI), “dietary fibre is defined as the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation” (AACC International 2000).

For many years, dietary fibre has been known to provide health benefits. In 1976, however, dietary fibre was redefined to include all indigestible polysaccharides, including gums, modified celluloses, mucilages, oligosaccharides, and pectins. This new definition came after a modification of the definition developed by scientists who defined dietary fibre as plant cell wall material that is resistant to hydrolysis by the enzymes of the human gastrointestinal tract (Roberfroid, 2005). This definition became widely accepted (AACC International 2000).

Recommended dietary fibre intake in the United States ranges between 20 and 35 grams per day, but the average intake is between 14 and 15 grams per day (Tunland and Meyer, 2002). In other developed countries, such as European countries, the average consumption of fibre is also below recommended intakes. Inulin as dietary fibre, the average daily consumption has been estimated to be 1-4 g in the USA and 3-11 g in Europe (Loo et al., 1999). Inulin has GRAS status in the USA and in most of the European countries it is recognized as “natural food ingredients” (Roberfroid, 2005).

Based on published data implicating the role of dietary fibre in reducing the risks of degenerative diseases, the Food and Drug Administration (FDA) of the United States approved a number of claims that certain types of fibre may reduce the risk of cancer and coronary heart disease (FDA 2002).

## 2.6 Health Benefits of Inulin

Inulin possesses several nutritional and functional properties that may be used to formulate innovative healthy foods for today's consumers (Rodríguez-García et al., 2013). It offers an alternative sweetener that is calorie poor and classified as a soluble dietary fibre because it is not metabolized in the small intestine (Maghaydah et al., 2013). Both the dietary fibre properties of inulin and also the prebiotic properties, are important as they are linked to a variety of beneficial physiological effects as improving bowel habits, increasing calcium absorption, lowering of serum lipids, a positive effect on feeling of satiety, and stimulating the immune system (Meyer et al., 2011).

The chains of fructose units in inulin cannot be broken in the small intestine but are fermented by beneficial bacteria in the colon. Indeed, several studies that investigated the effects of inulin and oligofructose on the human gut microbiota both *in vitro* and *in vivo*, a selective stimulation of growth of the beneficial flora, namely bifidobacteria, which are necessary for proper digestive function and to lesser extent *Lactobacilli* and possibly other species like *Clostridium coccooides*–*Eubacterium rectale* cluster known to be a butyrate producer (Roberfroid, 2005). Low-carb diets can create an inhospitable environment for beneficial bacteria in the colon. This can lead to digestive problems, such as constipation and diarrhoea, and it can also leave the colon susceptible to infection by harmful bacteria and yeast. Bifidobacteria are not only essential to proper digestive function, they also contribute to many other health benefits, inhibiting carcinogens, decreasing intestinal pH, reducing harmful bacteria and yeast, and decreasing toxic substances in the body (Buddington et al., 2002). Furthermore, Carvalho et al. (2013) illustrated that inulin caused an improvement to bowel functions and increased production and composition of short chain fatty acids (SCFA) which has been associated with reducing the risk of colon cancer and facilitate the immune system.

Inulin has much lower calorie values than typical carbohydrates due to the  $\beta(2,1)$  bonds linking the fructose molecules. Since these bonds render them non-digestible by mammalian digestive enzymes, this statement was proved in a human study by Coudray et al., (1997) who found 86-88 % of the dose (10, 17, 30 g) of inulin was recovered in the ileostomy effluent. The energy derived from fermentation

is largely a result of the production of short chain fatty acids and lactate, which are metabolized and contribute 1.5 kcal/g (6.3 kJ/g) of useful energy, rather than 4 kcal/g from its monosaccharide composition. This allows the manufacturer to replace fat containing 37.6 kJ/g with an inulin/water combination, which has an energy value of 2.09 kJ/g or less, resulting in significant calorie reduction (Nair et al., 2010). According to above properties, inulin can reduce the risk of several diseases including cardiovascular disease, diabetes, and is suitable for individuals attempting to lose weight. Clinical studies have shown that inulin can help lower serum triglycerides and low density lipoprotein (LDL) cholesterol in individuals with raised cholesterol levels. It is because the dietary fibre present in these functional sugars can bind to the bile acids and prevent its reabsorption in the liver, which inhibits cholesterol synthesis (Loo et al., 1999).

Inulin as a soluble DF has been shown to slow the release of glucose to in the blood due to its viscose and fibrous structure, thus helping in the control and management of diabetes mellitus and obesity (Qiang et al., 2009). Glycaemic index is a classification of food based on their blood glucose response relative to a starchy food (Brennan, 2005). Therefore, low glycaemic index food, such as highly concentration inulin food, has been proposed as a therapeutic principle for diabetes mellitus by slowing carbohydrate absorption (Qiang et al., 2009). Ziobro et al. (2013) observed a lowering of postprandial blood glucose and insulin responses when inulin was added to a meal of healthy human subjects, and improve the overall blood glucose and lipid concentrations for diabetic patients. Swennen et al. (2006) expanded on this by explaining how low calorie sugars such as the various forms of inulin reduce the peak-levels of the blood glucose spike after a meal, thus inhibiting and / or reducing insulin and glucose lipogenic enzymes. Researchers found no influence on serum glucose, no stimulation of insulin secretion and no influence on glucagon secretion.

Consuming inulin with calcium may help prevent osteoporosis. Loo et al. (1999) demonstrated in their research that inulin was a precursor for the improved bioavailability of calcium in the large intestine, thus reducing the risk of osteoporosis. In a clinical study by Griffin et al. (2002), it was shown that inulin

helped to boost the body's absorption of calcium by 18 % when consumed at relatively low levels. An overview of the benefits of inulin has been given in Table 2.4.

**Table 2. 4 Inulin offers nutritional and technological advantages.**

| Technical Inulin Benefits       |                        |  | Nutritional Inulin Benefits            |                             |                               |
|---------------------------------|------------------------|--|--|-----------------------------|-------------------------------|
| Easy processing                 | Sugar reduction        | Fat reduction                            | A healthy digestive system             | Strong bones                | Easier weight management      |
| High solubility; Dispersibility | Natural-like sweetness | Better texture; creaminess and mouthfeel | Overall fibre intake; Prebiotic effect | Improved calcium absorption | Reduced overall energy intake |

## 2.7 Sucrose Functions in Bakery Products

Sugar functionality is of great importance in baked products not only in terms of contributing to the sweetness of the product but also for the colour, flavour, rheological and textural roles.

Most breads have small amounts of sugars that are important in fermentation processes and non-enzymatic browning reactions. In cakes, quick breads, and cookies, sugar is included to provide sweetness and viscosity, control macromolecular transformations, influence air incorporation and retention, and is important in the emulsive-colloidal stability of the system (Davis, 1995). For surface characteristics, the type and amount of sucrose impacts the sensory and technology on sweet bakery products.

During baking-cookie, creaming and spreading are influenced by the granulation of sugar. For instance, less dough spreading and cracking on the baking surface results from coarse sugar which dissolves more slowly than fine granulated sugar (Nip, 2006). In addition to this sugar in moderate amounts causes a softening and a reduction in viscosity of the dough because it aids in the retention of moisture (Manisha et al., 2012). Olewnik and Kulp (1984) reported that increasing sugar content reduced consistency and cohesion in cookie dough.

Sucrose also acts as a hardening agent due to crystallization when products such as cookies are cooled to room temperature after baking. Similarly, according to the concentration in the formulation, sucrose controls hydration making products crisp. The formation of product colour is also affected by sucrose resulting from the occurrence of heat driven chemical reactions. Davis (1995) identified that even though sucrose inverts to glucose and fructose at higher temperatures, it is classified as non-reducing. Both monosaccharides take part in Maillard reactions with amino acids, which contributes to the development of important flavour components and browning compounds.

## **2.8 Other ingredients: Cocoa powder and Vanilla**

### **2.8.1 Cocoa powder**

Cacao originated in Mexico, but the Spanish found that the cacao tree (*Theobromacacao*) would grow in any tropical region within about 20° of the equator.

Chocolate and cocoa are derived from cocoa or cacao beans. When the beans are fermented, roasted, and ground, the resulting product, which contains a white or yellowish fat (cocoa butter), is called chocolate liquor. Cocoa butter is 50 % to 57 % of the weight of cocoa beans and gives chocolate its characteristic melting properties. Pure chocolate and its blends can be used to top or coat bakery products, or they may become an integral part of the product formula.

The colour of chocolate cake depends on the colour of the cocoa or chocolate used as well as on certain other variables. The colour of cocoa and chocolate is influenced by the variety of cacao beans from which they are produced, including the extent of roasting of the beans, the addition of alkali, and oxidation. As would be anticipated, the deeper the roast, the darker the colour of the resulting chocolate or cocoa. On the basis of processing, naturally processed cocoas and chocolates range between pH 5.1 and 6.2 (Miller et al., 2008), but Dutch-processed products range between pH 6.0 and 7.8 (Brown, 2009). Using cocoa powder in baked product applications can also impart chocolate flavour.

Cocoa powder is the powder remaining after most of the cocoa butter is removed from chocolate liquor, but it can have a wide range (0-24 %) of cocoa butter content.

Furthermore, the pH of chocolate or cocoa-containing cakes differs as a result of the cocoa or chocolate as well as the presence of leavening ingredients. For a desirable flavour, the pH of the batter should be no higher than pH 7.9. Chocolate-containing cakes range in colour from a definite brown at a pH between 6.0 and 7.0 to mahogany between pH 7.0 and 7.5, with increasing redness above pH 7.5 (Brown, 2009).

Cocoa powder contains several minerals including calcium, copper, magnesium, phosphorus, potassium, sodium and zinc. All of these minerals are found in greater quantities in cocoa powder than either cocoa butter or cocoa liquor. It is also rich in flavonoids, a subset of polyphenols (Steinberg, Bearden, & Keen, 2003). The phlobaphene is responsible for the reddish colour seen in cocoa and chocolate to varying degrees, depending on the extent of oxidation (Minifie, 1989). The darker the chocolate is, the higher the level of flavonols such as procyanidins, catechins, and epicatechins. Cocoa powder ranks highest in antioxidant content, followed by dark chocolate and milk chocolate (Steinberg, Bearden, & Keen, 2003). The presence of oxygen also inferences the shelf life of cocoa and chocolate because of the potential for oxidative rancidity of the fat in these products. The major fatty acids in cocoa butter are stearic acid (35 %), oleic acid (35 %), palmitic acid (25 %), and linoleic acid (3 %).

Cocoa and cacao powders products may contain cadmium, a toxic heavy metal and probable carcinogen. From January 1 2019, the European Union will impose a limit for cadmium in cocoa powders of 0.6 µg per gram of cocoa powder, and 0.8 µg per gram for chocolate with ≥ 50 % total dry cocoa solids (EU Commission 2010). Title 21 of the U.S. Code of Federal Regulations (CFR), part 163, states the industry has “specified requirements for specific standardized cacao products”. Standards are defined for cacao nibs, chocolate liquor, breakfast cocoa, cocoa, low-fat cocoa, cocoa with diacetyl sodium sulfosuccinate for manufacturing, sweet chocolate, milk chocolate, buttermilk chocolate, skim milk chocolate, mixed dairy product chocolates, sweet cocoa and vegetable-fat coating, sweet chocolate and vegetable-fat coating, and mild chocolate and vegetable-fat coating. These definitions



include specific required ingredients, optional ingredients, and labelling requirements for certain exceptions.

### **2.8.2 Vanilla**

Vanilla is one of the most important overall flavours used in both bakery products and ice cream. It also serves as a flavour enhancer, especially in premium and super-premium products, where it is commonly used in conjunction with cocoa or chocolate liquor in ice cream to provide a richer, fuller taste. Pure bourbon vanilla from Madagascar, if made properly, is considered the finest vanilla available. It presents a very clean flavour, and it does not over flavour, as would artificial flavours (Hui and Corke, 2006).

## **2.9 Bakery Products**

The most common baked products available on the world market include breads, cakes, pastries, cookies, crackers, and muffins. It is important to note that all the products mentioned above have ingredients which are wheat flour and sugar in common. Most of the sweet baked products have sugar as a major ingredient.

Current trends have seen consumers tending to seek foods that are not only healthy and nutritious, but also appetising and flavourful. This represents a considerable challenge for the baked product industry where consumers are interested in consuming sugar reduced or sugar free products based on health reasons, and yet wish to have the sweet flavour from a hedonic view point.

Stevia or inulin have been reported as potential ingredients to imitate the functional and sensorial properties of sugar or fat in baked products. Their use may have an effect on colour, flavour, volume and texture of the final product, however, replacing sugar constituents without affecting the quality characteristics poses a significant technical challenge.

### **2.9.1 Stevia or Inulin as a Sugar Replacer in Muffins**

Baked products have been subjected to chemical, colour and texture analysis, and sensory characteristics by a number of researchers. Zahn et al. (2010) indicated that a combination of inulin with rebaudioside-A results in products with characteristics close to a reference muffin formulation by multivariate analysis of instrumental and sensory data. Apart from providing sweetness and its contribution to aroma and crust colour formation via caramelisation, sucrose in cake batters facilitates air incorporation and leads to a more stable foam because of the viscosity increase of the continuous phase (Kocer et al., 2007). Sucrose in cake batters is also responsible for an elevation of starch gelatinisation temperature, which ensures a more thorough expansion of air bubbles in the first stage of baking and improves cake microstructure, porosity and volume increase (Zahn et al., 2013). Therefore, the reduction of sucrose levels in a cake system affects structural and sensory properties. Manisha et al. (2012) highlighted that an increase in the number of air bubbles was observed in the batter with increase in percentage of sorbitol from 25 to 100 along with stevioside. Also, cake structure is highly aerated and has a large volume.

Several research papers have reported the use of inulin to replace fat for muffins or cakes, which have laid the groundwork for this research. Psimouli and Oreopoulou (2013) reported that fat was replaced at 35 % to 100 % by inulin and oligofructose in cakes. The study showed that fat replacement by 35 % did not induce significant differences in general. Above 65 % fat replacement resulted in statistically significant decreased viscosity that was followed by significant decrease in air incorporation and broader bubble size distribution. The replacement of fat by carbohydrate- or protein-based replacers affected the batter and cake properties. The batter consistency and specific gravity appear to be controlling factors of the development of the cake volume and textural properties, while the ability to set starch gelatinization to higher temperatures also contributes to volume development. Therefore, an increase in starch gelatinization temperature by oligofructose is expected, while granulated inulin (inulin GR) with a low degree of polymerization (DP) seems to induce a similar effect. Oligofructose led to cake with the darkest crust colour, as expected, while the 2 types of inulin did not differ significantly

from the crust colour of control. The increase of the fat replacement led to cakes of increased hardness and elasticity, and decreased specific volume, and received lower scores on taste and flavour. However, a 65 % fat replacement resulted in cakes with acceptable textural, physical, and sensorial properties. Rodríguez-García et al. (2012) illustrated that oil substitution with inulin decreased batter viscosity, which led to an increase in air bubble size. As fat replacement levels increased, starch granules appeared as detached structures, thus the inulin appeared to disrupt the product structure. Cakes with fat replacement of up to 70 % had a high crumb air cell values; they were softer and rated as acceptable by an untrained sensory panel. Therefore, a good quality cake with a 70 % of oil replacement could be achieved. With increasing amounts of added inulin, product moisture and crumb density increased significantly, whereas muffin volume decreased. Zahn et al. (2010) found that the replacement of 50 % baking fat in a formulation resulted in muffins that were comparable or slightly higher in crumb firmness. However, the complete elimination of baking fat with inulin and water led to products which were unsuitable because of high toughness, low volume and the lack of a product-typical taste. The authors suggested that this may have been as a result of the thermal impact during baking resulting in a partial degradation of inulin. Böhm et al. (2004) showed that dry heating of inulin from chicory for up to 60 min at temperatures between 135 and 195 °C resulted in a significant degradation of the fructan ranging from 20 to 100 %. Using high-performance anion-exchange chromatography with pulsed amperometric detection as well as high-performance thin-layer chromatography, it was found that thermal treatment of inulin leads to a degradation of the long fructose chains and formation of new products, most likely di-D-fructose dianhydrides. Upon thermal treatment of sugars, dehydration and self-condensation reactions occur, giving rise to volatiles principally 2-hydroxymethylfurfural (HMF), pigments (melanoidins) and oligosaccharide material, among which di-D-fructose dianhydrides (DFAs) and glycosylated DFA derivatives of different degrees of polymerization (DP) have been identified (Suárez-Pereira et al., 2010). Therefore, these degradation products of inulin are cleavable by acid to fructose monomers, but their glycosidic bonds are no longer accessible for  $\beta$ -fructosidase, thus explaining the discrepancies in inulin quantification with respect to the method used (Böhm et al., 2004).

## 2.9.2 Stevia or Inulin as a Sugar Replacer in Breads

Being a source of proteins, dietary fibre, vitamins, micronutrients and antioxidants, bread is considered to be of global importance in nutrition (Dewettinck et al., 2008). Contrary to whole grain bread, which is relatively high in fibre content (7-8 % of dry matter), white bread contains only 2-3 % fibre on a dry matter basis (Poinot et al., 2010). A new formula enriched in fibres like inulin can be developed to improve the nutritional quality of white bread. The influence of inulin on the formation and release of white bread volatiles was studied during baking by Poinot et al. (2010). It was demonstrated that Inulin accelerated the formation of the bread crust and the Maillard reaction, they follow the development of crust physical properties and the formation of volatiles responsible for the flavour of breads having different amounts of inulin by kinetic studies. Therefore, it led to breads with an overall quality similar to that of non-enriched breads, but baked for a shorter time. Also, they showed that crust water activity, moisture and clearness could be good indicators of the Maillard reaction during the baking of bread. However, manufacturing high-DF products has challenges regarding technological changes and maintenance of desired sensory properties. The main problem with dietary fibre (DF) supplementation in baking is the detrimental effects on dough handling and on consumer acceptance, due to changes in dough rheological properties, loaf volume reduction, increase of crumb hardness, undesirable taste and mouthfeel (Peressini and Sensidoni, 2009). A study by Peressini and Sensidoni (2009) indicated that addition of inulin to both weak and moderately strong flours resulted in a strengthening effect. Raftiline® HP (inulin HP) reduced expansion during fermentation and baking due to the fact that the protein-starch matrix, important in governing dough strength, was weakened with the inclusion of inulin. Enrichment with Raftiline® ST (inulin ST) led to lower changes in linear viscoelastic properties of dough than inulin HP and had no negative effects on crumb hardness and volume of bread prepared with flour suitable for bread-making. Nevertheless, addition of inulin ST over 5 % was not recommended because of the sweet taste.

Morais et al. (2014) presented a novel concept for making gluten-free breads using sugar substitutes. The main aim of this study was to evaluate the influence of sugar substitutes, as sweeteners and

prebiotics, in gluten-free breads. For instance, the quality of gluten-free breads analysis showed that the sample added with stevia presented a greater intensity of yeast aroma, salty taste, yeast taste, chewiness and hardness, and a lower intensity of crust colour, sour aroma, and adhesiveness. The sample developed with inulin presented a higher intensity of sweet aroma and chewiness, and a lower intensity of crumb colour, crust colour and porosity. In addition, Ziobro et al. (2013) illustrated that an addition of HSI inulin ( $DP < 10$ ) and GR inulin ( $DP \geq 10$ ) to gluten-free formulation based on starch, pectin and guar gum resulted in an increase of loaf volume and reduction of crumb hardness. However, internal structure of the obtained loaves was less uniform and more open than in control bread. Texture of the crumb was also significantly changed both on the day of baking and during storage, as the samples with inulin were generally softer than control. A decrease in staling (measured as the rate of crumb hardening) was observed, which was caused by the presence of inulin. Also, inulin preparations with lower DP had stronger effect on all analysed parameters than that with higher DP. Therefore, the addition of prebiotic and sweetener opens up new opportunities to develop gluten-free breads that may present similar properties to those of wheat-based breads.

Rößle et al. (2011) used inulin and oligofructose as fat and sugar replacers in quick breads (scones). They found that higher concentrations of inulin and oligofructose in quick breads lead to a slight increase in crust and crumb hardness, and that loaf volume significantly increased with inulin. Furthermore, the crust colour was increased by replacement of inulin. Overall, it was found that quick breads (scones) containing inulin and oligofructose can show similar quality characteristics to a control which contains 10 % fat and 10 % sugar from a consumer's point of view.

### **2.9.3 Stevia or Inulin as a Sugar Replacer in Cookies**

Cookies are one of the most popular bakery items consumed due to their ready to eat nature, availability in different varieties and affordable cost (Sudha et al., 2007). Among cereal-based products, cookies are characterized by their enrichment with two major ingredients, sugars and fats, and by their low final water content. However, high sugar intake mentioned above is associated with increased risk

of diseases (Rößle et al., 2011). Several studies dealing with the addition of inulin or other dietary fibres as fat replacers have been conducted to evaluate physical, chemical, and sensory properties of cookies.

Rodríguez-García et al. (2013) evaluated the effects of inulin as fat replacer on short dough biscuits where 10, 20, 30 and 40 % of the shortening was replaced with inulin. The experiment results showed that biscuit texture, dimensions, and weight loss during baking were strongly related to microstructure of dough and biscuit. During baking, the biscuit structure was more continuous than dough structure in that a continuous fat layer coated the matrix surface, where starch granules were embedded. This confining of the starch granules, limited hydration thus water loss during baking increased.

Their research also demonstrated that high fat content improved biscuit expansion and aeration. For instance, biscuits with high fat content were brittle and soft, in contrast to dough with higher fat replacement (30 %) where the absence of fat enables a higher hydration of the component, giving harder biscuits. However, when fat replacement by inulin was 40 %, there was not enough fat to lubricate the system; thus, a fragile structure was obtained by Rodríguez-García et al. (2013). Therefore, sensory panellists found biscuits with 20 % of fat replacement slightly harder than the control biscuits. It can be concluded that fat may be partially replaced, up to 20 %, with inulin. These low-fat biscuits are similar to the control biscuits, and they can have additional health benefits derived from inulin presence.

In addition, Laguna et al. (2013) studied the effect of sucrose replacement by inulin in short-dough cookies using instrumental and sensory analysis. From the collected data of this study, it was concluded that sucrose replacement affects the appearance of cookie as well as the cookie matrix. As a consequence, changes in sensory and instrumental data were observed. They found the 25SI (25 % sucrose replacement by inulin) cookies were the closest sample to the control sample. The instrumental data showed that the 25SI cookies were softer and more brittle compared to control cookie, and without having a detrimental effect on consumer perception of the product.

Maghaydah et al. (2013) researched the possibility to produce gluten-free cookies with inulin. The study focused on using inulin as a source of both prebiotics and fibre used to enhance the nutritional value in gluten-free cookies. Results indicated that the total dietary fibre content increased with the inulin level, but the spread factor of the cookies decreased with increasing inulin level. Sensory evaluation of the products demonstrated that the addition of dietary fibre (inulin) at different concentrations did not compromise the sensory characteristics and incorporation of 4 % inulin had satisfactory consumer acceptance. It was concluded that enhancing gluten-free cookies with a new fibrous prebiotic substance met the nutritional demands in relation to practical demand.

Kulthe et al. (2011) highlighted the use of stevia to develop a high protein and low calorie cookie. The results showed that the thickness and hardness of cookies decreased; diameter, spread ratio and spread factor with increasing stevia leaves powder while there was an increase in protein, crude fibre and ash content and decrease in fat and carbohydrate content. They concluded that the cookies with 20 % substitution stevia leaves powder scored maximum for all the sensory quality attributes. However, the data of this experiment revealed that cookies with 25 and 30 % stevia leaf powder obtained low scores of texture, flavour and overall acceptability as compared to control and other samples. It was observed that, the score for colour, crispiness and taste were lower in the control sample. Therefore, data showed that a reduction of sensory quality in cookies was caused by increasing stevia leaf powder content.

Previous research has indicated the potential of using stevia, inulin and erythritol to replace sugar in muffin products. The aim of this research was to build upon this previous knowledge base and understand the effect of sugar reduction on the physical, sensory and nutritional quality of muffins.

## **Chapter 3**

### **Materials and Methods**

#### **3.1 Raw Materials of Muffin**

The standard formulation of muffins used Wheat flour (Medal Premium baker flour, Champion, Auckland, New Zealand), white sugar (Chelsea, Auckland, New Zealand), baking powder (Edmonds, Auckland, New Zealand), iodised table salt (Cerebos, Auckland, New Zealand), skim milk powder (Pams, Auckland, New Zealand), canola oil (Pams, Auckland, New Zealand), and fresh whole egg (obtained from the local New World supermarket, Food Stuffs, Christchurch, New Zealand). Inulin Frutafit IQ, an inulin with DP 5-7 and sweetness of 10 % compared to 100 % sucrose (Sensus, Amsterdam, Netherlands). Stevianna® (product code ST001\_SE; Stevianna®, Auckland, New Zealand), Stevianna® utilises organic Reb-A 98 % stevia as the main sugar substitute along with erythritol as bulking agent. Cocoa powder (Cadbury, Dunedin, New Zealand) and vanilla (Hansells, Auckland, Australia) were used in Recipe 2.

#### **3.2 Muffin Batter Preparation and Baking Procedure**

A control recipe was prepared according to the literature (Hui and Corke, 2006). Two muffin batter recipes were innovated in this research, and are given in Table 3.1 and Table 3.2.

Recipe 1 muffin batter contained 69.2 g sugar, 8.7 g skim milk powder, 5.8 g baking powder, 1.4 g salt, 34.6 g liquid whole egg, 57.6 g oil, 57.6g water and 115.3 g wheat flour. Addition of Stevianna® or inulin was used to replace sugar at 25 %, 50 %, 75 % and 100 %. Liquid whole egg was beaten in a plastic bowl by a wire whisk. Dry ingredients and prebeaten egg, oil and water were weighed using a Ohaus SP602 Scout Portable Scales (Bradford, MA, USA). After weighing, batter was mixed in a mixer (Delta Food Equipment, Savannah, GA, USA) with the following mixing steps. Firstly, prebeaten egg, oil and water were mixed with the mixer for 60 s (10 s at speed 4; 50 s at speed 8). Next step, dry ingredients were



added to the premixed liquid in the mixing unit and whisked for another 120 s (10 s at speed 2; 110 s at speed 8).

The ingredients used in the preparation of the Recipe 2 muffin batter were 138.4 g wheat flour, 92.2 g sugar, 8.7 g skim milk powder, 6.5 g baking powder, 1.4 g salt, and 34.6 g liquid whole egg, 77.6 g oil and 97.6 g water. Stevianna® was used as a sugar replacer at 50 % and 100 %. Additional batters were made by adding 23.1 g cocoa powder or 3 g vanilla to recipe 2 muffin batter. Liquid ingredients were mixed for 60 s (10 s at speed 4; 50 s at speed 8). Then, dry ingredients were added into the premixed liquid and mixed for 10 s at speed 2 then 170 s at speed 8.

After mixing step, the batter ( $43 \pm 0.1$  g aliquots) was placed into paper baking cases in a muffin pan, the muffins were baked in a Simpson gemini atlas series oven. Heat was set to 180 °C at fan bake, and baking time was 18 min. Baked muffins were cooled at room temperature for 1 h, then packed in plastic resealable bags and stored in the refrigerator (4 °C) overnight for further analysis.

**Table 3. 1 Ingredients (g) measured in the muffin recipe 1.**

| <b>Samples<sup>a</sup></b> | <b>C</b> | <b>I50</b> | <b>S50</b> | <b>I100</b> | <b>S100</b> |
|----------------------------|----------|------------|------------|-------------|-------------|
| <b>Ingredients</b>         |          |            |            |             |             |
| Wheat flour                | 115.3    | 115.3      | 115.3      | 115.3       | 115.3       |
| Sugar                      | 69.2     | 35         | 35         | 0           | 0           |
| Baking powder              | 5.8      | 6.25       | 6.25       | 5.8         | 5.8         |
| Salt                       | 1.4      | 1.4        | 1.4        | 1.4         | 1.4         |
| Skim milk powder           | 8.7      | 8.7        | 8.7        | 8.7         | 8.7         |
| Oil                        | 57.6     | 57.6       | 57.6       | 57.6        | 57.6        |
| Liquid whole egg           | 34.6     | 34.6       | 34.6       | 34.6        | 34.6        |
| Tap water                  | 57.6     | 65         | 65         | 72          | 57.6        |
| Inulin SENSUS              | 0        | 35         | 0          | 69.2        | 0           |
| Stevianna®                 | 0        | 0          | 35         | 0           | 69.2        |

<sup>a</sup> samples name: C, Control sample; I50, 50% of sucrose replacement by inulin; S50, 50% of sucrose replacement by stevia; I100, 100% of sucrose replacement by inulin; S100, 100% of sucrose replacement by stevia.

**Table 3. 2 Recipe 2 for muffins at two stevianna levels, with or without cocoa powder and/or vanilla.**

| <b>Samples <sup>a</sup></b><br><b>Ingredients</b> | <b>C</b> | <b>V</b> | <b>CP</b> | <b>CP+V</b> | <b>50S</b> | <b>50S+V</b> | <b>50S+CP</b> | <b>50S+CP+V</b> | <b>100S</b> | <b>100S+V</b> | <b>100S+CP</b> | <b>100S+CP+V</b> |
|---|----------|----------|-----------|-------------|------------|--------------|---------------|-----------------|-------------|---------------|----------------|------------------|
| Wheat flour                                       | 138.4    | 138.4    | 115.3     | 115.3       | 138.4      | 138.4        | 115.3         | 115.3           | 138.4       | 138.4         | 115.3          | 115.3            |
| Sugar   | 92.2     | 92.2     | 92.2      | 92.2        | 46.1       | 46.1         | 46.1          | 46.1            | 0           | 0             | 0              | 0                |
| Baking powder                                     | 6.5      | 6.5      | 6.5       | 6.5         | 6.5        | 6.5          | 6.5           | 6.5             | 6.5         | 6.5           | 6.5            | 6.5              |
| Salt  | 1.4      | 1.4      | 1.4       | 1.4         | 1.4        | 1.4          | 1.4           | 1.4             | 1.4         | 1.4           | 1.4            | 1.4              |
| Skim milk powder                                  | 8.7      | 8.7      | 8.7       | 8.7         | 8.7        | 8.7          | 8.7           | 8.7             | 8.7         | 8.7           | 8.7            | 8.7              |
| Oil   | 77.6     | 77.6     | 77.6      | 77.6        | 77.6       | 77.6         | 77.6          | 77.6            | 77.6        | 77.6          | 77.6           | 77.6             |
| Liquid whole egg                                  | 34.6     | 34.6     | 34.6      | 34.6        | 34.6       | 34.6         | 34.6          | 34.6            | 34.6        | 34.6          | 34.6           | 34.6             |
| Top water   | 97.6     | 97.6     | 97.6      | 97.6        | 97.6       | 97.6         | 97.6          | 97.6            | 97.6        | 97.6          | 97.6           | 97.6             |
| Cocoa Powder                                      | 0        | 0        | 23.1      | 23.1        | 0          | 0            | 23.1          | 23.1            | 0           | 0             | 23.1           | 23.1             |
| Vanilla   | 0        | 3        | 0         | 3           | 0          | 3            | 0             | 3               | 0           | 3             | 0              | 3                |
| Stevianna®  | 0        | 0        | 0         | 0           | 46.1       | 46.1         | 46.1          | 46.1            | 92.2        | 92.2          | 92.2           | 92.2             |

<sup>a</sup> Sample name of formulation: Control (C); Vanilla (V); Cocoa Powder(CP); Cocoa+Vanilla (CP+V); 50% Stevianna (50S); 50% Stevianna+Vanilla (50S+V); 50% Stevianna+Cocoa (50S+CP); 50% Stevianna+Cocoa+Vanilla (50S+CP+V); 100% Stevianna (100S); 100% Stevianna+Vanilla (100S+V); 100% Stevianna+Cocoa (100S+CP); 100%Stevianna+Cococa+Vanilla (100S+CP+V)

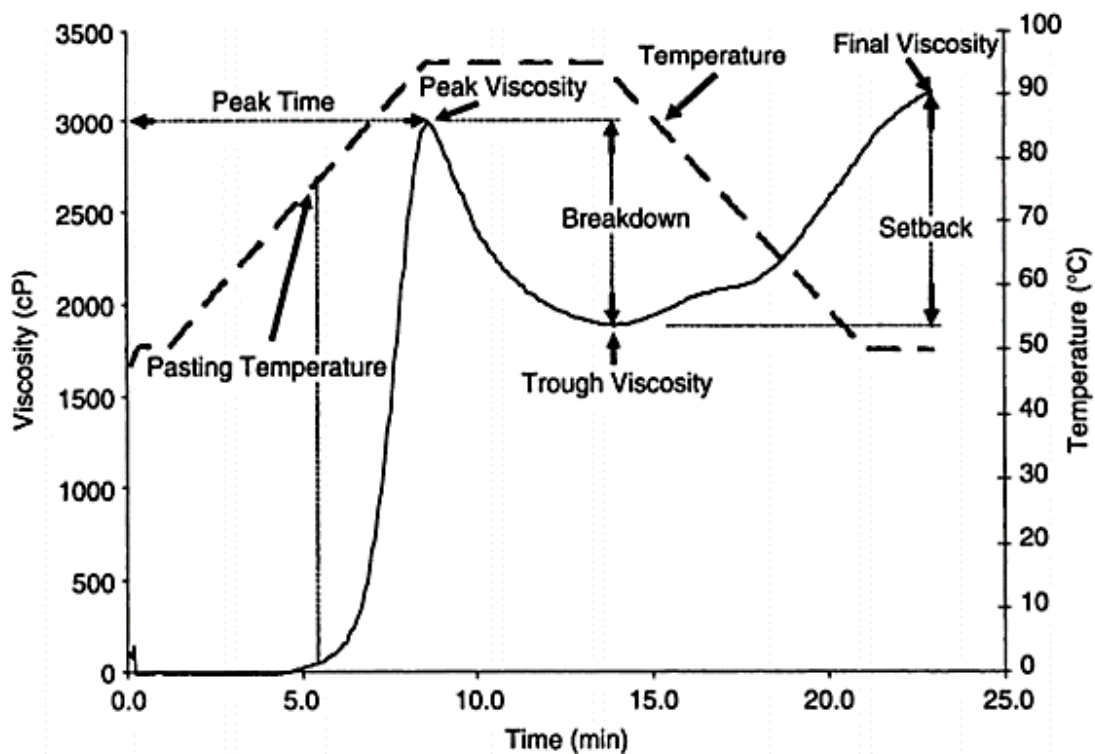
### **3.3 Properties of the Muffin Batter**

#### **3.3.1 Pasting Properties of Batter**

Rheological properties of starch pasting are traditionally studied by an instrument called the Rapid Visco Analyzer. The working principle of this type of equipment is that the rheology is directly related to the microstructure of starch and is influenced by many factors such as the amylose/amylopectin ratio, the chain length of amylose and amylopectin molecules, the concentration of starch, shear and strain, and temperature. The sample is heated over a range of temperatures and the viscosity is recorded over a period of time. Starch granules are generally insoluble in water below 50 °C, so the viscosity of the starch mixture is below this temperature. When the starch granules are heated, the granules absorb a large amount of water and swell to many times of their original size.

As the instrument generates shear conditions, the viscosity increases when the swollen starch granules squeeze past each other. The temperature at which the rise in viscosity is seen is known as the pasting temperature, which indicates the minimum temperature required to cook a sample. As a sufficient amount of starch granules are heated, there is a period of time where there is a rapid increase in viscosity as the temperature increases. The peak viscosity occurs at the equilibrium point where starch granules are completely swollen and just as they begin the retrogradation process. The peak viscosity and temperature indicates the water binding capacity of the starch. As the temperature is held constant over a period of time, the starch granules begin to rupture and polymer realignment occurs, which is evident by the decrease in apparent viscosity of the paste and is known as the breakdown viscosity, which occurs at the beginning of the cooling phase. The viscosity at this stage of heating also gives an indication of paste stability. As the sample is cooled to the starting temperature, re-association between the starch molecules, especially amylose, occurs to varying degrees, which results in an increase in viscosity once again as a gel begins to form. This phase of the pasting curve is referred to as the setback region, and occurs due to the retrogradation of the starch molecules. The final viscosity gives an indication of the stability of the cooled, cooked paste under low shear conditions (Cui and Liu,

2005). Below is a typical pasting curve used to illustrate the specific points in the pasting profile determined during the duration of the run.



**Figure 3. 1 Typical RVA pasting profile of a normal maize starch for viscosity ( — ) and temperature ( - - - ) as a function of time (Cui and Liu, 2005).**

A Rapid Visco Analyser (RVA Super 4; Newport Scientific, Warriwood, Australia) was used to study the viscosity properties of the muffin batters to characterize the behaviour of Stevianna® or inulin concentration on the viscosity properties of the batter and resulting changes occurring during processing. The analysis data can be calculated by a computer running ThermoCline for Windows v3 (TCW3) software.

The RVA studies were carried out using 20 g of batter sample in an aluminium canister. The temperature profile started with a holding step at 25 °C for 5 min, followed by a linear temperature increase from 25 °C to 95 °C at 2 °C /min and a holding step of 25 min at 95 °C. The paddle speed was 75 rpm (rotations per min). The TCW3 software continuously recorded the viscosity and calculated the peak viscosity and final viscosity.

### **3.3.2 Differential Scanning Calorimetry**

Calorimetry involves the determination of the temperature and or the quality of heat absorbed or emitted when a material undergoes a specific chemical or physicochemical change such as baking. Differential scanning calorimetry (DSC) was performed using a DSC 8000 (PerkinElmer, Waltham, USA) to investigate the thermal parameters of muffin batter and to evaluate the changes in starch at the molecular level during baking. Batter (3 mg) was mixed with distilled water, to a total weight of 10 mg, in a gold DSC pan and then left to equilibrate for 2 hours prior to the testing. The samples were heated from 0 to 110 °C at 10 °C /min, together with an empty reference pan, and indium was used for calibration. The thermal parameters associated with the gelatinisation process, onset temperature ( $T_{\text{onset}}$ ), peak temperature ( $T_{\text{peak}}$ ), end temperature ( $T_{\text{endset}}$ ) and the change of enthalpy ( $\Delta H$ ), were measured by heating the crystalline material at 10 °C/min rate to a temperature.

## **3.4 Determination of the Physiochemical Characteristics of Muffin**

### **3.4.1 Image Analysis of Cellular Structure in the Muffins**

For bubble measurements, the muffins were cut on a horizontal plane at a distance of 4 cm from the base and images of the freshly cut surface of the crumb were captured using a EOS-1000D super steady shot camera (Canon, Japan). Image processing was performed using the software ImageJ (Natl. Inst. Of Health, Bethesda, Md., U.S.A.). The image was cropped in a 1300-pixel section and converted to an 8-bit greyscale. After threshold, the contrast was enhanced and finally the image was binarized. Computed parameters included air cell area within the crumb and air cell average size. The data were obtained by measuring air cells in three different images for each formulation.

### **3.4.2 Scanning Electron Microscopy**

Samples of baked muffin were frozen in liquid nitrogen and freeze-dried. A scanning electron microscope (EVO 18 SEM, Carl Zeiss, Germany) was used to examine the morphologies of the freeze-

dried muffins. The samples were transversely fractured to expose interior surfaces, and separately mounted on metal (molybdenum) stub using double sided tape. Because the freeze-dried cakes were good electrical insulators, they charged upon exposure to the electron beam; this resulted in loss of resolution. To reduce the charging effects on exposure of the samples to the electric beam, all samples were coated with a thin layer of gold by a sputter sputtering equipment (E306A, Edwards Coating System). The microscope scanned across the surface of the samples with an ultrafine beam of electrons at the accelerating voltage of 30 kV. The images of the sample surfaces were displayed at magnification of 50K to 100K. The Zeiss-EVO 18 is a low vacuum SEM, which has a high-performance SEM for fast characterisation and imaging of fine structures. The selectable low vacuum mode allows for the observation of specimens that cannot be viewed at high vacuum due to excessive water content or because they have a nonconductive surface as the case of muffin samples.

### **3.4.3 Muffin Height**

The muffin product was taken out from the paper baking case, and the muffin height was measured with an electronic caliper (INSIZE) from the highest point of the muffin to the bottom of the muffin.

### **3.4.4 Muffin Volume**

The volume of the muffins was measured by the rapeseed displacement method. Each muffin was placed in a plastic beaker of known volume (total volume,  $V_t$ ), the remaining space in the plastic beaker was then filled with rapeseed; the volume of the rapeseed required ( $V_s$ ) was then determined by graduated cylinder. Muffin volume was calculated as the difference between the total volume and volume of rapeseed, the muffin volume =  $V_t - V_s$  (Lin et al. 2003).

### **3.4.5 Textural Characteristics of the Muffin**

As a measurement of food quality, texture is important for observing both defective and acceptable food products. Texture can be defined as a group of physical characteristics that arise from the structural elements of the food and are assessed primarily by the feeling of touch, are related to the

deformation, disintegration and flow of the food when a force is applied (Taub and Singh, 1997). A group of properties based on physical structure, sense of touch, and functions of mass, distance, and time comprise the definition of texture (Bourne, 2002). The classifications of this testing are puncture, compression-extrusion, cutting-shear, compression, tensile, torsion, bending and snapping and deformation. A comprehensive definition of food texture analysis and methods for evaluation can be found at Bourne (2002).

The various methods for food texture analysis depend on the properties of the food. A common texture instrument or universal testing machine measures force as a function of time and distance. A compression test will measure the larger surface area of the food sample by forcing it to flow or fracture and deform dependent on its composition. This type of a compression test is widely used in the industry as a measure of food quality during shelf-life studies and to observe changes occurring due to ingredient modifications. When the direction of the force applied to the sample is parallel to the direction it is sliding, this is known as shear. A food product can also be measured for the force to be divided into two sections, bent or pulled apart (Tabilo-Munizaga and Barbosa-Cánovas, 2005). Using any test, the most accurate data depends on a consistent sample temperature, size, shape, speed, distance and direction.

Instrumental techniques do not completely indicate textural quality of a product since they lack the uniqueness of consumers' perception. A sensory texture analysis is needed to measure the quality of a food dependent on its acceptability. However, human experience of a trained expert can be compared to physical properties results for insight on the reaction of texture differences. Using the human senses to manipulate the food product by eating allows for many different variables to be identified.

The instrumental texture measurements of the muffin samples were determined using a TA.XT.plus Texture Analyzer (Stable Microsystems, Godalming, UK) provided with Texture expert software. Firstly, a 50-kg load cell was used for force calibration, and the upper probe was previously calibrated at 75 mm above the lower fixture. The texture parameters were determined with a test speed of 1.0 mm/s,

and strain of 50 % of the original cube height, a 5 s time was elapsed between compression cycles. The compression was performed using a 75 mm diameter flat-ended cylindrical aluminum probe (P/75) which can be attached to the arm of texture analyser. Next, measurements were performed on four muffins from each sample. Each muffin was removed from paper cup and placed on a hollow planar base and the force was then applied to the sample by a 75 mm cylindrical aluminum probe with a strain of 25 % of the original height at a constant speed of 1 mm/s with a 5 s waiting time was compressed, followed by a return to the original position. Finally, the compression test was obtained the two primary textural parameters from the curves, which were firmness (the maximum value in the compression), springiness (the distance until the detected height in the compression), as calculated by the Texture expert software.



**Figure 3. 2 Apparatus and set-up of TA.XT.plus Texture Analyzer for measurement of the baked muffin samples.**

### **3.4.6 Muffin Colour**

Surface colour is one of the important characteristics of baked products and is considered as a critical index for judging baking quality. Baked products develop colour in the later stages of baking, simultaneously with crust formation and occur through chemical processes including the Maillard



reaction and sugar caramelization. It will be important to monitor these colour changes differences that occur due to the removal of sugar from the product, which may affect the caramelization reactions occurring at the higher temperature stages of the baking process.

The colour of the surface and interior of muffin samples were analysed using a Tristimulus Colour Analyzer (Minolta Chroma Meter CR200, Minolta Camera Co., Japan). The instrument was equipped with a CR200 measuring head connected to a microcomputer and was calibrated using the standard white tile ((L\* — 98.03, a\* — -0.23, b\* — 0.25)). After preparing muffin samples as described above, measurements were taken for surface colour on top of the muffin and the interior crumb surface colour from muffins that were cut longitudinally from top to bottom. Measurements were taken as an average of three locations across the surface of the same muffin, and the results were expressed as means for L\*, a\* and b\* values of the illuminant C system (CIE, standard, 6774 K). In addition, results were expressed as total colour difference ( $\Delta E^*$ ) between control sample and sugar-reduced products according to the following equation (Maskan, 2001):

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

where L\* is brightness and ranges from 0 (black) to 100 (white), a\* is redness from +100 (redness) to -100 (greenness), and b\* is the yellowness from +100 (yellowness) to -100 (blueness).

The perception of the colour difference  $\Delta E^*$  has been observed to vary according to the colour and the sensitivity of the human eye (Bodart et al., 2008). When  $\Delta E^*$  is less than 1 no colour difference is obvious to the human eye;  $1 < \Delta E^* < 3$  minor colour differences could be appreciated by the human eye depending on the hue, and when  $\Delta E^* > 3$  colour differences are obvious for the human eye (Martínez-Cervera et al., 2011).

The three-dimensional L\*, a\* and b\* colour were also expressed as a browning index (BI), as shown in the equation below (Maskan, 2001):

$$BI = 100 (X - 0.31) / 0.172$$

Where  $X = a^* + 1.75L^* / (5.645L^* + a^* - 3.012b^*)$

### 3.4.7 Moisture Content

A domestic kitchen food chopper (Zyliss®) was used to chop and homogenize the muffin (crust and crumb) of each formulation. Approximately 4 g was dried in an air oven at 105 °C for 16 h, until no further weight change.

The moisture content (MC) was calculated using the following equation:

$$\text{MC (\%)} = (\text{W before drying} - \text{W after drying} / \text{W before drying}) \times 100$$

where W denotes weight (g).

### 3.4.8 Muffin Total Starch

Total starch analysis was carried out in triplicate according to the official AACC International method 76.13 (AACC International, 1995). To determine the starch construction of all samples, this step was required in order to establish a consistent level of starch for the *in vitro* digestion process, thus enabling comparative assessments of starch digestion between samples to be conducted.

## 3.5 *In vitro* Predictive Glycaemic Response Digestion Analysis

Each muffin recipe was analysed for potential glycaemic response in triplicate following the method reported previously by Woolnough et al. (2010).

### 3.5.1 *In vitro* Digestion Analysis for Muffin Samples

Whole muffins were chopped with a domestic kitchen food chopper (Zyliss®) to stimulate particle size reduction which occurs during natural mastication for at least one minute of steady chopping until a fine crumb was achieved. Using data obtained from the starch analysis step (3.4.8) muffin sample weights were used at equation starch concentration as follows:

$$\text{Sample mass} = 0.25 / (\% \text{Starch} / 100)$$

Thus, total sample weight was approximately 1.3 g of material depending upon starch content. Triplicate samples of product (approximate 1 g of cooked muffin) were each placed into the 60 mL plastic pots and 30 mL of distilled water added, and duplicate blank samples. These pots were inserted to a pre-heated 15 place magnetic heated stirring block (IKAMAG® RT15, IKA®-WERKE GmbH & Co., Staufen, Germany) preheated to 37 °C, on each pot one magnetic stirrer, followed by 0.8 mL of 1 M aqueous HCl. Then 1 mL of a 10 % pepsin (Acros Organics, New Jersey, USA CAS:901-75-6) solution in 0.05 M HCl was added in order to replicate gastric digestion. The sample incubated at 37 °C for 30 min with slow constant stirring (130 rpm) to simulate gastric digestion conditions. Stomach digestion was halted by the addition of 2 mL NaHCO<sub>3</sub>. Small intestine digestion was mimicked by the addition of 5 mL 0.1 M Na maleate buffer (pH 6). An aliquot (1 mL) was withdrawn (Time 0) and added to 4 mL absolute ethanol to stop any further enzyme reaction. A 0.1 mL dose of amyloglucosidase (*A. niger*, Megazyme, E-AMGDF; 3260 U/mL) was added to prevent end-product inhibition of pancreatic amylase. A 5 mL 2.5 % pancreatin (EC: 232-468-9, CAS: 8049-47-6, activity: 42362 FIP-U/g, Applichem GmbH, Darmstadt, Germany) in 0.1 M Na maleate buffer (pH 6) followed by adjusting the volume being to 53 mL with continued stirring and heat maintained at 37 °C for 120 min. Aliquots (1 mL) were taken at 20, 60 and 120 min and placed into ethanol (4 mL) to stop digestion. The samples were stored at 4 °C for reducing sugar analysis by dinitrosalicylic (DNS) colourimetry. These samples were then analysed for their reducing sugar content using 3,5-dinitrosalicylic acid by the method of Woolnough et al. (2010).

### **3.5.2 *In vitro* Digestion Analysis for Muffin Batters**

*In vitro* digestion was conducted on all of the RVA gels to determine the predictive glycaemic response in the “cooked” muffin mixture.

This procedure measures the breakdown of carbohydrates to sugars by the action of amylase enzymes added to the “cooked” muffin mixture. A 3.5 g sample of “RVA cooked” mix was used to determine the predictive glycaemic response. The procedure used pancreatin to digest the food and the amount of reducing sugars released (RSR) over a 120 min digestion process was determined. Samples were

incubated at 37 °C with constant stirring and aliquots were withdrawn at 0, 20, 60, 120 min. Triplicate 1 mL aliquots were added to 4 mL absolute alcohol and stored for later analysis using DNS.

### **3.5.3 Reducing Sugar Determination Using DNS Colourimetry**

The test tubes containing 1 mL digesta aliquots in 4 mL absolute ethanol were centrifuged (ROTINA 380 R, Massachusetts, United States) at 1,000 rpm for 5 min. Aliquots of 0.05 mL were withdrawn from each tube and transferred into a fresh set of tubes, as well as water blanks, standards (5 mg/mL glucose) and standards (10 mg/mL glucose). To each tube, 0.25 mL of enzyme solution A (1% invertase, 1% amyloglucosidase in 0.1 M Na acetate buffer (pH 5.2) was added. Tubes were gently shaken and left to sit at room temperature for 20 min while any incompletely hydrolysed starch fragments were broken down to measurable glucose (secondary digest). DNS mixture (0.75 mL) was added to all tubes, which contained 0.5 mg/mL glucose: 4 M NaOH: DNS reagent, mixed in the ratio 1:1:5, the tubes covered and boiled in a water bath for 15 min at 95-100 °C. Following boiling, samples, reagent blanks and glucose standards were diluted with 4 mL water before transferring to cuvettes and their absorbance (abs) read by colourimetry at 530 nm wavelength (V-1200, Leicestershire, England). Glucose release was plotted against time and area under the curve (AUC) was calculated by dividing the graph into trapezoids as described elsewhere (Matthews et al., 1990). Predicted glycaemic response were determined by the *in vitro* digestion analysis.

## **3.6 Sensory Evaluation**

Sensory evaluation is a scientific discipline used to evoke, measure, analyse, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing (Hui and Corke, 2006). Sensory evaluation is a technique that food scientists use the human body and its perception of the five basic senses as a tool to measure differences and intensities of food characteristics. The objective of the sensory panels pertaining to this research included looking at key differences occurring due to the replacement of sugar from the muffin formulations.

Sensory evaluation was conducted in the food sensory suite at Lincoln University. The trial was approved by the Lincoln University human ethic committee (2015-38). A copy of the final approval letter can be found in Appendix B.1. Panellists were recruited by email and were not informed of the treatments. Potential panellists who had a history of serious anaphylactic reaction to any food or a history of significant bowel disease (including Crohn's; ulcerative colitis; Coeliac's disease) were excluded (Appendix B.1). Each participant received an invitation letter explaining the research; its purpose, responsible researchers and a list of allergens contained in the test products (see Appendix B.2).

The experiment involved three sessions which were conducted over 3 weeks. A consumer panel of 40 untrained panellists (staff and students of Lincoln University) completed the sensory evaluation study. In this study, muffins were cut into quarters, revealing both crust and crumb. Panellists received four samples of muffins at the first tasting session, and 5 samples were provided at each of the following two sessions. All samples were coded with random 3-digit numbers and were served simultaneously on white plastic trays. Additionally, each panellist was given two unsalted crackers, a cup of water, pencil, napkin and the sensory evaluation form (See Appendix B.3)

The sensory line scales form in Appendix B.3 was used to collect information from panellists' evaluation. Line scales of 15 cm were used to record panellists' opinions. Line scales are more common than category scales in contemporary sensory studies. Their advantages are that they avoid decisions by the experimenter about category labels and spacing and that they are less constraining in actual use by the panellists. Panellists indicate their judgments by placing a mark at any point on the line and so may indicate minor differences between products which may have been grouped together under a category scale (Warner, 1995). The detailed information of scale anchors are shown in Table 3.3. The response categories were appearance, colour, texture, mouth-feel, sweetness, and overall liking of the muffin. Panellists were asked to respond directly about the presence of an aftertaste.

**Table 3. 3 Description of the sensory scale anchors used.**

| <b>Variable category</b> | <b>Scale anchors</b>                        |
|--------------------------|---|
| Visually                 | Not at all appealing to extremely appealing |
| Colour                   | Extremely light to extremely dark           |
| Texture                  | Extremely soft to extremely hard            |
| Mouth-feel               | Extremely moist to extremely dry            |
| Sweetness                | Not sweet at all to extremely sweet         |
| Overall liking           | Dislike extremely to like extremely         |

### **3.7 Statistical Analysis**

#### **3.7.1 One-way ANOVA**

All parameters were measured with three major replications of each sample. Results were examined to determine if significant differences existed. Analysis of variance (one-way ANOVA) were performed on the data, and the significance at Tukey's comparison test ( $P < 0.05$ ) were determined. Mean values and standard errors of the mean are reported in the text. These analyses will be performed using Minitab 17.

#### **3.7.2 Data Analysis of Sensory Evaluation**

For all products, participant ratings on the labelled 15 cm line scale were measured geometrically to produce factor values (cm). In the data processing procedure, the control value was subtracted from the sample value for each parameter for each participant before the data was analysed. The control sample values were used as the relative value (0) for each parameter in this study, as the control muffin was presented at each session with the rest of the samples and was evaluated in random order among panellists. This gives a positive or negative value which can be interpreted as being "more" or "less" than the control as shown in Table 3.4. Thus, figures obtained in the present work are relative values.

**Table 3. 4 Description of calculated factor values.**

| Variables category | Factor values after calculation* |                 |                         |
|--------------------|----------------------------------|-----------------|-------------------------|
|                    | Positive (+)                     | 0               | Negative (-)            |
| Visually           | Better than control              | Same as Control | Worse than control      |
| Colour             | Darker than control              | Same as Control | Light than control      |
| Texture            | Harder than control              | Same as Control | Softer than control     |
| Mouth-feel         | Dryer than control               | Same as Control | Moister than control    |
| Sweetness          | Sweeter than control             | Same as Control | Less sweet than control |
| Overall liking     | Better than control              | Same as Control | Worse than control      |

\* These values are relative to control muffin.

Data from assessment of appearance, colour, texture, mouthfeel, sweetness, and overall liking were evaluated separately by analysis of variance (one-way ANOVA) using Minitab 17. A value of  $P < 0.05$  was selected for statistical significance using Tukey's comparison test. Responses to a question about presence of aftertaste were coded as "0" for no-aftertaste and "1" for an aftertaste. The aftertaste data were evaluated using nominal logistic regression of Minitab 17.

### **3.7.2.1 PCA**

In order to analyse the relationship between different products types based on the individual response categories, principle component analysis (PCA) was performed on individual data using Minitab 17. Briefly, this method attempted to explain the relationship between variables and each major axis produced is a result of their joint contribution. In order to produce meaningful results, the first two or three axes must account for a considerable percentage of the total variance (Petridis et al., 2014).

## Chapter 4

### Effect of Sugar Replacement with Stevianna® or Inulin on the Texture and Predictive Glycaemic Response of Muffins

(This is derived from data presented in a published paper: Gao, J., Brennan, M. A., Mason, S. L., & Brennan, C. S. (2016). Effect of sugar replacement with stevianna and inulin on the texture and predictive glycaemic response of muffins. *International Journal of Food Science & Technology*, 51(9), 1979-1987.)

**Abstract:** The application of sugar replacers used in bakery products is of growing interest to the food industry, as it provides the possibility of delivering products with reduced energy and sugar. The aim of this study was to investigate the textural properties and glycaemic responses of muffins made using Stevianna® and inulin. Two levels of sugar replacer were used (50 % and 100 %). Total replacement of sucrose gave muffins with a firmer texture than the control ( $P < 0.05$ ); 50 % replacement, however, gave a similar texture to control. The predicted glycaemic response was reduced in sugar-replaced muffins compared to control samples. In particular, the replacement of sucrose with 100 % Stevianna® caused a significant decrease in the standardised area under the curve values. Therefore, there exists the potential to regulate the glycaemic response of muffins by the incorporation of 50 % Stevianna® or 50 % inulin without affecting their textural properties.



## 4.1 Introduction

Muffins are a popular snack product with high consumer acceptance. Sugar is one of the main ingredients of muffins and contributes to the product structure as well as the characteristic taste and soft texture. However, sugar has a high glycaemic index, which can cause an acute increase in postprandial plasma glucose and insulin levels after eating these foods (Grigor et al., 2016). Excessive consumption of sugar leads to high energy intakes may cause dental problems, obesity, type 2 diabetes, high blood cholesterol and coronary heart disease (Rößle et al., 2011).

Sucrose performs multiple functions in muffins. It is included to provide sweetness, control moisture retention, influence air incorporation, stabilise air bubbles and limit the swelling of starch during baking, all of which help to create a finer texture (Nip, 2006).

The structural and sensory properties of muffin system have been reported to be influenced by the reduction in sucrose levels (Martínez-Cervera et al., 2014). Researchers have investigated the sugar substitutes needed to replace all major functions of sucrose, such as sweetness, colour, texture and flavour (Kocer et al., 2007; Nip, 2006; Struck et al., 2014). High-intensity sweeteners usually provide only sweetness to a product, that is why it is common practice to use bulking agents combined with them to provide the functional properties of sugar and act as structure-building substances in foods. This represents a considerable challenge for the cereal food industry where consumers are interested in consuming sugar-reduced or sugar-free products based on health reasons and yet wish to have the sweet flavour and good texture from a hedonic point of view.

Stevia is a typical sucrose replacer in food products that satisfies the requirements for low-calorie and high-intensity sweeteners in these products (Azevedo et al., 2015). Several researchers have studied the possibility of using stevia in the formulation of various baked goods, such as muffins (Zahn et al., 2013), cakes (Manisha et al., 2012) and cookies (Kulthe et al., 2014). In our study, we used Stevianna® (product code ST001\_SE supplied by Stevianna NZ) in muffin products. This sweetener is extracted from stevia (*Stevia rebaudiana*), a small, herbaceous, perennial shrub of the Asteraceae family. Stevia has zero calories, so does not affect blood glucose and insulin levels, as shown in human studies

(Gregersen et al., 2004); it is a natural sweetener with a relative sweetness 250–300 times sweeter than table sugar (Manisha et al., 2012). In safety studies, stevia has been approved as a safe supplement by JECFA, WHO and FDA. Moreover, FSANZ (Food Safety Australia and New Zealand) have set the value for the acceptable daily intake (ADI) at 0–4 mg steviol equivalents (Geuns, 2010).

Inulin is a natural dietary fibre derived from chicory roots, garlic, wheat, bananas and artichokes and, as such, has always been part of the human diet (Rodríguez-García et al., 2013). Chemically, native inulin is a mixture of oligomer and polymer chains with a variable number of fructose molecules joined by  $\beta$  (2-1) bonds, and usually includes a glucose molecule at the end of the chain (Bayarri et al., 2011). The degree of polymerisation (DP) of chicory fructans varies from 2 to 60 (average DP = 12). Inulin offers a unique combination of nutritional and technological advantages (Rodríguez-García et al., 2013). It is not only a dietary fibre but also a prebiotic that is linked to a variety of beneficial physiological effects, such as improved bowel habits, increased calcium absorption, lowered serum lipids, a positive effect on the feeling of satiety and stimulation of the immune system (Meyer et al., 2011). Regarding its technological properties, inulin can be used for partial sucrose replacement or to give structure to low-fat foods (Tárrega et al., 2011).

Different researchers have studied inulin and stevia and other sugar replacers in bakery products (Shevkani and Singh, 2014; Colla and Gamlath, 2015; Rumiya et al., 2015). The addition of inulin to gluten-free layer cakes is believed to slow the release of reducing sugars and, hence, lower postprandial blood glucose levels (Gularte et al., 2012). Due to the structure of inulin, it resists digestion in the human intestines (Aravind et al., 2012) and delays gastric emptying (Gularte et al., 2011). Apart from these health benefits, inulin has also been used as a food substitute, and a bulking and structure-forming agent in food processing (Meyer et al., 2011). Zahn et al. (2010) found that the replacement of 50% fat with inulin in a formulation resulted in muffins that were comparable or slightly higher in crumb firmness than the control muffin. Moreover, Zahn et al. (2013) indicated that a combination of inulin with rebaudioside-A resulted in products with characteristics close to a reference muffin formulation by multivariate analysis of the instrumental and sensory data. The effects from a

mixture of stevioside and liquid sorbitol on the rheological, microstructural and quality characteristics of cakes were observed by Manisha et al. (2012). However, while several studies have illustrated the benefit of consuming inulin or stevia-rich products, the results are still not fully understood. There is a paucity of studies documenting stevia or inulin additions and their effect on the quality, texture and *in vitro* starch digestion of muffins.

Therefore, the aim of this study was to substitute sucrose in muffin production with different levels of Stevianna® and inulin, added individually, in order to investigate the possible mechanisms involved in the modulation of postprandial glycaemia responses by delayed starch digestibility. Furthermore, the textural properties of the muffins, as affected by the different types and levels of sugar replacement, were measured and compared with a control muffin.

## **4.2 Materials and Methods**

### **4.2.1 Raw Materials**

Raw materials were used as outlined in 3.1.

### **4.2.2 Muffins Preparation**

Muffin recipe 1 was prepared and manufactured as described 3.2. Sugar was replaced by either Stevianna® or inulin for making sugar-reduced muffin, shown in Table 3.1.

### **4.2.3 Textural Characteristics of the Muffin**

All muffin textures were measured for the firmness and springiness as described in 3.4.5.

### **4.2.4 Muffin Total Starch**

Total starch analysis was determined according to the official AACC International method 76.13 as outlined in 3.4.8.

#### 4.2.5 *In vitro* Predictive Glycaemic Response Digestion Analysis

Each muffin recipe was analysed for potential glycaemic response in triplicate following the method reported by Woolnough et al. (2010) as outlined in 3.5.1 and 3.5.3.

#### 4.2.6 Statistical Analyses

One-way ANOVA was used to compare the characteristics of each parameter between control and modified muffin samples with a significance of  $P < 0.05$ . Significant differences between means were determined using at Tukey's comparison test as outlined in 3.7.1.

### 4.3 Results and Discussion

#### 4.3.1 Textural Properties of Muffins

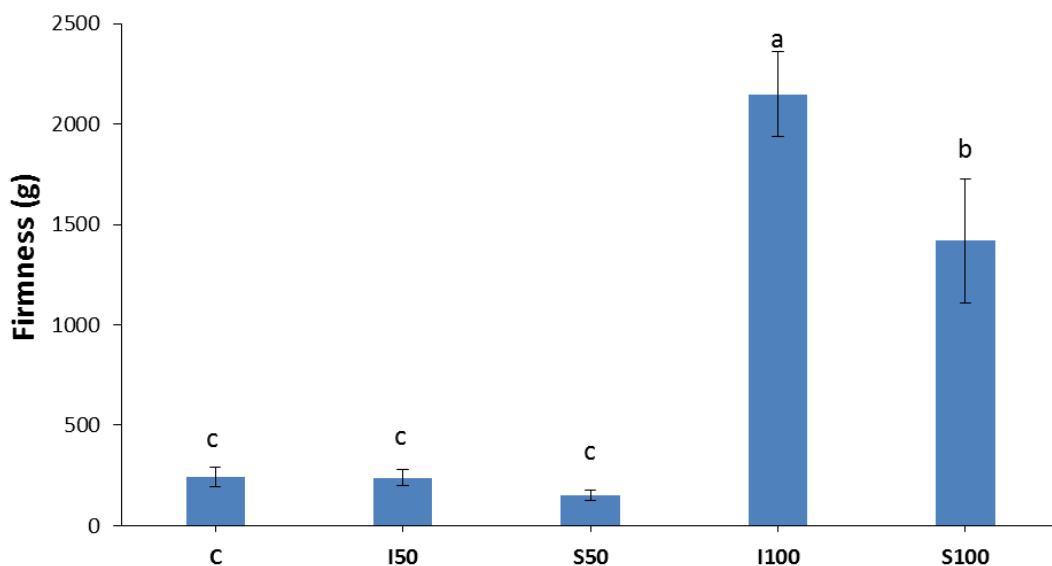
The textural characteristics of the muffins were investigated by conducting a texture profile analysis in terms of the firmness and springiness of the muffins. The mean values of the texture profile parameters are shown in Table 4.1.

**Table 4. 1 Effect of inulin and stevianna on texture profile analysis and *in vitro* starch digestion profile in low-sugar muffins.**

| Product | Texture parameters            |                             | <i>In vitro</i> starch digestibility |                           |                             |
|---------|-------------------------------|-----------------------------|--------------------------------------|---------------------------|-----------------------------|
|         | Firmness (g)                  | Springiness (%)             | RDS (mg/g sample)                    | Area RDS (mg/g sample)    | Total AUC (mg/g sample)     |
| C       | 243.43 ± 49.10 <sup>c</sup>   | 56.343 ± 2.63b <sup>c</sup> | 503.22 ± 23.75 <sup>a</sup>          | 58.58 ± 2.26 <sup>a</sup> | 453.00 ± 28.55 <sup>a</sup> |
| I50     | 237.97 ± 39.02 <sup>c</sup>   | 48.95 ± 1.71 <sup>cd</sup>  | 370.48 ± 15.92 <sup>b</sup>          | 40.90 ± 1.97 <sup>b</sup> | 358.09 ± 6.52 <sup>b</sup>  |
| S50     | 152.24 ± 24.94 <sup>c</sup>   | 58.53 ± 0.51 <sup>b</sup>   | 340.06 ± 2.78b <sup>c</sup>          | 36.13 ± 0.33 <sup>b</sup> | 327.71 ± 15.40 <sup>b</sup> |
| I100    | 2149.20 ± 213.78 <sup>a</sup> | 42.27 ± 0.18 <sup>d</sup>   | 266.59 ± 69.73 <sup>cd</sup>         | 25.87 ± 6.23 <sup>c</sup> | 256.92 ± 34.42 <sup>c</sup> |
| S100    | 1418.87 ± 307.98 <sup>b</sup> | 68.79 ± 4.60 <sup>a</sup>   | 185.26 ± 12.56 <sup>d</sup>          | 19.40 ± 2.04 <sup>c</sup> | 189.90 ± 7.61 <sup>d</sup>  |

All measurements are mean values ± SD of triplicate determinations. Means in the same column with different letters are significantly different ( $P < 0.05$ ). Control (C); 50% inulin (I50); 100% inulin (I100); 50% Stevianna<sup>®</sup> (S50); 100% Stevianna<sup>®</sup> (S100).

Muffins with total sucrose replacement showed significantly ( $P < 0.05$ ) higher firmness values compared to the control (Figure 4.1). In particular, the sample with 100 % inulin showed significantly different firmness ( $P < 0.05$ ) values compared to the 100 % Stevianna® sample. Inulin (100 %) had significantly higher firmness values ( $P < 0.05$ ) of all the samples analysed. However, the replacement of sucrose by 50% Stevianna® or inulin gave the muffins similar firmness values in comparison with the control. As shown in Figure 4.1, no significant differences were found between the 50 % Stevianna® and 50 % inulin muffins. Similar observations have been made in the case of sugar replacers, where using inulin or fibre revealed significantly ( $P < 0.05$ ) higher firmness in the fat or sucrose-free muffins, and the higher concentrations of inulin led to higher hardness values (O'Brien et al., 2003; Psimouli and Oreopoulou, 2013).



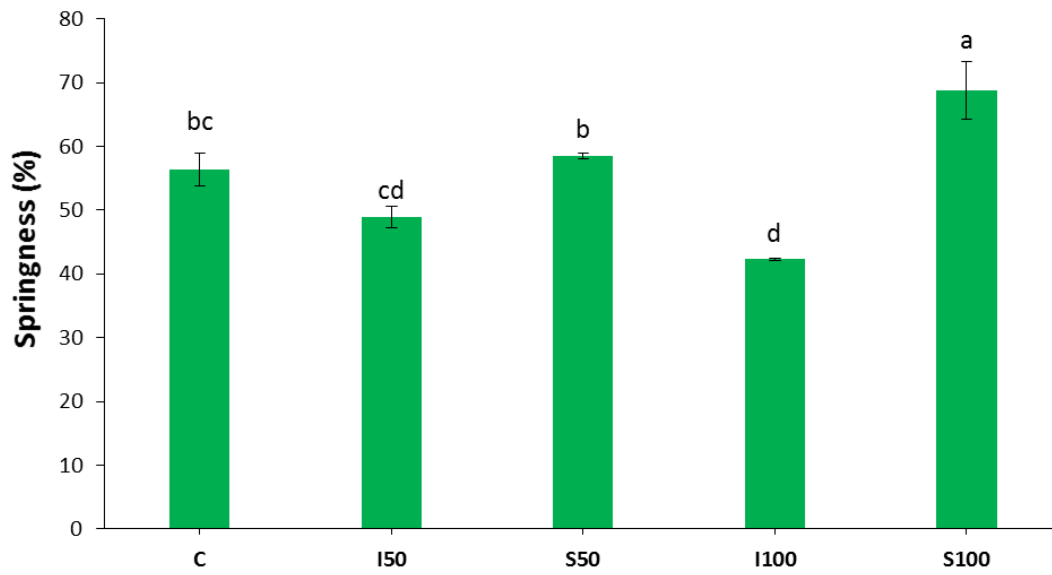
**Figure 4. 1 Firmness values for muffins containing different levels of inulin and Stevianna® as sugar replacer. Control (C); 50% inulin (I50); 100% inulin (I100); 50% Stevianna® (S50); 100% Stevianna® (S100).**

Those results agreed with the findings of Coleman and Harbers (1983), who added high levels of high fructose corn syrup to sugar-free cakes. The increased firmness might be due to either the decreased stiffness of the foams or premature starch gelatinisation. Rodríguez-García et al. (2014a) have observed that in general, crumb hardness is significantly related to the volume and total air cell area, so that as the gas cell size increases, the product has a reduced density and a softer crumb structure. Manisha et al. (2012) showed that the sugar provided a considerable part of the bulking agent during cake baking, and this can delay starch gelatinisation and, consequently, improve the size of air bubbles due to carbon dioxide and water vapour before the cake sets. Furthermore, a previous study found that the cause of hardness and the rising and falling of volume could be attributed to a reduction in the aeration of the cake batter and the heat-assisted coagulation of proteins, leading to a very dense crumb structure (Kalinga and Mishra, 2009). Struck et al. (2016) suggested that fibre-enriched muffin firmness was highly sensitive to the density of the crumb, implying that the fibre affected the incorporation of air cells and contributed to mechanical resistance during compression. Martínez-Cervera et al. (2014) also found total sucrose replacement with erythritol caused a significant ( $P < 0.05$ ) increase in muffin firmness. These effects are mainly related to the differences in water-binding capacities when the sucrose replacer competes for water with the starch, as noted by Juszczak et al. (2012), who considered that the water-binding ability of inulin played a key factor in modifying the dough properties of the system. This may be due to the influence of solvent availability on the other dough constituents, which would affect the retrogradation of the starch (Juszczak et al., 2012).

Moreover, Rosell et al. (2010) reported an improvement in the stability and gas-holding capacity of inulin when it was solubilised and integrated into the cellular structure of bread. In our case, the results were consistent with those of Akesowan (2009) who observed that no significant differences in chiffon cake firmness were found when 50 % sucrose was replaced by a sucralose-erythritol mixture. When inulin has been used to partially replace sucrose, no significant differences in firmness were observed by Röβle et al. (2011). However, Zahn et al. (2010) found the 50 % replacement of fat by inulin significantly affected muffin crumb firmness, this was in contrast to our results. Therefore, this study

indicated that partial sucrose replacement with inulin was accountable for keeping the muffins soft and close to the firmness of the control muffins.

Figure 4.2 shows that no differences were observed among the I50 (50 % Inulin) and S50 (50 % Stevianna®) muffin formulations for springiness, but I100 (100 % Inulin) had significantly lower values than the other samples, reflecting a more compact muffin texture (Figure 4.3). The most surprising aspect of the data was in the springiness of S100 (100 % Stevianna®) ( $P < 0.05$ ), which increased when 100 % of the sucrose was replaced by Stevianna®. Martínez-Cervera et al. (2014) showed a decrease in the springiness of sugar-free muffins prepared with erythritol, isomalt or sorbitol. This differs from the findings presented in our results. A correlation obtained by Akesowan (2009) showed that the properties of sugar can retard the gelatinisation of starch and, consequently, lead to a tenderising effect on the muffin texture. This inconsistency may be due to the different types of constituents in the muffin recipes and indicated that muffin springiness can be improved by replacing sucrose with Stevianna®. However, several similar results were found in cakes when fat was partially replaced by inulin (Kalinga and Mishra, 2009), or sugar was partially replaced with sorbitol (Martínez-Cervera et al., 2014). A possible explanation for this result may be because the same total solid content was maintained in each of these samples. In addition, Zahn et al. (2013) also found that springiness values fell as the fat was increasingly replaced by dietary fibre (DF). This can be related to a decrease in the strength of the hydrogen bonds in the three-dimensional protein network in cakes (Kalinga and Mishra, 2009). Furthermore, in our study, the effects on the muffin texture might depend on the type and concentration of the sucrose replacer used.



**Figure 4. 2 Springiness values for muffins made from different levels of inulin and Stevianna® as sugar replacers. Control (C); 50% inulin (I50); 100% inulin (I100); 50% Stevianna® (S50); 100% Stevianna® (S100).**





**C (Control)**

**I50 (50 % Inulin)**

**S50 (50 % Stevia)**

**I100 (100 % Inulin)**

**S100 (100 % Stevia)**

**Figure 4. 3 Effect of different levels of inulin and Stevianna® in low-sugar muffins: Control (C); I50, 50% inulin; I100, 100% inulin; S50, 50% Stevianna® ; S100, 100% Stevianna®.**

### 4.3.2 *In vitro* Predictive Glycaemic Response

The nutritional quality of the Stevianna® or inulin-enriched muffins, in terms of their starch digestibility and predictive glycaemic response, was determined by an *in vitro* enzymatic digestion that mimics the human digestive track. This investigated the effect of the starch gelatinisation properties on starch digestion and reducing sugar release (Foschia et al., 2015). Values for reducing sugar release during *in vitro* digestibility studies varied according to the type and quantity of sugar replacer used in the muffins. The effects of sugar replacer (Stevianna® or inulin) on the *in vitro* starch digestion were investigated by measuring the reducing sugars released during the 120-min starch digestion process. Figure 4.4 illustrates that reducing sugar release was significantly decreased ( $P < 0.05$ ) in muffins containing Stevianna® or inulin, compared with the control sample. In particular, the amount of reducing sugars in samples containing inulin or Stevianna® was significantly lower after 20 and 60 min of digestion. The strongest decrease was registered after the addition of 100 % Stevianna® followed by I100, S50 and I50 muffin samples, and this trend was maintained for 120 min.

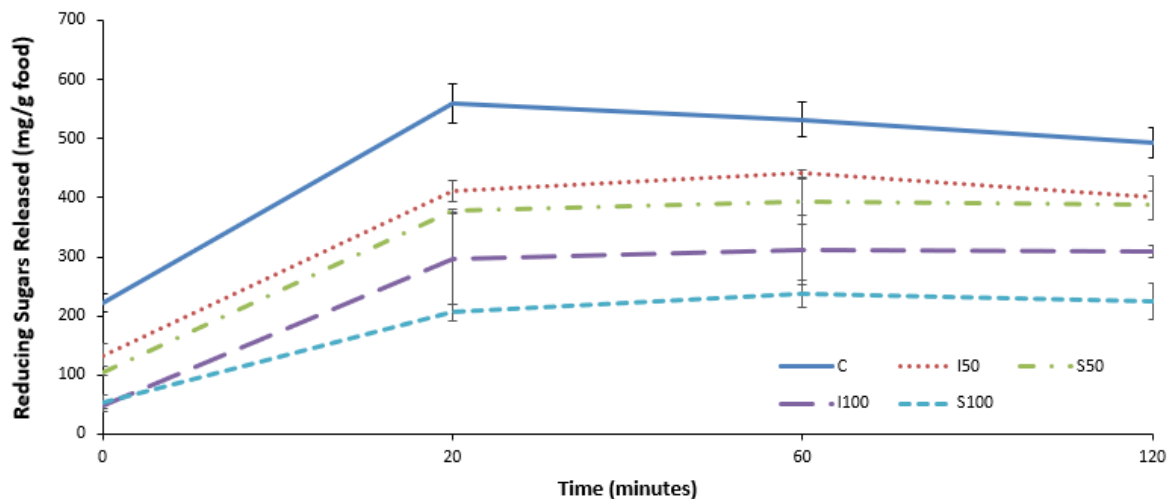
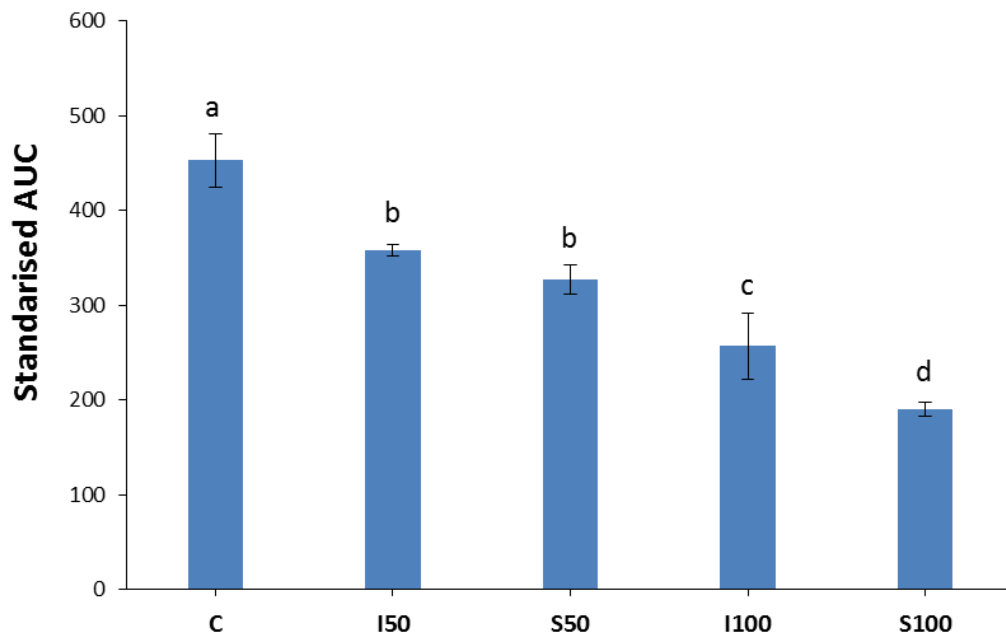


Figure 4. 4 Amount of reducing sugars released (mg / g of starch) during *in vitro* digestion. Control (C); 50% sugar replaced by inulin (I50); 50% sugar replaced by Stevianna® (S50); 100% sugar replaced with inulin (I100); 100% sugar replaced with Stevianna® (S100).

The reducing sugar release measurements using enzymatic assays were divided into rapidly digestible starch (RDS) and slowly digestible starch (SDS) based on the amount of reducing sugar released by *in vitro* digestion. The amounts of RDS and area under the curve values of RDS in the control and Stevianna® or inulin-enriched muffins are presented in Table 4.1. RDS was the predominant fraction in muffin samples that were measured as glucose after 20 min of digestion, to reflect the rate of absorption in the small intestine. From Table 4.1, it can be seen that muffin products containing Stevianna® or inulin had significantly lower RDS ( $P < 0.05$ ) than the control. In addition, the amounts of RDS reduced as the replacement sugar levels increased. Table 4.1 shows another parameter, total AUC (area under the curve), which was always lower in the 100 % sugar replacement than in the 50 % sugar replacement for the corresponding Stevianna® or inulin formulations. The effect of the presence of Stevianna® in reducing total AUC contents was more pronounced than the samples containing inulin.

Standardised AUC values more clearly illustrated in Figure 4.5 for all samples and treatments. The effects of the replacement of sucrose in muffin preparation with 50 %, 100 % inulin or Stevianna® on standardised AUC values are shown in comparison with the control sample. In all samples, a clear decrease in AUC reducing sugar levels after the addition of Stevianna® or inulin was observed. In particular, the replacement of sucrose with 100 % Stevianna® in the muffin samples caused a major decrease in the standardised AUC values.

In summary, for this study, the addition of Stevianna® and inulin to muffins was found to depress reducing sugar release by digestive enzymes and, thus, reduce the potential glycaemic impacts with increasing amounts of sugar replacer.



**Figure 4. 5 Values for area under the curve (AUC) comparing the control and other low-sugar muffins made with different levels of inulin and Stevianna®.**

The *in vitro* digestion of inulin or Stevianna® inclusions into muffin products clearly illustrated that the type and quantity of sugar replacer reductions on the rate and extent of reducing sugar release may be due to the reduction in the starch content. Several researchers have studied the effect of dietary fibre (DF) and polysaccharides on starch digestibility in a range of food products (Brennan, 2005; Cleary and Brennan, 2006; Oh et al., 2014). Their results were similar to our observations, which showed that inulin has a rate regulatory role in reducing sugar release. This consistent result may be due to the inulin preferentially hydrating, aggregating and forming a matrix to encase starch granules in a semisolid gel (Tolstoguzov, 2003). The encasing of starch granules could be attributed to the limitation of water movement during the hydrolysis process of DF, which leads to a reduced degree of starch gelatinisation (Oh et al., 2014). Other studies indicated that the accessibility of starch-degrading enzymes in the partially gelatinised starch granules may also be interfered with by a reduction in water movement (Foschia et al., 2015). In addition, studying the activity of inulin in reducing starch digestibility was a necessary and important step in developing an understanding of the mechanisms of action of inulin in reducing glycaemic responses in actual food systems. Brennan and Samyue (2004)

suggested that the low glycaemic response of an individual was attributable to a decrease in the accessibility of  $\alpha$ -amylase to starch within a food matrix, accounting for the inclusion of DFs. This could be possibly explained by the function of DF, which was recognised as encapsulating the starch granules in a protective coat, resulting in the suppression of enzymic degradation and, consequently, the reduced potential for starch degradation and sugar liberation (Bae et al., 2016; Tudorică et al., 2002). Brennan et al. (1996) observed a similar effect on starch degradation according to the incorporation of guar galactomannan in bread. They observed that the fibre formed a physical barrier around starch granules and protected them from enzymic degradation, thus decreasing starch hydrolysis. Regarding the specific effect on the DP of the inulin fraction, Aravind et al. (2012) clarified that higher DP will make it more likely to form a cohesive encapsulating layer. In our case, a higher DP inulin was used and that indicated that the attenuation of reducing sugars release was possibly attributed to its well-formed polysaccharide matrix with a strong entrapment of starch.

As a result, the variations in RDS values could be good indicators of the glycaemic responses of *in vitro* starch digestibility in muffin products. More recently, Gularte et al. (2012) illustrated that inulin, as a fibre source in cakes had a significantly lower RDS fraction when compared to the control cakes. Brennan et al. (2012) showed a similar occurrence in mushroom coproduct material (MCM) extruded products, which indicated that it did, indeed, restrict the amount of readily digestible carbohydrates from the fibre-fortified extruded products. As shown in Figure 4.5, this further illustrated a trend for decreased starch digestion with increasing inulin levels for the average AUC relating to the release of sugar over a hydrolysis period of 120 min. These results demonstrated that Stevianna® or inulin as a source of sugar replacer in the muffins was extremely effective in lowering the predicted glycaemic response and overall AUC. Starch digestibility in cakes (Oh et al., 2014) and pasta (Brennan et al., 2004) with added inulin also reduced the predicted glycaemic index values. The theory exists to explain the effect of soluble polysaccharides on the digestibility of cereal products *in vivo* and they support the previous observations of Brennan et al. (1996). The reduction in blood glucose levels in guar-enriched breakfast cereal products has been proposed to increase the viscosity in the small intestine, possibly resulting in dietary fibres adhering to starch granules (Brennan et al., 1996). As mentioned above, the

addition of inulin to the cake led to lower starch hydrolysis, and hence, one could postulate that lower sugar liberation would occur under *in vivo* conditions. Previous work has shown that the reduced glycaemic response was more likely to be due to a slowing down of gastric emptying and a reduced rate of intestinal absorption of glucose (Gularte et al., 2012).

Previous reports using different *in vivo* digestion methods showed that the interaction between stevia and other food components has the potential to influence postprandial glucose and insulin levels in humans (Alizadeh et al., 2014; Anton et al., 2010). However, none of the studies assessed the mechanism by which stevia was related to the release of reducing sugar during *in vitro* digestion and, thus, the glycaemic response in muffin products. In our case, the addition of Stevianna® to muffins revealed two important factors-showing the slowest release of sugars during *in vitro* starch digestion and, therefore, having a reduction in the predicted glycaemic response by up to hundred per cent. Stevia does not contribute to the available carbohydrate and glycaemic responses in food products as it is a natural sweetener that contains no glucose. Similar trends have been observed in previous research. Alizadeh et al. (2014) produced ice cream by replacing sucrose with stevia and that resulted in significant reductions in postprandial insulin levels compared to those of sucrose-based formulations, and this indicated that stevia can decrease a large part of the calorie and carbohydrate intake effect on postprandial glucose levels (Anton et al., 2010). Standardised AUC (Figure 4.5), observations may also support the hypothesis that Stevianna® would have a beneficial effect in terms of weight management and the potential glycaemic impacts of such foods. Therefore, Stevianna® could be used to create a model of lower calorie content of traditionally readily digestible starchy foods.

#### **4.4 Conclusions**

On the basis of the results obtained, the evaluation of the usefulness of Stevianna® or inulin as improvers for replacing sugar in muffins has focused on the texture and glycaemic response effects on these muffins compared with the control muffins. *In vitro* digestion analysis conducted in this study has highlighted that the inclusion of Stevianna® or inulin in muffins can significantly reduce the predicted glycaemic response from the muffin material. The results suggested that increasing levels of

the Stevianna® or inulin in muffins resulted in a decrease in RDS. Moreover, when comparing the different sugar replacement level formulations, sugar release was lower in the 100 % muffins than in the 50 % muffins. In particular, the 100 % Stevianna® formulation was significantly more efficient in reducing AUC values when compared with the control muffins. We believe that the Stevianna® can be used for the production of appropriate food samples with low calories and low glycaemic responses.

The textural properties of muffins significantly depended on the level of sugar replacement. Increased additions of Stevianna® or inulin led to muffins with increased firmness. However, replacement of 50 % sugar resulted in muffins similar to the control muffins for texture, firmness and springiness. Therefore, good quality muffins with a 50 % sugar replacement can be obtained using Stevianna® or inulin at low levels. Further work to conduct a sensory evaluation to assess consumer acceptability of the low-sugar muffins produced by the addition of Stevianna® is underway.

## Chapter 5

### Effects of Sugar Substitution with Stevianna® on the Sensory Characteristics of Muffins

(This is derived from data presented in a published paper: Gao, J., Brennan, M. A., Mason, S. L., & Brennan, C. S. (2017). Effects of sugar substitution with “stevianna” on the sensory characteristics of muffins. *Journal of Food Quality*, 2017.)

**Abstract:** Sugar is a main ingredient of muffins and other baked products, so removal or reduction of sucrose negatively affects product appearance, texture, and mouthfeel. The aim of this study was to investigate the colour, textural properties, and sensory characteristics of sugar replaced muffins made using Stevianna® in combination with cocoa powder and/or vanilla. Optimal results were obtained with 50 % Stevianna®, leading to muffins similar to the control products and having a high level of acceptance in sensory evaluation. Sugar-free muffins (100 % Stevianna®) were harder in texture and more compact in crumb compared to the control. Results from sensory evaluation also illustrated that 100 % Stevianna® addition led to muffins with poorer acceptance, harder texture, and a drier mouthfeel when compared against the control. This study also investigated the use of cocoa powder and/or vanilla to mask the Stevianna® bitterness in terms of aftertaste.



## 5.1 Introduction

Consumers are becoming increasingly aware of the nutritional quality of food products and the link with health. The prevalence of obesity and overweight has increased dramatically with suggestions that in Europe the prevalence of obesity had risen threefold since the 1980s (Branca et al., 2007). As a result, the food industry has focused on reducing calorie content by production of sugar-free foods. However, continued consumption of low-calorie foods is difficult to achieve as these products are often evaluated as having poor organoleptic qualities (Devereux et al., 2003). Sucrose in bakery products makes a major contribution to providing sweetness, controlling moisture retention, influencing air incorporation, stabilising air bubbles, and limiting the swelling of starch during baking, all of which help to create a finer texture (Nip, 2006). There are many reports that show reduced sucrose products to be less acceptable than their full-sucrose counterparts (Abdel-Salam et al., 2009; Drewnowski et al., 1998; Edelstein et al., 2007; Psimouli and Oreopoulou, 2012). The structural and sensory properties of the muffin system have been shown to be influenced by the reduction in sucrose levels (Martínez-Cervera et al., 2014). Therefore, intense sweeteners cannot solely replace sugar and the food industry is facing the challenge of developing new bakery products where reducing sucrose content of baked goods would reduce calories while maintaining the sensory quality and the acceptability of the product. It is important to find alternative sugar replacers for traditional sugars in order to improve the quality of low-sugar muffins.

Stevia is the generic term used for food ingredients that are a group of intensely sweet compounds extracted and purified from the herb *Stevia rebaudiana* (Bertoni). A more precise term for these compounds is steviol glycoside. The main sweetening components in *Stevia* leaves are stevioside and rebaudioside-A (Carakostas et al., 2008). Rebaudioside-A is a high-intensity sweetener with a relative sweetness 350–450 times that of sucrose; however the bitterness that presents as an aftertaste affects the sensory quality of the final product (Struck et al., 2014). Stevia has been indicated for use as a sweetener by diabetics (Gasmalla et al., 2014). Safety studies have shown no side effects and stevia has been approved as a safe ingredient by JECFA, WHO, and FDA, with FSANZ (Food Safety Australia and New Zealand) setting an acceptable daily intake (ADI) at 0-4 mg steviol equivalents (Geuns, 2010).

Stevianna® combines the main sugar substitute of rebaudioside-A (98 % steviol glycoside; 1 %) with erythritol (99 %) to provide one time sweetness of sucrose (product code ST001 SE supplied by Stevianna® NZ). Erythritol, a four-carbon sugar alcohol with sweetness intensity varying from 60 % to 80 % that of sucrose (Goossens and Roper, 1994), is a useful functional food ingredient because it has a high digestive tolerance (daily at doses of 1 g/kg body weight), is non-calorie, noncariogenic, and non-glycaemic, and has been reported to have antioxidant properties (Storey et al., 2006). It is the only sugar alcohol produced commercially by fermentation of wheat or corn starch (Struck et al., 2014). Erythritol has been classified as nontoxic from acute and subchronic studies in animals (Munro et al., 1998), and consequently the FDA has declared erythritol generally recognized as safe (GRAS) for use in foods.

In bakery products, using stevia to replace sucrose causes an increase in hardness, cohesiveness, and toughness of cake structure and has therefore been evaluated as being suitable for high sweetness intensity but it does not support texture characteristics (Abdel-Salam et al., 2009). Similarly, Edelstein et al. (2007) found that compared with other artificial sweeteners stevia produced least desirable cupcakes when replacing sucrose on a w/w basis. They also reported that stevia had a distinct bitterness in flavour or strong aftertaste that could limit its application in foods. However, adding hydrocolloids, sugar alcohols, or plant fibres may have a positive effect on the loss of volume and bulk when the amount of sucrose is reduced in bakery products (Storey et al., 2006). Lin et al. (2003) reported that use of erythritol as the bulking agent for sugar replacement in chiffon cakes resulted in desirable physical quality characteristics but indicated that if 100 % sucrose was replaced by erythritol there was a significant loss of sweetness.

Zahn et al. (2013) used a combination of inulin with rebaudioside-A to make reduced sugar muffins and illustrated that the resulting products had characteristics close to a reference muffin formulation as determined by sensory evaluation. Baeva et al., (2000) also demonstrated that complete sucrose substitution could be achieved by replacing sugar with aspartame and bulking agents (sorbitol, wheat starch, and wheat germ) in sponge cakes for diabetics. Additionally, replacement of 50 % sucrose by a

mixture of erythritol and sucralose in reduced fat chiffon cakes resulted in no negative influences on the sensory and physical quality characteristics (Akesowan, 2009). It is well known that consumers are highly sensitive to even small variations in sweetness (Drewnowski et al., 1998). Martínez-Cervera et al. (2014) studied the effect of polyols on the acceptability of muffins and showed no differences for sensory acceptance. Similar results have been obtained in studies using sweeteners in cakes, where overall acceptance followed closely the scores of textural properties and taste (Psimouli and Oreopoulou, 2012). Manisha et al. (2012) conducted research using a mixture of stevioside and liquid sorbitol in cakes illustrating that hedonic response profiles ascended gradually with increasing sucrose replacement content.

The research carried out in Chapter 4 (Gao et al., 2016) illustrated the effect of sucrose replacement by Stevianna® in muffins and concluded that replacement of up to 50 % of sugar resulted in products with textural qualities similar to full-sugar muffins. This manuscript focuses on the effect of sugar replacement by two levels of Stevianna® in muffin products with the addition of cocoa powder and/or vanilla. The usefulness of cocoa powder and/or vanilla to mask any potential aftertaste that may result from the incorporation of Stevianna® was evaluated. The formulated muffins were evaluated for their physical properties (colour analysis and textural properties) via instrumental analysis. A sensory panel was also used to compare the effect of sugar replacement on the product's sensory properties. All muffins were compared to a control muffin formulation with no added Stevianna®, cocoa powder, or vanilla.

## **5.2 Materials and Methods**

### **5.2.1 Raw Materials**

Raw materials were used as outlined in 3.1.

### **5.2.2 Muffin Preparation**

Muffin recipe 2 was prepared and manufactured as described 3.2. Sugar was replaced by Stevianna® with/without cocoa powder and/or vanilla for making sugar-reduced muffin, shown in Table 3.2. Muffins for sensory evaluation were prepared on the early morning of each trial.

### **5.2.3 Physical Measurement on Muffins**

Muffins were assessed for their physical characteristics based on their texture and colour. The determinations of the texture muffins were described in 3.4.5. Colour measurements were made on the crumb and crust of muffin with a Tristimulus Colour Analyzer (Minolta Chroma Meter CR200, Minolta Camera Co., Japan). The method of colour measurement was described in 3.4.6.

### **5.2.4 Sensory Evaluation**

Sensory evaluation was assessed by the panellists using a line scale of 15 cm as described in 3.6.

#### **5.2.4.1 Data Analysis of Sensory Evaluation**

Line scales analysis and principal component analysis (PCA) were performed as outlined in 3.7.2 and 3.7.2.1.

The data was evaluated separately by Minitab 17 (one-way ANOVA) to determine the appearance, colour, texture, mouthfeel, sweetness, and overall liking.

PCA is a useful statistical method for visualising and interpreting large datasets by forming fewer composite variables (principal component). PCA was used to obtain a simplified overview of the relationships between data sets and to double check the results from sensory evaluation. The Varimax rotation method was also used in PCA to maximise the sum of the variances of the squared loading to obtain all the coefficients which can be either extremely larger or small with few intermediate values (Kaiser, 1958).

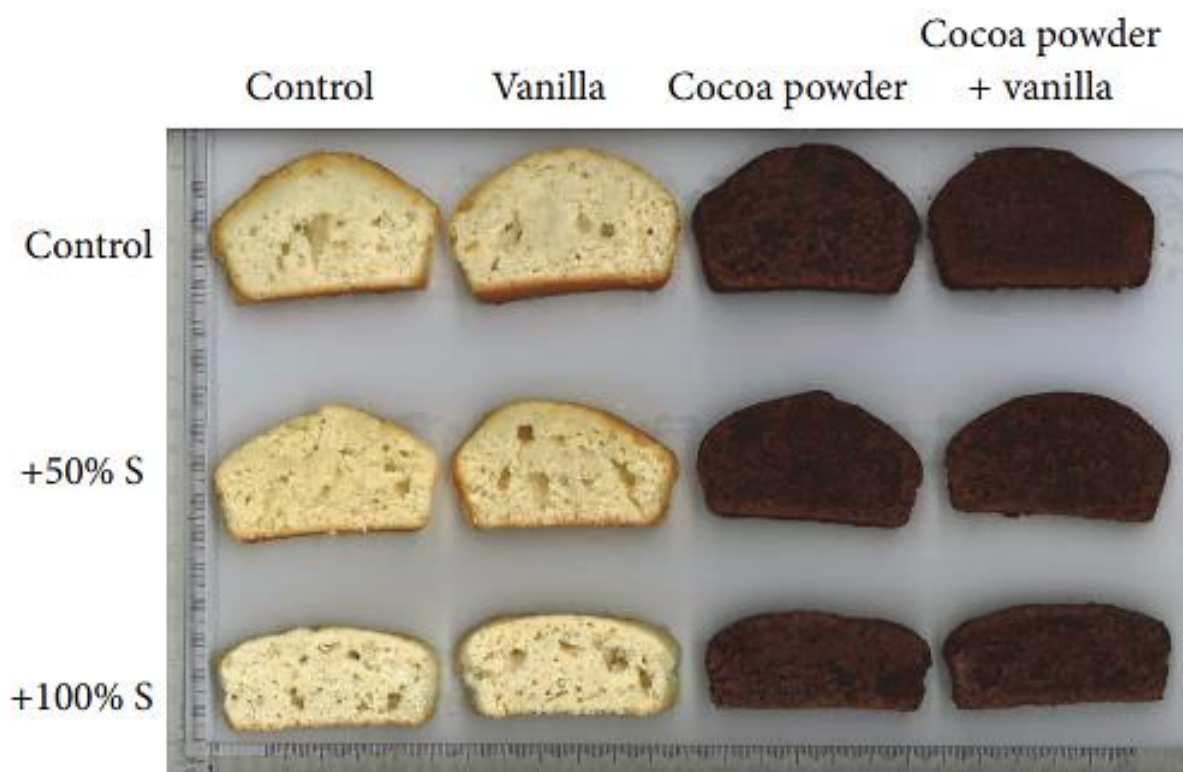
## 5.2.5 Statistical Analysis

One-way ANOVA was performed as described in 3.7.1. Each analysis was conducted in triplicate.

## 5.3 Results and Discussion

### 5.3.1 Colour Analysis of the Muffins

The colour of muffins is an important factor which affects the acceptability of the product and is directly influenced by the raw materials used in the formulation. Figure 5.1 is a photo of samples of the muffins and demonstrates the colour changes with each treatment. Figures 5.2 and 5.3 show L\*, a\* and b\*: the lightness, the redness, and the yellowness, respectively. The samples were divided into two groups: without cocoa powder and with cocoa powder (Table 5.1).



**Figure 5. 1** Effect of Stevianna® without/with cocoa powder and/or vanilla on the crumb colour of muffin. Muffins are, from left to right and top to bottom, control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

### 5.3.1.1 Crust Colour

In the group without cocoa powder,  $L^*$  values of muffins were not affected by the replacement of sucrose with Stevianna®. Samples containing Stevianna® had higher  $a^*$  values (redness) when compared to the control while samples with 100 % Stevianna® (100S and 100S + V) had significantly lower ( $P < 0.05$ ) mean value for the yellowness ( $b^*$ ) than the controls. The changes in  $a^*$  and  $b^*$  values may be because Stevianna® is thermo-stable and contains nonreducing substances, does not react with amino acids by Maillard reaction (Edelstein et al., 2007), and has limited caramelization. This is in keeping with the findings of Martínez-Cervera et al. (2012a), which showed the addition of erythritol in muffins appeared not to influence the crust colour. The addition of vanilla also failed to change the colour of the muffin crust. Within the group with cocoa powder, crust  $L^*$ ,  $a^*$  and  $b^*$  values were not significantly different for 0 %, 50 %, and 100 % Stevianna® with or without vanilla muffins (Figure 5.2).

In the group without cocoa powder (Table 5.1), the Stevianna® containing samples had a  $\Delta E^* > 3$  compared to the control samples and were appreciably different by the human eye. The crust  $\Delta E^*$  values of muffins with cocoa powder were notably higher than those of the control muffin (Table 4). The dark colour of cocoa powder used in this study influenced the overall colour of the muffins. Akesowan (2009) showed a similar result in that the inclusion of cocoa powder affected the crust colour of the muffins. No significant differences were found in crust  $\Delta E^*$  due to the use of Stevianna® in the group with cocoa powder. The results indicated that the cocoa powder diminished the crust colour change from Stevianna®.

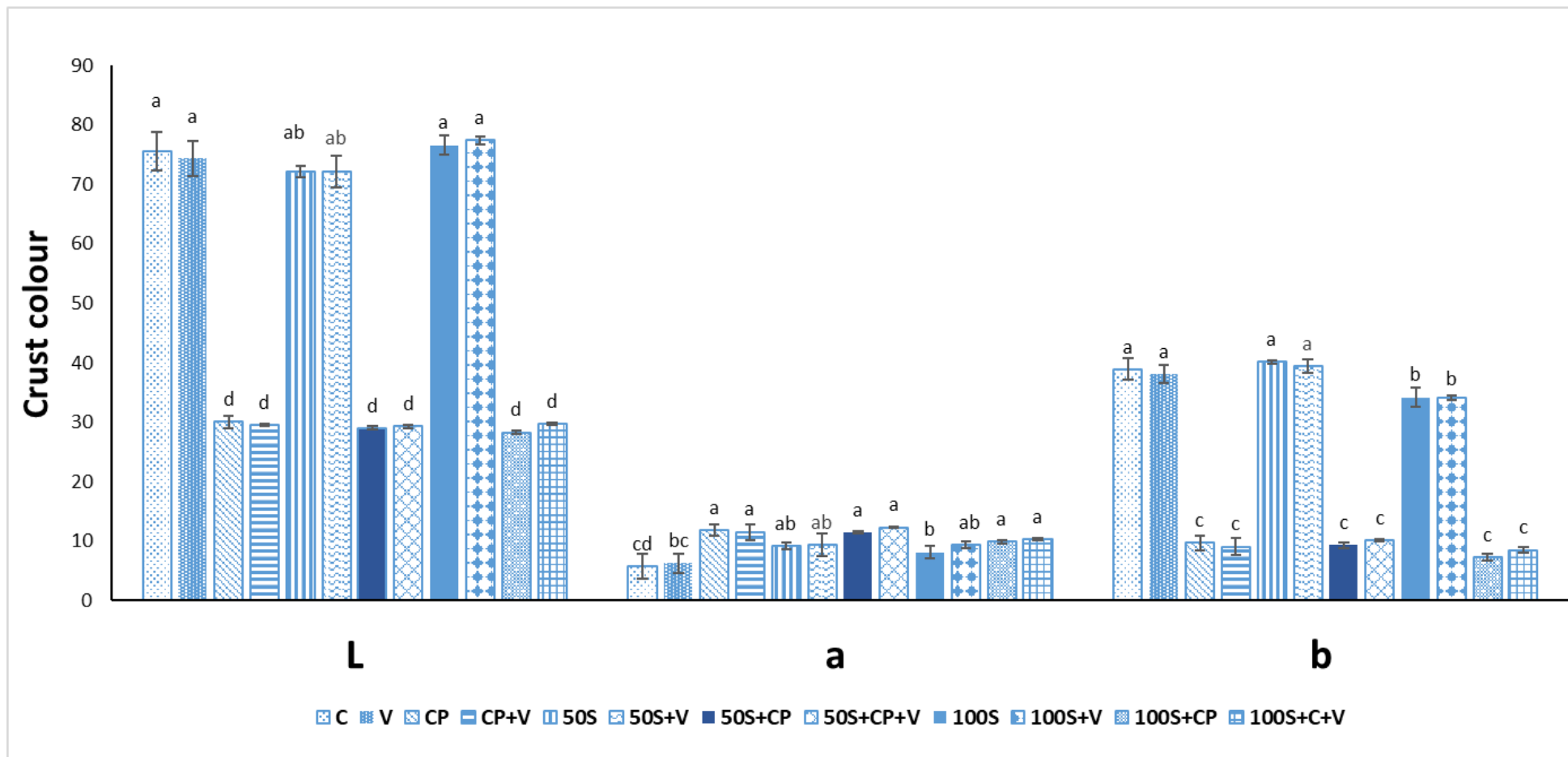


Figure 5. 2 Effect of Stevianna® without/with cocoa powder and/or vanilla on the crust colour of muffin. Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

### 5.3.1.2 Crumb Colour

For the group without cocoa powder, Figure 5.3 presents  $b^*$  values which indicate greater yellowness ( $P < 0.05$ ) of the crumb of 50 and 100 % Stevianna® with vanilla muffin, but the measured  $L^*$  and  $a^*$  values of colour did not show any significant differences.

The crumb  $\Delta E^*$  values for 100 % Stevianna® samples without cocoa powder were in excess of 3 units; however these values were lower than the crust  $\Delta E^*$  values (Table 5.1). The difference between the crumb and crust colour was due to the fact that the crumb temperature does not get as high as the crust temperatures and therefore caramelization reaction does not occur in the crumb (Lebesi and Tzia, 2009). Within the group containing cocoa powder, as the level of Stevianna® increased, the crumb  $\Delta E^*$  value showed a decreasing trend, indicating a slightly lighter crumb was obtained as a result of the Stevianna® substitute.  $a^*$  values and  $b^*$  values from the muffin crumb indicated that both the red and yellow colour did not change significantly due to different amounts of Stevianna® with cocoa powder and/or vanilla. However, the lightness of muffin crumb was affected ( $P < 0.05$ ) by the 100 % Stevianna® replacement in muffins with cocoa powder (Figure 5.3). Lin et al. (2003) reported that the addition of erythritol caused  $L^*$  values to increase in the crumb colour.

### 5.3.1.3 Browning Index

BI is presented in Table 5.1. The BI is an appropriate index for investigating the colour differences in Stevianna® muffins due to the brown colour observed after the cocoa powder addition (Table 5.1). Overall, in muffins containing cocoa powder, colour changes observed due to the different Stevianna® levels were less intense in the crust than in the crumb. This is because the crust colour is affected mainly by Maillard and caramelization reactions, while the crumb colour depends to a higher extent on raw materials (Lebesi and Tzia, 2012). The addition of cocoa powder resulted in significantly higher values ( $P < 0.05$ ) of BI than the control sample.



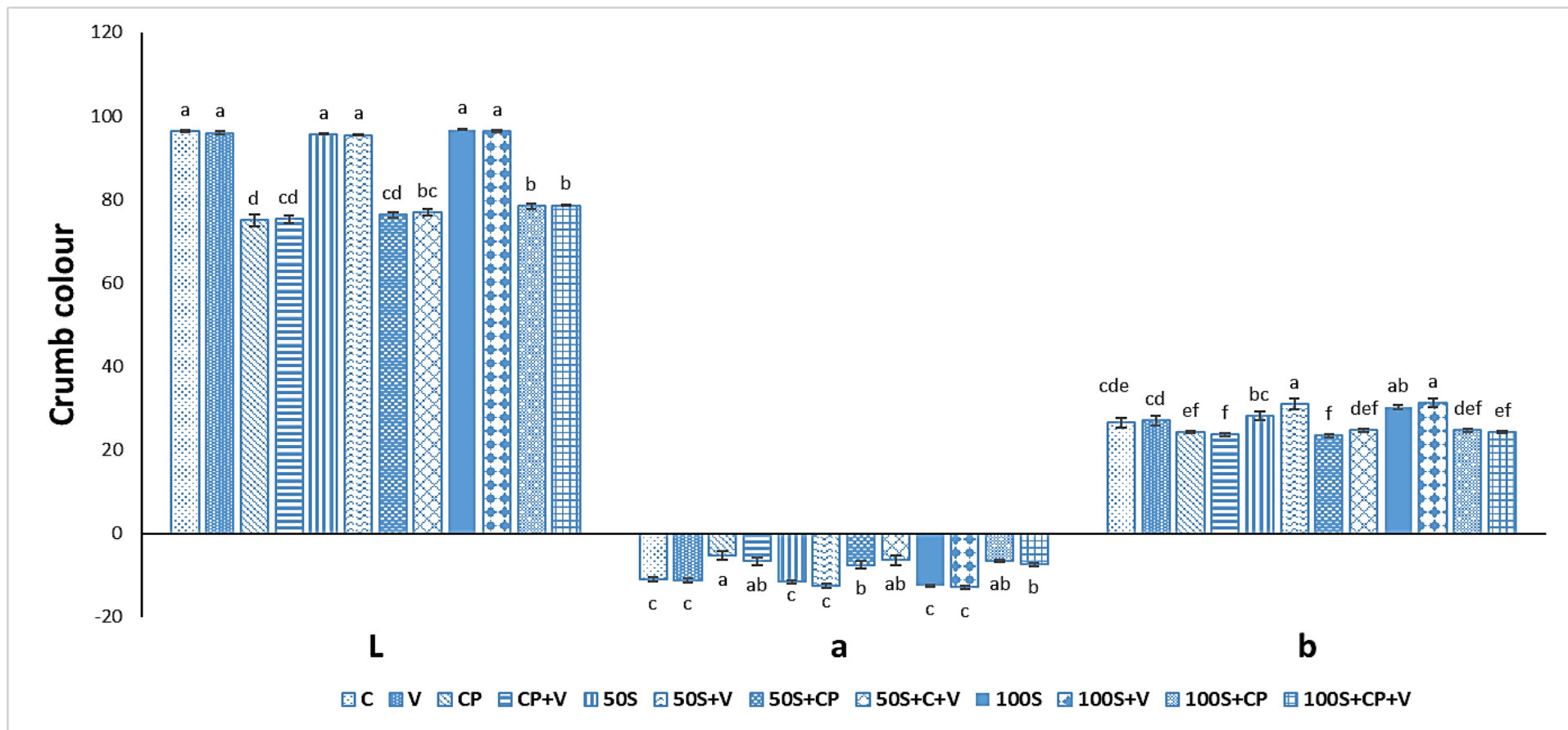


Figure 5. 3 Effect of stevianna without/with cocoa powder and/or vanilla on the crumb colour of muffin. Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

**Table 5. 1 Total colour difference ( $\Delta E^*$ ) and the browning index (BI) of muffin determined using different Stevianna® levels without/with cocoa powder and/or vanilla.**

| Product                    | Colour                    |                           |                           |              |                             |                            |                            |                             |              |                              |
|----------------------------|---------------------------|---------------------------|---------------------------|--------------|-----------------------------|----------------------------|----------------------------|-----------------------------|--------------|------------------------------|
|                            | Crust                     |                           |                           |              |                             | Crumb                      |                            |                             |              |                              |
| Without cocoa powder group | L*                        | a*                        | b*                        | $\Delta E^*$ | BI                          | L*                         | a*                         | b*                          | $\Delta E^*$ | BI                           |
| C                          | 75.63 ± 3.23 <sup>a</sup> | 5.68 ± 2.09 <sup>cd</sup> | 38.90 ± 1.81 <sup>a</sup> | 0            | 75.56 ± 11.12 <sup>ab</sup> | 96.27 ± 0.26 <sup>a</sup>  | -11.05 ± 0.49 <sup>c</sup> | 26.51 ± 1.11 <sup>cde</sup> | 0            | 22.28 ± 1.14 <sup>e</sup>    |
| V                          | 74.35 ± 2.92 <sup>a</sup> | 6.25 ± 1.67 <sup>bc</sup> | 38.10 ± 1.54 <sup>a</sup> | 1.62         | 75.79 ± 9.74 <sup>ab</sup>  | 95.96 ± 0.35 <sup>a</sup>  | -11.25 ± 0.59 <sup>c</sup> | 26.99 ± 1.23 <sup>cd</sup>  | 0.60         | 22.86 ± 1.25 <sup>e</sup>    |
| 50S                        | 72.08 ± 0.97 <sup>a</sup> | 9.18 ± 0.57 <sup>ab</sup> | 40.12 ± 0.27 <sup>a</sup> | 5.13         | 87.28 ± 2.22 <sup>a</sup>   | 95.61 ± 0.14 <sup>a</sup>  | -11.60 ± 0.40 <sup>c</sup> | 28.17 ± 1.04 <sup>bc</sup>  | 1.87         | 24.30 ± 1.16 <sup>de</sup>   |
| 50S+V                      | 72.13 ± 0.82 <sup>a</sup> | 9.39 ± 1.94 <sup>ab</sup> | 39.47 ± 1.16 <sup>a</sup> | 6.12         | 85.66 ± 5.48 <sup>a</sup>   | 95.46 ± 0.21 <sup>a</sup>  | -12.58 ± 0.53 <sup>c</sup> | 30.98 ± 1.23 <sup>a</sup>   | 4.79         | 27.52 ± 1.44 <sup>bcd</sup>  |
| 100S                       | 76.61 ± 1.54 <sup>a</sup> | 8.12 ± 0.41 <sup>b</sup>  | 34.16 ± 1.64 <sup>b</sup> | 5.42         | 65.47 ± 5.55 <sup>bc</sup>  | 96.75 ± 0.10 <sup>a</sup>  | -12.49 ± 0.26 <sup>c</sup> | 30.28 ± 0.46 <sup>ab</sup>  | 4.06         | 26.14 ± 0.41 <sup>cd</sup>   |
| 100S+V                     | 77.39 ± 0.62 <sup>a</sup> | 9.38 ± 0.60 <sup>a</sup>  | 34.12 ± 0.39 <sup>b</sup> | 6.30         | 65.66 ± 1.81 <sup>bc</sup>  | 96.28 ± 0.25 <sup>a</sup>  | -12.87 ± 0.42 <sup>c</sup> | 31.94 ± 1.14 <sup>a</sup>   | 5.10         | 28.37 ± 1.41 <sup>abcd</sup> |
| With Cocoa powder group    | Crust                     |                           |                           |              |                             | Crumb                      |                            |                             |              |                              |
| CP                         | 30.01 ± 1.03 <sup>d</sup> | 11.86 ± 0.92 <sup>a</sup> | 9.71 ± 1.23 <sup>c</sup>  | 54.52        | 66.73 ± 6.24 <sup>bc</sup>  | 75.02 ± 1.36 <sup>d</sup>  | -5.04 ± 1.03 <sup>a</sup>  | 24.28 ± 0.20 <sup>ef</sup>  | 22.10        | 32.35 ± 2.13 <sup>a</sup>    |
| CP+V                       | 29.48 ± 0.16 <sup>d</sup> | 11.52 ± 1.35 <sup>a</sup> | 9.05 ± 1.41 <sup>c</sup>  | 55.27        | 64.16 ± 10.11 <sup>bc</sup> | 75.33 ± 0.90 <sup>cd</sup> | -6.70 ± 0.96 <sup>ab</sup> | 23.64 ± 0.43 <sup>f</sup>   | 21.57        | 29.58 ± 2.21 <sup>abc</sup>  |
| 50S+CP                     | 28.99 ± 0.31 <sup>d</sup> | 11.41 ± 0.20 <sup>a</sup> | 9.31 ± 0.46 <sup>c</sup>  | 55.53        | 66.29 ± 1.96 <sup>bc</sup>  | 76.28 ± 0.69 <sup>cd</sup> | -7.56 ± 0.84 <sup>b</sup>  | 23.41 ± 0.44 <sup>f</sup>   | 20.52        | 27.79 ± 1.95 <sup>bcd</sup>  |
| 50S+CP+V                   | 29.29 ± 0.25 <sup>d</sup> | 12.27 ± 0.13 <sup>a</sup> | 10.06 ± 0.21 <sup>c</sup> | 54.98        | 71.34 ± 0.69 <sup>abc</sup> | 76.96 ± 0.70 <sup>bc</sup> | -6.48 ± 1.10 <sup>ab</sup> | 24.71 ± 0.36 <sup>def</sup> | 19.93        | 30.97 ± 1.87 <sup>ab</sup>   |
| 100S+CP                    | 28.28 ± 0.34 <sup>d</sup> | 9.90 ± 0.31 <sup>a</sup>  | 7.30 ± 0.53 <sup>c</sup>  | 57.09        | 54.43 ± 3.17 <sup>c</sup>   | 78.36 ± 0.57 <sup>b</sup>  | -6.62 ± 0.31 <sup>ab</sup> | 24.74 ± 0.41 <sup>def</sup> | 18.54        | 30.18 ± 0.42 <sup>abc</sup>  |
| 100S+CP+V                  | 29.64 ± 0.20 <sup>d</sup> | 10.36 ± 0.22 <sup>a</sup> | 8.49 ± 0.48 <sup>c</sup>  | 55.33        | 58.35 ± 2.95 <sup>bc</sup>  | 78.55 ± 0.08 <sup>b</sup>  | -7.41 ± 0.37 <sup>b</sup>  | 24.27 ± 0.38 <sup>ef</sup>  | 18.23        | 28.45 ± 1.03 <sup>abcd</sup> |

All measurements are mean values ± SD of triplicate determinations.

Means in the same column with different letters are significantly different ( $P < 0.05$ ).

Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

### 5.3.2 Textural Properties

Firmness and springiness are the main textural properties of a muffin, which are related to physical properties. Textural analysis provides an accurate estimation of firmness through measurement of the maximum force during the 1st compression. Springiness provides information about the sample's recovery from deformation, with springiness referring to the recovery between 2 compressions (Psimouli and Oreopoulou, 2013).

With respect to sucrose replacement, the 100 % stevianna (100S) muffin showed firmness values significantly higher ( $P < 0.05$ ) than the control (Figure 5.4), while springiness decreased when 100 % of the sucrose was replaced by Stevianna® (Figure 5.5). Overall, these results indicate that the addition of 100 % Stevianna® as a sugar replacer in muffins gave harder and more crumbly muffins with a more compact, less aerated crumb.

These results could be related to the ability of sugar to retard the gelatinization of starch, which has been found to lead to a softening effect on bakery products (Barndt and Antenucci, 1993). Therefore, removing sugar from the muffin was responsible for the effect on muffin firmness and springiness. Similarly, Martínez-Cervera et al. (2012a) showed significantly higher firmness values in the sucrose-free muffins when using 100 % sucrose replacement with erythritol than in control muffins. Akesowan (2009) also found an increase in the firmness of sugar-free chiffon cakes prepared with an erythritol-sucralose mixture.

No significant differences in firmness or springiness were found at 50 % sucrose replacement with Stevianna® compared with the control sample (Figures 5.4 and 5.5). The results were consistent with the research carried out in Chapter 4 (Gao et al., 2016). When only 50 % of sugar is removed, there is still sufficient sugar present to support better texture.

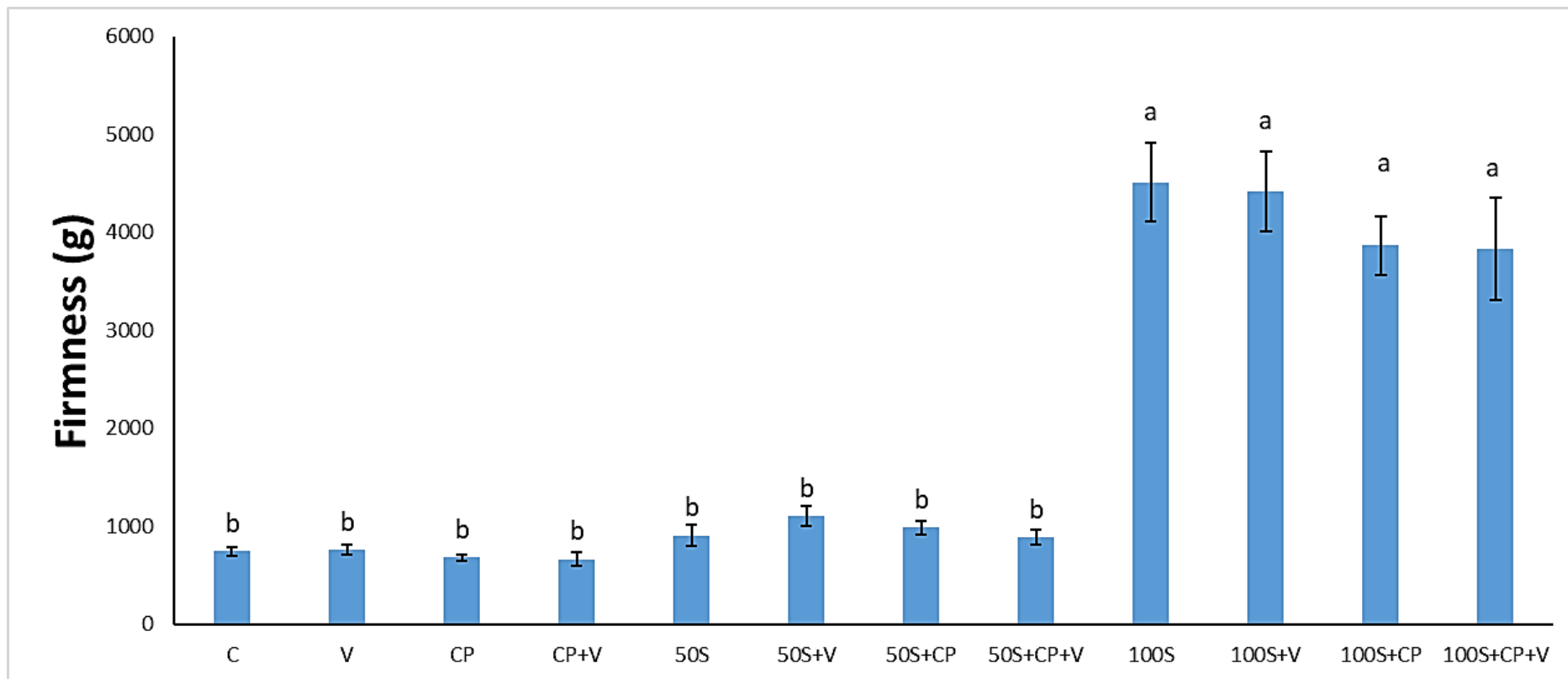


Figure 5. 4 Firmness values for muffins containing two levels of stevianna as sugar replacer with or without cocoa powder and vanilla. Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

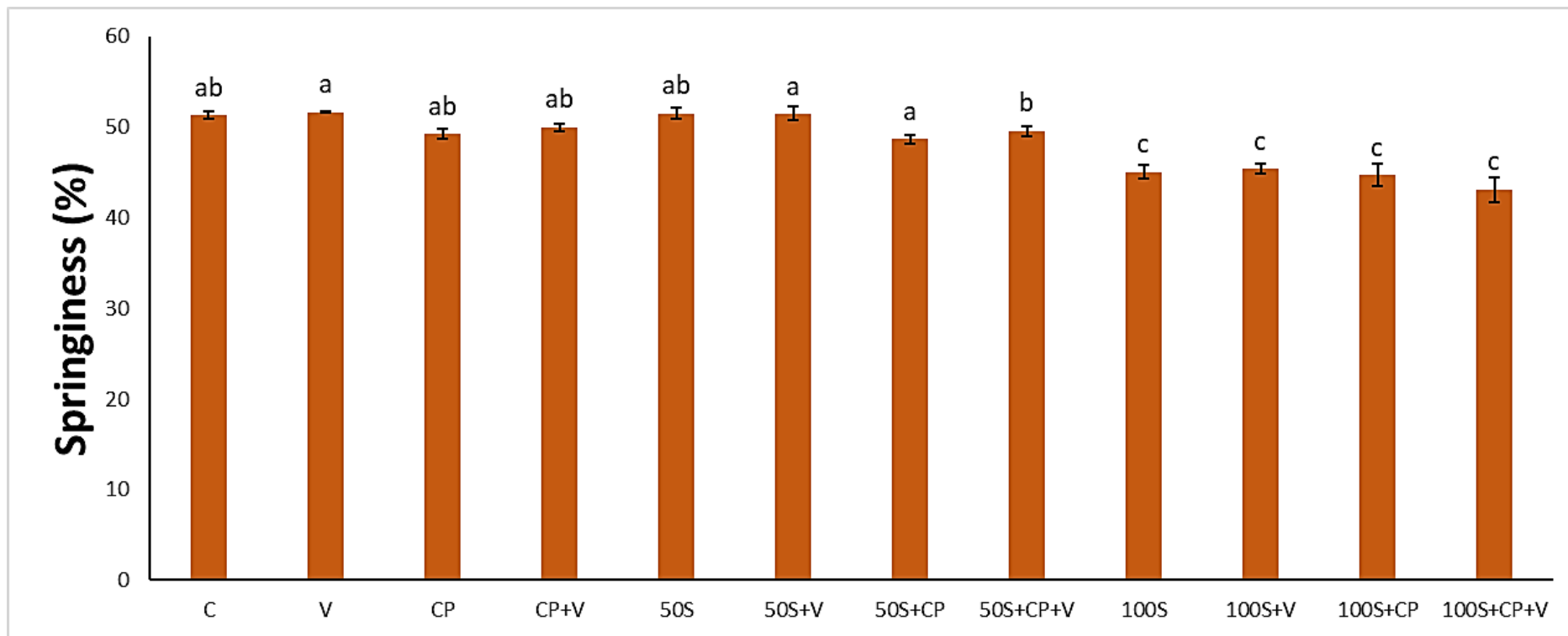


Figure 5. 5 Springiness values for muffins made from two levels of stevianna as sugar replacer with or without cocoa powder and vanilla. Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

### 5.3.3 Sensory Evaluation

In order to assess the acceptability of the muffins formulations, sensory evaluation was carried out. The transformed data of crust colour, mouthfeel, texture, sweetness, appearance, and overall liking of the low-sugar muffins with/without cocoa powder and/or vanilla are presented in Table 5.2.

**Table 5. 2 Sensory evaluation of half-sugar/sugar-free muffins in comparison with the control (C) muffin which was taken as relative value and processed factor values for each experimental sample.**

| Type of product | Colour             | Mouthfeel            | Sweetness          | Texture            | Visually            | Overall liking       |
|-----------------|--------------------|----------------------|--------------------|--------------------|---------------------|----------------------|
| V               | -0.82 <sup>b</sup> | -0.18 <sup>cd</sup>  | 0.08 <sup>a</sup>  | -0.35 <sup>b</sup> | -0.31 <sup>ab</sup> | -0.24 <sup>ab</sup>  |
| CP              | 4.14 <sup>a</sup>  | -0.24 <sup>cd</sup>  | -0.63 <sup>a</sup> | -0.57 <sup>b</sup> | 0.39 <sup>a</sup>   | -0.23 <sup>ab</sup>  |
| CP+V            | 4.09 <sup>a</sup>  | -0.4 <sup>d</sup>    | -0.61 <sup>a</sup> | -0.88 <sup>b</sup> | 0.31 <sup>a</sup>   | -0.50 <sup>a</sup>   |
| 50S             | 0.38 <sup>b</sup>  | -0.42 <sup>d</sup>   | -0.03 <sup>a</sup> | -1.43 <sup>b</sup> | -0.28 <sup>a</sup>  | 0.02 <sup>a</sup>    |
| 50S+V           | 0.3 <sup>b</sup>   | -0.46 <sup>d</sup>   | 0.61 <sup>a</sup>  | -1.09 <sup>b</sup> | 0.03 <sup>a</sup>   | -0.18 <sup>a</sup>   |
| 50S+CP          | 4.99 <sup>a</sup>  | 0.18 <sup>bcd</sup>  | -0.82 <sup>a</sup> | -1.07 <sup>b</sup> | 0.5 <sup>a</sup>    | -1.1 <sup>abc</sup>  |
| 50S+CP+V        | 5.15 <sup>a</sup>  | 0.4 <sup>abcd</sup>  | -0.96 <sup>a</sup> | -1.14 <sup>b</sup> | 0.59 <sup>a</sup>   | -0.93 <sup>abc</sup> |
| 100S            | -1.01 <sup>b</sup> | 1.74 <sup>abc</sup>  | 0.39 <sup>a</sup>  | 2.63 <sup>a</sup>  | -2.66 <sup>c</sup>  | -2.46 <sup>c</sup>   |
| 100S+V          | 0.34 <sup>b</sup>  | 1.49 <sup>abcd</sup> | -0.41 <sup>a</sup> | 2.81 <sup>a</sup>  | -3.06 <sup>c</sup>  | -2.75 <sup>c</sup>   |
| 100S+CP         | 4.36 <sup>a</sup>  | 2.31 <sup>a</sup>    | -1.14 <sup>a</sup> | 3.12 <sup>a</sup>  | -2.59 <sup>bc</sup> | -2.52 <sup>c</sup>   |
| 100S+CP+V       | 4.21 <sup>a</sup>  | 1.92 <sup>ab</sup>   | -1.19 <sup>a</sup> | 2.71 <sup>a</sup>  | -2.63 <sup>c</sup>  | -2.41 <sup>bc</sup>  |

Mean values with the same superscript letter within the same column are not significantly different at P < 0.05.

Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

### **5.3.3.1 Crust Colour**

Muffin samples containing Stevianna® at levels 50 % and 100 % with or without vanilla were judged not to be significantly different in crust colour compared to the control. This result was consistent with the instrumental analysis. Understandably, muffins containing cocoa powder showed significantly darker crust colour ( $P < 0.05$ ). Panellist ratings agreed with  $\Delta E^*$  values, which indicated that the crust of muffins became darker when the cocoa powder was added. Martínez-Cervera et al. (2011) evaluated the effects of cocoa addition on sensory characteristics of crust colour, and samples were perceived to have a stronger chocolate colour than the control muffin. Generally, the sensory evaluation of crust colour followed the instrumental measurements, while the panellists did not distinguish the minor differences detected by the colorimeter.

### **5.3.3.2 Mouthfeel and Texture**

The sensory evaluation of texture was in good agreement with the instrumental measurement of firmness. The muffins with 50% sugar replacement were evaluated as not significantly different to the control muffin in terms of texture and mouthfeel. At 100 % Stevianna® replacement levels, all muffins, with/without cocoa powder and/or vanilla, were perceived as being significantly harder and having a drier mouthfeel ( $P < 0.05$ ) when compared to control. The trend in mouthfeel is counter to the moisture content of the muffins (according to the comments of panellists but data not shown) and may reflect the humectant effect of the erythritol holding water content. Instrumental texture profile analysis also indicated that higher levels of Stevianna® had a negative effect on the texture quality of the muffin. Several authors have obtained similar results in other lowered sugar products. For instance, Akesowan (2009) included differing levels of erythritol-sucralose in cake formulations and found that with increasing content of erythritol-sucralose the cake texture became harder than the control. Martínez-Cervera et al. (2012a) also found significantly lower texture scores in low-sucrose muffins prepared with sucralose than controls.

### **5.3.3.3 Appearance**

The 100 % Stevianna® muffins with/without cocoa powder and/or vanilla were significantly ( $P < 0.05$ ) less appealing than those made with 0 % and 50 % Stevianna®, showing that when higher levels of Stevianna® were used muffins lost visual appeal. It is likely that the flat upper surface of the muffins resulted in the lowest panellist visual ratings for 100S muffins.

### **5.3.3.4 Sweetness**

Compared with control samples, there was no significant difference in panellist ratings for sweetness of the sugar-free or sugar-reduced muffins without cocoa powder. It appears that the amount of Stevianna® added to the formulations is theoretically equal to the amount of sucrose in the basic formulation of the muffins, since Stevianna® product is 1 time sweeter than sucrose. Table 5.2 illustrates that the use of increasing amounts of Stevianna® with cocoa powder resulted in a slightly lower perceived sweetness when compared against other muffin samples; however this was not significant. The bitterness of cocoa powder could have affected the perceived sweetness of the muffin. The result is in agreement with previous findings which showed that sugar replacement by different polyols in sponge cakes did not affect the overall sweetness of the product when sucrose was replaced by xylitol, sorbitol, and maltitol (Ronda et al., 2005).

### **5.3.3.5 Overall Liking**

The panellists' ratings for overall liking tended to decrease with increasing sucrose replacement level, following the trend observed for the other sensory parameters. Those muffins prepared with 50 % Stevianna® were not significantly different to the control muffin and were more highly appreciated by panellists than the 100 % sugar-free muffins. The lowest overall liking ratings were obtained when 100 % Stevianna® was in the muffin products; these muffins had a poor appearance, hard texture, and dry mouthfeel. The sensory result shows the poor overall liking ratings of 100S muffin products were mainly due to the effects of appearance, mouthfeel, and texture. Struck et al. (2016) reported similar



observations, illustrating that partial sucrose replacement by rebaudioside-A resulted in products having similar overall liking to the control muffin used.

The sample with 50 % of the sugar replaced by the Stevianna® had similar visual appearance, colour, texture, mouthfeel, and overall liking to the control muffin.

### **5.3.3.6 Aftertaste**

In a preliminary study with muffins containing Stevianna®, a bitter aftertaste was noted so the added flavoured ingredients were tested for their masking effect on this negative taste. Cocoa powder and vanilla were chosen as classic muffin flavour with a natural bitterness and sweetness, respectively. Table 5.3 presents the aftertaste results obtained by analysing the descriptions given by panellists over the three sensory analysis sessions conducted for all types of muffin sample. It can be seen that 8.6 % of panellists noted some aftertaste and used words such as “little bitterness,” “artificial sweetness,” “sour,” and “flour taste,” in muffins without cocoa powder. According to the panellist descriptions, Stevianna® substitution in control muffins resulted in the occurrence of a little bitterness which is attributed to the inherent bitterness of steviol glycosides (Carakostas et al., 2008). Of the total number of participants, 6.6 % of the panellists expressed an aftertaste of bitterness that was associated with the cocoa powder containing muffins and hence may be related to the flavour of cocoa. When a nominal logistic regression was fitted to the data it showed the Stevianna® muffins had a bitter response, with the main bitterness derived from the presence of cocoa powder ( $P < 0.05$ ). In this group of panellists, the overall liking improved when vanilla was added to the cocoa formulation (Table 5.3). This implied that the addition of cocoa powder could mask the Stevianna® bitterness in terms of taste and that the addition of vanilla enhanced the flavour in muffins. This observation is similar to results obtained by Nip (2006) and Belščak-Cvitanović et al. (2015) who recorded that the presence of vanilla in cereal products serves to enhance the sweetness of products through both flavour and odour receptors.

**Table 5. 3 Panellist descriptors and frequency of aftertaste in muffin products.**

| Aftertaste       | Descriptors  | Total count<br>(number of times) |
|------------------|--|----------------------------------|
| No aftertaste    | NA   | 475                              |
| Bitterness       | Chocolate flavour, dark chocolate, bitter chocolate taste, cocoa taste                               | 37                               |
| Other aftertaste | Artificial sweetness, egg, baking soda, sour, flour taste, not good aftertaste, little bitter, plant | 48                               |

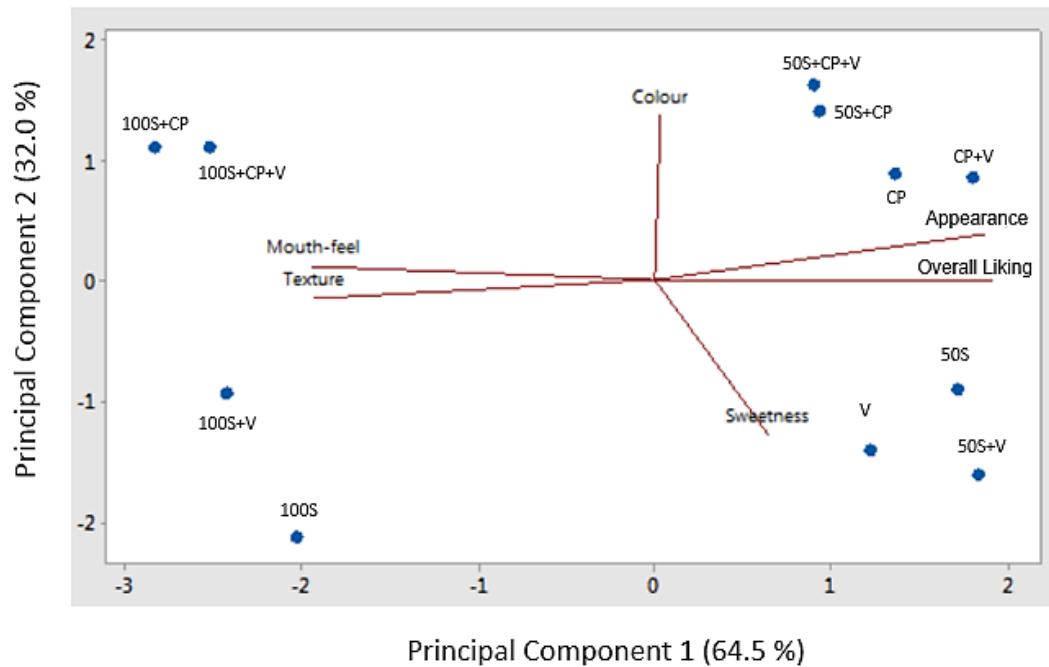
### 5.3.4 Principal Component Analysis

In order to illustrate the differences between product types based on individual panellist perceptions of sensation, principal component analysis (PCA) was utilized. The group average plot (Figure 5.6) shows that all the muffin samples were separated, and the replicates of each muffin analysis were close to each other indicating that the panel evaluation was consistent (Díaz-Maroto et al., 2002). PCA extracted two components that explained 96.5 % of the variation. The first component which segregated the samples based on sugar replacement and addition of cocoa powder/vanilla was positively correlated with the attributes of appearance, overall liking, and sweetness and negatively correlated with mouthfeel and texture (explaining 64.5 % of the variation). The second component, which explained 32.0 % of the variation, was mainly positively correlated with crust colour.

Consequently, the sensory characteristics of all the samples are mainly explained by the positive side of principal component 1 (PC1). These samples (V, CP, CP + V, 50S, 50S + V, 50S + CP, and 50S + CP + V) possessed the highest rating for appearance, overall liking, and sweetness. Muffins made by the addition of 100 % Stevianna® showed negative coordinates along PC1 and were mainly characterized by attributes mouthfeel and texture as an index of the textural properties of the sample. The main descriptor which was associated with PC2 is crust colour, when with-cocoa-powder samples were more distant from the without-cocoa-powder samples corresponding to differentiations in chocolate colour.

Therefore, the results of this study indicate the potential of using Stevianna® to completely replace sugar when combined with cocoa powder and vanilla to achieve the desired sweetness of a food

product. However, further optimizing is required to obtain muffins with satisfactory textural properties and mouthfeel and an appealing appearance that would satisfy consumer preference.



**Figure 5. 6** Principal component analysis of muffin attributes. Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

## 5.4 Conclusions

The results of this investigation show that an encouraging option and novel formulation of muffin production with Stevianna® and cocoa powder/vanilla were developed. Muffins formulated with partial replacement of sucrose with up to 50 % Stevianna® had sensory and texture characteristics comparable with muffins prepared with 100 % sucrose. When 100 % Stevianna® replaced sugar in the sugar-free formulation some negative sensory ratings were observed, namely, bitter aftertaste, poor appearance, hard texture, and dry mouthfeel leading, to reduced acceptability. Cocoa powder and vanilla were added to the formulation with Stevianna® in an attempt to mask any bitter aftertaste arising from the Stevianna® and to enhance product flavour. While this was successful in some sensory properties, 100 % Stevianna® muffins possessed poor physical qualities and were associated with texture failure. Further work is required to conduct an *in vitro* digestion analysis to assess whether the sugar replacement of the muffins can lead to a reduction in the predicted glycaemic response from the muffin material.

## Chapter 6

### Replacement of Sucrose with Stevianna® Based Sweetener Lowers Predicted Glycaemic Impact in Muffins

**Abstract:** Muffins are popular bakery products, however they generally contain high amounts of sugar. Over consumption of muffins may therefore result in a high calorie intake and could lead to increased health risks. For this reason, muffins were prepared substituting sucrose with two levels of Stevianna®. In addition, cocoa powder and vanilla were added to the muffin formulation with and without Stevianna® to mask any potential off-flavours. Results illustrate that muffins with 50 % Stevianna® replacement of sucrose were similar to the control samples in terms of volume, density and texture. However, replacement of sugar with 100 % Stevianna® resulted in reductions in height, volume, and texture compared to the control sample. Sugar replacement significantly affected the *in vitro* predictive glycaemic response of muffins. This work illustrates the importance of sugar in maintaining muffin structure as well as controlling the rate of glucose release during simulated digestions.

## 6.1 Introduction

In recent years, consumers have gained an increasing awareness regarding the effect of dietary carbohydrates on the nutritional quality of foods. In particular, attention has been focused on the relationship between the various types of carbohydrate containing foods and the different postprandial glucose responses by these foods post ingestion (Brennan, 2005; Jenkins et al., 1980; Monro, 2002). The glycaemic index (GI) is a physiological classification widely accepted for carbohydrate foods based on their ability to raise the concentration of glucose in the blood (Monro and Shaw, 2008). Bakery foods, muffins for example, are regarded as a high glycaemic impact food due to the high concentration of sugar contained in the muffins. Previous research (Barros et al., 2007) has shown that over-consumption of sucrose can lead to a number of metabolic complications including hyperinsulinemia, hyperglycaemia, hypertension and insulin resistance, as well as being related to dyslipidaemia and ectopic lipid deposition in healthy subjects with diabetes (Lê et al., 2009). Indeed, high GI food products are quickly digested and their carbohydrate rapidly absorbed resulting in higher blood glucose levels (Burton and Lightowler, 2006). On the contrary, the health benefits of the low GI products are thought to be derived from the slower rate of carbohydrate absorption, consequently leading to a gradual rise in blood glucose level and better glycaemic control (Bae et al., 2016).

The food industry has focused on reducing the calorific content of food to promote a healthier diet. Therefore, different natural sweeteners have been used in sugar-reduced or sugar-free products based on their multiple potential health benefits and functional properties, including maintaining sweetness and acceptable texture (Baeva et al., 2000; Karp et al., 2017; Kulthe et al., 2014; Livesey, 2003).

Steviol glycosides have been extracted and purified from the leaves of *Stevia rebaudiana Bertoni*, commonly known as stevia; they are naturally sweet-tasting, have good solubility in water, good temperature and pH stability (Kroyer, 2010) as well as having no calorific value (Gregersen et al., 2004) allowing them to be used as a sugar substitute or natural sweetener. Stevioside and rebaudioside-A are the major glycoside constituents responsible for sweetness, and are the most abundant glycosides

in the *Stevia rebaudiana Bertoni* plant (Carakostas et al., 2008). They are very useful as a food additive due to their relative sweetness being 250-300 times sweeter than table sugar (Manisha et al., 2012).

Extracts from stevia have broad health-promoting properties for blood glucose and insulin levels in human studies (Chang et al., 2005). Steviol glycosides are not hydrolyzed by human digestive enzymes of the mouth, stomach, and small intestine (O'Donnell and Kearsley, 2012), however, rebaudioside-A and stevioside are hydrolyzed (*in vitro* and *in vivo*) to aglycone steviol by colon microflora through the successive removal of glucose units (Wheeler et al., 2008). Chang et al. (2005) reported insulin sensitivity is increased due to stevia consumption in rodent models, and thus does not increase blood glucose and insulin levels (Gregersen et al., 2004). Furthermore, the research carried out in Chapter 4 (Gao et al., 2016) has found that a reduction in the predicted glycaemic response was observed due 50 % or 100 % replacement of sucrose with Stevianna® in muffins during *in vitro* digestion experiments. Therefore, stevia has the potential to be a low-cost natural sweetener due to important pro-health properties, such as being non-calorific, non-fermentable and non-toxic as well as having a high intensity sweetness (Geuns, 2010), it is also recommended as a treatment for diabetics and obese persons (Goyal and Goyal, 2010).

Several studies have shown that the utilization of stevia as a sugar replacer in baking leads to a negative effect on appearance, compactness, moisture and texture of the bakery products structure (Abdel-Salam et al., 2009; Edelstein et al., 2007; Kulthe et al., 2014). These results have indicated that stevia is not acceptable to replace sucrose completely in bakery products as stevia exhibits high-intensity sweetness but does not possess the necessary bulking characteristics (Struck et al., 2014). That is why Stevianna® (product code ST001 SE supplied by Stevianna® NZ) is used for our study, as it incorporates rebaudioside-A (98 % steviol glycoside; 1 %) with erythritol (99 %).

Erythritol is a 4-carbon sugar alcohol or polyol with about 60 to 80 % of the sweetness of sucrose (Goossens and Roper, 1994). It is not only a sweetener but also a bulking agent, and thus can be as a sugar replacer is used in bakery products. Partial replacement of sucrose with erythritol had no negative influence on physical quality characteristics in a baked product (Lin et al., 2003; Struck et al.,

2014). In addition, previous studies reported erythritol is useful as a non-glycaemic and low-calorie sweetener that is safe for diabetics (Bornet, 1994; Lin et al., 2010). Erythritol has been demonstrated to have a small molecular size thus it is rapidly absorbed by the small intestine and does not undergo systemic metabolism by the human body (Bornet, 1994; Bornet et al., 1996). Some research has shown that the combination of a high-intensity sweetener with bulking agents or fibres in sugar-reduced formulations of food resulted in bakery products with acceptable physical quality (Manisha et al., 2012; Wang et al., 2016; Wheeler et al., 2008; Zahn et al., 2013).

However, none of these previous studies assessed a complex food sweetener to replace traditional sugar in bakery products. The aim of the study was to evaluate the replacement of sugar with Stevianna® (1 x sweetness of sucrose) and the addition of cocoa powder and/or vanilla to muffins for their physical properties and glycaemic response, compared with a control muffin formulation with no added Stevianna®, cocoa powder, or vanilla.

## **6.2 Materials and Methods**

### **6.2.1 Raw Materials**

Raw materials were used for this chapter are in Chapter 3.1.

### **6.2.2 Muffin Preparation**

Muffin recipe 2 was prepared and manufactured as described 3.2. Sugar was replaced by Stevianna® with/without cocoa powder and/or vanilla for making sugar-reduced muffin, which is shown in Table 3.2.

### **6.2.3 Muffin Height**

Muffin height was measured as outlined in 3.4.3.



#### **6.2.4 Moisture Content**

Moisture content assay was performed as described in 3.4.7.

#### **6.2.5 Muffin Volume**

Volume of muffin is measured as described in 3.4.4.

#### **6.2.6 Muffin Texture**

Muffin texture was determined using a texture analyzer as outlined in 3.4.5.

#### **6.2.7 Muffin Total Starch**

The total starch was determined by the method described in 3.4.8.

#### **6.2.8 *In vitro* Predictive Glycaemic Response Digestion Analysis**

Whole muffins were chopped until a fine crumb was achieved, which was determined using *in vitro* digestion analysis as outlined in 3.5 (3.5.1 and 3.5.3). This procedure measures the breakdown of carbohydrates to sugars by the action of amylase enzymes added to the baked muffin.

#### **6.2.9 Statistical Analyses**

One-way ANOVA was performed as described in 3.7.1.

### **6.3 Results and Discussion**

#### **6.3.1 Moisture Content**

Table 6.1 shows that the moisture content of muffin samples ranged from 19 to 27 %. The moisture content of the muffin samples produced was higher when cocoa powder or/and vanilla was used. In addition, Figure 6.1 (A, B, and D) shows that moisture content increased significantly ( $P < 0.05$ ) when

sucrose was replaced by Stevianna<sup>®</sup>, in particular, the moisture content of 100 % Stevianna<sup>®</sup> samples were higher than the full-sucrose muffin samples. Sucrose plays an important role in water retention that results in moisture loss during baking of the muffins (Martínez-Cervera et al. 2012a). However, the moisture content increased when sucrose was replaced because the Stevianna<sup>®</sup> acted as a humectant and prevented water from escaping during baking. Research using other types of sugar replacers has shown similar results, Martínez-Cervera et al. (2012b) used erythritol in muffins for its water retention properties. Ghosh and Sudha (2012) showed that the use of the polyol sorbitol was reflected in a significantly higher moisture content ( $P < 0.05$ ). Due to the high water-binding capacity of formulations with carbohydrate-based sugar replacers a greater amount of water is required in the cereal products.

Moisture content in bakery products is an important factor as it has a direct impact on the texture attributes and a strong correlation has been found between moisture content and firmness (Morris & Morris, 2012). As can be seen from the Table 6.1, muffin firmness increased as moisture content increased. As reported by Rößle et al. (2011), this must be related to the replacement of the sugar by Stevianna<sup>®</sup>, affecting the formation of muffin structure.

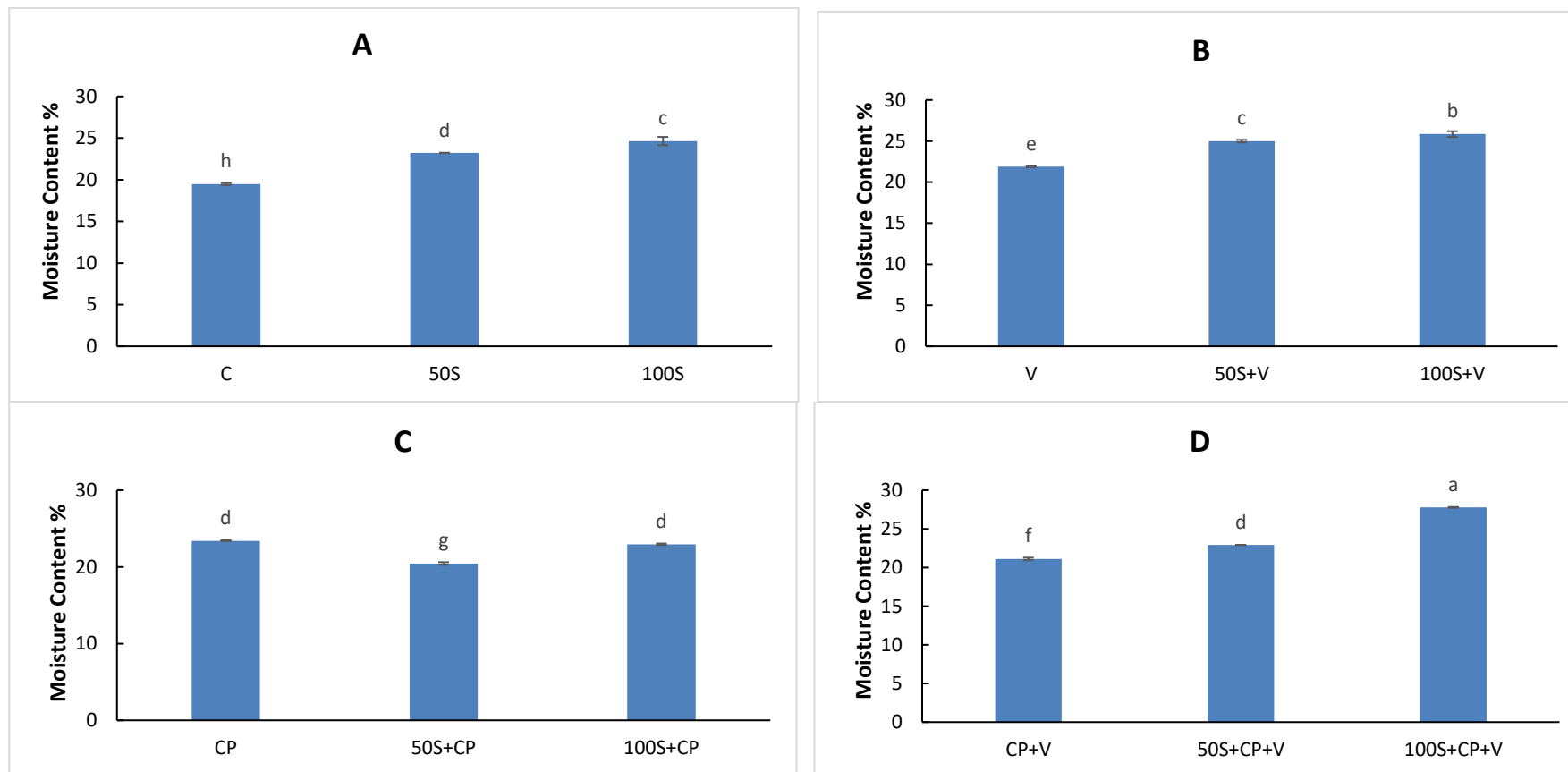
**Table 6. 1 Effect of stevianna on texture profile analysis and total starch in muffins with or without cocoa powder and/or vanilla.**

| Product   | Moisture (%)              | Height (mm)               | Volume (mL)               | Density (g/mL)           | Firmness (g)                  | Springiness (%)            | Total starch (%)             |
|-----------|---------------------------|---------------------------|---------------------------|--------------------------|-------------------------------|----------------------------|------------------------------|
| C         | 19.49 ± 0.12 <sup>h</sup> | 41.07 ± 0.45 <sup>b</sup> | 63.33 ± 2.89 <sup>a</sup> | 0.59 ± 0.03 <sup>b</sup> | 746.06 ± 44.10 <sup>b</sup>   | 51.29 ± 0.44 <sup>ab</sup> | 26.83 ± 1.92 <sup>abc</sup>  |
| V         | 21.89 ± 0.07 <sup>e</sup> | 40.69 ± 0.34 <sup>b</sup> | 63.33 ± 2.89 <sup>a</sup> | 0.59 ± 0.03 <sup>b</sup> | 763.51 ± 51.48 <sup>b</sup>   | 51.66 ± 0.09 <sup>a</sup>  | 27.93 ± 0.42 <sup>ab</sup>   |
| CP        | 23.40 ± 0.04 <sup>d</sup> | 43.30 ± 0.10 <sup>a</sup> | 63.33 ± 2.89 <sup>a</sup> | 0.59 ± 0.03 <sup>b</sup> | 680.99 ± 30.33 <sup>b</sup>   | 49.26 ± 0.54 <sup>ab</sup> | 26.14 ± 0.60 <sup>abcd</sup> |
| CP+V      | 21.11 ± 0.17 <sup>f</sup> | 43.21 ± 0.07 <sup>a</sup> | 66.67 ± 2.89 <sup>a</sup> | 0.56 ± 0.02 <sup>b</sup> | 662.97 ± 68.46 <sup>b</sup>   | 49.99 ± 0.43 <sup>ab</sup> | 24.43 ± 1.06 <sup>bcde</sup> |
| 50S       | 23.21 ± 0.03 <sup>d</sup> | 36.73 ± 1.30 <sup>c</sup> | 66.33 ± 5.77 <sup>a</sup> | 0.60 ± 0.05 <sup>b</sup> | 906.07 ± 111.09 <sup>b</sup>  | 51.51 ± 0.62 <sup>ab</sup> | 28.50 ± 0.85 <sup>a</sup>    |
| 50S+V     | 24.99 ± 0.16 <sup>c</sup> | 35.85 ± 1.02 <sup>c</sup> | 63.33 ± 2.89 <sup>a</sup> | 0.59 ± 0.03 <sup>b</sup> | 1102.18 ± 102.10 <sup>b</sup> | 51.49 ± 0.78 <sup>a</sup>  | 29.03 ± 0.36 <sup>a</sup>    |
| 50S+CP    | 20.46 ± 0.17 <sup>g</sup> | 35.83 ± 0.64 <sup>c</sup> | 63.33 ± 2.89 <sup>a</sup> | 0.06 ± 0.02 <sup>b</sup> | 987.03 ± 68.00 <sup>b</sup>   | 48.67 ± 0.52 <sup>a</sup>  | 22.72 ± 0.39 <sup>de</sup>   |
| 50S+CP+V  | 22.92 ± 0.20 <sup>d</sup> | 37.02 ± 0.50 <sup>c</sup> | 63.33 ± 2.89 <sup>a</sup> | 0.59 ± 0.03 <sup>b</sup> | 890.78 ± 76.18 <sup>b</sup>   | 49.59 ± 0.54 <sup>b</sup>  | 23.40 ± 0.09 <sup>cde</sup>  |
| 100S      | 24.64 ± 0.49 <sup>c</sup> | 28.35 ± 0.62 <sup>d</sup> | 51.7 ± 2.89 <sup>b</sup>  | 0.74 ± 0.04 <sup>a</sup> | 4512.78 ± 399.65 <sup>a</sup> | 45.07 ± 0.71 <sup>c</sup>  | 26.60 ± 0.94 <sup>abc</sup>  |
| 100S+V    | 25.85 ± 0.35 <sup>b</sup> | 26.63 ± 0.77 <sup>d</sup> | 51.7 ± 2.89 <sup>b</sup>  | 0.75 ± 0.04 <sup>a</sup> | 4419.70 ± 409.69 <sup>a</sup> | 45.44 ± 0.56 <sup>c</sup>  | 29.09 ± 2.56 <sup>a</sup>    |
| 100S+CP   | 22.95 ± 0.09 <sup>d</sup> | 27.99 ± 0.71 <sup>d</sup> | 53.3 ± 2.89 <sup>b</sup>  | 0.71 ± 0.03 <sup>a</sup> | 3868.00 ± 300.87 <sup>a</sup> | 44.74 ± 1.12 <sup>c</sup>  | 22.62 ± 1.42 <sup>e</sup>    |
| 100S+CP+V | 27.77 ± 0.06 <sup>a</sup> | 27.85 ± 0.79 <sup>d</sup> | 51.7 ± 2.89 <sup>b</sup>  | 0.74 ± 0.04 <sup>a</sup> | 3839.94 ± 522.34 <sup>a</sup> | 43.11 ± 1.36 <sup>c</sup>  | 26.17 ± 1.14 <sup>abcd</sup> |

All measurements are mean values ± SD of triplicate determinations.

Means in the same column with different letters are significantly different (P < 0.05).

Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).



**Figure 6. 1** Moisture content for muffins of formulation made from two levels of Stevianna® without/with cocoa powder and/or vanilla. Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

### 6.3.2 Height

The height of the muffins prepared with the different levels of Stevianna® with/without cocoa powder and/or vanilla is shown in Figure 6.2. The full-sucrose muffin was significantly higher ( $P < 0.05$ ) than the muffins that were prepared using Stevianna®. The lowest height was found in the 100 % Stevianna® muffin samples. The full-sucrose muffin with cocoa powder and/or vanilla group had greater height than the control and other samples (Figure 6.2). These results indicate that the decrease in muffin height was associated with an absence of interconnectivity of a more compact structure and with a low number of air cells for levels of sucrose replacement higher than 50 % (Figure 6.3).

Photographs of vertical cross-sections of the different muffin formulations are shown in Figure 6.3. As the Stevianna® content increased, in the formulations, the air bubbles became smaller and the air channels gradually diminished. This could be due to the fact that muffins with a full sucrose content gained an increased number of air bubbles during the beating of the batter, and these air bubbles are then expanded by carbon dioxide and water vapour pressure generated during baking, resulting in the formation of air channels, which influence the texture of the finished muffin product. The lack of air channels as the sucrose was replaced may also be associated with earlier thermosetting of the batter during the heating process in the oven, therefore, not allowing enough time for bubble expansion and formation of air channels (Martínez-Cervera et al. 2012a). Martínez-Cervera et al. (2012b) also found that the number of small air bubbles increased, air channels diminished and circular bubbles increased with an increase in sucrose replacement by polydextrose and sucralose in a muffin product.

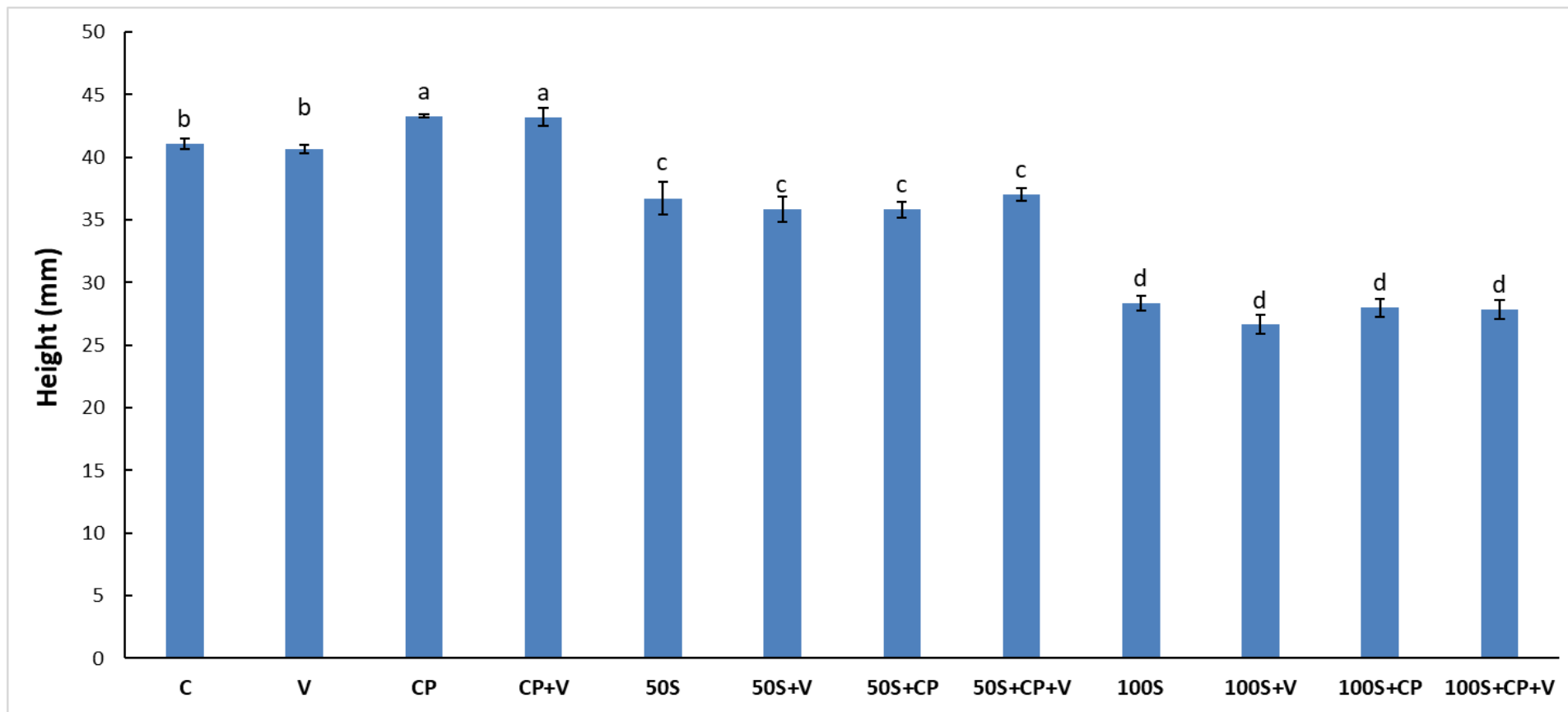


Figure 6. 2 Effect of Stevianna® without/with cocoa powder and/or vanilla on the height of muffin. Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

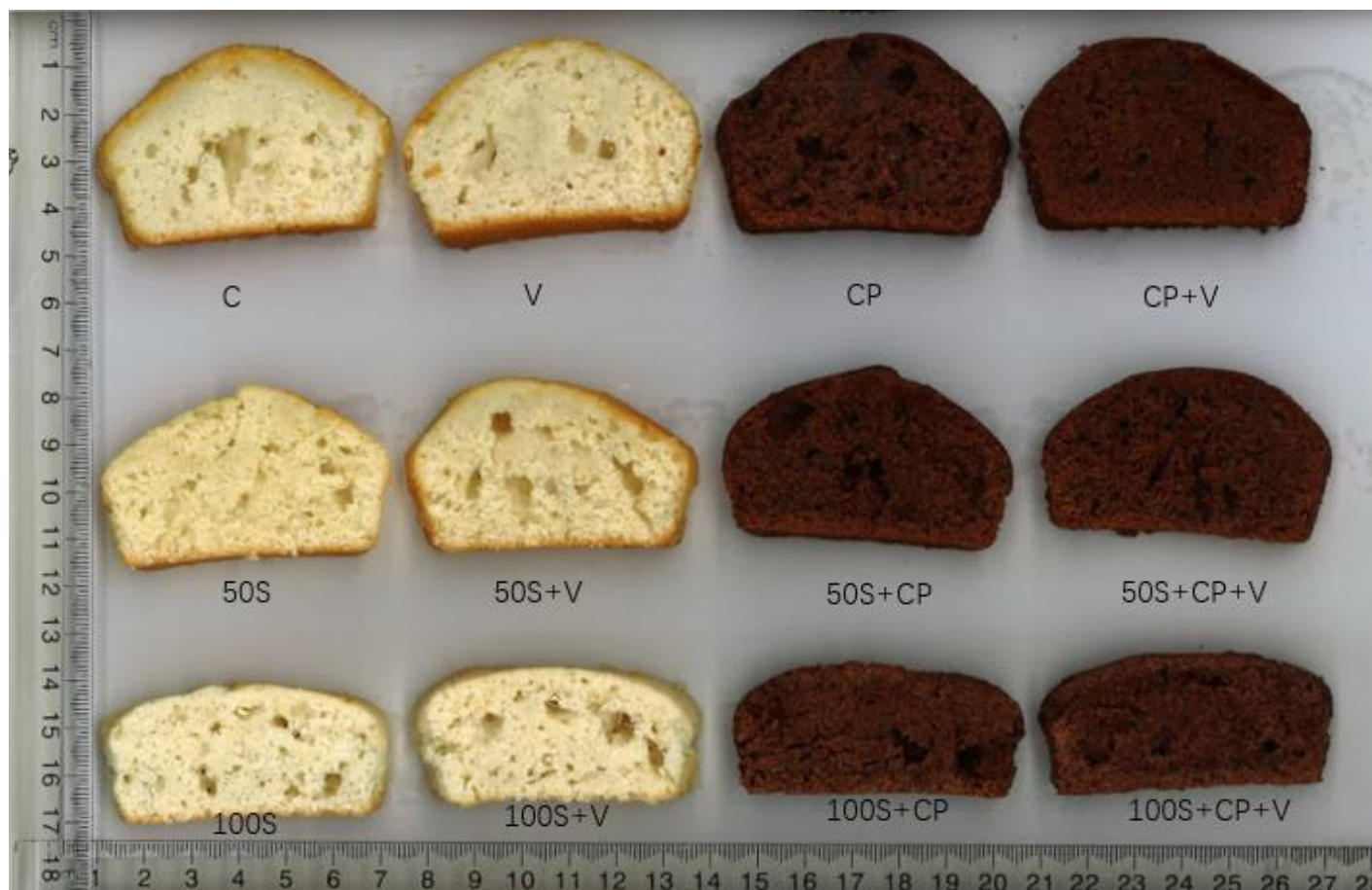


Figure 6. 3 Effect of two levels of Stevianna® with/without cocoa powder and/or vanilla in muffins: Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

### 6.3.3 Volume, Density and Texture

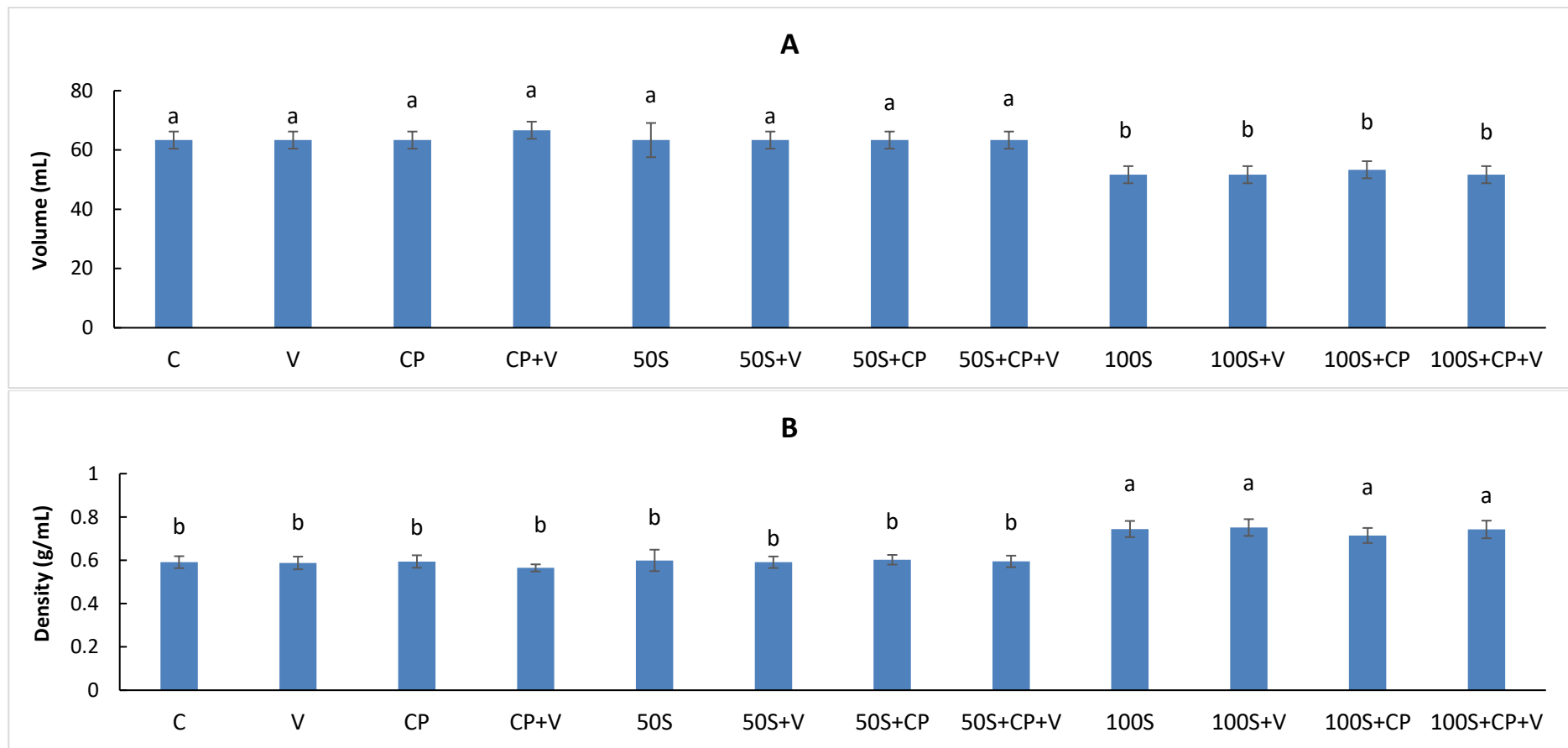
The volume of the muffin is an important indicator of air bubble expansion during baking and consequently also of the porous structure of the product. The volumes of muffins prepared with different levels of Stevianna® with/without and/or vanilla along with the control muffin are presented in Figure 6.4A. The samples with 100 % Stevianna® muffin group had significantly lower volumes ( $P < 0.05$ ) compared to those of the full-sucrose muffin products. Muffin density appeared to be negatively correlated with muffin volume (Figure 6.4B). Density of the muffins was calculated from mass and volume after baking. Table 2 illustrates that when sugar was completely substituted with Stevianna® there was a significant increase ( $P < 0.05$ ) in muffin density. Additionally, product characteristics such as springiness and firmness were affected ( $P < 0.05$ ) (Table 6.1). These results indicate that an increase in the level of Stevianna® had an adverse effect on volume, density and texture of the muffin. Manisha et al. (2012) also reported that replacement of sucrose with 100 % stevioside and liquid sorbitol caused a significant deterioration in physical properties which decreased volume and increased firmness in cake.

A function of sugar during cake baking is that it delays starch gelatinization thus contributing to the aeration of the batter and the optimum quality of sugar will affect formation of the cake structure and improve crumb texture and tenderness (Manisha et al., 2012). The decrease in sugar-free muffin expansion is the result of less air bubble incorporation and reduced air holding capacity during baking (Psimouli and Oreopoulou, 2013). In addition, starch gelatinization temperature seems to contribute to volume development due to different interactions between the Stevianna® and starch and proteins of the batter, these interactions affect starch gelatinization and protein denaturation temperatures. These results are in agreement with Ronda et al. (2005) findings that showed a decrease of starch gelatinization and protein denaturation temperatures in sorbitol cakes is expected to cause a premature thermosetting of protein or starch matrix, this process will start at the crust due to direct contact with the heating medium. Therefore, this lowers the heat transfer rate, and produces a vapour pressure build-up, resulting in inadequate expansion of individual bubbles. Additionally, Ronda et al.



(2005) found that high fructose corn syrup (HFCS) mainly contributed to the early gelatinization of starch during baking process and restricted the volume of baked products compared to sucrose.

However, 50 % Stevianna® used had no significant effect on the volume and density of muffin compared to the full-sucrose muffin samples (Figure 6.4). These results suggest that muffin samples containing the half amount of Stevianna® have a similar ability, to muffins with full sucrose, to retain air. These results are consistent with those of Lin et al. (2003) who found no significant differences among the volume estimates for 50 % erythritol cakes. Furthermore, addition of the 50 % Stevianna® in muffin samples exhibited a texture close to that of the full-sucrose muffin samples (Table 6.1), which conferred an appearance of firmness and springiness. The results were consistent with the research carried out in Chapter 4 (Gao et al., 2016).



**Figure 6. 4** Volume and density values for muffins containing two levels of Stevianna® as sugar replacer with or without cocoa powder and vanilla. Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

#### 6.3.4 *In vitro* Predictive Glycaemic Response

The total starch content of modified muffins was measured and compared with control sample (Table 6.1). Compared to the control muffin, 50 % or 100 % sucrose replacement with Stevianna® with added cocoa powder samples had significantly lower amounts of total starch. Similar levels of total starch were observed in control and full-sucrose muffin samples, 50 % and 100 % Stevianna® with/without cocoa powder and/or vanilla muffin samples. Thus, the presence of cocoa powder with Stevianna® in muffin had a significant effect on total starch contents.

The effects of Stevianna® on *in vitro* starch digestion in muffin and chocolate muffin products were investigated by measuring the glucose released during starch digestion. Figure 6.5 shows the reducing sugars curves of two levels of Stevianna® with/without cocoa powder and/or vanilla muffin samples that were compared with full-sucrose with/without cocoa powder and/or vanilla samples, respectively. These two levels of Stevianna® used in this study were found to decrease reducing sugars released by digestive enzymes, compared with the full-sucrose muffin samples. The rate and extent of reducing sugars released were the highest in the control muffin, followed by 50 % Stevianna® with/without cocoa powder and/or vanilla muffin products, and 100 % Stevianna® with/without cocoa powder and/or vanilla muffins (Figure 6.5). In particular, muffins with Stevianna® showed a significant decrease in terms of reducing sugars released throughout 120 min starch digestion process.

The total area under the hydrolysis curve (AUC) relates the total glucose release to the digestion time of 120 min. The concentration of the Stevianna® had a significant effect on the AUC values ( $P < 0.05$ ), which demonstrated that the replacement of sucrose with 100 % Stevianna® resulted in the lowest AUC value of muffin samples in a dose response (Figure 6.6). It is of interest that the additions of vanilla and/or cocoa powder with muffin production did not lead to significant reduction of *in vitro* digestion values compared to the full-sucrose, 50 % Stevianna®, and 100 % Stevianna® samples respectively. These results are consistent with the research carried out in Chapter 4 (Gao et al., 2016).

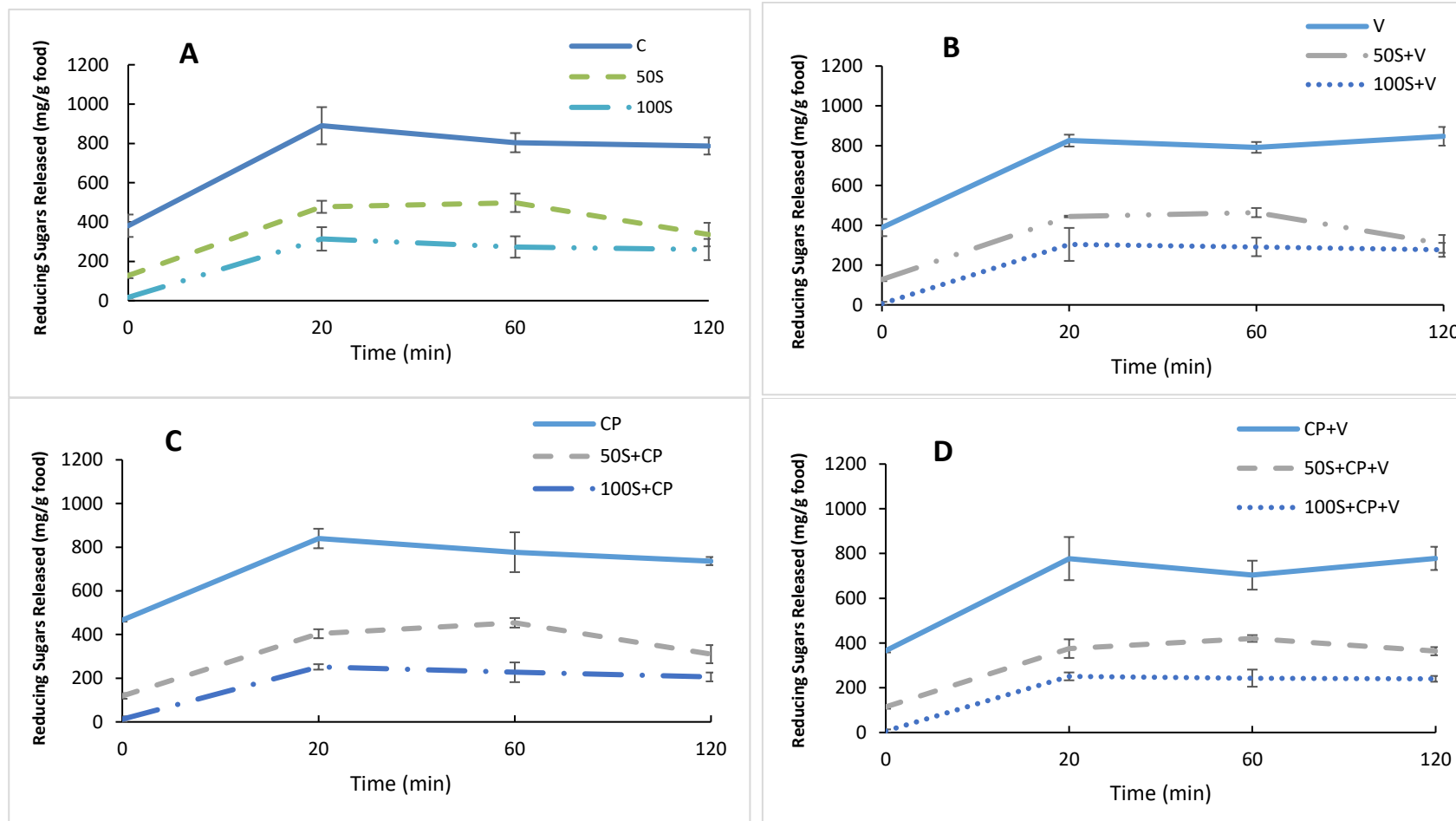


Figure 6. 5 Amount of reducing sugars released during *in vitro* digestion. Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

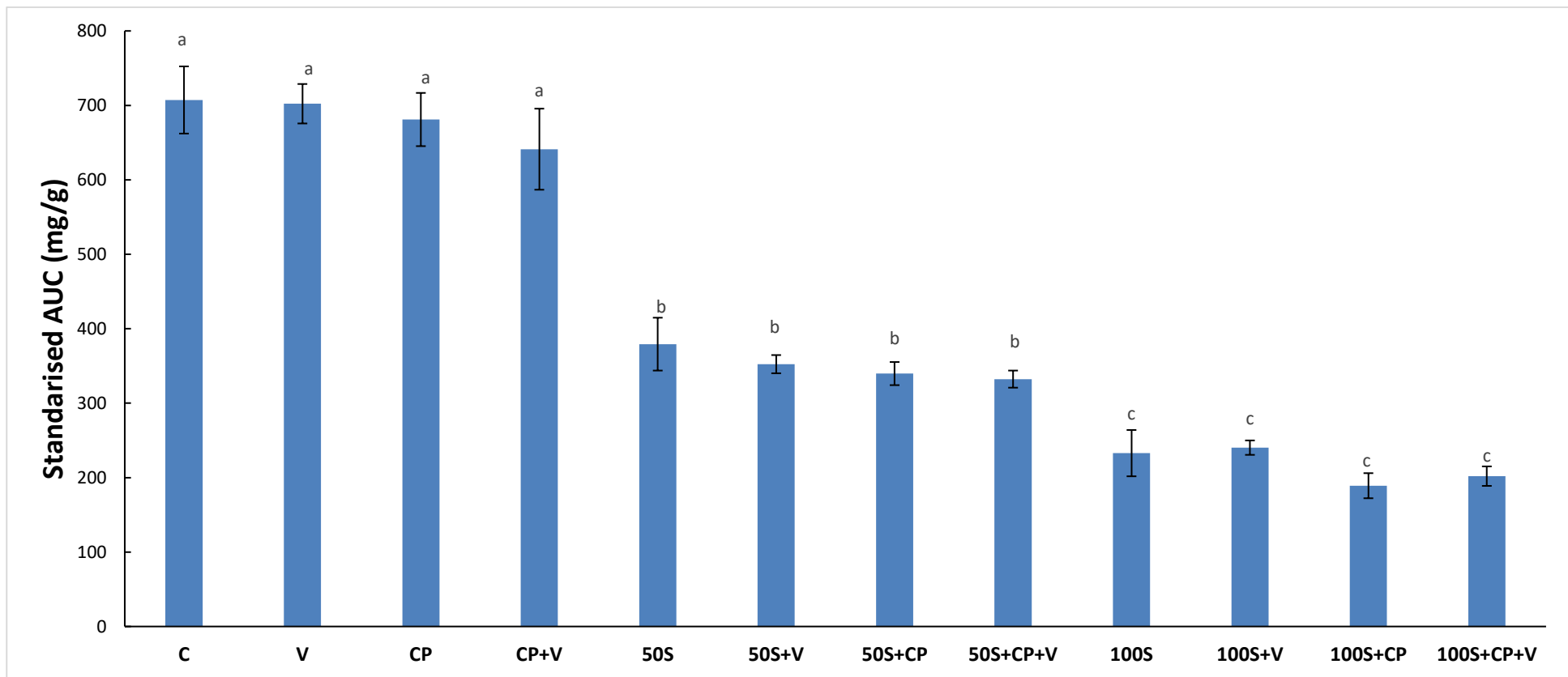


Figure 6. 6 Values for area under the curve (AUC) comparing the control and other low-sugar muffins made with two levels of Stevianna® with/without cocoa powder and/or vanilla. Control (C); vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

This study did not focus on the impact of sweeteners on *in vitro* starch digestion analysis of bakery products. However, several research projects have been designed to test the effects of the stevia or erythritol on postprandial glucose and insulin levels *in vivo* and *in vitro* digestion methods as compared to sucrose (Alizadeh et al., 2014; Bornet 1994; Ishikawa et al., 1996; Lin et al., 2010; Wheeler et al., 2008).

Breakdown or disruption of starch granules that results from salivary amylase causes a greater susceptibility of the granule to further enzyme degradation. This process will lead to more readily digestible starch, and hence create a higher blood glucose response (Granfeldt et al., 2000). The level of postprandial blood glucose is a major factor in predicting the profile of insulin resistance (Monro and Shaw, 2008). Alizadeh et al. (2014) found that there were differing effects on postprandial blood insulin levels that were dependent on the type and amount of sweetener consumed. The effect of consumption of beverages containing stevia has been tested by measuring the *in vivo* glycaemic impact (Anton et al., 2010), it was found that postprandial glucose and insulin levels were significantly reduced in the stevia beverages compared to the sucrose beverages. These effects on postprandial glucose levels are mainly due to the lack calories and carbohydrate content of Stevianna<sup>®</sup>, thus there are no reducing sugars released. A similar trend has been observed in that the postprandial insulin levels were reduced in stevia ice cream samples compared to full-sucrose ice cream samples (Alizadeh et al., 2014), this is most likely due to the functional properties of stevia that results in no contribution to the available carbohydrate and glycaemic response in food products (Alizadeh et al., 2014). In addition, Roberts and Renwick (2008) proved that steviol glycosides are not readily absorbed by the upper small intestine when it is administered orally to normal rat or human subjects. There are no human digestive enzymes present in the small intestine to hydrolyze the  $\beta$ -glycosidic linkages resulting in limited small intestine digestion (Wheeler et al., 2008).

Lin et al. (2010) illustrated that 0-100 % sugar replacement with erythritol in cookies decreased the carbohydrate contents by *in vivo* digestion. Since the calorie value of erythritol is approximately 0.4 kcal/g (Bornet et al., 1996), it provides no energy to the body and thus is not systemically metabolized

nor fermented in the colon (Bornet, 1994). It has been suggested that the consumption of erythritol does not raise postprandial glycaemic and insulin levels by oral ingestion in healthy human subjects (O'Donnell and Kearsley, 2012). In a study carried out by Bornet et al. (1996), more than 90 % of erythritol is rapidly absorbed by the small intestine when eaten and is excreted unchanged in the urine.

The Stevianna® used in our study was composed of rebaudioside-A (stevia) and erythritol, therefore, the observations made are consistent with those made by the above studies. Our experiment results showed that under *in vitro* conditions a lower reducing sugar liberation took place when sucrose was replaced by Stevianna® in muffins, and consequently this can be beneficial as it will decrease the postprandial blood glucose. Additionally, it is probable that the intake of these muffins decreases the rate of intestine absorption of glucose and delays gastric emptying (Burton and Lightowler, 2006).

## 6.4 Conclusion

The stevia containing product, Stevianna®, has been shown to be a suitable sucrose replacement for low-sucrose formulation of muffins. The results showed that 50 % sugar replacement with Stevianna® had similar physical characteristics in terms of volume, density and texture to a control muffin. However, when the sugar was replaced by 100 % Stevianna® the muffin showed a reduction in volume, an increase in textural firmness and a correspondingly high density of the product when compared to the control muffin samples. Furthermore, Stevianna® was able to simulate sucrose functionality in muffins, producing an increase in moisture content in comparison with the full-sucrose muffins. The negative effect of Stevianna® on muffin properties can be associated with the fact that as the Stevianna® level was increased it led to a reduction of air bubble expansion during the heating process (possibly due to the weakening of the starch-protein-sugar interface of the muffin allowing for greater structural collapse) and thus a corresponding reduction in height. This research illustrates that Stevianna® is a major factor impacting on physical characteristics of muffins. The addition of cocoa powder and/or vanilla did not affect the physical characteristics of muffins significantly.

In relation to the nutritional quality of the muffin products, the effect of Stevianna® inclusion on the predicted glycaemic impact as determined by *in vitro* digestion illustrated the role of sugar in elevating the glycaemic response during digestion. The replacement of sugar with increasing levels of Stevianna® was found to significantly decrease the potential glycaemic response values, this is most likely to be attributed to the fact that Stevianna® was not degraded into glucose units and acted as an inert filler within the muffin samples. No significant changes to the predicted glycaemic response values were observed due to either the cocoa powder and/or vanilla addition.

Finally, it can be seen that a partial replacement of Stevianna® for sucrose with/without cocoa powder and/or vanilla in muffins gave a product with physical characteristics close to that of the full-sucrose muffin sample. At the same time, the reduction in potential glycaemic response values was greater than would have been expected with 50 % sucrose reduction and consequently providing a better muffin that produces a lowered postprandial response with the potential associated health benefits.



## Chapter 7

# The Effect on Starch Pasting Properties and Predictive Glycaemic Response of Muffin Batters using Stevianna® or Inulin as a Sucrose Replacer

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**Abstract:** Different levels of sugar replacers (inulin or Stevianna®) were used in two muffin batter recipes differing in sugar : flour ratios. The properties of these sugar replacers were linked to differences in batter viscosity, starch gelatinisation and *in vitro* predictive glycaemic response of batters. The replacement of sugar with Stevianna® had no significant effect on the viscosity of the batter (Recipe 1) or the starch gelatinisation (Recipe 1 and 2). Replacement of 50 % or more of the sugar with inulin increased the viscosity. The starch gelatinisation properties were altered with the incorporation of inulin. Batters incorporating Stevianna® and cocoa powder (Recipe 2) had significantly different viscosity compared to the batters incorporating Stevianna® without cocoa powder. *In vitro* starch hydrolysis of the modified batter illustrated that the inclusion of inulin or Stevianna® significantly reduced the rate and extent of carbohydrate hydrolysis during digestion.

## 7.1 Introduction

Muffin batters are complex fat-in-water emulsion systems containing flour, starch, sugar, fat, eggs and baking powder. The biochemical and physicochemical reactions which occur during baking are complex and involve water evaporation, protein denaturation, starch destruction, browning and Maillard reactions, dough expansion by production and thermal expansion of gas during batter processing (Chevallier et al., 2000). Starch gelatinisation plays an important role in baked foods with swelling and pasting influencing product structure. Differential scanning calorimetry (DSC) is a powerful tool for changing the characteristics of starch gelatinization in thermal properties being heated or cooled at a constant rate. The batter viscosity is investigated with a rapid visco analyser (RVA) and related to the quality of baked products. The batter needs to be sufficiently viscous to trap gas bubbles during mechanical mixing and during heating (Wilderjans et al., 2008). Another fundamental requirement for baked products is that most of the swollen starch granules retain a recognisable granular shape, and be strong enough to be self-supporting when the baked product is removed from the oven (Donovan, 1977).

Sugar functions as main ingredient in muffin formulation. It can increase the temperature at which starch gelatinises by interacting with the starch and forming bridges between starch chains. Psimouli and Oreopoulou (2012) reported that sugar limits the available water, thereby lowering water activity. Starch gelatinisation increases the viscosity of the batter considerably, which strengthens the batter structure leading to the depression of bubbles (Wilderjans et al., 2010). Therefore, more air bubble development may occur when starch gelatinisation occurs at higher temperatures thus allowing the development of a porous structure in the final product (Psimouli and Oreopoulou, 2012).

However, high sugar levels are associated with increased health problems including obesity and chronic diseases. Therefore, ingredients which can lead to calorie reduction such as sugar replacers can be considered as instrumental in weight control strategies. Stevianna® (product code ST001 SE supplied by Stevianna® NZ) was used for our study, as it incorporates rebaudioside-A (98 % steviol glycoside; 1 %) with erythritol (99 %). Rebaudioside-A is extracted from stevia, has broad health-

promoting properties for blood glucose and insulin levels (Chang et al., 2005). Erythritol is a sugar alcohol which is absorbed very slowly and provides reduced calorie sweetening (Storey et al., 2006).

Inulin is a dietary fibre (Tudorică et al., 2002) and has prebiotic properties, hence it is a nutraceutical ingredient that is extensively used in the food products (Meyer et al., 2011). Inulin is a carbohydrate of the fructan family with  $\beta$  (2-1) linked fructose residues and a terminal glucose residue (Giri et al., 2014). Short-chain inulin can be used as a sugar substitute in bakery products (Gonzalez-Tomás et al., 2008) and is useful in the treatment of obesity and diabetes (Swennen et al., 2006).

Several researchers have reported about the importance of the formulation and processing parameters on the functional properties of sugar substitutes. Partial replacement of sucrose with erythritol in a baked product resulted in positive influence on physical characteristics (Alencar et al., 2017; Grigor et al., 2016; Lin et al., 2003). Manisha et al. (2012) conducted research using a mixture of stevioside and liquid sorbitol in reduced sugar cakes and obtained effects on the rheological, microstructural and physical characteristics of the modified cakes.

Inulin is typical a sucrose or fat replacer in baked goods and their properties have been studied along with other fibre components, by many researchers (Herranz et al., 2016; Karp et al., 2017; O'Brien et al., 2003; Tunland and Meyer, 2002). The rheological properties and the sensory properties of a product will not be affected strongly due to the neutral or slightly sweet taste and the limited effect on viscosity of this ingredient.

Apart from the above sugar replacers functional role in low-calorie baked products, they have also been used in bakery products for the control of blood glucose and for the control of body weight or energy balance (Gregersen et al., 2004; Gularte et al., 2012; Lin et al., 2010). Furthermore, the research carried out in Chapter 5 (Gao et al., 2017) has found that the predicted glycaemic response was reduced when sugar was replaced with inulin or Stevianna® in muffins compared to full-sugar samples.

The aim of this study was to explore whether inulin or Stevianna® could replace sugar in muffin batter and whether the Rapid Visco Analyzer (RVA) could be a tool for evaluating muffin chemical properties

in relation to the results reported in Chapter 4 (Gao et al., 2016). Sugar was replaced by different levels of each substitute in two kinds of muffin recipes. The effect of sugar replacers on starch gelatinisation and batter viscosity during baking were separately studied through differential scanning calorimetry (DSC) and RVA assays, as well as *in vitro* starch digestibility of the batter was investigated.

## **7.2 Materials and Methods**

### **7.2.1 Raw Materials**

Raw materials used for this chapter are in Chapter 3.1.

### **7.2.2 Preparation of Muffin Batter**

Two muffin batter recipes (recipe1 and recipe2) were prepared using a mixer as described in 3.2.

### **7.2.3 Pasting Properties of Batter**

The Rapid Visco Analyzer (RVA) was used to study the viscosity properties of muffin batter during simulated baking as outlined in 3.3.1.

### **7.2.4 Differential Scanning Calorimetry**

For starch gelatinization measurements, DSC method was performed as described in 3.3.2.

### **7.2.5 *In vitro* Predictive Glycaemic Response Digestion Analysis**

*In vitro* digestion was conducted on all of the RVA gels to determine the predictive glycaemic response in the “cooked” muffin mixture. The procedure followed the method described in 3.5.2 and 3.5.3.

### **7.2.6 Statistical Analysis**

Each analysis was conducted in triplicate. Analysis of variance (one-way ANOVA) was performed as described in 3.7.1.

## 7.3 Results and Discussion

### 7.3.1 Pasting Properties of the Batter

Pasting properties of muffin batter were measured using an RVA which measures changes in viscosity during heating from 25 °C to 95 °C.

Table 7.1 shows peak and final viscosity for muffin batters. There were no significant differences between the control and samples containing Stevianna® for either peak viscosity or final viscosity for recipe 1. In this regard, the Stevianna® was successful in mimicking the effect of sucrose on the viscosity properties of batter during heating. The sucrose-induced delay in starch gelatinisation has been demonstrated to be a result of anti-plasticisation by sugar-water co-solvents as compared to water alone (Manisha et al., 2012). Struck et al. (2016) reported that intermolecular interactions of sucrose with starch chains in the amorphous regions of the starch granule led to the stabilisation of those regions. Therefore, Stevianna® appears to have simulated the effect of sucrose as the viscosity was similar to the control sample. This indicates delayed starch gelatinisation and thermosetting, thus time was allowed for appropriate air and vapour expansion during baking.

The peak and final viscosity of batter containing inulin increased significantly ( $P < 0.05$ ) compared to the control apart from 25 % replacement, and each increased level of replacement had significantly ( $P < 0.05$ ) higher viscosity. Increase batter viscosity in might be attributed to the high water-holding capacity of the fibre and a tendency to form a networked gel structure (Gularte et al., 2012). Inulin is highly hydrophilic resulting in a decrease in water availability swelling starch granules, thus reducing the formation of structural hydrocolloids in the batter (Gonzalez-Tomás et al., 2008). A similar relationship was reported by Zahn et al. (2010) whereby batter flowability increased with an increasing amount of inulin as a fat replacement. Batter flowability is significantly related to the volume of the final product, as Frutafit IQ inulin is a prefabricated gel, the reduction of bakery product volume was attributed to excessive batter consistency limiting the batter expansion in the fat-free product recipe by Zahn et al. (2010). The highest viscosity was observed with inulin replacing 100 % of sugar as the

presence of inulin inhibited the hydration of the starch granules by bonding to the available water and thus also reduced aeration of the cake batter (Oh et al., 2014). Inulin is capable of forming entangled networks with other food components when mixed with water and forming a highly viscous polymer. Final viscosity is related to the formation of viscoelastic gel. The 25 % inulin replacement of sugar did not cause a significant increase of viscosity during heating in comparison to control sample, which was probably due to its low amount of substitution. This result agrees with Gularte et al. (2012) who reported the batter viscosity displayed no significant differences when 20 % inulin added was in layer cake.

Four batters were made without Stevianna® using recipe 2, (original, with vanilla, with cocoa powder and with cocoa powder and vanilla) replacement of sugar with Stevianna® at 50 % or 100 % in each of these batters resulted in a significant reduction in peak viscosity. Although it is noted that the two batters that included cocoa powder always had a significantly higher peak viscosity than the comparative batters without cocoa powder. The final viscosity of the four batters was significantly ( $P < 0.05$ ) reduced when sucrose was replaced with 100 % Stevianna®. The final viscosity of batters without cocoa powder were also significantly reduced when sucrose was replaced with 50 % Stevianna®. This indicates that Stevianna® had an effect on the viscosity of recipe 2 whereas it did not in recipe 1 when compared to control samples (Recipe 1). This difference could be attributed to the interactions between the recipe components and the ratio of the batter constituents of flour, sugar, egg, water and oil. Manisha et al. (2012) reported that decreased batter stability, of sugar-free cake, during heating led to a decrease in expansion. It was found that the inclusion of sorbitol and stevioside affected the viscosity of the starch, as they interrupted the usual starch protein interactions that occur during gelatinization which then caused changes in the thermosetting mechanism.

Batter viscosity during baking affects the retention of air and leavening gases (Bath et al., 1992). The peak viscosity increased significantly ( $P < 0.05$ ) with the addition of cocoa powder (Table 1). Do et al. (2011) found a higher apparent viscosity when chocolate was formulated with standard cocoa powder. The cocoa particles swelled and led to a perception of coarse texture in water-based applications (Dyer,

2003). Martínez-Cervera et al. (2011) reported that using cocoa as a fat replacer in muffins profoundly modified the batter viscosity, possibly due to cocoa powder interfering with leavening agents, affecting the capacity of the batter to retain air bubbles during beating and heating (Dyer, 2003).

**Table 7. 1 Pasting properties of batter enriched sugar replacers as measured by the rapid visco analyzer (RVA) and *in vitro* starch digestion profile in low-sugar batters.**

| Sample                      | RVA                       |                             | <i>In vitro</i> starch digestibility |
|-----------------------------|---------------------------|-----------------------------|--------------------------------------|
| Recipe 1                    | Peak Viscosity (cP)       | Final Viscosity (cP)        | Total AUC (mg/g)                     |
| Control                     | 10559 ± 274 <sup>d</sup>  | 5669 ± 127 <sup>c</sup>     | 456.52 ± 9.16 <sup>a</sup>           |
| 25% Stevianna <sup>®</sup>  | 10371 ± 343 <sup>d</sup>  | 5517 ± 209 <sup>c</sup>     | 420.16 ± 7.11 <sup>a</sup>           |
| 50% Stevianna <sup>®</sup>  | 10393 ± 242 <sup>d</sup>  | 5555 ± 333 <sup>c</sup>     | 339.13 ± 9.05 <sup>bc</sup>          |
| 75% Stevianna <sup>®</sup>  | 10557 ± 350 <sup>d</sup>  | 5587 ± 262 <sup>c</sup>     | 316.99 ± 8.21 <sup>cd</sup>          |
| 100% Stevianna <sup>®</sup> | 10471 ± 525 <sup>d</sup>  | 5379 ± 323 <sup>c</sup>     | 237.27 ± 21.90 <sup>e</sup>          |
| 25% Inulin                  | 11329 ± 368 <sup>d</sup>  | 5518 ± 412 <sup>c</sup>     | 427.74 ± 17.34 <sup>a</sup>          |
| 50% Inulin                  | 13907 ± 242 <sup>c</sup>  | 18810 ± 389 <sup>b</sup>    | 364.36 ± 14.69 <sup>b</sup>          |
| 75% Inulin                  | 22306 ± 307 <sup>b</sup>  | 20499 ± 424 <sup>a</sup>    | 336.27 ± 8.29 <sup>bc</sup>          |
| 100% Inulin                 | 31697 ± 525 <sup>a</sup>  | 20424 ± 202 <sup>a</sup>    | 284.34 ± 13.70 <sup>d</sup>          |
| Recipe 2                    | Peak Viscosity (cP)       | Final Viscosity (cP)        | Total AUC (mg/g)                     |
| Control                     | 5747 ± 309 <sup>c</sup>   | 5609.7 ± 45.2 <sup>ab</sup> | 431.27 ± 19.24 <sup>a</sup>          |
| Vanilla                     | 5712 ± 109.3 <sup>c</sup> | 5787 ± 296 <sup>ab</sup>    | 413.16 ± 8.85 <sup>ab</sup>          |
| Cocoa powder                | 7492 ± 232 <sup>a</sup>   | 6373 ± 186 <sup>a</sup>     | 374.47 ± 16.20 <sup>c</sup>          |
| Cocoa powder+Vanilla        | 7447 ± 364 <sup>a</sup>   | 6356 ± 541 <sup>a</sup>     | 386.86 ± 13.27 <sup>bc</sup>         |
| 50% Stevianna <sup>®</sup>  | 4479 ± 104 <sup>d</sup>   | 4560 ± 222 <sup>c</sup>     | 287.14 ± 6.72 <sup>d</sup>           |
| 50S+V                       | 4361 ± 218 <sup>d</sup>   | 4563 ± 358 <sup>c</sup>     | 285.92 ± 10.13 <sup>d</sup>          |
| 50S+CP                      | 6574 ± 176 <sup>b</sup>   | 6157 ± 223 <sup>ab</sup>    | 273.53 ± 4.96 <sup>d</sup>           |
| 50S+CP+V                    | 6655 ± 246 <sup>b</sup>   | 6106 ± 403 <sup>ab</sup>    | 266.99 ± 9.28 <sup>d</sup>           |
| 100% Stevianna <sup>®</sup> | 4847 ± 329 <sup>d</sup>   | 3303 ± 224 <sup>d</sup>     | 213.49 ± 3.69 <sup>e</sup>           |
| 100S+V                      | 4601 ± 448 <sup>d</sup>   | 3503 ± 223 <sup>d</sup>     | 211.48 ± 7.15 <sup>e</sup>           |
| 100S+CP                     | 6587.3 ± 149 <sup>b</sup> | 5408 ± 368 <sup>bc</sup>    | 187.34 ± 6.88 <sup>e</sup>           |
| 100S+CP+V                   | 6569 ± 172 <sup>b</sup>   | 5409 ± 382 <sup>bc</sup>    | 188.17 ± 10.41 <sup>e</sup>          |

All measurements are mean values ± SD of triplicate determinations.

Means in the same column with different letters are significantly different (P < 0.05).

50% Stevianna<sup>®</sup> + vanilla (50S + V); 50% Stevianna<sup>®</sup> + cocoa (50S + CP); 50% Stevianna<sup>®</sup> + cocoa + vanilla (50S + CP + V); 100% Stevianna<sup>®</sup> + vanilla (100S + V); 100% Stevianna<sup>®</sup> + cocoa (100S + CP); 100% Stevianna<sup>®</sup> + cocoa + vanilla (100S + CP + V).



### 7.3.2 Effect of Starch Gelatinisation on Batter

The DSC results are shown in Table 7.2, these results are similar to that expected when considering results of batter viscosity during baking (Table 7.1).

All batters with Stevianna® replacement, both recipes 1 and 2 showed no significant differences to the control in any of the measured parameters ( $T_{\text{onset}}$ ,  $T_{\text{peak}}$ ,  $T_{\text{endset}}$ ). The enthalpy of gelatinisation ( $\Delta H$ ) was not observed to be significantly different between samples. Martínez-Cervera et al. (2014) suggested polyols such as sorbitol, maltitol, and erythritol, as total sucrose replacers in muffins, due to the starch gelatinisation temperature being very similar when using sucrose or polyols. Use of the polyols, sorbitol and lactitol, as sugar substitutes in cake batter had no significant ( $P < 0.05$ ) influence on the gelatinisation enthalpies by Psimouli and Oreopoulou (2012).

The addition of cocoa powder and vanilla had no significant effect on  $T_{\text{onset}}$ ,  $T_{\text{peak}}$ ,  $T_{\text{endset}}$  or the enthalpy of gelatinisation ( $\Delta H$ ) compared to the control. Although the RVA analysis of the gelatinising batter properties illustrated that there were significant differences ( $P < 0.05$ ) between control sample and those including cocoa powder (Table 7.1), this observation was not clear in the DSC measurement (Table 7.2). Such a result illustrates that the DSC and RVA protocols examine different physiological properties of starch-based systems and are therefore not directly comparable.

Replacing 100 % sugar with inulin, significantly ( $P < 0.05$ ) increased  $T_{\text{onset}}$ ,  $T_{\text{peak}}$  and  $T_{\text{endset}}$ , it was the only sample to show significant difference ( $P < 0.05$ ) to the control at the measured parameters ( $T_{\text{onset}}$ ,  $T_{\text{peak}}$ ,  $T_{\text{endset}}$ ). However, replacement of 75 % sugar and 100 % sugar with inulin showed a significant ( $P < 0.05$ ) reduction in the enthalpy of gelatinisation ( $\Delta H$ ). Replacing the sugar with inulin caused the crystalline regions of starch to become more stable leading to higher  $T_{\text{onset}}$ ,  $T_{\text{peak}}$  and  $T_{\text{endset}}$  values. The results are consistent with research that shows the inclusion of inulin leads to an increase in gelatinisation temperature in gluten-free dough (Juszczak et al., 2012). This may be attributed to thermal transition temperatures being higher after inulin incorporation, as it forms a gel structure. Another factor that could be concerned with inulin decreasing the water activity and hence limit starch

swelling and gelatinisation (Tudorică et al., 2002). Psimouli and Oreopoulou (2013) have reported that the presence of inulin profoundly modified starch gelatinisation in cake batter, due to its ability to bind water and act as a stabiliser of the amorphous region in the starch granule. Aravind et al. (2012) also observed that inulin in starch-water systems raised starch gelatinisation temperature. The reduction in the enthalpy of gelatinisation ( $\Delta H$ ) is likely to be related directly to the concentration of inulin within the system. Tudorică et al. (2002) indicated that the enthalpy of a system is an indicator of the amount of starch gelatinisation within a starch base and should be related to the gelatinisation temperature of the starch within the system. This can be explained by pockets of higher fibre concentrations where cross-linked gums form resulting in less encapsulation of individual starch granules (Tudorică et al., 2002).

**Table 7. 2 Effect of sugar replacer addition with/without cocoa powder and/or vanilla on gelatinization parameters (DSC Measurements) of batter samples.**

| Sample                | DSC                         |                            |                            |                          |
|-----------------------|-----------------------------|----------------------------|----------------------------|--------------------------|
| Recipe 1              | Tonset (°C)                 | Tpeak (°C)                 | Tendset (°C)               | enthalpy (DeltaH, J/g)   |
| Control               | 66.27 ± 0.90 <sup>bc</sup>  | 69.82 ± 1.57 <sup>b</sup>  | 75.44 ± 1.43 <sup>bc</sup> | 1.59 ± 0.09 <sup>a</sup> |
| 25% Stevianna®        | 65.41 ± 1.94 <sup>c</sup>   | 69.87 ± 1.79 <sup>b</sup>  | 73.79 ± 0.80 <sup>c</sup>  | 1.53 ± 0.08 <sup>a</sup> |
| 50% Stevianna®        | 65.78 ± 1.76 <sup>bc</sup>  | 70.93 ± 1.08 <sup>ab</sup> | 75.61 ± 0.79 <sup>bc</sup> | 1.47 ± 0.05 <sup>a</sup> |
| 75% Stevianna®        | 66.31 ± 2.02 <sup>bc</sup>  | 70.49 ± 2.06 <sup>ab</sup> | 76.27 ± 1.01 <sup>bc</sup> | 1.53 ± 0.16 <sup>a</sup> |
| 100% Stevianna®       | 67.42 ± 3.98 <sup>abc</sup> | 69.90 ± 3.90 <sup>b</sup>  | 73.34 ± 1.90 <sup>c</sup>  | 1.45 ± 0.09 <sup>a</sup> |
| 25% Inulin            | 65.72 ± 0.69 <sup>bc</sup>  | 70.55 ± 0.49 <sup>ab</sup> | 75.03 ± 0.82 <sup>bc</sup> | 1.56 ± 0.08 <sup>a</sup> |
| 50% Inulin            | 65.08 ± 1.73 <sup>c</sup>   | 70.67 ± 0.83 <sup>ab</sup> | 75.19 ± 1.26 <sup>bc</sup> | 1.47 ± 0.08 <sup>a</sup> |
| 75% Inulin            | 70.99 ± 0.92 <sup>ab</sup>  | 73.49 ± 0.97 <sup>ab</sup> | 77.74 ± 0.48 <sup>ab</sup> | 1.06 ± 0.03 <sup>b</sup> |
| 100% Inulin           | 72.56 ± 0.67 <sup>a</sup>   | 75.42 ± 0.03 <sup>a</sup>  | 79.39 ± 0.24 <sup>a</sup>  | 0.49 ± 0.05 <sup>c</sup> |
| Recipe 2              | Tonset (°C)                 | Tpeak (°C)                 | Tendset (°C)               | enthalpy (DeltaH, J/g)   |
| Control               | 66.43 ± 0.81 <sup>a</sup>   | 70.24 ± 1.36 <sup>a</sup>  | 74.80 ± 1.03 <sup>a</sup>  | 1.57 ± 0.06 <sup>a</sup> |
| Vanilla               | 65.58 ± 1.38 <sup>a</sup>   | 71.64 ± 0.70 <sup>a</sup>  | 74.85 ± 0.73 <sup>a</sup>  | 1.60 ± 0.02 <sup>a</sup> |
| Cocoa powder          | 66.06 ± 2.64 <sup>a</sup>   | 69.08 ± 2.76 <sup>a</sup>  | 74.39 ± 1.89 <sup>a</sup>  | 1.57 ± 0.33 <sup>a</sup> |
| Cocoa powder +Vanilla | 65.80 ± 1.84 <sup>a</sup>   | 72.82 ± 2.05 <sup>a</sup>  | 76.13 ± 0.41 <sup>a</sup>  | 1.55 ± 0.45 <sup>a</sup> |
| 50% Stevianna®        | 66.97 ± 1.04 <sup>a</sup>   | 71.74 ± 0.89 <sup>a</sup>  | 74.36 ± 0.90 <sup>a</sup>  | 1.57 ± 0.25 <sup>a</sup> |
| 50S+V                 | 66.77 ± 4.00 <sup>a</sup>   | 71.52 ± 3.56 <sup>a</sup>  | 75.35 ± 2.84 <sup>a</sup>  | 1.50 ± 0.25 <sup>a</sup> |
| 50S+CP                | 65.61 ± 2.54 <sup>a</sup>   | 71.30 ± 1.65 <sup>a</sup>  | 75.93 ± 0.37 <sup>a</sup>  | 1.55 ± 0.05 <sup>a</sup> |
| 50S+CP+V              | 65.80 ± 2.44 <sup>a</sup>   | 70.81 ± 0.95 <sup>a</sup>  | 74.91 ± 2.44 <sup>a</sup>  | 1.41 ± 0.39 <sup>a</sup> |
| 100% Stevianna®       | 66.59 ± 1.66 <sup>a</sup>   | 70.50 ± 1.65 <sup>a</sup>  | 74.66 ± 1.81 <sup>a</sup>  | 1.56 ± 0.11 <sup>a</sup> |
| 100S+V                | 65.27 ± 0.92 <sup>a</sup>   | 70.47 ± 0.81 <sup>a</sup>  | 75.29 ± 1.32 <sup>a</sup>  | 1.51 ± 0.14 <sup>a</sup> |
| 100S+CP               | 65.81 ± 2.18 <sup>a</sup>   | 70.12 ± 1.17 <sup>a</sup>  | 74.93 ± 1.56 <sup>a</sup>  | 1.52 ± 0.17 <sup>a</sup> |
| 100S+CP+V             | 65.98 ± 2.65 <sup>a</sup>   | 70.85 ± 0.66 <sup>a</sup>  | 75.25 ± 1.20 <sup>a</sup>  | 1.52 ± 0.11 <sup>a</sup> |

All measurements are mean values ± SD of triplicate determinations.

Means in the same column with different letters are significantly different (P < 0.05).

50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

### 7.3.3 *In vitro* Predictive Glycaemic Response for Batter

For recipe 1, the amount of reducing sugar present at time zero was significantly lower in all samples containing Stevianna® and inulin when compared with full sugar samples as expected due to less sugar in the recipe. The amount of reducing sugars released at 20 min was reduced with the replacement of sugar with 50 %, 75 % and 100 % inulin or Stevianna® (Figure 7.1). A similar pattern was observed at 60 and 120 min, the reducing sugar release in samples with more than 50 % sugar replacer samples were significantly lower than the control. There was a significant decrease in AUC values when inulin or Stevianna® levels were used to replace sugar at 50 %, 75 % or 100 % (Table 7.1).

These results are consistent with data obtained in the research carried out in Chapter 4 (Gao et al., 2016), indicating the inclusion of Stevianna® or inulin in baked muffins can significantly ( $P < 0.05$ ) reduce the predicted glycaemic response. The reduction in glycaemic response level in inulin replaced muffin products has been thought to be related to the added viscosity that inulin provides during digestion, resulting in the entrapment of starch granules within a viscous fibre-starch network. For instance, Brennan et al. (2008) reported a similar occurrence in fibre-enriched breakfast cereals, where the postprandial glucose impact of these foods was reduced compared with non-fibre cereal products. In addition, Foschia et al. (2015) found that starch gelatinisation properties have an effect on starch digestion and reducing sugar released. In this study, low levels of inulin sample ( $< 50\%$ ) did not cause significant viscosity differences. However, the gelatinisation temperatures of inulin sample indicate that the inulin had a protective effect on the starch granules. Oh et al. (2014) also found that the addition of inulin to cake, restricted starch hydrolysis, hence lowering reducing sugar release under *in vitro* conditions. Tudorică et al. (2002) observed a similar result in pasta, it was found that inulin becomes incorporated into the structure of pasta, resulting in a significantly reduced value of glucose release with the inclusion of inulin.

Recipe 2 (Figure 7.2) which only used Stevianna® to replace sugar showed similar results to the Stevianna® replacement in recipe 1 (Figure 7.1). Stevianna® concentration had a significant ( $P < 0.05$ ) effect on the AUC values ( $P < 0.05$ ) (Table 7.1). However, the mechanism of action may be different to

that of inulin as Stevianna® is composed of rebaudioside-A, for sweetness, and erythritol, as a filler, thus it has virtually no calorific value and probably does not interact with starch and water similar to inulin, as noted by the viscosity results.

Other work has been carried out using stevia in ice cream and beverages that have shown a reduced postprandial glucose response when compared with a control (Alizadeh et al., 2014). Stevia contains no glucose thus, stevia does not contribute to the available carbohydrate and glycaemic responses in food products thereby giving it very different functional properties to sucrose. Carakostas et al. (2008) studies provide further evidence that purified rebaudioside-A has no effect on either blood pressure or glucose homeostasis for use in food and beverages.

The main component of Stevianna® is erythritol. When erythritol has been used by other researchers as a sucrose replacement, they have found that postprandial glucose and insulin levels were reduced (Lin et al., 2010). These results are due to lack of calorie and carbohydrate content of erythritol, thus there are no raising postprandial glycaemic and insulin levels by oral ingestion in healthy human subjects (Grigor et al., 2016).

Batters with Stevianna® that included cocoa powder and/or vanilla in recipe 2, also had relatively lower reducing sugars released and AUC values (Figure 7.2 and Table 7.1) than the control batter, but no significant difference of Stevianna® samples without cocoa powder and/or vanilla was observed. These results are consistent with Chapter 6 findings which showed the additions of vanilla and/or cocoa powder with baked muffin production did not lead to significant reduction of *in vitro* digestion compared to the 50 % and 100 % Stevianna® samples. However, the full-sucrose batter samples containing cocoa powder had significantly lower AUC values than the control (Table 7.1). This inconsistency may be due to fibre components in cocoa powder, which may interact with the starch and therefore reduce the potential glycaemic impacts.

In summary, the results of batter samples with sugar replacer are consistent with data obtained from baked muffin products (same recipe) by *in vitro* starch digestion, indicating that the baking process did

not have an impact on the predictive glycaemic response. There are two main sugar replacers that could be important in reducing the glycaemic response of muffin batter. The first one is that the inulin formed a matrix to encase starch granules, resulting in the limitation of starch swelling and gelatinisation and, consequently, the reduced potential for starch degradation and sugar liberation (Tudorică et al., 2002). The second aspect could be the Stevianna® evaluated the lost calories value that provides no energy to the body and thus is not systemically metabolised nor fermented in the colon (Carakostas et al., 2008). Furthermore, stevioside has been reported by Manisha et al. (2012) who shown good stability under normal conditions of application, and no interaction between the individual low calorie sweeteners.

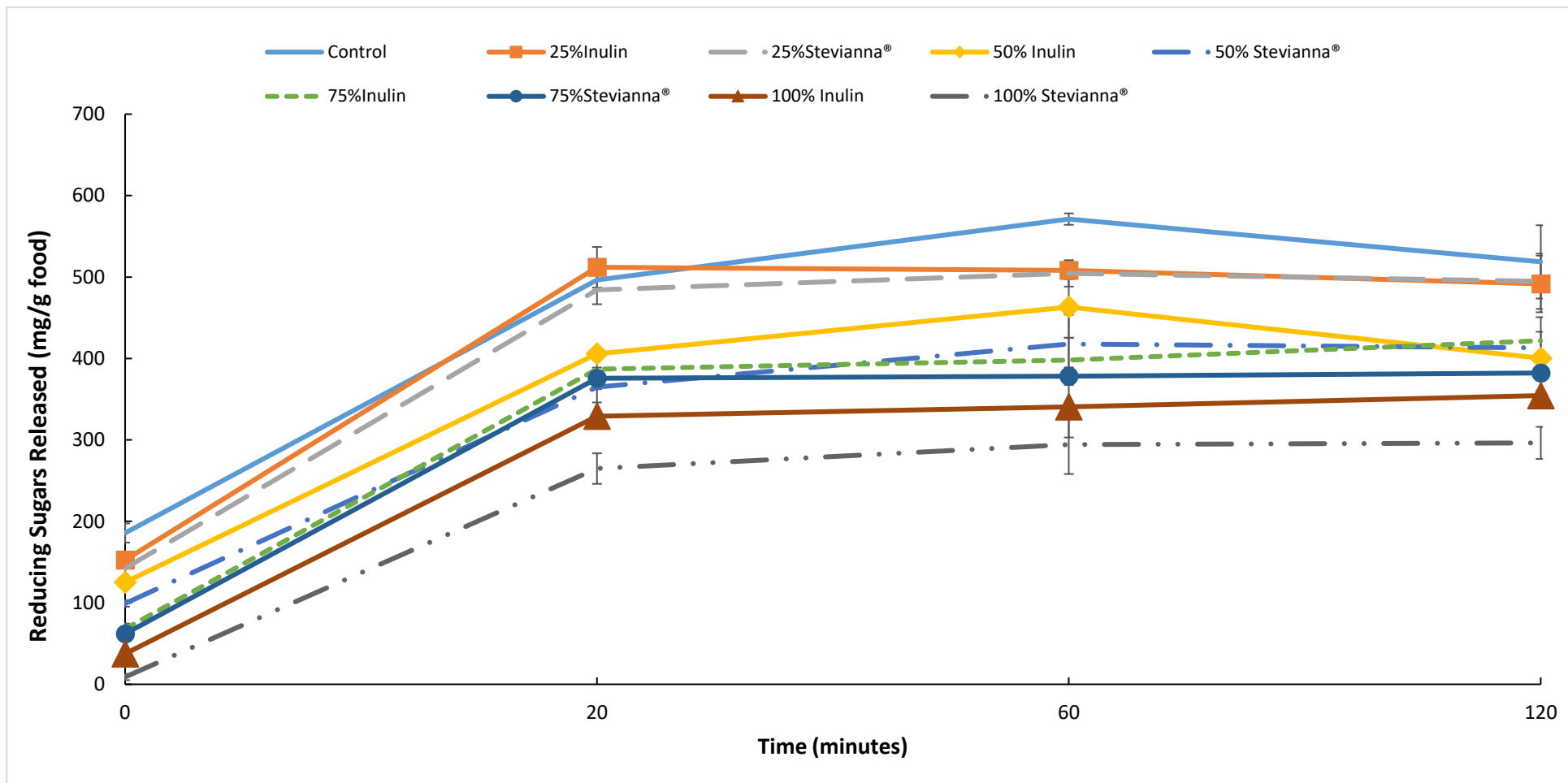


Figure 7. 1 Amount of reducing sugars released during *in vitro* digestion for Recipe 1.

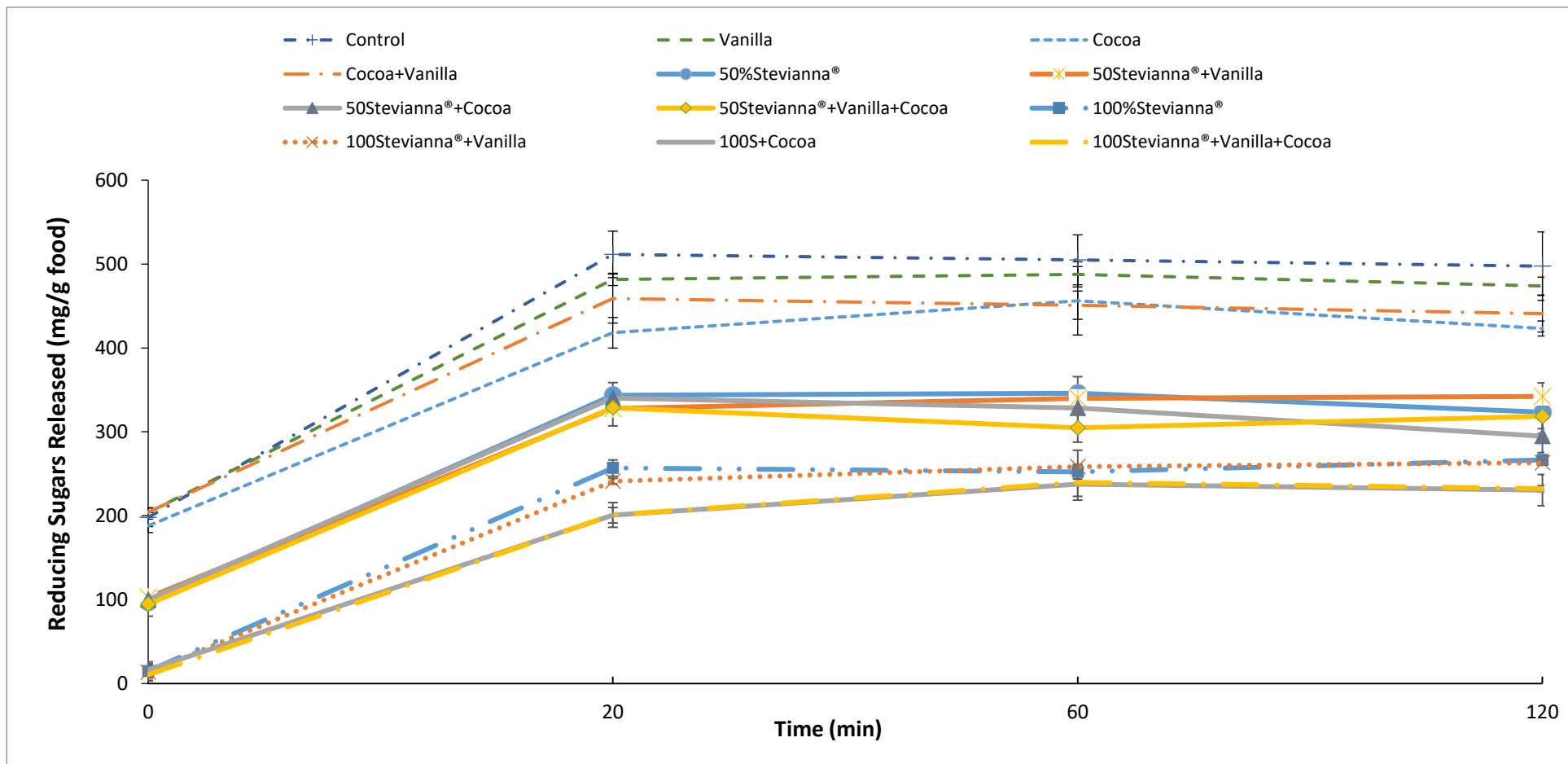


Figure 7. 2 Amount of reducing sugars released during *in vitro* digestion for Recipe 2.



## 7.4 Conclusions

This study on muffin batters shows that the results of pasting properties, starch gelatinisation and potential nutritional quality are intrinsically linked to the ability of different levels of sugar substitutes into batter systems.

The positive effect of Stevianna® on recipe 1 batter properties is associated with the fact that Stevianna®, like sucrose, did not differ significantly from the full-sugar batter in viscosity during heating. However, the inclusion of Stevianna® with/without cocoa powder in recipe 2 batter products showed different effects on the pasting properties, suggesting interactions between ingredients which were not present in recipe 1. At the same time, sugar replacement with inulin led to recipe 1 batters with increased viscosity during heating, resulting in the water-binding ability of inulin and forming a networked gel structure.

Differential scanning calorimetry (DSC) analysis showed that the inclusion of inulin increased starch gelatinisation temperature and decreased  $\Delta H$  in the recipe 1 batters, whereas no significant differences were found with Stevianna® addition in recipe 1 and 2 batters. Therefore, it can be concluded that addition of inulin has significant effect on the starch gelatinisation properties of muffin batters.

These findings confirm that inulin and Stevianna® can act as a good sugar replacers to inhibit predicted glycaemic impact, and the baking process no significant effects from these sugar replacers for starch digestion.

## Chapter 8

### Image Analysis of the Sugar-reduced Muffin Formulated with Stevianna® or Inulin as a Sugar Replacer

**Abstract:** Sugar replacement and/or reduction in cereal products is important in relation to consumer appeal for nutritionally balanced, calorie reduced, products. However when using sugar replacers, product structure can be negatively altered. The effect on air-cell development and microstructure with the use of Stevianna®, or inulin, as sugar replacer was evaluated using muffins as a model food. The total replacement of sugar using Stevianna® or inulin resulted in the development of non-uniform air cells leading to poor muffin microstructure. However, formulations involving partial sugar replacers gave similar air cell characteristics and hence microstructural characteristics, to that of the control sample. The results indicated that sugar is an important factor for the development of muffin microstructure and is difficult to replace completely.

## 8.1 Introduction

The structural development of muffins depends on the type of water-retaining agent used in the system, as these agents regulate the colloidal processes during baking (Psimouli and Oreopoulou, 2012; Ronda et al., 2005). Starch gelatinisation and protein denaturation start at a low temperature and proceed only in the presence of adequate moisture (Sanchez-Pardo et al., 2008; Zaroni et al., 1991). Hesso et al. (2015) suggested that sucrose is a foam stabilizer which regulates gelatinisation processes causing a shift towards higher temperatures. This has been shown to be due to the water-retaining effect of sucrose and its ability to bind starch chains together (Frye and Setser, 1992). Therefore, the critical functions of sucrose are not easy to mimic by sucrose replacers.

The substitution of sugar have been reported to affect the physical and chemical transformations in the bakery product system (Baeva et al., 2003; Rodríguez-García et al., 2014b; Sidhu et al., 2003). The research carried out in Chapter 4 (Gao et al., 2016) has indicated that reduction of sugar in muffins might be achieved by partial replacement with inulin to provide acceptable structure as well as functional properties that resemble control muffin. In addition, the desired sweetness of cupcakes could be achieved by the addition of intense sweeteners (Edelstein et al., 2007). Stevia is stable at baking temperature, non-fermentable and approximately 300 times sweeter than sucrose (Gasmalla et al., 2014). Sugar alcohols have been proposed as bulking agents for low-sugar baked goods, as intense sweeteners do not perform bulk functions similar to sugar (Ronda et al., 2005). Lin et al. (2003) obtained comparable physical characteristics of chiffon cake, when 100% sucrose was replaced by erythritol.

Starch partially takes over the functions of sucrose. However, unlike sucrose, it acts as a stabilizer only during baking and in particular during its swelling in the process of gelatinisation (Tolstoguzov 2003). Sorbitol has been used as a bulking agent by Manisha et al. (2012), and has water-absorption properties similar to those of sucrose, affecting starch gelatinisation in the batter and the formation of cake with a porous structure similar to the control.

Inulin and Stevianna® may be considered as sucrose substitutes with advantageous properties. Inulin possesses the advantage that may act as dietary fibre with prebiotic effects (Meyer et al., 2011).

In recent years image analysis, based on a large variety of microscopic techniques, has been applied for characterization of bakery product crumbs. Size, distribution, wall thickness, and number of air cells were determined in these studies (Datta et al., 2007; Farrera-Rebollo et al., 2012; Kocer, Hicsasmaz et al., 2007). Scanning electron microscopy (SEM) is one of the most important image analysis technique, since it provides a combination of higher magnification, greater depth of focus, greater resolution, and ease of sample observation. SEM studies have been assessed to determine the changes that occur during baking qualitatively (Ozge et al., 2009; Polaki et al., 2010). Hsu et al. (1980) used SEM to compare the crumb structure of cakes with different emulsification systems.

The objective of this study was to explore and evaluate the effect of sucrose, inulin and Stevianna® on the structure of muffin and the suitability of using sugar substitutes to replace sugar in a muffin.

## **8.2 Materials and Methods**

### **8.2.1 Raw Materials**

Raw materials used for this chapter are described in Chapter 3.1.

### **8.2.2 Muffins Preparation**

Two kinds of muffin recipe (recipe 1 and recipe 2) were prepared and baked as described 3.2, the detailed information of ingredients was shown in Table 3.1 and Table 3.2.

### **8.2.3 Image Analysis of Cellular Structure in the Muffins**

Image analysis was used to detect the internal appearance of baked muffins, and this is described in section 3.4.1.

### **8.2.4 Scanning Electron Microscopy**

SEM experiment was conducted as described in section 3.4.2.

### **8.2.5 Statistical Analysis**

Each analysis was conducted in triplicate. Analysis of variance (one-way ANOVA) was performed as described in 3.7.1.

## **8.3 Results and Discussion**

### **8.3.1 Image Analysis of the Cellular Structure**

In order to obtain an aerated structure in the final baked product, air bubbles have to be present in the batter. Two mechanisms permit air bubble entrapment: the primary mechanism involves the incorporation of such bubbles directly from the headspace due to the rotation of the mixer whisks and the secondary one involves the formation of entrapped bubbles through the breakup of larger ones (Massey et al., 2001). Foam is formed when air is incorporated into the liquid phase upon agitation, which occurs during beating and mixing. The larger air cells incorporated into the batter matrix are then further broken during mixing, reducing the mean bubble size and increasing the number of air cells (Rodríguez-García et al., 2014b). During baking, a second step takes place: the air cells are expanded by CO<sub>2</sub> and the vapour pressure generated, resulting in the formation of the final gas cells, which influence the texture of the finished product.

#### **8.3.1.1 Image Analysis of Longitudinal**

Images of the longitudinal cut surface of the baked muffins of recipe 1 are shown in Figure 8.1. When more than 50% sucrose was replaced with inulin, a significant decrease in the tunnels (or diffusion pathways) was observed as compared to the full-sugar muffin (Figure 8.1). As the sucrose was partially replaced with Stevianna<sup>®</sup>, tunnels and air cell were more evident compared to those containing inulin samples. However, the muffin where 100 % of the sucrose was replaced with Stevianna<sup>®</sup> contained tunnels with non-uniform air cells.

Surface images of Figure 8.2 showed that when the muffin of recipe 2 was vertically cut, the internal appearance deteriorated as the level of sugar substitution with Stevianna<sup>®</sup> increased with/without

cocoa powder and/or vanilla. Therefore, when the sugar was partially replaced with Stevianna® the results of muffin air cell was similar to the control sample.

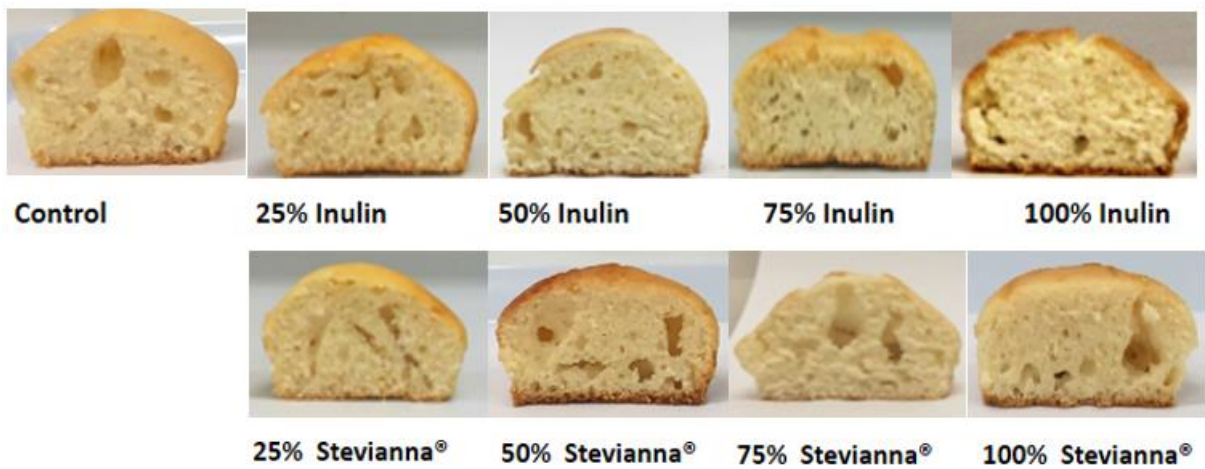


Figure 8. 1 Images of the longitudinal cut surface of Recipe 1 muffin samples.



Figure 8. 2 Images of the longitudinal cut surface of Recipe 2 muffin samples.

### 8.3.1.2 Image Analysis of Transversal from Recipe 1

Figure 8.3 shows the cellular structure characteristics (air cell area within the crumb and average air cell size) measured using image analysis from the binarized images of the different formulations muffin crumbs. Air cell area is determined as the percentage of air cells in the crumb area analyzed. The muffins with higher sugar replacement with inulin had significantly ( $P < 0.05$ ) lower air cell area percentage (Figure 8.4). Average air cell size is calculated using the size of the air cells in mm. When sugar was replaced by more than 50 % inulin, a change in the average air cell size was observed (Figure 8.4). These observations could be related to inulin viscosity properties from the results of Chapter 7. Compared with control batter, a significant increase ( $P < 0.05$ ) in viscosity was found with sugar replacement by the inulin. As the beating energy applied to all the batter formulations was the same, a lower viscosity may have allowed the larger air cells to coalesce and escape while retaining the small ones (Hanselmann and Windhab, 1998). In principle, a larger number of small gas nuclei in the batter is a positive factor for final quality, as it will favour the formation of tiny air cells that can enlarge during baking (Martínez-Cervera et al., 2012b). However, the findings of the current study do not support this principle in sugar replaced muffin mixer. Our results indicated that inulin substitution caused thinner air cells seen in the batters, which were lost later during heating and that not all of them could expand appropriately during heating, resulting in a more compact structure with an absence of interconnectivity for the sugar-replaced samples. A further reason for this decrease in crumb structure was that inulin contributes to excessive batter consistency which in turn might limit the batter expansion. Similar results were found by Rodríguez-García et al. (2012), where differences between the cellular structure of the control cake (C0), partially fat-substituted cakes (C35, C50, C70), and totally fat substituted cake (C100) were attributed to an inadequacy in bubble expansion in batters with high levels of inulin.

The images of the crumb showed that the Stevianna® samples also differed from the control sample, with the air cells size becoming larger as the amount of sugar replacement increased; the 100 % Stevianna® samples contained larger air cells than the control sample (Figure 8.3). Air cell area has also

been found to increase in muffin on replacing full sucrose with Stevianna® (Figure 8.4). These results show evidence for decrease in expansion with increasing levels of sugar-replacement. Two main reasons that affect expansion are a decrease in batter stability during the heating stage, and changes in the thermosetting mechanism due to sugar-replacement. Martínez-Cervera et al. (2012a) illustrated that the excessively early thermosetting is associated with the crumb structure. Since the water vapour did not have enough time to expand during the heating process in the oven, it collapsed and led to the appearance of large bubbles. The second is the lack of effect of the Stevianna® on the protein denaturation temperature. As the premature thermosetting of the protein matrix starts in the crust region, the heat transfer mechanism is gradually converted from convection to conduction starting from the surface (Martínez-Cervera et al., 2012b). Kocer et al. (2007) reported that faster thermosetting of the crust acted as a barrier against vapour release causing the vapour from the crumb to accumulate in the interfacial zone resulting in inadequate expansion of individual air cells with less interconnectivity. However, no significant difference in air cell area and average air cell size were observed up to the 50 % sugar-replacement level (Figure 8.4). This improvement in the crumb structure associated with the use of Stevianna® can be related to the Stevianna® and control batters, which have similar viscoelastic properties from Chapter 7. Therefore, the effect of Stevianna® on the 50 % sugar batter structure during heating was similar to that of full-sugar sample. This result is in agreement with Martínez-Cervera et al. (2012a) findings which showed the crumb structure bore a greater resemblance between partially sugar replacement with erythritol and full-sugar samples, implying that erythritol was conferring some of the structural effects provided by sucrose.



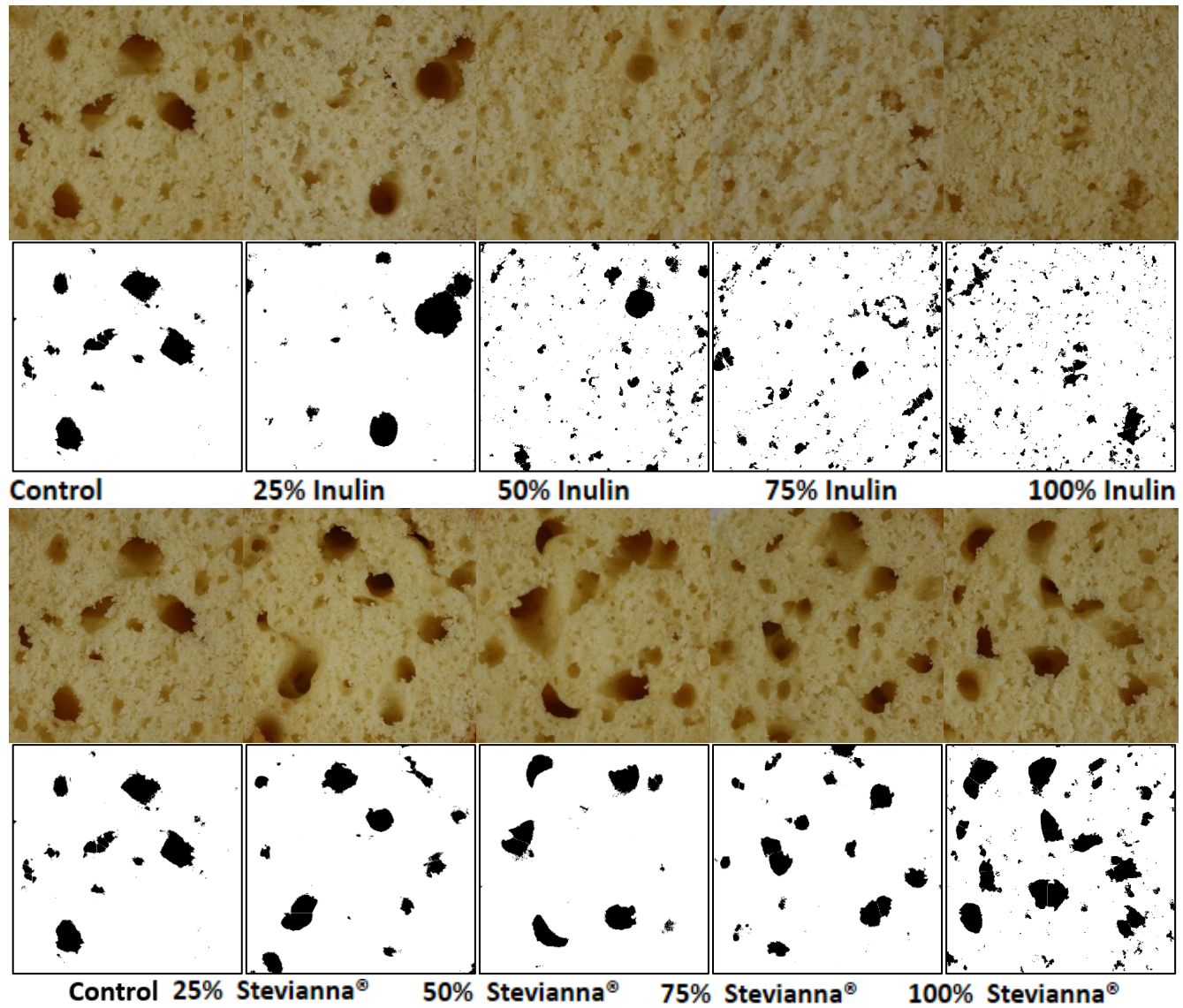


Figure 8. 3 Cellular structure of the crumb of the recipe 1 muffins. Top line: photographs of transverse sections. Bottom line: binarized images of photographed crumbs.

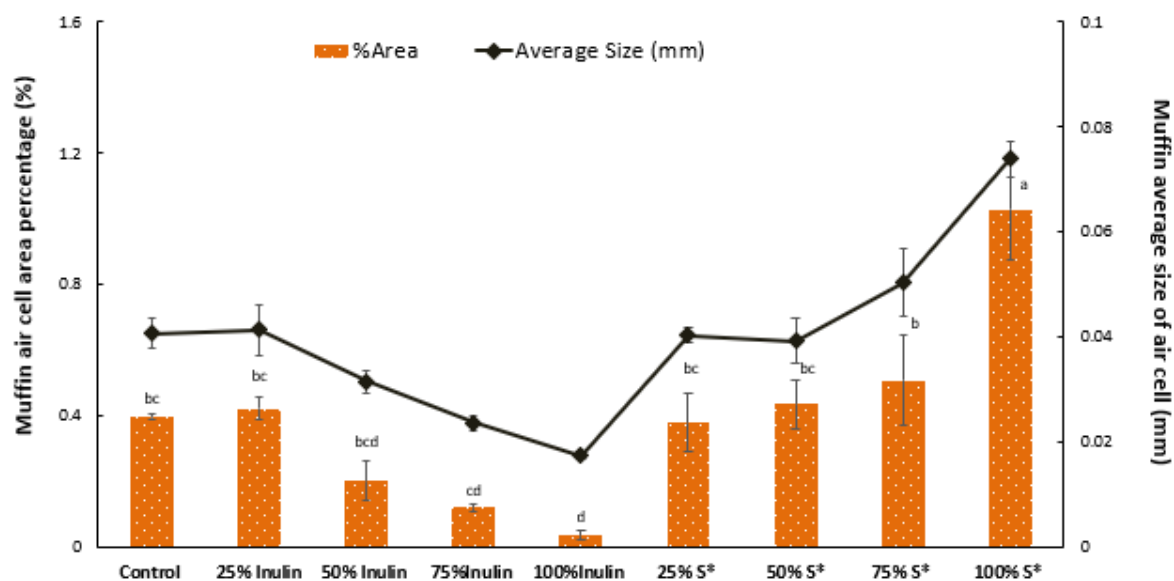
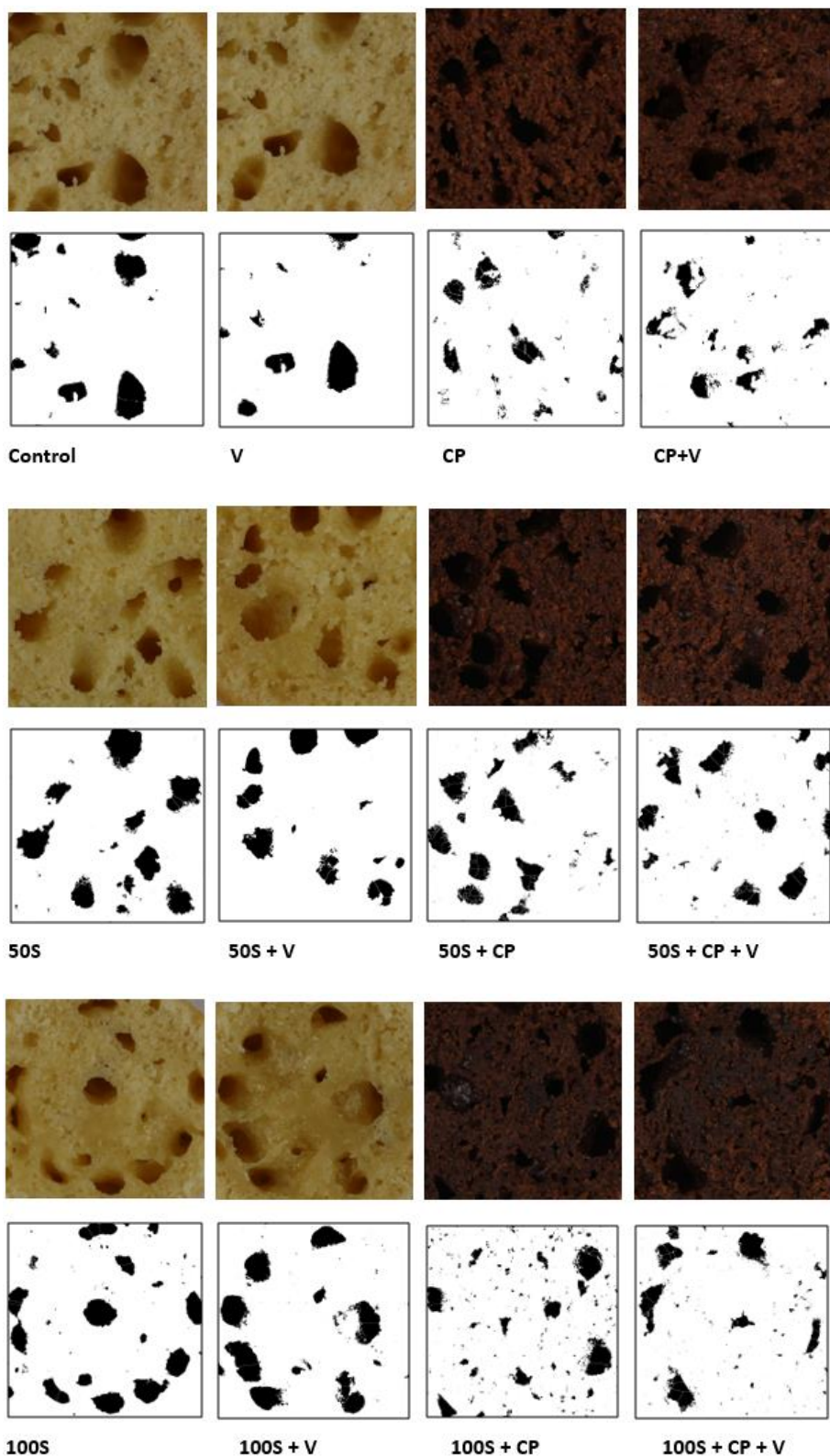


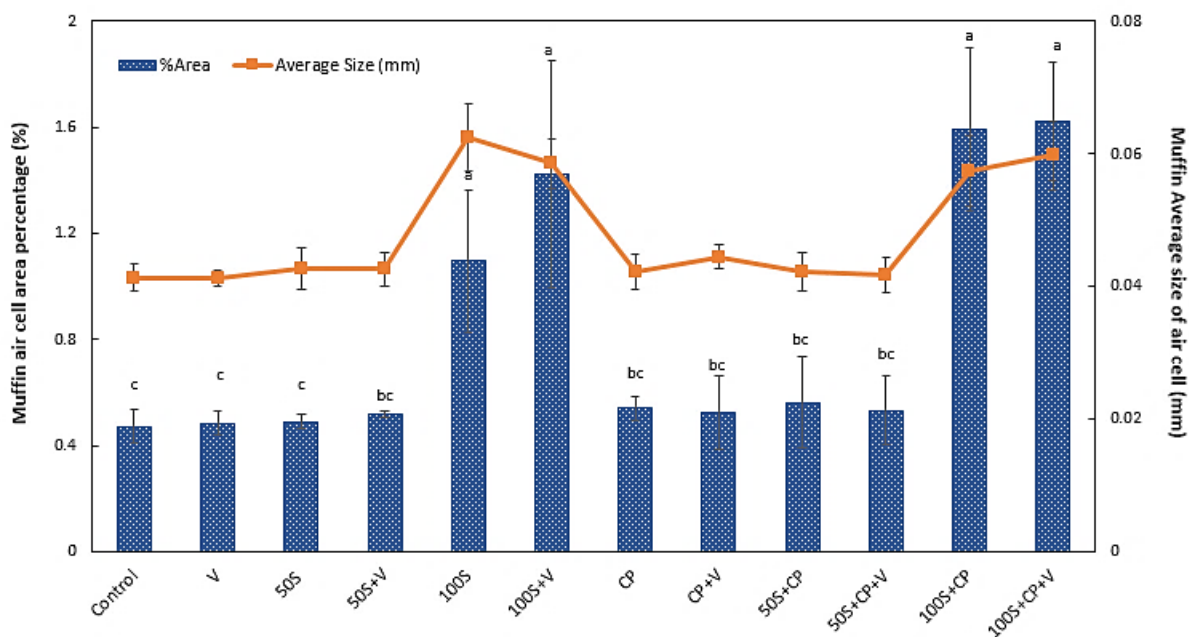
Figure 8. 4 Based on image analysis, air cell and average air size of muffins prepared with recipe 1 formulations. \*Abbreviated sample names: 25% Stevianna®; 50% Stevianna®; 75% Stevianna®; 100% Stevianna®.

### 8.3.1.3 Image Analysis of Transversal from Recipe 2

Photos of the baked muffins of recipe 2 can be seen in Figure 8.5. Image crumb analysis (Figure 8.6) revealed the effect of sucrose replacement with Stevianna® and addition of cocoa powder and/or vanilla in air cell area and average air cell size of muffins. More specifically 50 % Stevianna® replacement seems not to affect the crumb characteristics of muffin compared to control sample. Stevianna® concentration up to 100 % differed significantly compared to the control sample. Furthermore, cocoa powder and/or vanilla was added the control, 50 % Stevianna® and 100 % Stevianna® samples, and exhibited no significant differences between them. These results are consistent with air cell structure of recipe 1 that deteriorated, as the amount of sucrose replaced by Stevianna® increased. Martínez-Cervera et al. (2012a) reported an increase in size of air cells and air cell area for cakes prepared with increasing amounts of erythritol in cake samples.



**Figure 8. 5 Cellular structure of the crumb of the recipe 2 muffins. Top line: photographs of transverse sections. Bottom line: binarized images of photographed crumbs. Control and vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).**



**Figure 8. 6** Based on images analysis, air cell and average air size of muffins prepared with recipe 2 formulations. Control and vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).

### 8.3.2 Scanning Electron Microscopy

SEM technique was used to investigate the structural integrity of baked muffin products. Figure 8.7 represents the scanning electron micrographs of muffin crumbs with different levels of inulin or Stevianna® to replace sugar in muffin recipe 1. The micrograph of the crumb of control muffin with sugar made wholly with wheat flour shows the small and large starch granules are gelatinized and embedded in a continuous matrix formed mainly by denatured proteins. Ashwini et al. (2009) described the protein components of wheat flour as a network to cover the starch granules, and the mixed wheat flour dough was also reported as a random mixture of protein fibrils with adhering starch granules by Lee et al. (2001). A few partial outlines of starch granules are also visible. The starch granules are distorted owing to the gelatinization process. During baking, the oil melted and coated

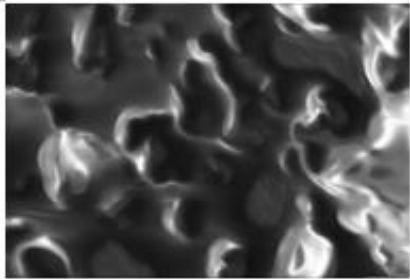
the surface creating a smooth appearance (see Figure 8.7: control, 25 % Stevianna® and 25 % Inulin), and oil form large interconnected masses between the starch-protein masses (Flint et al., 1970).

As the sugar replacement level increased, the muffin matrix became more irregular, the starch granules were not fully embedded and they were observed as detached structures on the matrix surface. In Figure 8.7, which are the micrographs of muffin with 75 % and 100 % inulin, a rather ruptured, discontinuous, gluten protein matrix can be seen. These facts might be due to the presence of inulin which would limit gluten and starch hydration during mixing and baking giving rise to a less developed structure (Rodríguez-García et al., 2012). A few starch granules can be seen wrapped in thick protein matrix owing to the use of inulin. In particular, in the 100 % inulin sample, the gluten matrix appears disrupted to a greater degree. The starch granules may not be visible, due to being totally wrapped by inulin, and only the thick protein matrix can be seen. Tudorică et al. (2002) reported the fibre component within the highly developed protein-fibre-starch network may decrease the starch-protein binding. For instance, inulin thickens the crumb air cell walls and its increased concentration can enhance this phenomenon resulting in more compact structure (Rodríguez-García et al., 2014a). This result was consistent with Chapter 4 (Gao et al., 2016) which investigated the effect of sugar replacer muffin volume and texture results (especially at high levels of inulin replacer). However, the replacement of sugar with 50 % inulin appear not to affect muffin structure compared with that of the control. The protein-fibre-starch network is highly developed with discrete starch granules (showing signs of gelatinization) visible. Demirkesen et al. (2013) reported that fibre and sugar are effective in incomplete disintegration of starch granules, because of their high hygroscopicity reduces the availability of water for starch and proteins. Therefore, starch gelatinization was probably inhibited due to water competition between sugar, inulin, protein and starch (Rodríguez-García et al., 2013). The similarity in structure between inulin and control samples may explain the similarity in muffin texture as observed in Chapter 4 (Gao et al., 2016).

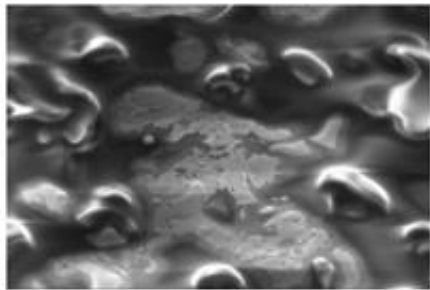
As the amount of sugar replaced by Stevianna® increased, a rougher surface was observed by Figure 8.7. Some of the large starch granules appear shrivelled, shrunken owing to gelatinization and are seen

trapped in protein matrix. Struck et al. (2016) discussed that sucrose lower water activity so that less water remains available for the starch granules, causing a delay in starch gelatinization and protein denaturation. Furthermore, the sugar lead to the stabilization of that regions by intermolecular interactions of sucrose with starch chains in the amorphous regions of the starch granule. The water in the muffin batter in this study was constant and originated with oil and eggs. When Stevianna® was used to completely replace sugar, the water enters the amorphous regions of starch granule and causes increasing solubilization of amylose and amylopectin. Therefore, starch granules were more easily identified in 100 % Stevianna® sample; the gluten protein matrix appeared discontinuous and most of the starch granules were gelatinized. This disruption to the muffin structure may explain the reduced overall texture observed in Chapter 6. A potential problem with erythritol has been reported that it does not attract water (Gao et al., 2017; Rodríguez-García et al., 2013), and stevia with low water solubility was also observed by Yadav and Guleria, (2012). Ronda et al. (2005) reported that starch gelatinization and protein denaturation temperatures was decreased in sorbitol cakes causing a premature thermosetting of protein or starch matrix, this process will start at the crust due to direct contact with the heating medium. However, the starch granules swelling inside the muffin crumb observed by SEM was not marked different, but the characteristics of the inter-granule matrix appeared to be related to the concentration of Stevianna®. SEM cake-crumb reported that the degree of swelling of starch granules is difficult to assess based on SEM studies of cake crumb, due to the limited number of granules viewed, the random orientation of the granules, the wide range of initial sizes, and the presence or absence of leached materials.

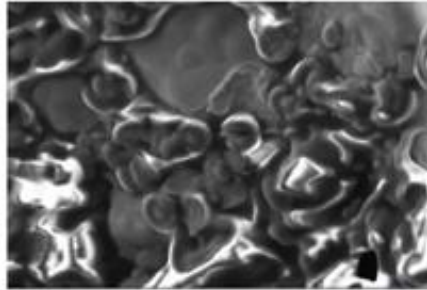




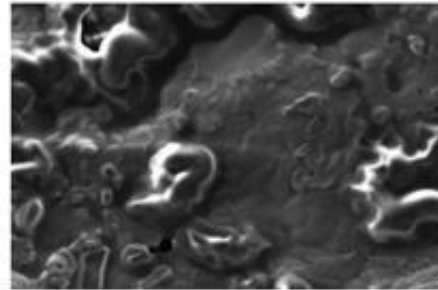
a) Control ↵



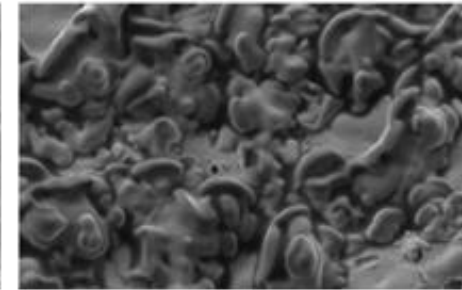
b) 25 % Inulin



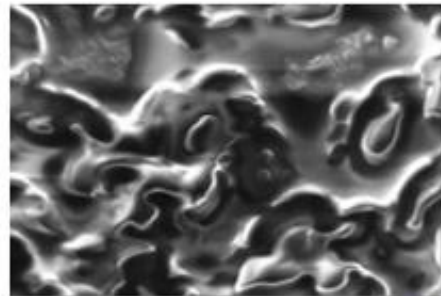
c) 50 % Inulin



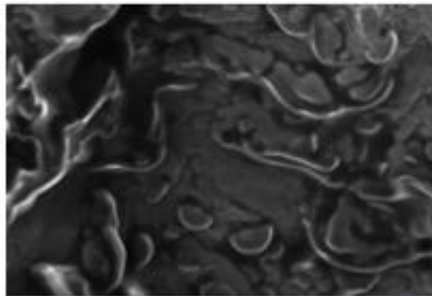
d) 75 % Inulin



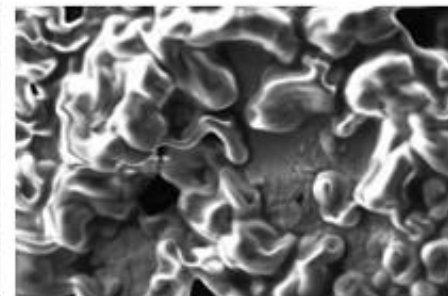
e) 100 % Inulin



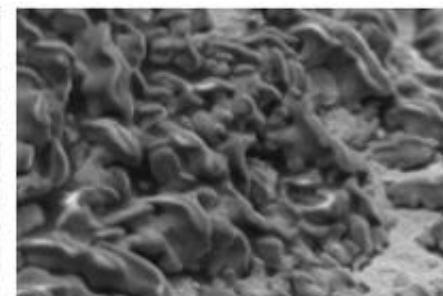
f) 25% Stevianna®



g) 50% Stevianna®



h) 75% Stevianna®



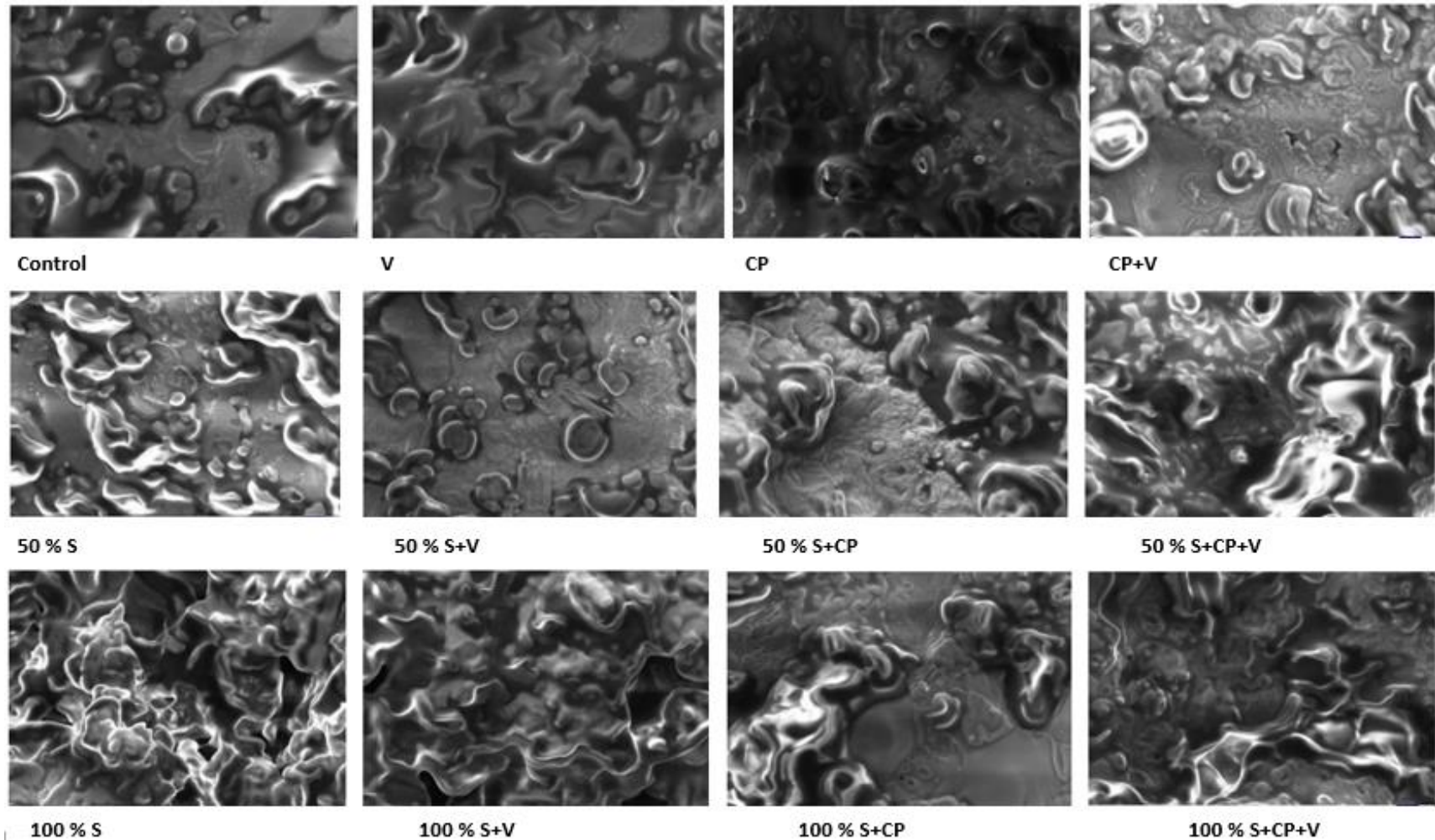
i) 100% Stevianna®

Figure 8. 7 SEM micrographs of recipe 1 muffin samples: a) Control; b) 25 % Inulin; c) 50 % Inulin; d) 75 % Inulin; e) 100 % Inulin; f) 25% Stevianna®; g) 50% Stevianna®; h) 75% Stevianna®; i) 100% Stevianna®.

Figure 8.8 shows the micrographs of muffin crumb for recipe 2. In muffin crumb with 100 % Stevianna®, it can be observed that starch granules are enmeshed in the discontinuous gluten protein matrix. A similar effect can be seen in the microstructure of 100 % Stevianna® muffin crumb without/with cocoa powder and/or vanilla (Figure 8.8), respectively.

This is in agreement with the findings of recipe 1 results that the microstructure crumb appeared a rougher surface when sucrose was totally replaced by Stevianna®. However, there was no significant difference between control and 50% Stevianna® samples by SEM analysis. Starch granule silhouettes were visible beneath the veiling proteins. The proteins draped finely over the mass of granules. A similar effect was noticed when a combination of additives (cocoa powder or vanilla) was used (Figure 8.8). Additionally, a continuous matrix appears as 'lakes' that assume irregular shape around starch granules. Zahn et al. (2010) stated that in muffin components with oil, protein and starch granules prevent hydration and formation of a continuous gluten-starch network. Results indicated that cocoa powder or vanilla used had no significant effect on the appearance of muffin crumbs compared to those without cocoa powder or vanilla muffin samples by SEM. Therefore, a right amount of sucrose ensures a sufficient product rise, a factor which has to be compensated when sucrose is replaced by non-sugar ingredients.





**Figure 8. 8 SEM micrographs of recipe 2 muffin samples: Control and vanilla (V); cocoa powder (CP); cocoa + vanilla (CP + V); 50% Stevianna® (50S); 50% Stevianna® + vanilla (50S + V); 50% Stevianna® + cocoa (50S + CP); 50% Stevianna® + cocoa + vanilla (50S + CP + V); 100% Stevianna® (100S); 100% Stevianna® + vanilla (100S + V); 100% Stevianna® + cocoa (100S + CP); 100% Stevianna® + cocoa + vanilla (100S + CP + V).**

## 8.4 Conclusions

Image analysis technique used to evaluate muffin characteristics allowed the detection of relative differences among formulations. The method is fast and easy to use, and it can be effective in evaluating the air cell structure of muffin crumbs. Muffins prepared with Stevianna® as full-sugar replacer had a more open air cell structure in recipe 1. However, air cell area and average air cell size decreased when more than 50 %sucrose was replaced by inulin. In the recipe 2, results indicated that the air cell structure of the muffin sample with cocoa powder and/or vanilla were similar to those of the muffin samples without cocoa powder and/or vanilla, air cell area and average air cell size increased as the sugar-replacement level increased by Stevianna®.

Based on SEM images, recipe 1 showed a rather ruptured, discontinuous, gluten protein matrix due to the use of inulin as sugar replacer in muffin samples. Stevianna® also produced a rougher surface in the full-sucrose replacement of muffin crumb, resulting in gelatinization and are seen trapped in protein matrix. The recipe 2 had similar results with Stevianna® samples of recipe 1, although cocoa powder and/or vanilla were added into the recipe 2 formulations.

The major outcome of partial sugar-substitution was close to the control sample in the crumb structure, but air cell or crumb structure decreased as the sugar-replacement level increased. Addition of cocoa powder and vanilla did not effect on the muffin structure in the recipe 2.

## Chapter 9

### General Discussion and Conclusions for Future Work

#### 9.1 Aims and General Discussion

In this chapter, the main findings with regard to the research questions are summarised and general conclusions based on the findings of the studies presented in this thesis are described.

The directions of future work have been discussed in this chapter as well. The aim of this research was to evaluate the impact of Stevianna® or inulin as partial or complete sugar replacers in muffin products, and to explore the following objectives:

Objective 1: Comparison of muffin structure characteristics between the control sample and Stevianna® / inulin samples, evaluated by physical analysis.

Objective 2: Sensory characteristics were determined by forty untrained panellists.

Objective 3: *In vitro* carbohydrate digestibility was used to measure predictive glycaemic impact in control muffins and compared to muffin samples containing Stevianna® or inulin.

##### 9.1.1 Comparing of Muffin Structure Characteristics Between Control and Inulin Samples

Inulin, as a sugar replacer, was used in muffin product. Chapter 4 illustrated that increased levels of inulin led to muffins with increased firmness and decreased springiness. However, replacement of 50 % of sucrose with inulin resulted in muffins close to the firmness and springiness of control muffins. Rößle et al. (2011) reported inulin could be used to replace 50 %

of sucrose in scones without having a detriment at effect on firmness of the scone product. Zahn et al. (2010) found that 50 % replacement of fat by inulin significantly affected muffin crumb firmness, this differs from the findings presented in our results. This difference may be due to different roles of fat and sugar in the muffin recipes.

A associate obtained in Chapter 8 showed that crumb hardness was significantly related to the air cell area, so that as the air cell size decreases, the muffin product had a more compact crumb structure. These observations could be related to inulin viscosity properties noted in the results of Chapter 7. A higher viscosity of batter was observed with inulin replacing more than 50 % of sugar as the presence of inulin inhibited the hydration of the starch granules by bonding to the available water and thus also reduced the aeration properties of the cake batter (Oh et al., 2014). Kalinga and Mishra (2009) agreed with the findings which showed that the increased firmness might be due to either the decreased stiffness of the foams or premature starch gelatinization, leading to a very dense crumb structure. Our research of DSC analysis (Chapter 7) has indicated that the presence of inulin significantly altered starch gelatinization in muffin batter, due to its ability to bind water and act as a stabiliser of the amorphous region in the starch granule (Psimouli and Oreopoulou, 2013). That is why in the micrograph of muffin crumb illustrated by SEM analysis (Chapter 8) can be seen a few starch granules are visible wrapped in thick protein matrix owing to the use of high levels of inulin. The thermal transition temperatures are higher after inulin incorporation, due to the formation of a gel structure. Furthermore, this decrease in sugar-replaced muffin crumb structure is that inulin contributes to excessive denseness of batter which in turn might limit batter expansion. Juszczak et al. (2012) considered that the water-binding ability of inulin

played a key factor in modifying the properties of the system. However, the similarity of structure between inulin (25 % & 50 %) and control samples may explain the similarity in muffin texture by air cell structure and SEM analysis proved (Chapter 8).

### **9.1.2 Sensory Evaluation Between Control and Stevianna® Samples**

Only Stevianna® was used as sugar replacer in sensory evaluation for our research. This is because Stevianna® has low calories and is a more intense sweeter than inulin. Furthermore, cocoa powder and vanilla, as extra ingredients, were added into muffin formulations in order to improve the sweetness of the sugar-reduced muffin. Chapter 5 described that sensory evaluation with forty panellists showed an overall preference for the 50 % Stevianna® muffins with/without cocoa powder and/or vanilla samples. Kulthe et al. (2014) illustrated that the cookies with 20 % substitution stevia leaves powder scored maximally for all the sensory properties attributes. However, a lowest overall liking ranking of samples which were prepared with 100 % Stevianna®, and a bitter aftertaste was reported by panellists. This bitterness maybe attributed to the inherent bitterness of steviol glycosides (Carakostas et al., 2008). The muffin with inclusion of cocoa powder also exhibited an aftertaste of bitterness, but which was described as acceptable bitterness by panellists. This can be related to the flavour of cocoa powder. Additionally, panellists showed the overall liking improved when vanilla was added to the cocoa formulation. Belščak-Cvitanović et al. (2015) indicated that the presence of vanilla in cereal products serves to enhance the sweetness of products through both flavour and odour receptors. Our research showed that the addition of cocoa powder could mask the Stevianna® bitterness in terms of taste and that the addition of vanilla

enhanced the flavour of muffins. Therefore, 100 % Stevianna® with cocoa powder and/or vanilla samples gave a similar sweetness to 50 % Stevianna® samples and control, while inclusion with 100 % Stevianna® samples still presented poorer appearance, texture and mouthfeel than other samples.

### **9.1.3 Comparing of Muffin Physical Characteristics Between Control and Stevianna® Samples**

Chapter 4 and Chapter 6 demonstrated the muffin physical properties by instrumental analysis.

Chapter 4 showed that 50 % of sucrose could be replaced in bakery products by using Stevianna® without any negative effects on texture of the product. However, 100 % sucrose replacement with Stevianna® gave a similar texture to 100 % inulin muffin samples (harder and more crumbly texture than the control muffin). Martínez-Cervera et al. (2012a) reported significantly higher firmness values in the 100 % sucrose replacement with erythritol muffins as compared to the control muffins. Chapter 6 supported the texture results of Chapter 4, and further studied the textural properties of muffin samples with cocoa powder and/or vanilla. Our research indicated that the addition of cocoa powder and/or vanilla did not affect the muffin texture. Chapter 6 illustrated that in general, textural properties were significantly related to the volume and density. The 100 % Stevianna® muffin with/without cocoa powder and/or vanilla samples had significantly lower volumes and higher density compared to those muffin samples including sugar, this led to the firmer texture in muffin properties. Muffin density appeared to be negatively correlated with muffin volume. A similar significant overall

deterioration in cake physical properties were observed by Manisha et al. (2012) when 100 % of sucrose was replaced with stevioside and liquid sorbitol.

The muffin volume is an important indicator of air cell expansion during baking. Therefore, Chapter 8 evidenced a decrease in air cell expansion with increasing levels of Stevianna® in muffin samples. In addition, muffin height and air cell numbers were related to muffin products. Chapter 6 indicated that the decrease in muffin height was associated with absence of interconnectivity of a more compact structure and with the development of large air cell size for levels of sucrose replacement higher than 50 % Stevianna®. Two main reasons that affect expansion are decrease in batter stability during the heating stage, and changes in the thermosetting mechanism due to sugar replacement with Stevianna®, which were described by Chapter 7.

In addition, the moisture content in bakery products is an important factor as it has a direct impact on the textural attributes. A strong associate has been found in Chapter 6, when 100 % Stevianna® muffins firmness increased as moisture content increased. The muffin textural properties were also explored by SEM images (Chapter 8), which showed a rougher surface as the amount of sugar replaced by Stevianna® increased.

According to the above summary, our research found that Stevianna® is a major factor impacting on physical characteristics of muffins. The addition of cocoa powder and/or vanilla did not affect the physical properties of muffins significantly.

Colour testing was determined in muffin products with Stevianna® (Chapter 5), the control sample was more yellow than the 100 % Stevianna® muffin samples. Since the Stevianna® is

white in colour and does not participate in Maillard browning this might explain the lighter yellow hue of crust and crumb as replacement levels increased. In Stevianna® muffin sample containing cocoa power resulted in significantly higher values of browning index (BI) than the control sample. In sensory evaluation, muffin crust colour was also judged by panellists but no significantly different between each sample. Due to the colour testing generally followed the instrumental measurements, while the panellists did not distinguish the minor differences detected by the colorimeter.

#### **9.1.4 Use *in vitro* Carbohydrate Digestibility to Measure Glycaemic Impact in Baked Muffins**

The nutritional quality of the baked muffin products are highlighted in Chapter 4. The effect of Stevianna® or inulin inclusion on the predicted glycaemic impact as determined by *in vitro* digestion and illustrated the role of sugar in elevating the glycaemic response during digestion. The potential glycaemic response values of the Stevianna® or inulin muffins were significantly lower than the control muffin. Our research observed that the inulin formed a matrix which encased starch granules in a protective coat (Chapter 8), resulting in the suppression of starch hydrolysis and, consequently, the reduced potential for starch degradation and sugar liberation. This finding is consistent with that of Oh et al. (2014) who reported starch digestibility in cakes with added inulin also reduced the predicted glycaemic index values.

In particular, the replacement of sucrose with 100 % Stevianna® caused a significant decrease in the standardized area under the curve when compared with control sample. This result revealed two important factors-showing the slowest release of sugars during *in vitro* starch



digestion and, therefore, having a reduction in the predicted glycaemic response by up to hundred per cent. It may be because stevia does not contribute to the available carbohydrate and glycaemic responses in food products as it is a natural sweetener that contains no glucose. Roberts and Renwick (2008) reported that steviol glycosides are not readily absorbed by the upper small intestine when it is administered orally to normal rat or human subjects. Stevianna® is composed at rebaudioside-A (stevia) and erythritol, O'Donnell and Kearsley (2012) had proved that erythritol does not raise postprandial glycaemic and insulin levels by oral ingestion in healthy human subjects.

Chapter 6 focused on the glycaemic response results of 100 % Stevianna® samples, which are consistent with data obtained in Chapter 4, and inclusion of cocoa powder and/or vanilla muffin samples were observed by *in vitro* digestion. There were no significant changes to the predicted glycaemic response due to either the added of cocoa powder and/or vanilla in Stevianna® samples.

### **9.1.5 Use *in vitro* Carbohydrate Digestibility to Measure Glycaemic Impact in Muffin Batters**

The glycaemic response of all muffin batters (Recipe 1 and Recipe 2) from this research were also determined by *in vitro* digestion and using RVA gels as a “cooked” sample. This experiment observed the predicted glycaemic response relationship between batter and baked muffins during baking process. Chapter 7 indicated that the results of batter samples with sugar replacer are consistent with data obtained in the glycaemic response of baked muffin, indicating that Stevianna® or inulin can significantly reduce the predicted glycaemic

response in batter samples. Our research also indicated that the baking processing did not have an impact on the predictive glycaemic response.

## **9.2 General Conclusions**

Demand for low calorie bakery products market is on the rise and people are becoming more conscious of their health and nutrition. In such a scenario, it is necessary that the sugar replacement with functional sugar in bakery products which are designed and developed for either improving general health and well-being or targeting specific health conditions, are safe to the consumer.

In summary, this study was undertaken with the intention of providing optimal levels of sugar substitutes to imitate sugar in maintaining muffin structure as well as depressing the rate of reducing sugar release during simulated digestions. A partial replacement of Stevianna® or inulin for sucrose in muffins gave a product with quality characteristics close to that of the full-sucrose muffin sample. At the same time, the reduction in potential glycaemic response was greater than would have been expected with 50 % sucrose reduction and consequently providing a muffin that produces a lowered postprandial response with the potential of associated health benefits. Compared to inulin, Stevianna® may be a suitable sucrose replacement for low-sucrose formulation of muffins due to the intense sweetener. Our study also investigated the use of cocoa powder and/or vanilla to mask the Stevianna® bitterness in terms of aftertaste and to enhance muffin product flavour, which was successful in the sensory evaluation. Therefore, innovation of muffin products by natural sweeteners, which are not only nutrient-rich but are also acceptable to the panellist, was achieved.

### 9.3 Recommendations for Future Work

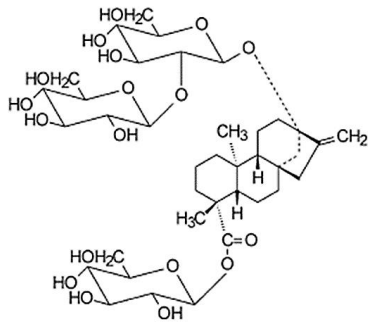
Future research may be aimed at optimizing the ratio of sucrose to Stevianna® possibly with 55, 60 or 65% replacement into other bakery products, such as bread or biscuit. Meanwhile, combinations of inulin and Stevianna® will lead to a 50-75 % reduction of sugar and possibly the development of a sugar free product. This mixture may prove to be a suitable all-natural competitor that has a similar texture to a conventional baked product.

In the present study, a sensory evaluation of these products had been investigated by untrained panellises. In future, the sensory evaluation could be conducted by a trained panel. These sensory attributes may prove to be helpful in reducing some negative attributes, when correlated with some of the instrumental measurements. With more availability data of sensory properties, it is expected that increase market requirement for these bakery products may soon take place.

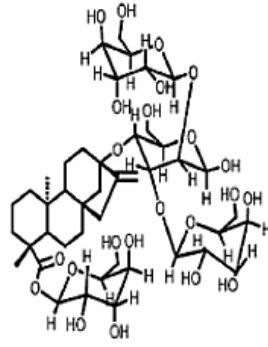
Our research also suggested a relationship with the effect of replacing sugar with Stevianna® or inulin on the nutrition analysis. Future research should focus on measurement of fat, protein and antioxidants in different sugar substitutes level, and thus possible formulation adjustment for nutrient-rich bakery products may be explored.

## Appendix A

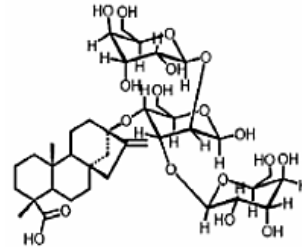
### A.1 Diagrams of Steviol Glycosides



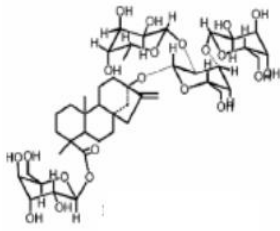
Stevioside



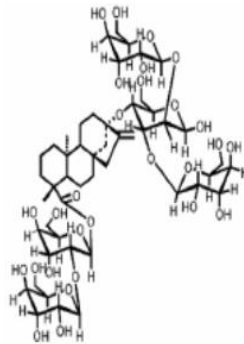
Rebaudioside-A



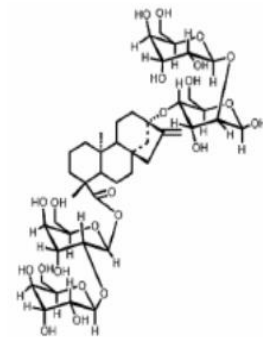
Rebaudioside B



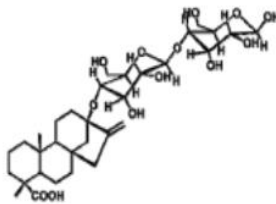
Rebaudioside C



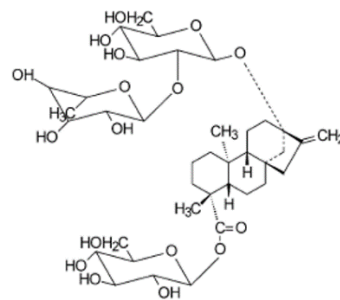
Rebaudioside D



Rebaudioside E



Steviolbioside



Dulcoside A

(Carakostas et al., 2008)

## **Appendix B**

### **Sensory Evaluation**

#### **B.1 Human Ethics Committee Exempt Status Approval and Invitation Letter**

**Application No: 2015-38**

28 September 2015

**Title:** Sensory evaluation of muffins BICH651

**Applicant:** J Gao

---

*The Lincoln University Human Ethics Committee has reviewed the above noted application.*

Thank you for your response to the questions which were forwarded to you on the Committee's behalf.

I am satisfied on the Committee's behalf that the issues of concern have been satisfactorily addressed. I am pleased to give final approval to your project.

Please note that this approval is valid for three years from today's date at which time you will need to reapply for renewal.

Once your field work has finished can you please advise the Human Ethics Secretary, Alison Hind, and confirm that you have complied with the terms of the ethical approval.

May I, on behalf of the Committee, wish you success in your research.

Yours sincerely



Grant Tavinor  
Chair, Human Ethics Committee

**PLEASE NOTE:** The Human Ethics Committee has an audit process in place for applications. Please see 7.3 of the Human Ethics Committee Operating Procedures (ACHE) in the Lincoln University Policies and Procedures Manual for more information.

## Invitation Letter

Hi Everyone

We are doing a muffin sensory evaluation as part of developing a “healthier” muffin mix.

We need people to try the muffins and formally evaluate them for us.

Would you be willing to help?

The tasting will take be 3 sessions each of 15-30 minutes of your time and is in the sensory suite in Room 087 RFH.

This week: Thursday (01/10/2015) at these time 10.30, 11, 11.30, 12, 12.30, 13, and 13.30

Next week: Thursday (08/10/2015) at these time 10.30, 11, 11.30, 12, 12.30, 13, and 13.30

After next week: Wednesday (14/10/2015) at these time 10.30, 11, 11.30, 12, 12.30, 13, and 13.30

Reply with your preferred time above these time.

Please do not drink strong coffee or eat spicy foods within the hour before your tasting time.

The Muffins contain wheat and egg and may contain ingredients from chicory and stevia.

You should not volunteer if you have an allergy or intolerance to any of these food ingredients, and you are vegan or vegetarian.

Other people who should exclude themselves are anyone who has a history of serious anaphylactic reaction to any food

or a history of significant bowel disease (Includes Chronn`s; Ulcerative Colitis; Coeliac`s.)

Otherwise we welcome everyone.

Thank you in anticipation

Candy and Sue

## **B.2 Sensory Evaluation of Muffin Consent Form**

### **Sensory evaluation of muffin Consent Form**

I have read and understood the description of the above-named project. On this basis I agree to participate as a subject in the project, and I consent to publication of the results of the project with the understanding that anonymity will be preserved. I understand also that I may withdraw from the project at any time including withdrawal of any information I have written until the data is pooled for analysis on Friday (date).

Name: \_\_\_\_\_

Signed: \_\_\_\_\_ Date: \_\_\_\_\_



## **B.3 Sensory Evaluation Form**

# Muffin Sensory Evaluation

Welcome!

Today you will be evaluating muffins

Please assess each muffin for the various attributes given.

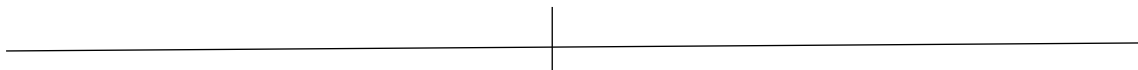
Please test the samples from left to right.

- Please attend **(Please note that)**: Firstly, use the water to freshen your mouth before begins to taste of the first sample.

Secondly, waiting 30 seconds when you swallow each sample and then use the water to freshen your mouth.

All in all, waiting 30s and use the water to freshen your mouth between each sample.

Please make One vertical mark on each horizontal line to show your decision. An example decision will look like this:



You are then asked to rank the samples in order of your overall preference.

All information you provide today is anonymous and is only used as pooled data.

If you have any questions, please ask.

**Before tasting the samples, please fill in the information below:**

Ethnicity:

Gender:

Panellist Number:

Enter your **First sample number** here \_\_\_\_\_

**1. Visually**

How visually appealing do you find this muffin?

Not at all  
appealing

Extremely  
appealing

---

**2. Colour**

What is your impression of the colour intensity of the top of the muffin?

Extremely light

Extremely dark

---

**3. Texture**

What is your impression of the texture?

Extremely soft

Extremely hard

---

**4a. Mouth-feel**

What is your impression of the mouth-feel?

Extremely moisture

Extremely dry

---

**4b. Are there any other mouth feel attributes that you notice?**

**5. Sweetness**

How would you rate the sweetness of the sample?

Not sweet at  
all

Extremely sweet

---

**6. After taste**

Is there any after taste in the sample?

YES

NO

**If yes, please describe**

**7. Overall liking**

Considering everything. Overall how much do you like or dislike the muffin?

Dislike  
Extremely

Like  
Extremely

---

Enter your **Second sample number** here \_\_\_\_\_

**1. Visually**

How visually appealing do you find this muffin?

Not at all  
appealing

Extremely  
appealing

---

**2. Colour**

What is your impression of the colour intensity of the top of the muffin?

Extremely light

Extremely dark

---

**3. Texture**

What is your impression of the texture?

Extremely soft

Extremely hard

---

**4a. Mouth-feel**

What is your impression of the mouth-feel?

Extremely moisture

Extremely dry

---

**4b. Are there any other mouth feel attributes that you notice?**

**5. Sweetness**

How would you rate the sweetness of the sample?

Not sweet at  
all

Extremely sweet

---

**6. After taste**

Is there any after taste in the sample?

YES

NO

If yes, please describe

**7. Overall liking**

Considering everything. Overall how much do you like or dislike the muffin?

Dislike  
Extremely

Like  
Extremely

---

Enter your **Third sample number** here \_\_\_\_\_

**1. Visually**

How visually appealing do you find this muffin?

Not at all  
appealing

Extremely  
appealing

---

**2. Colour**

What is your impression of the colour intensity of the top of the muffin?

Extremely light

Extremely dark

---

**3. Texture**

What is your impression of the texture?

Extremely soft

Extremely hard

---

**4a. Mouth-feel**

What is your impression of the mouth-feel?

Extremely moisture

Extremely dry

---

**4b. Are there any other mouth feel attributes that you notice?**



**5. Sweetness**

How would you rate the sweetness of the sample?

Not sweet at  
all

Extremely sweet

---

**6. After taste**

Is there any after taste in the sample?

YES

NO

If yes, please describe

**7. Overall liking**

Considering everything. Overall how much do you like or dislike the muffin?

Dislike  
Extremely

Like  
Extremely

---

Enter your **Fourth sample number** here

---

**1. Visually**

How visually appealing do you find this muffin?

Not at all  
appealing

Extremely  
appealing

---

**2. Colour**

What is your impression of the colour intensity of the top of the muffin?

Extremely light

Extremely dark

---

**3. Texture**

What is your impression of the texture?

Extremely soft

Extremely hard

---

**4a. Mouth-feel**

What is your impression of the mouth-feel?

Extremely moisture

Extremely dry

---

**4b. Are there any other mouth feel attributes that you notice?**

**5. Sweetness**

How would you rate the sweetness of the sample?

Not sweet at  
all

Extremely sweet

---

**6. After taste**

Is there any after taste in the sample?

YES

NO

If yes, please describe

**7. Overall liking**

Considering everything. Overall how much do you like or dislike the muffin?

Dislike  
Extremely

Like  
Extremely

---

**Please rank the samples in order of your preference: one (1) being your most preferred sample and (3) being your least preferred sample.**

Most preferred

1. \_\_\_\_\_

2. \_\_\_\_\_

Least preferred

3. \_\_\_\_\_

4. \_\_\_\_\_

**Do you have any other comments about any one the samples you have tasted today?**

**Thanks for your taking part!**

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