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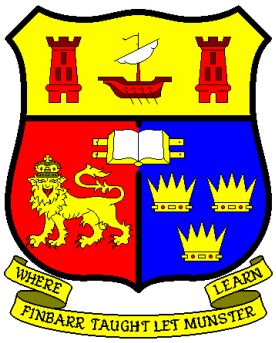
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School of Food and Nutritional Sciences



SUGAR REDUCTION IN SWEET BAKERY PRODUCTS: SOURDOUGH TECHNOLOGY AS A NOVEL TECHNOLOGICAL APPROACH TO OVERCOME QUALITY LOSS

Thesis presented by

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Master of Science Pharmaceutical Bioprocess Engineering

Under the supervision of

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To obtain the degree of

Doctor of Philosophy - PhD in Food Science and Technology

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DECLARATION

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

_____

Aylin Sahin

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"Equipped with his five senses, man explores the universe around him and calls the adventure Science" (Edwin Powell Hubble).

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ABSTRACT

Sugar reduction in food and beverages represents one of the major trends followed by consumers. Sugar is one factor, which promotes non-communicable diseases, such as type-2-diabetes, obesity and cardiovascular health issues. This doctoral dissertation, firstly, highlights the need for sugar reduction in bakery products in form of a literature review. Commonly known strategies, such as the replacement of sugar by bulking agents and artificial sweeteners, are discussed and the potential of sourdough technology to overcome techno-functional limitations is introduced. Several lactic acid bacteria and yeast strains are natural polyol producers and, furthermore, are able to produce exopolysaccharides, which makes sourdough a functional ingredient. The essential role of sugar is shown by the simple reduction of sugar by wheat starch, a non-sweet bulking agent, in a burger bun system. Sugar reduction increased specific volume (+0.99 ml/g) and changed the texture and structure of the burger buns significantly. Furthermore, sweetness intensity decreased, and microbial shelf life was shortened (-6 days). The replacement of sugar by commercially available polyols, such as xylitol, maltitol or mannitol, instead of wheat starch, revealed a significantly lower specific volume (-30 to -48%) and a harder crumb texture (+135% to +678%) compared to the full-sugar control. Moreover, polyols did not contribute to Maillard browning resulting in lighter crust colour. Among all polyols tested, mannitol showed the most promising results as a partial sugar replacer. An alternative approach to overcome quality loss during sugar reduction is the application of mannitol-rich sourdough. The heterofermentative lactic acid bacteria (LAB) strain *Leuconostoc citreum* TR116 was isolated from a yellow pea sourdough and characterised as a high mannitol producing strain. Wheat sourdough fermented with this LAB strain was performed with fructose addition, which was converted to mannitol (yield: 87%). The optimal fermentation time for high mannitol production was 30 h and mannitol-rich sourdough showed a production of metabolites in the ratio 1:0.34:0.15 (mannitol:lactate:actate). The incorporation of sourdough into a 50%-sugar-reduced burger bun system caused the same effect on gluten network development and viscoelastic properties, as the full-sugar control. Furthermore, no differences in specific volume and crumb hardness occurred, and mannitol-rich sourdough also contributed to sweetness and flavour. Additionally, a prolonged microbial shelf life was achieved. The addition of 10% mannitol-rich sourdough in a burger bun system is recommended. Since mannitol-rich sourdough could compensate quality loss in sugar-reduced burger buns, its effect on a 50% -sugar-reduced cake was investigated. Mannitol-rich sourdough increased the specific volume and softened the crumb texture (-8.6 N) of a 50%-sugar-reduced cake. Furthermore, it increased the sweetness intensity by 93% and contributed to aroma (+30%) and flavour (+25.5%). In sugar-reduced cakes a sourdough addition level of less than 10% is recommended. The positive impact of sourdough technology on buns and cakes led to the investigation of the effect of mannitol-rich sourdough in low-sugar biscuits. Since *Leuconostoc citreum* TR116 is a multifunctional strain, which is also able to produce exopolysaccharides, a mannitol-rich sourdough and a mannitol-rich sourdough with exopolysaccharides was fermented and incorporated in the biscuits. Sourdough addition caused an improvement in biscuit spreading and hardening. Furthermore, it contributed to colour and increased sweetness and flavour intensity (+140%, +139% respectively). It has to be noted that sourdough incorporation did not negatively influence the predicted glycemic index of low-sugar biscuits. The outcome of this research thesis provides a starting point for the development of sugar-reduced, low-sugar, or even no-sugar, highly consumer accepted bakery products using natural functional ingredients.

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ABBREVIATIONS

a_w	Water activity
BU	Brabender units, 1 BU = 0.01 Nm
C1	Full-sugar control
C2	Sugar reduced control using wheat starch
C3	Sugar reduced control using commercially available mannitol
CLSM	Confocal laser scanning microscope
DM	Dry matter
DNL	*De novo* lipogenesis
DNS	3,5-dinitrosalicylic acid
DY	Dough yield
EPS	Exopolysaccharide(s)
Foxo-1	Forkhead protein-1
Fru	Fructose
G*	Complex modulus
G′	Storage modulus
G″	Loss modulus
Gal	Galactose
Glu	Glucose
GLUT2	Glucose-transporter-2
GLUT-5	Glucose-transporter-5
HePS	Heteropolysaccharide(s)
HFCS	High fructose corn syrup
HI	Hydrolysis index
Hm	Maximum height of dough during proofing
Hm′	Maximum height of gaseous release during proofing
HoPS	Homopolysaccharide(s)
IMP	Inosine monophosphate
LAB	Lactic acid bacteria
MDH	Mannitol-dehydrogenase

PCA	Principal component analysis
pGI	Predicted glycemic index
PMT	Peak maximum time of gluten network development
RSR	Reducing sugar release
RVA	Rapid visco analyser
SD	Sourdough containing wheat flour and water (dough yield 200)
SD_{FRU}	Sourdough containing fructose as trigger for mannitol production
SD_{FS}	Sourdough containing fructose as trigger for mannitol production and sucrose as trigger for exopolysaccharide production
SEM	Scanning electron microscope
SGLT-1	Sodium/glucose-symporter-1
SSL	Sodium Stearoyl Lactylate
Spp.	Species
T1	Time required to reach maximum height of dough during fermentation
Ti	Time of inflection point of dough during heating
TM	Torque maximum of gluten network
TPA	Texture profile analyser
TTA	Total titratable acids
V_{tot}	Volume of CO_2 production
WHO	World Health Organisation
ΔE	E-value as an expression of colour changes compared to a control sample
μ_{max}	Maximum microbial growth rate
ν_{max}	Maximum acidification rate

Chapter 1

INTRODUCTION

The number of people suffering from obesity, type-2-diabetes and cardiovascular disease increased in the last decades significantly. The high sugar-intake in the Western World is one of many factors which contributes to these heath issues. The average consumption of added sugar per day in Europe is 50 g, which makes double the amount recommended by the World Health Organisation (WHO) published in the "global action plan for non-communicable diseases 2013-2020" (Azais-Braesco et al., 2017; WHO, 2015). Furthermore, several countries introduced sugar taxation on sugar-sweetened beverages and other food products to decrease sugar consumption and promote a healthier life-style of the population. In the UK the government advised the baking industry to reduce the amount of sugar in their products by 20% by 2020. The awareness of consumers caused a high demand for sugar-reduced, low sugar, or no-sugar products. However, the reduction of sugar by functional ingredients without lowering the product quality is challenging. Hence, novel approaches to overcome quality loss in sugar reduced bakery products are urgently needed.

The most commonly used sweetening agent in the food industry, especially in the bakery sector, is sucrose. Sucrose does not only contribute to sweetness and flavour, it is very unique in its physicochemical properties and influences volume, texture, structure and shelf life.

Chapter 2 of this thesis describes, firstly, the mechanisms of sugar absorption and metabolism by the human body, followed by the effect of sugar-overconsumption on human health. Secondly, the role of sugar in bakery products is explained by the illustration of the interactions of sugar with other ingredients, and the impact of sugar on the techno-functional properties of cakes, biscuits and sweet-yeast-leavened products. Furthermore, commonly known strategies for sugar reduction in sweet bakery products are discussed and the potential of sweet proteins as sugar replacers is presented. As an alternative approach to successfully replace sugar, sourdough technology is introduced, in particular, the application of naturally produced polyols by lactic acid bacteria (LAB) or yeast in a sourdough system as a functional ingredient. Additionally, the production of exopolysaccharides (EPS) to compensate structural and textural changes by sugar reduction is discussed.

Chapter 3 provides a deep insight of the role of sugar in a sweet-yeast-leavened product using the example of burger buns. Wheat starch as a non-sweet bulking agent was used as a sugar replacer and the effect on dough properties and burger bun quality was

investigated. Sugar replacement by 30% was feasible, while a reduction of more than 30% caused an increased specific volume, a more open crumb structure and a lighter crust colour, promoted staling, shortened microbial shelf-life and decreased sweetness and flavour significantly.

Based on the fundamental understanding of the role of sugar in bakery products, **Chapter 4** reveals the impact of three different commercially available polyols, xylitol, mannitol and maltitol, as sugar replacers on the quality parameters of burger buns. The replacement caused a decrease in CO_2 production by yeast, and, thus, resulted in a lower specific volume and a harder crumb texture. Moreover, polyols caused a lighter crust colour due to less Maillard browning but contributed to taste and flavour. Overall, polyols can be considered as partial replacement, but a total sugar substitution led to an enormous loss of product quality and is not recommended. Among all tested polyols, mannitol showed the best overall results.

Since mannitol, as a partial sugar replacer, showed acceptable results, **Chapter 5** of this thesis represents the investigation of *in-situ* mannitol production by the heterofermentative LAB strain *Leuconostoc citreum* TR116 in a sourdough system. Certain LAB are able to convert fructose into mannitol. With a yield of 87%, *Leuconostoc citreum* TR116 can be considered as a high-mannitol producing strain. Firstly, two different sourdoughs, one where the mannitol production was triggered by the addition of fructose and one without fructose, were fermented by *Leuconostoc citreum* TR116. Sourdough characteristics, such as pH, total titratable acids (TTA), microbial cell count and sugar-, mannitol- and acid profile, during fermentation (48 h) were determined. Afterwards, the effect of the individual sourdoughs on the dough and product quality of burger buns reduced in added sugar by 50% was investigated. The use of sourdough technology as a novel technological approach showed promising results, especially sourdough including naturally produced mannitol. A low specific volume and a dense crumb structure was achieved, as well as a darker crust colour and a prolonged microbial shelf life. Furthermore, sourdough contributed to flavour and increased the sweetness perceived by a trained sensory panel.

The application of sourdough improved the techno-functional properties of burger buns. In **Chapter 6** sourdough technology was used to ameliorate the product quality of cakes reduced in added sugar by 50%. The same sourdoughs as mentioned in Chapter 5 were used and added in three different concentrations (5%, 10% and 20%) and the batter and

cake properties were investigated. An addition of 5% mannitol-rich sourdough to the cake ameliorated the techno-functional properties reflected by a higher specific volume, softer crumb, lower staling rate and a microbial shelf life comparable to the full-sugar control.

Sourdough technology represents a useful tool to overcome product quality loss in sugar reduced burger buns and cakes. **Chapter 7** reveals the effect of sourdough on the properties of low-sugar biscuits. The same sourdoughs used in Chapter 5 and 6 were applied. Additionally, a sourdough, in which EPS-production was triggered by the incorporation of sucrose in the sourdough, was tested. The production of EPS by *Leuconostoc citreum* TR116 was relatively low (0.71 g/kg). The incorporation of sourdough in a low-sugar biscuit enhanced biscuit spreading, contributed to the biscuit snap-firmness and increased biscuit colour. Furthermore, sourdough, in particular mannitol-rich sourdough without EPS, enhanced sweetness and flavour.

The research of this doctorate dissertation highlights the unique properties of sugar and emphasis the faced challenges occurring by sugar reduction in sweet bakery products. Additionally, sourdough technology is introduced as a promising novel technological approach to overcome quality loss in sugar-reduced baked goods, such as burger buns, cakes and biscuits. The outcome of this research thesis provides a starting point for the development of sugar-reduced, low-sugar, or even no-sugar, highly consumer accepted bakery products using natural functional ingredients.

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Chapter 2

SUGAR REDUCTION IN BAKERY PRODUCTS: CURRENT STRATEGIES AND SOURDOUGH TECHNOLOGY AS A POTENTIAL NOVEL APPROACH

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Abstract

The world is facing a big problem of non-communicable diseases, such as obesity, cardiovascular disease and diabetes. An excessive sugar consumption is considered as a main factor, which triggers these diseases. The two main sources of sugar in processed products on the market are sugar-sweetened beverages and sweet bakery products. Sugar reduction is challenging, especially in baked goods, since it interacts significantly with all ingredients. These interactions cause an increase in gelatinization temperature, a delay in gluten network development, an increase or decrease in yeast activity depending on the sugar concentration, as well as an enhancement of emulsification. Reflecting the molecular interactions on the product quality characteristics of different types of baked goods, sugar also contributes to browning reactions and extension of microbial shelf life. During cake preparation, sugar supports the batter aeration which results in the typical soft cake crumb. Furthermore, it contributes to the spreading process of biscuits during baking and enhances surface cracking due to recrystallization. Sugar reduction requires the development of different strategies; Two well-known strategies are the replacement of added sugar by the combination of bulking agents and high-intensive sweeteners, or by sweet bulking ingredients, such as polyols. The *in-situ* production of polyols to enhance sweetness, and exopolysaccharides to improve texture, in a sourdough system shows high potential as sugar replacement. *Lactobacillus sanfranciscensis*, *Leuconostoc mesenteroides* and *Leuconostoc citreum* are high mannitol producing strains with yields of 70-98% and *Leuconostoc oenos* was found to produce erythritol. Several yeast strains produce xylitol, erythritol, mannitol or arabitol under aerobic conditions. Exopolysaccharides are known to improve the texture and structure of bakery products and, thus, have high potential as natural functional ingredients to compensate quality loss in sweet bakery goods.

2.1. Introduction

The number of people suffering from obesity, cardiovascular disease and type-2-diabetes, increased enormously. The published statistic is shocking: 1.9 billion adults over the age of 18 and 41 million children under the age of 5 are overweight or obese (World Health Organization, 2017). The consumption of sugar increased during the last 20 years and, additionally, new sugar sources, such as high-fructose-corn-syrup (HFCS), became available (Johnson et al., 2007). A governmental reaction to the increased cases of obesity, is the introduction of tax on sugary beverages and foods, which is currently under discussion. Furthermore, the government in the UK expressed its concern for public health in form of an advice for the baking industry to reduce the sugar content of their products by 20% by 2020 (Tedstone et al., 207).

The reduction of sugar in sweet bakery products is challenging, however, since sugar fulfils more functions than only sweetness and flavour (Clemens et al., 2016). It is essential to investigate the interactions of sugar with the main ingredients in baked goods and to understand the role of sugar in different products. By now, two main strategies to reduce sugar with a promising result have been introduced: Firstly, the replacement of sugar by a sweet bulking agent, such as polyols, and secondly, sugar substitution by a combination of non-sweet bulking agents and high-intensive sweeteners. Furthermore, certain sweet proteins can also be considered as sugar replacers.

One novel approach is the *in-situ* production of sweet polyols and exopolysaccharides (EPS) in a sourdough system in order to, firstly, convert monosaccharides to calorie reduced sweet agents (polyols), and, secondly, to reduce the amounts of monosaccharides by polymerisation to long chain carbohydrates (EPS). Certain lactic acid bacteria (LAB) and yeast strains are able to produce polyols and/or EPS naturally using their specific metabolic pathways, and this sourdough can be applied in a sugar reduced bakery product. Since this approach to overcome quality loss in sugar reduced baked goods is novel, a proper understanding about polyol/EPS producing microorganisms is necessary.

This review introduces the need for sugar reduced bakery products and explains the essential role of sugar in cakes, biscuits and sweet leavened products. Furthermore, it describes strategies for sugar reduction in baked goods and discusses the novel approach of incorporating sourdough. Thus, the natural production of polyols/EPS by certain LAB and yeast strains is reviewed and their application in a controlled sourdough system as a sugar substitute is discussed.

2.2. The need for sugar reduction in sweet baked goods

The roots of sugar are located in New Guinea and India as a very rare and expensive raw material. In the late 15th century Spain and Portugal started planting sugar cane on some of their islands and increased the wealth of their empires (Galloway, 2005). By exporting this raw material, the consumption of sugar worldwide began to rise, and it became popular as an additional carbon source to barley, wheat, oats and rye. However, as salt, sugar was highly taxed and very expensive. In 1700 the yearly average sugar intake per person in England was determined as 1.8 kg and increased to 8.1 kg in 1800. The production of sugar and its availability increased, due to its popularity, and also, because the British Prime Minister, William Gladstone, decided to remove the sugar tax in 1874, which led to a further increase in sugar consumption to 45 kg per capita in England in 1950 (Johnson et al., 2007).

After the British Navy blocked the import of sugar to Europe in the 18th century, the Europeans discovered a way to compensate their sugar shortage and extracted sugar from sugar beets. Since then Germany, France and Austria established sugar beet industries and the worldwide production of sugar increased from 250 000 tons in 1800 to 8 million tons in 1900 (Mintz, 1986). The introduction of high-fructose-corn-syrup (HFCS) in the United States of America in the 1970s, offered an additional source of sugar for the industry and end-consumer. The two reducing monosaccharides glucose and fructose (usually in a ratio of 45:55 respectively) are not bond, and hence readily bioavailable in the body (Johnson et al., 2007). HFCS has several advantages compared to sucrose, such as a longer shelf life and lower costs and is mainly used in sugary drinks, fruit punches, pastries and processed foods. Johnson et al. (2007) mentioned the consumption of HFCS and sugar being 67.6 kg per capita per year in the United States.

The main source of added sugar in food products are sugar sweetened beverages. The consumption of these kind of beverages increased from 36 litres per person per year in 1997 to 43.5 litre per person per year in 2010. A study by Bray and Popkin (2014) about the effect of the consumption of sugar sweetened beverages on the body weight showed that people with the highest intake gained 1.55 kg in one year compared to people with the lowest intake.

Besides sugary drinks, baked goods, such as biscuits and cakes, are another important source of added sugar in food products (Guallar-Castillon et al., 2013). Sugar is one of the essential ingredients in biscuits and cakes, not just to ensure taste and flavour, but also

to give products their unique texture. Biscuits contain between 10 and 30% sugar and cakes are usually characterized by a sugar content between 27 and 45%, depending on the type (Wilderjans et al. 2013). The need for sugar reduction in the bakery sector is justified by the fact that 14.7% of the sugar intake among children in the US comes from sweet bakery products, with cookies and brownies being the main products (Bailey et al., 2018).

The demand for sugar in the Western World significantly increased in the last 20 years. In concern of the public health, several countries introduced or reintroduced a sugar tax on sugary food and/or beverages. Australia, for example, taxes soft drinks, confectionary and bakery products since the year 2000. In Europe Finland, Norway, Hungary and, most recently, the UK and Ireland introduced tax on sugar containing drinks and/or sweets and bakery products (Mytton et al., 2014).

The following subsections give an overview about the introduction of sugar tax as an action of the government to lower the sugar consumption of the population. Additionally, the sugar metabolism in a human body is illustrated, and the effects of an overconsumption of sugar on the human body are explained.

2.2.1. An action of the government: sugar tax

In 2015 the World Health Organisation published a guideline about the daily sugar intake, in order to prevent and control non-communicable health issues. A reduced consumption of free sugars to less than 10% of the daily calorie intake was recommended (World Health Organization, 2015).

As a measure for the high sugar consumption of the population, governments introduced a sugar tax on sugar-rich food and/or beverages. The taxation varies between countries and, thus, influenced the sugar consumption differently (Jou and Techakehakij, 2012). In the US sugar-rich soft drinks are taxed with 1-8%, but the introduction of the tax did not reduce the consumption. Mytton et al. (2012) suggested that this tax rate might be too low to influence consumers' behaviour, and referred to a modelling study, which predicted a minimum tax rate of 20% to achieve significant changes (Mytton et al., 2012).

In Hungary, the introduction of tax on convenience food caused a significant decrease by 3.4% in consumption of processed food, whereas the intake of unprocessed food increased by 1.1% (Biro, 2015). In this case the taxation of certain, as unhealthful considered, food products changed the consumes' behaviour. In general, the effect of

sugar tax on health depends on many factors, such as consumption of sugary drinks and obesity predominance within the country, as well as rate of tax (Jou and Techakehakij, 2012).

Lustig et al. (2012) reported a cost of $ 150 billion spent on essential medical health care for patients suffering from metabolic syndrome, which accounts 75% of the whole US health care budget to fight this disorder. Most recently, Ireland and the UK introduced a sugar taxation on sugar sweetened beverages. On 6th of April 2018 the UK taxed beverages containing 5-8% sugar with 18 pence per litre, and drinks with more than 8% sugar with 24 pence per litre. Furthermore, on the 1st May 2018 The Republic of Ireland introduced taxation on sugar sweetened beverages as the first country in the European Union. Beverages including 5-8% sugar are taxed with 20 cents per litre, and drinks with higher sugar content than 8% are taxed with 30 cents per litre. The introduction of tax on sugar-sweetened beverages might be a first step to reduce health issues, but governments have to consider that beverages are just one source of sugar. The second main source is sweet bakery products. The British government already paid attention to this food category by advising the bakery industry to reduce the sugar content in their products by 20% by 2020 (Tedstone et al., 2017).

2.2.2. Sugar absorption and metabolism

In order to understand the effect of sugar on health, it is essential to know, how the human body absorbs and metabolises different sugar molecules. Sucrose degradation by invertase occurs to a small extent already in the mouth (Makinen, 1989). In the stomach, some sugars are hydrolysed due to the high acidity. After passing the stomach, disaccharides, such as sucrose, lactose or maltose, are broken down by enzymes (sucrase, lactase or maltase), resulting in their monosaccharides glucose, fructose and galactose, before their absorption in the small intestine (Clemens et al., 2016).

The absorption of glucose and galactose occurs together with Na^+ from the intestinal lumen into the epithelial cell through sodium/glucose-symporter-1 (SGLT-1) (Wright et al., 2007). In order to maintain the active glucose and galactose transport, a Na^+K^+-ATPase is needed to ensure a Na^+-gradient in the epithelial cells (Figure 2.1).

Different to the glucose and galactose absorption, fructose is transported from the lumen into the epithelial cells via the glucose-transporter-5 (GLUT-5), a uniporter which actively transports fructose depending on a concentration gradient. Due to the lower

number of GLUT-5 in the membrane, the fructose transport is slower compared to glucose and galactose (Latulippe and Skoog, 2011). After the absorption of these three monosaccharides, they are all exported across the basolateral membrane into the portal circulation by the glucose-transporter-2 (GLUT2) (Figure 2.1).

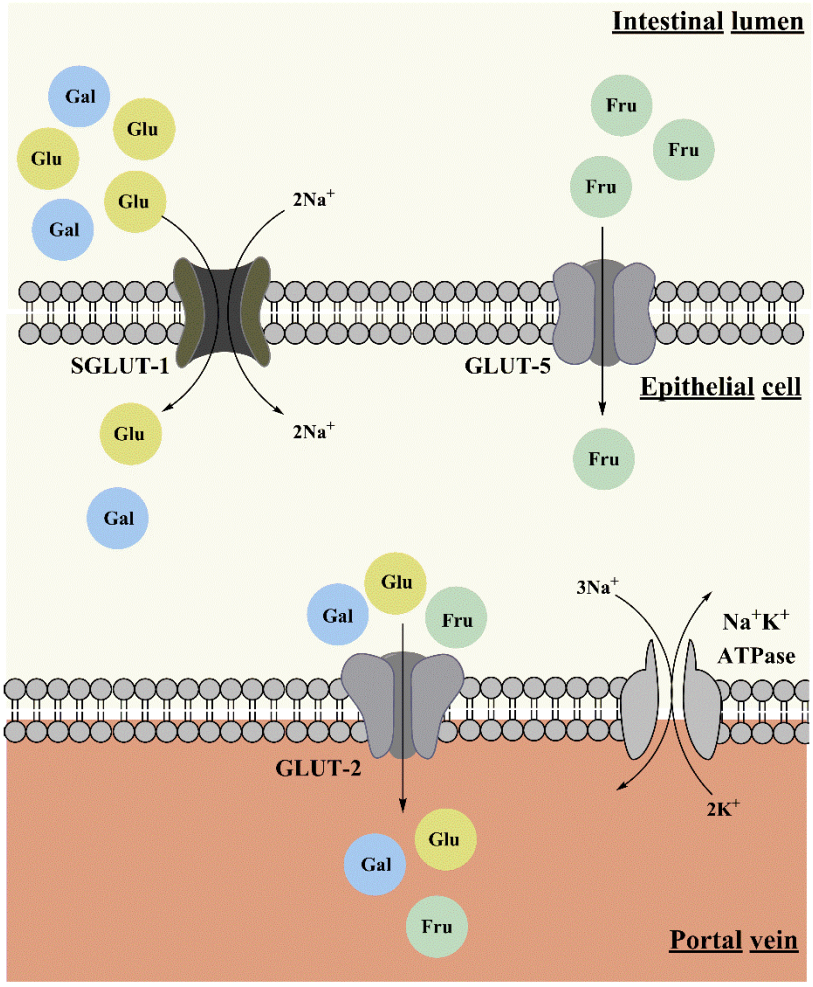


Figure 2.1 Absorption of glucose (Glu), galactose (Gal) and fructose (Fru) from the intestinal lumen through the epithelial cell into the portal vein. SGLUT-1 is the sodium/glucose-symporter-1, and GLUT-5 and GLUT-2 are active transporters

Arrived in the liver, galactose is converted to glucose by the Leloir pathway, and glucose can either be used by the liver itself or will be further transported to the systemic circulation. Glucose, which is not immediately metabolised to provide energy for the body, is stored in form of glycogen in muscles and liver cells (Clemens et al., 2016).

Fructose also needs to be converted to other molecules in order to be finally metabolized. There are two ways fructose can be processed by the human body: (a) phosphorylation by hexokinase to fructose-6-phosphate; and (b) phosphorylation by fructokinase to fructose-1-phosphate. Even though both pathways are active, most of the absorbed

fructose is metabolized via the ketohexokinase (b). Thereby, adenosine triphosphate (ATP) gets depleted into adenosine diphosphate (ADP) and further to adenosine monophosphate (AMP), which is broken down by adenosine monophosphate deaminase and results in inosine monophosphate (IMP). Further enzymatic reaction causes the conversion of IMP to uric acid (Johnson et al., 2010). Only 60 to 70% of absorbed fructose is metabolized in the liver. The rest is further transported to the kidneys, adipose tissue and other organs (Johnson et al., 2010).

2.2.3. The effect of excess sugar consumption on the human body

The majority of the world's population lives in countries, where death due to overweight and obesity occurs more often than due to underweight (World Health Organization, 2017). Several factors such as lack of exercise, predisposition and an intake of too many calories are likely to contribute to certain diseases, among which are obesity, diabetes mellitus, cardiovascular disease and dental decay. Scientists agree that the Western diet, which includes products containing high levels of high-fructose-corn-syrup (HFCS), artificial sweeteners and low calorie sugar alcohols, causes a reduction of bacterial diversity in the gut. This, in turn, results in changes of gene and metabolic pathway expressions, which is associated with obesity (Payne et al., 2012; Tilg, 2010). Furthermore, excess sugar consumption has been linked to mental health problems such as poor cognitive function and depression (Bassoli, 2006).

The most common used sugar is sucrose, also called table sugar. Sucrose is a non-reducing disaccharide comprising of 50% glucose and 50% fructose which are covalently bond (Johnson et al., 2010). This disaccharide can be hydrolysed into its monomers glucose and fructose either chemically by enzymes or acids, or physically by heat.

An overconsumption of sugar over a long period can lead to glucose toxicity. Glucose toxicity is a medical term defined as the dysfunction of pancreatic β-cells to synthesise and secrete insulin due to chronical hyperglycaemia (blood glucose level ≥ 200 mg/dL), which may become irreversible (Umpierrez et al., 2002; Robertson et al., 2003). Basically, hyperglycaemia may lead to the generation of reactive oxidative species, which cause oxidative stress and result in a dysfunction of β-cells in the pancreas. As a consequence, insulin production decreases, which leads to a permanent hyperglycaemia and eventually to diabetes (Robertson et al., 2003).

In addition, an overconsumption of sugar could also lead to an insulin resistance. In this case the human body produces insulin in excess due to hyperglycaemia, in order to decrease the blood sugar level (Leahy, 2005). As a long term effect, the communication between insulin and the insulin-receptor in target cells fails, and glucose cannot be transported into the cells. Hence, insulin as well as glucose remain in the blood. The high concentration of insulin in the blood, in turn, promotes the synthesis of triglycerides in adipocytes and may cause overweight as a long term effect (Defronzo, 2004).

Compared to other monosaccharides, fructose affects the human body differently. Firstly, fructose does not trigger an insulin secretion from the pancreas, which causes an insufficient level of leptin in the blood; a hormone which inhibits the feeling of being hungry and controls further food intake (Friedman, 2010; Teff et al., 2004).

Secondly, the intake of fructose triggers an increased expression of the forkhead protein Foxo1. In its phosphorylated form, Foxo1 inhibits the expression of enzymes which are active in the gluconeogenesis. An overexpression of Foxo1 leads to its accumulation in the dephosphorylated form in the nucleolus and, hence, causes an increase of glucose concentration in the blood. The insulin secretion for these high amounts of glucose is insufficient, and, thereby, hyperglycaemia and type-2-diabeter can develop (Lustig, 2010).

Thirdly, an excess of fructose causes an accumulation of uric acid in the blood and triggers an inhibition of nitric oxide synthase, which, in turn, decreases the amount of nitric oxide in the body and causes hypertension (Johnson et al., 2010; Nakagawa et al., 2006).

Lastly, fructose is the main driver of *de novo* lipogenesis (DNL), the synthesis of fatty acids from excess carbohydrates, in the liver (Stanhope et al., 2009). Fructose can enter the glycolysis after phosphorylation and can form xylulose-5-phosphate, when combined with glyceraldehyde. Xylulose-5-phosphate in turn stimulates the expression of all three DNL enzymes and causes an increase in acytl-CoA and free fatty acids, which can lead to steatosis, the accumulation of fat in the cell (Lustig, 2010).

2.3. Key role of sugar in bakery products

The term “sugar” is defined as all mono- and disaccharides present in foods, excluding polyols (Regulation (EU) No 1169/2011, 2011). The most common mono- and disaccharides in food are, sucrose, lactose and maltose, glucose, galactose and fructose. They all differ in their physicochemical properties as well as in relative sweetness. Sugar is one of the main ingredients in sweet backed goods. Besides sweetness, it also contributes to texture and shelf life and influences the structure formation of the backed product (Cauvain and Young, 2006).

In this section, the interactions of sugar and other main ingredients in sweet cereal-based products are illustrated. Furthermore, the impact of sugar on the quality characteristics of different baked goods, such as biscuits, cakes and sweet-leavened bakery products, is discussed.

2.3.1. Interaction of sugar with main ingredients in baked products

Sugar ensures the sweet taste, enhances flavour, but also contributes to technological properties, such as structure, mouthfeel, elasticity, colour and microbial shelf life of sweet bakery products. During the baking process, sugar interacts with other ingredients, such as water, starch, protein, yeast and fat. These interactions are explained in the following subsections.

Sugar and water

Sugar is known to have a certain affinity to water. As a consequence, sugar molecules bind to water immediately by the formation of hydrogen bonds. This interaction causes a decrease of the water activity of products rich in sugar. A lower water activity, in turn, favours a prolonged microbial shelf life, since freely available water is required for microbial growth (Cauvain and Young, 2006).

Furthermore, sugar influences the thermodynamic properties of water. The boiling point of a sugar-water-solution is elevated, and the freezing point is depressed with increasing amounts of sugar under atmospheric conditions. This is based on the fact, that hydrogen bonds between sugar molecules and water stabilize the water molecules (Clemens et al., 2016). These bonds need to be broken down before water can reach the state of boiling, and, lower temperatures are needed to realign the water molecules during freezing (Cauvain and Young, 2006; Clemens et al., 2016). Moreover, the addition of sugar to

water changes its consistency and leads to an increase in viscosity of the solution (Clemens et al., 2016).

Sugar and starch

Starch is a long chain of glucose molecules connected with each other by α-(1-4)-glycosidic bonds in amylose and additional α-(1-6)-glycosidic bonds in amylopectin.

In the pasting process, starch granules depend on free available water, in order to swell and finally gelatinize. The presence of sugar in a starch solution decreases the degree of starch hydration and swelling, due to the high affinity of sugar to water. Consequently, higher temperatures are required to achieve starch gelatinisation. Cauvain and Young (2006) reported an increase of starch gelatinization temperature from 60 °C to 80 °C by the addition of 50% sucrose. At low sugar concentrations the increase in gelatinization temperature is explained by the stabilization of the crystalline regions of starch by sugar. The addition of high sugar concentrations (>20%) shows the same effect, but the reason for the temperature increase is the lack of freely available water, which is needed for the hydration of the starch granules (Kohyama and Nishinari, 1991).

Additionally, sugar decreases retrogradation, with sucrose being the most effective type of sugar, followed by glucose and fructose. The inhibition of retrogradation is caused by the stabilization of the amorphous and entangled state of gelatinized starch by sugars binding between starch chains (Spies and Hoseney, 1982). The stabilisation of the starch molecules by sugar result in an extended shelf life reflected by a lower staling reaction in bakery products (Esteller et al., 2004).

Sugar and protein (gluten)

Wheat flour contains between 8-13.5% protein, which includes glycoprotein, globulin, gliadin, glutenin and albumin. The protein hydration causes an aggregation of gliadin and glutenin, resulting in gluten. Gluten is one of the most structure-giving raw materials in bakery products (Graveland et al., 1979; Cauvain and Young, 2006; Frazier, 2009;).

As already mentioned before, sugar has a high affinity to water. Macromolecules, such as starch, but also proteins, compete with sugar for water. The addition of sugar causes a delay of the gluten network development and weakens the protein network, due to less freely available water (Baxter & Hester, 1958; Wilderjans et al., 2013; Sahin, Axel, & Arendt, 2017).

During baking, in contrast to the interaction with starch, sugar does not influence the coagulation temperature of proteins significantly. However, reducing sugars, such as glucose and fructose, undergo a reaction with free amino acids, when temperatures between 80 °C and 140 °C are reached (van Boekel, 2001). This reaction is referred to as Maillard reaction and causes browning and formation of flavour compounds of cereal products. The intensity of the Maillard reaction depends on several parameters, such as pH, type and concentration of free amino acids and sugars, temperature, presence of buffer (such as phosphate or acetate) and water activity (Martins et al., 2000; van Boekel, 2001).

Sugar and yeast

Usually, the leavening of sweet bakery products is achieved by leavening agents, such as sodium bicarbonate, ammonium bicarbonate or baking powder (Indrani and Rao, 2008). In some sweet baked goods, such as Pandoro, burger buns or brioches, yeast, in particular *Saccharomyces cerevisiae*, is used to accomplish a dough rising. Dough fermentation is an anaerobic process in which the yeast metabolizes fermentable sugars, mainly glucose, to CO_2 and ethanol. Glucose as a substrate can either be added in form of sucrose, or it results from the enzymatic breakdown of damaged starch. Added sucrose is converted by yeast to glucose and fructose by an exocellular invertase. Damaged starch is part of the flour and is created, when granules are broken during milling. These broken granules absorb water and swell at ambient temperatures, which makes the starch susceptible for amylases. Amylases degrade starch to maltose molecules, which are subsequently converted into glucose in the yeast cells (Sluimer, 2005).

Sweet-yeast-leavened baked goods contain usually 5-20% sugar (Cauvain and Young, 2007; Pagani et al., 2014). These high amounts of sugar increase the osmotic pressure in the system and inhibit the yeast activity (Attfield, 1997). Inhibited yeast activity leads to a lower CO_2-production, and affects the texture and aroma of the final product. Hirasawa and Yokoigawa (2001) exposed baker's yeast *Saccharomyces cerevisiae* to hyperosmotic media characterized by a high sugar concentration. This treatment decreased the yeast growth, but affected only to a small extent the CO_2 production.

Sugar and fat

As already mentioned before, sugar disrupts the structure formation of starch as well as proteins. Interestingly, fat has the same effect as sugar on starch and gluten behaviour. It covers protein molecules and starch granules by hydrophobic interactions and, hence,

inhibits the hydrolysis of starch and protein by water (Ghotra et al., 2002). In the presence of high amounts of sugar, fat also coats sugar particles and prevents the solubilisation of sugar in water. Furthermore, sugar is able to enhance emulsification by forming linkages with lipids, especially in low moisture products, such as biscuits (Clemens et al., 2016; Pareyt and Delcour, 2008).

During the production of cakes, a creaming step is often conducted in order to incorporate air into the system and support the rising as well as the equal distribution of gas cells. This creaming procedure includes the mixing of sugar and fat. Thereby, sugar gets partially dissolved and stabilises the air cells, and, furthermore, undissolved sugar granules rub against the air incorporated by the fat and, thus, decrease the air cell size and a foam is formed (Wilderjans et al., 2013).

2.3.2. The effect of sugar on the quality characteristics of different baked goods

Sugar is a vital ingredient in sweet bakery products. Depending on the product, sugar fulfils different techno-functional roles. This section explains the function of sugar in different baked goods, such as cakes, biscuits and sweet yeast leavened products.

Cakes

Cakes usually contain between 27% and 45% sugar. These sugar concentrations weaken, or even prevent, the development of the gluten network, and delay starch gelatinisation. These effects on protein network and starch lead to a soft crumb texture, which is low in resilience and chewiness (Cauvain and Young, 2006).

In the cake production process, a creaming step is usually performed. This includes the high speed mixing of fat and sugar, before the addition of other ingredients. In this process, the fat surrounds the sugar crystals and protects the sugar from dissolving in the water phase. Thus, the air-fat-foam is stabilized, which leads to a higher gas holding capacity of the batter system. This ensures a homogeneous distribution of fine gas cells and increases the structure stability, which leads to a higher cake volume (Clemens et al., 2016).

Sugar also influences the colour of the cake. As mentioned before, reducing sugars, such as glucose and fructose, undergo Maillard reaction with free amino acids, resulting in an appealing brown colour. Interestingly, very high amounts of sugars in the system cause a decrease in browning due to the recrystallization (Cauvain and Young, 2006). Colour formation is also influenced by the degree of caramelisation on the crust. Different to

Maillard reaction, caramelisation is not a reaction with other macromolecules, such as proteins; the browning occurs due to the melting of sugars at high temperatures on the curst, which contributes to moisture retention. During both, Maillard reaction and caramelisation, flavour compounds are formed and influence the sensorial characteristics of the product (Frazier, 2009; Struck et al., 2014;).

Biscuits

The preparation of biscuits involves low amounts of water and high amounts of fat and sugar (up to 30%).

Biscuits contain low amounts of liquids, such as water or milk. Thus, starch granules are only partially hydrated and starch gelatinisation is limited (Chevallier et al., 2000). Furthermore, no gluten network development is observed due to the high amounts of fat and sugar as well as the low amounts of water present (Manohar and Rao, 1997).

Sugar influences the spreading of the biscuit during baking. With increasing dough temperature, the shortening and the sugar melt. This leads to a liberation of water and decreases the viscosity of the dough which results in spreading (Vetter, 1984; Sumnu and Sahin, 2008). When gluten reaches its glass transition temperature and, thus, the viscosity of the biscuits increases again, the spreading stops (Miller et al., 1996). The degree of spreading correlates positively with the amount of added sugar. This, in turn, affects the final diameter and height of the biscuit, as well as the setting time of the structure (Pareyt et al., 2009; Sman and Renzetti, 2018).

Furthermore, the recrystallization of sucrose increases the biscuit hardness after cooling and, hence, causes the characteristic fracturability of the biscuits (Pareyt et al., 2009). Caramelisation and Maillard reaction occur during baking and result in browning and flavour.

Burger buns and other sweet-yeast-leavened goods

Sweet leavened bakery products, such as burger buns, brioches, Pandoro, Colomba and Panettone, are one group of baked goods, characterized by sugar contents of 5 to 20% (Cauvain and Young, 2007; Pagani et al., 2014). While burger buns are manufactured by the addition of industrial produced yeast, brioche, pandoro and panettone are traditionally produced by the addition of sourdough as a leavening ingredient. Studies about the effect of sugar in sweet leavened bakery products are scarce. Burger buns contain up to 12% sugar, which leads to the typical dense crumb structure and contributes to flavour. The

dense crumb with equally distributed tiny cells is achieved by the inhibition of yeast activity, due to the osmotic stress triggered by the high sugar content (Sahin et al., 2017). Furthermore, burger buns have a dark crust, which is due to the high level of Maillard browning reaction (Esteller et al., 2006).

Brioches are a traditional French product and contain high amounts of butter and eggs. The sugar content in plain brioches is about 5%, which can also increase up to 8% by the addition of chocolate chips, raisins or icing after baking. Although brioches are prepared using strong flour, the dough is very sticky resulting in a soft texture of the end product (Amendola and Rees, 2003).

Panettone, Colomba and Pandoro are traditional sweet leavened baked products from Italy. They are characterized by their open structure and soft texture, due to the fermentation process and the ratio of the main ingredients. Leavening is achieved by the incorporation of natural fermented sourdough. The production procedure of these sweet leavened goods can take up to 24 h, considering sourdough refreshment and three stages of dough preparation with resting/leavening times in between. Up to seven sourdough refreshments, before dough preparation, are conducted. Each refreshment is characterized by a slightly increase in sugar content to adapt the microbial ecosystem to increased osmotic stress (Spicher and Stephan, 1987). While sourdough is the only leavening ingredient used for the production of Panettone and Colomba, additional baker's yeast *Saccharomyces cerevisiae* is sometimes used during the manufacture of Pandoro, due to the high quantities of fat (up to 27% in the final recipe). Furthermore, Pandoro shows a higher sugar amount than Panettone, which also inhibits the activity of microorganisms and affects the fermentation process (Pagani et al., 2014).

2.4. Strategies to reduce sugar in baked goods

The question how to replace sugar in bakery products by using healthy sugar alternatives challenges scientists for a few decades. The main problem with reducing the amount of sugar is to ensure the product quality in terms of appearance, sweetness, texture, volume and microbial shelf life. Sugar contributes to browning, crystallization, control of starch and protein thermal settings, structure, bulk, bodying and viscosity, fermentation, hygroscopicity, humectancy and moisture migration control, as well as freezing point depression and osmotic pressure control (Alonso and Setser, 1994). Hence, new approaches for the reduction of added sugar without losing product quality are urgently needed. The following subsections explain the most commonly used strategies of sugar reduction in bakery product, which are (2.4.1) the replacement by bulking agents in combination with artificial sweeteners, (2.4.2) the supplementation by sweet bulking agents and, additionally, (2.4.3) the potential of sweet proteins as sugar replacers is discussed.

2.4.1. Bulking agents and their use in combination with artificial sweeteners

Long chain polysaccharides, such as starches, polydextrose, maltodextrin, hydrocolloids as well as dietary fibres, are considered as bulking agents, and perform as functional ingredients. The incorporation of bulking agents compensates the loss in texture and volume, when sugar is reduced. Although these functional ingredients improve the physicochemical properties of the product, they do not contribute to sweetness, and have a negative effect on the sensorial characteristics. The incorporation of polydextrose, for example, contributes to more variation in cell size distribution in the cake batter, but, on the downside, it causes a bitter, astringent aftertaste and a mouth-drying effect (Frye and Setser, 1991; Pateras et al., 1994). The addition of high levels of polydextrose results in a high number of gas cells, which destabilise the cake batter during baking. Consequently, the final cake is low in height and volume (Hicsasmaz et al., 2003). Ronda (2005) replaced added sucrose by different non-digestible oligosaccharides as bulking agents in cakes. As an outcome, polydextrose and oligofructose contributed to browning reaction and showed a darker crust colour than the control.

Maltodextrin as a sugar replacer in sweet bakery products contributes to a creamy mouthfeel due to its very fine particle size (Setser and Racette, 1992). However, it causes a thick, leathery crust in cakes, when used in high amounts (Frye and Setser, 1991).

The use of non-sweet bulking agents as sugar replacers decreases the sweetness of the final product. In order to incorporate sweetness into the sugar-reduced product, the use of high-intensive sweeteners might be considered. High-intensive sweeteners are non-nutritive sweeteners and have a sweetness of 30-13000 relative to sucrose (Mooradian et al., 2017). They are agents which bind on the taste receptors on the tongue sending the brain the signal of sweetness. Most of the artificial sweeteners have zero calories, except aspartame (4 kcal/g) and alitame (1.4 kcal/g) (Schiffman and Gatlin, 1993).

A combination of non-sweet bulking agent and high-intensive sweeteners, in order to ensure the techno-functional properties and the sweetness of sugar-reduced bakery products, represents one approach of sugar reduction. Artificial sweeteners are allowed as food additives in fine bakery products only for special nutritional uses. The maximal dosage is between 50 and 1000 mg/kg depending on the type of sweetener (Regulation (EU) No 1129/2011).

A total sugar replacement in sponge cake by the combination of fructose, polydextrose and acesulfame-K or aspartame showed high consumer acceptance and reduced calories by 40% (Attia et al., 1993). On the contrary, Pong et al. (1991) showed that this combination causes a higher density in cupcakes, due to less incorporation of air during mixing. Sucralose together with polydextrose as sugar replacers also caused a denser crumb structure in muffins. Thus, polydextrose is not able to hold the incorporated air in the batter as effectively as sugar does (Martínez-Cervera, Sanz, Salvador, & Fiszman, 2012).

Polysaccharides in combination with high-intensive sweeteners as sugar replacers are very promising. However, thermal degradation of the polysaccharides into mono-and disaccharides has to be taken into account. For example, the total replacement of sugar by maltodextrin, xanthan gum and sucralose in a muffin resulted in 1.8% sugar in the end product, which is attributed to the degradation of maltodextrin during the process (Khouryieh et al., 2005).

Natural high-intensive sweeteners are the steviol glycosides, stevioside and rebaudioside A. They are 400 times sweeter than sucrose, cause a lower glycaemic response, are lower in calories and authorized as sweeteners in certain foods (Mooradian et al., 2017; Struck et al., 2014). The use of steviol glycosides in bakery products, such as cakes and biscuits, is not approved by the European union, yet (Regulation (EU) No 1131/2011, 2011). However, the effect of steviol glycosides on biscuits and muffins has been investigated.

Table 2.1 Characteristics of bulking agents and artificial sweeteners used as sugar replacers in sweet bakery products and their effect on the product quality characteristics

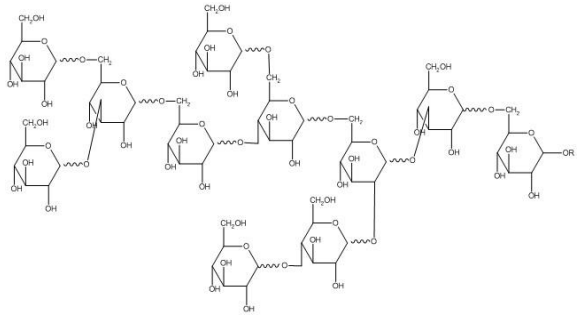
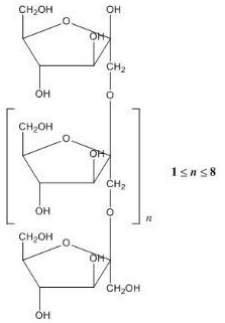
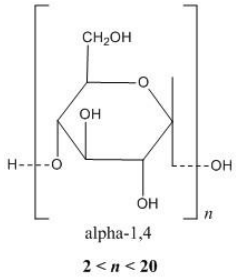
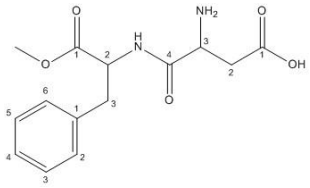
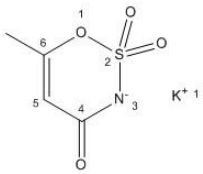
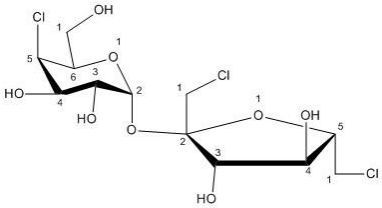
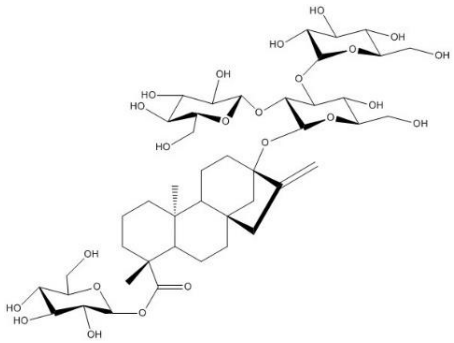
Food additive	Structure	Sweetness relative to sucrose=1	Used as sugar replacer in	Effect on the product	Reference
Polydextrose		0.2	High-ratio cake Chiffon cake Pound cake Muffin Cookies Hamburger buns	• Contributes to browning • Lowers specific volume • Increases mean air bubble size in cake batter • Decreases viscosity and viscoelasticity of batter • Decreases structure setting temperature • Increases cookie brittleness	Hicsasmaz et al. (2003) Martinez-Cervera et al. (2012) Zoulias et al. (2002) Esteller et al. (2006)
Oligofructose		0.25-0.5	Sponge cake Short dough Biscuits	• Contributes to browning • Maintains specific volume • Increases cake crumb firmness • Decreases biscuit snap force • Decreases biscuit dough hardness	Ronda (2005) Gallagher et al. (2003)
Maltodextrin		0	Biscuits	• Contributes to biscuit spreading • Contributes to browning	Pourmohammadi et a. (2017)

Table 2.1 continued

Food additive	Structure	Sweetness relative to sucrose=1	Used as sugar replacer in	Effect on the product	Reference
Aspartame		200	Sponge cake Cupcake	• Denser crumb structure • Lower specific volume • Bitter aftertaste	Attia et al. (1993) Pong et al. (1991)
Acesulfame-K		200	Sponge cake	• Lower specific volume • Less browning reaction • Harder crumb	Attia et al. (1993)
Sucralose		600	Muffins	• Denser crumb structure • Lower specific volume	Martinez-Cervera et al. (2012)
Rebaudioside A		250-450	Muffins	• Bitter aftertaste • Lower specific volume • Denser crumb structure • Harder crumb • Less browning reaction	Gao et al. (2017)

"Stevianna" a blend of 98-99% erythritol and 1-2% rebaudioside A, which is produced in New Zealand, showed promising results regarding texture and sensory properties in muffins, when sugar is partially replaced. A total replacement led to poor physicochemical properties in combination with a bitter aftertaste, a repulsive appearance, increased crumb hardness and dry mouthfeel (Gao et al., 2017, 2016).

2.4.2. Sweet bulking agents

The most common sweet bulking agents used in the area of sugar reduction are polyols. Polyols are sugar alcohols, which are either produced by chemical or biochemical reduction of sugars, or during fermentation using lactic acid bacteria or yeast. Generally, polyols are lower in calories and known to reduced postprandial glycaemia, the blood glucose level after a meal, since they do not cause insulin response (Ghosh and Sudha, 2012; Livesey, 2003). Although polyols seem to have health beneficial properties, some negative aspects need to be mentioned. The fact that some sugar alcohols cannot be fermented and are very slowly absorbed leads to the occurrence of osmotic diarrhoea, when consumed in high amounts (Bhise, 2013; Buttriss, 2017). The extent of laxative effect depends primarily on the type of polyol (Ghosh and Sudha, 2012). While mannitol and isomalt are reported to show high laxative effect, erythritol does not cause diarrhoea (Lin et al., 2010). The laxative effect caused by polyols in the gut is simplified in Figure 2.2.

Furthermore, replacing sugar by polyols results in a decrease in sweetness. Usually, depending on the type, polyols have a sweetness between 30% and 90% relatively to sucrose. Xylitol is an exception and is as sweet as sucrose. Table 2.2 shows the characteristics of some polyols and their effect as sugar replacers in specific sweet baked products.

Mannitol, sorbitol, maltitol, erythritol, isomalt, xylitol and lactitol are considered as food additives and listed as a 'E' number in the list of ingredients. They are only allowed to be added as sweeteners in products which are either 'energy-reduced' or have 'no additional sugar' (Regulation (EU) No 1129/2011, 2011). Foods containing more than 10% added polyols have to be claimed as 'excessive consumption may cause laxative effects'. Studies about partial sugar replacement by polyols in sweet baked goods showed promising results (Ronda et al., 2005; Sahin et al., 2018; Sun et al., 2014). They influence starch gelatinisation and viscosity in the same way as sugar does (Martínez-Cervera et al., 2014).

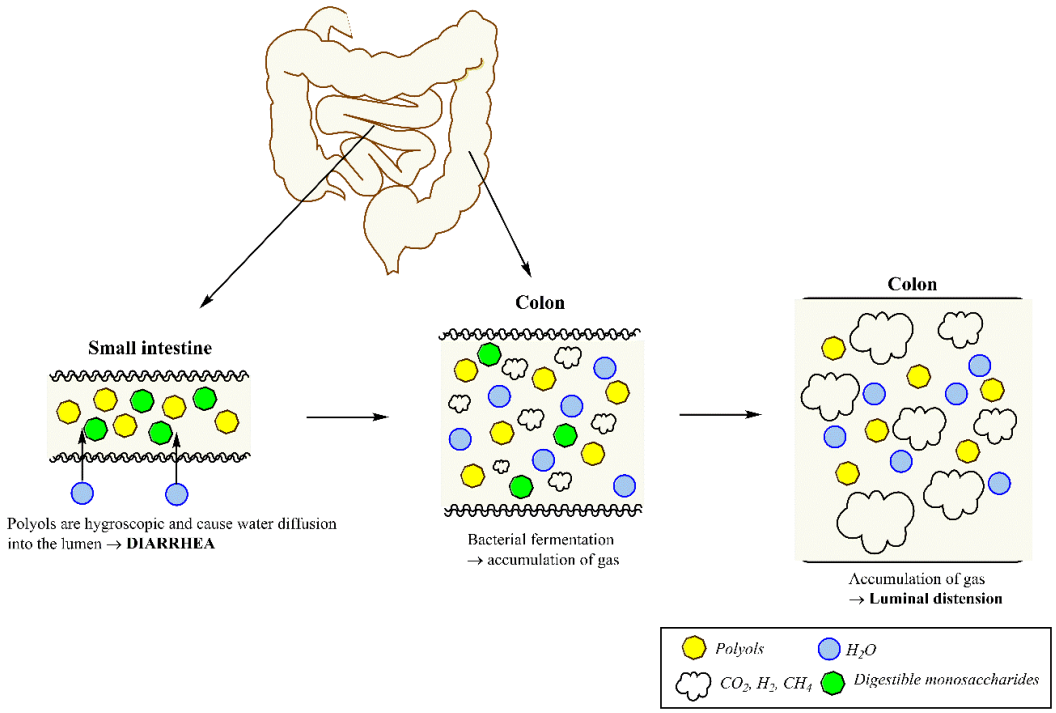


Figure 2.2 The effect of polyols on the human gut

On the downside, none of the sugar alcohols contributes to Maillard browning and causes paler crust colour, due to the missing reactive aldehyde group (Ronda et al, 2015). This is also the reason for some polyols being non-fermentable by microorganisms (Hough et al., 1979). Sugar substitution by polyols in sweet yeast leavened products decreases the yeast activity and results in reduced volume and denser crumb structure (Sahin et al., 2018; Winkelhausen et al., 2007). Additionally, sugar alcohols delay microbial growth, which leads to a prolonged shelf life. Some polyols are more suitable as sugar replacers in sweet baked goods than others, as outlined in the next paragraphs.

Xylitol, for example, has the same sweetness as sucrose and is highly accepted in terms of sensory properties by consumers (Winkelhausen et al., 2007). At the same time xylitol decreases batter stability of cakes and results in lower specific volumes (Kim et al., 2014).

Sugar replacement by mannitol in biscuits resulted in an increased firmness of the dough compared to the control. This may cause difficulties in sheeting the dough, which affects the baking process and could lead to restrictive spread (Zoulias et al., 2000). Mannitol as a sugar replacer in cakes increases batter stability. More gas cells are remained in the batter, which leads to higher specific volumes of the final product. However, mannitol reduces the adhesiveness, springiness, cohesiveness and chewiness of the cake crumb, and results in poor sensory attributes, such as dryness (Kim et al., 2014).

Table 2.2 Characteristics of polyols used as sugar replacers in sweet bakery products and their effect on the product quality characteristics

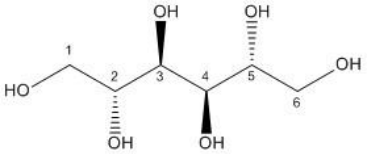
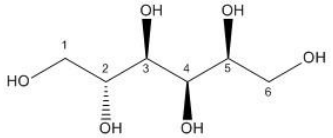
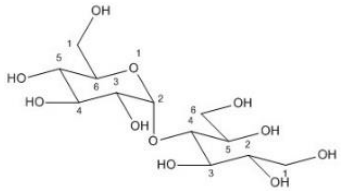
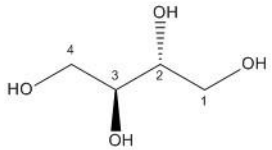
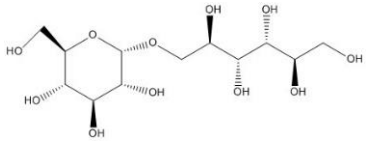
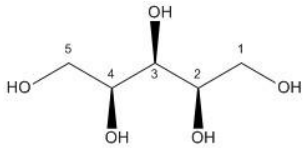
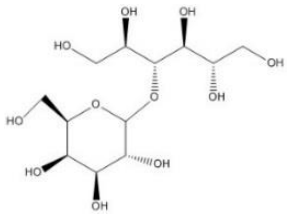
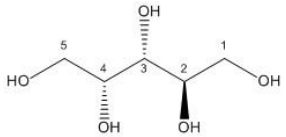
Food additive	Structure	Sweetness relative to sucrose=1	Used as sugar replacer in	Effect on the product	Reference
Mannitol		50-70	Hamburger buns Cookies Sponge cake	• Less CO_2 production by yeast • Less browning reaction • Harder crumb texture • Less cookie-spreading • Lower biscuit snap force • Higher cake batter stability • Higher specific volume in cakes	Sahin et al. (2018) Zoulias et al. (2000) Ronda et al. (2005)
Sorbitol		50-70	Cookies Sponge cake Muffins	• Lower crumb hardness in cakes and muffins • Lower snap firmness in cookies • Less biscuit spreading • Less browning reaction	Martinez-Cervera et al. (2014) Zoulias et al. (2000) Ronda et al. (2005)
Maltitol		75	Hamburger buns Cookies Short dough biscuit Muffins	• Less CO_2 production by yeast • Less browning reaction • Higher crumb hardness • Higher biscuit spreading • Higher biscuit snap force • Lower specific volume in cakes and muffins	Sahin et al. (2018) Zoulias et al. (2000) Ronda et al. (2005) Martinez-Cervera et al. (2014)
Erythritol		60-80	Short dough biscuit Danish cookies Muffins	• Lower biscuit snap force • Less cookie-spreading • Less browning reaction • Lower specific volume in muffins • Harder crumb texture in muffins	Martinez-Cervera et al. (2014) Lin et al. (2010) Laguna et al. (2013)

Table 2.2 continued

Food additive	Structure	Sweetness relative to sucrose=1	Used as sugar replacer in	Effect on the product	Reference
Isomalt		45-65	Biscuits Muffins	• Higher biscuit snap force • Lower biscuit spread+- • Lower specific volume in muffins • Lower crumb hardness	Pourmohammadi et a. (2017) Martinez-Cervera et al. (2014)
Xylitol		100	Hamburger buns Cookies Sponge cake	• Decreases CO_2 production by yeast in buns • Increases crumb hardness in buns • Decreases browning in buns • Contributes to sweetness • Lowers cookie-spreading • Decreases biscuit snap force • Decreases cake crumb hardness	Sahin et al. (2018) Zoulias et al. (2000) Ronda et al. (2005)
Lactitol		30-40	Cookies	• Contributes to cookie spreading • Decreased cookie snap force • Causes same colour as control	Zoulias et al. (2000)
Arabitol		70	Not listed in European Regulation for food additives	-	Regulation (EU) No 1129/2011

Maltitol is said to be the most similar to sucrose in the field of sweet baked goods. In biscuits and cookies, maltitol has been evaluated as the most suitable sugar replacer among all polyols. Admittedly, it increased the hardness, but it showed the highest acceptance in sensory attributes (Zoulias et al., 2000; Carocho et al., 2017;). In cakes, sugar substitution by maltitol resulted in a similar specific volume compared to the control, and was highly accepted in terms of sweetness (Kim et al., 2014; Di Monaco et al., 2018).

Erythritol is the only polyol which is absorbed in the intestine, transported to the kidneys and excreted with urine. Compared to other polyols, the absorption of erythritol is more efficient, and its intake results in a less to no laxative effect (Oku and Nakamura, 2007). Its melting behaviour differs significantly from sucrose and leads to a lighter crust colour in baked goods. An incorporation in biscuits increased dough strength and elasticity of the final product. Moreover, erythritol deceases fragility and showed lower texture quality (Laguna et al., 2013b). In cakes and muffins, a partial sugar replacement by erythritol led to a softer crumb (Cock, 2012; Lin et al., 2003). Martínez-Cervera et al. (2014) concluded that a total sugar substitution by erythritol in cakes and biscuits is not feasible. However, this polyol performed very well in combination with sucrose and can be used for a partial sugar replacement up to 75% (Lin et al., 2003; Lin et al., 2010).

A recently discovered sugar alternative called Mylose 351 in liquid syrup form and Glucodry 314 in powder form, manufactured by Tereos Syral in Belgium, showed promising results in cakes and biscuits as a sweet bulking agent. This sugar alternative is a syrup with a relative sweetness of 0.25 and a lower glycaemic index compared to mono- and disaccharides. It contains mainly maltotriose and maltotetrose and <10% mono-and disaccharides according to the manufacturer (Kweon et al., 2012a). A total replacement of sucrose by this sweet bulking agent resulted in cakes with superior product geometry compared to the full-sugar control (Kweon et al., 2012a). Mylose 351 and Glucodry 314 showed in combination with flour the desired retardation of starch gelatinization and performed very similar to sucrose (Kweon et al., 2012b).

It has to be noted that studies, which investigated the effect of Mylose 351 and Glucodry 314 as sugar replacers in cakes and biscuits, did not determine the sugar profile of the final products. Possible thermal degradation of maltotrioses and maltotetraoses into maltose or glucose was not considered, and the final product could be high in sugar after processing. The total amount of mono- and disaccharides in the final product would need

to be determined, if a “low-sugar”- (< 5 g/100 g) or “sugar free”- (<0.5 g/ 100 g) claim is aimed for (Regulation (EC) No 1924/2006).

2.4.3. Sweet proteins as potential sugar replacers

The incorporation of sweet tasting proteins represents a potential strategy to reduce sugar in sweet products. Studies about the effect of these natural sweeteners as functional ingredients in cereal based food products are scars. Currently, seven sweet tasting proteins are known: thaumatin, miraculin, curculin, monellin, mabinlin, pentadin, brazzein. All of them were identified in fruits originated in Africa or Asia. Their properties are listed in Table 2.3.

Among all, thaumatin is the only sweet protein approved as a flavour enhancer in certain food and listed as E 957 (Regulation (EU) No 2018/677). It is 2000-10000 times sweeter than sucrose and consists of 207 amino acids. According to Priya et al. (2011), thaumatin is thermostable up to 80 °C under acidic conditions and it is often used for masking astringency and off-flavours. However, thaumatin is not stable during baking or boiling (Kemp, 2006).

Curculin and miraculin modify sour taste into sweet taste. Miraculin does not possess any sweetness itself. It only turns citric acid, acetic acid and ascorbic acid into sweet taste. Curculin has a sweetness of 550 relatively to sucrose, and, additionally, modifies sour taste into sweet (Frazier, 2009).

Monellin and mabinlin are both heterodimers. While monellin is heat stable up to 50 °C, mabinlin can be heat stable during boiling for 48 h. Four different mabinlin peptides were extracted from mature seeds of *Capparis masakai*. One of them showed heat stability during boiling for 48 h, the others were thermostable to max 80 °C (Nirasawa et al., 1994; Priya et al., 2011). The thermostability was determined to be dependent on the amount of intermolecular disulphide bonds (Kant, 2005).

Another sweet protein is brazzein. Like thaumatin, brazzein is a single chain protein. It shows the highest heat stability (up to 98 °C), which is most likely due to the increased amount of disulphide bonds (Caldwell et al., 1998; Priya et al., 2011). Brazzein has already been expressed in yeast and grains. Lamphear et al. (2005) expressed the protein in corn and produced carbohydrate-reduced muffins. Sensory evaluation demonstrated a higher acceptance of the muffins with brazzein compared to the control.

Table 2.3 Properties of known sweet proteins

Sweet protein	Source	Listed as an ingredient in food	Relative sweetness to sucrose	Number of amino acids	Sequence part contacting the sweetness receptor	Active form	Thermostability
Thaumatin	*Thaumatococcus danielli*	Yes (E 957)	2.000-10.000	207	Y(57)FD	Monomer	80 °C for 4 h at pH 2
Curculin	*Curculingo latifolia*	No	Approx. 550	114	Unknown	Homodimer	50 °C for 1 h at pH 3-11
Brazzein	*Pentadiplandra brazzeana*	No	2000	54	Unknown	Monomer	98 °C for 2 h at pH 2.5-8
Pentadin	*Pentadiplandra brazzeana*	No	500	Unknown	Unknown	Unknown	Unknown
Miraculin	*Richadella dulcifica*	No	Only in combination with acids (up to 400.000)	191	Unknown	Homotetramer	Not thermostable
Monellin	*Dioscoreophyllum cumminsii*	No	3.000	45 (A-chain) 50 (B-chain)	Y(13)ASD (of A-chain)	Heterodimer	<50 °C
Mabinlin	*Capparis masakai*	No	100	33 (A-chain) 72 (B-chain)	Unknown	Heterodimer	1) not thermostable[1] 2) and 3) 80 °C for 1 h[1] 4) ≥100 °C for 48 h[1]

[1]Four different forms of Mabinlin which differ in heat stability

They were described as sweeter and more intense in flavour (Lamphear et al., 2005). Additionally, this protein is stable in beverages containing citric acid or phosphate (Hellekant and Danilova, 2005). However, brazzein is not approved as an additive in food products by the FDA.

When applied in baked goods, sweet proteins require thermostability in order to ensure the tertiary structure being responsible for the sweet taste. Since most of the sweet proteins are not heat stable, technological approaches, such as encapsulation using cyclodextrins might be worth investigating. Melting and thermal decomposition of cyclodextrins occur at temperatures of 300 °C, which are not reached during the baking process (Hedges et al., 1995).

Another possibility to increase heat stability of proteins is to change the amino acid composition. As already mentioned before, some homologues of mabinlin are more thermostable than others. The exchange of glutamine by arginine on the 47^{th} position in the B-chain increased the heat-stability significantly, while ensuring the sweet taste (Nirasawa et al., 1994).

2.5. Sourdough: How a controlled microbiota can contribute to sugar reduction in sweet bakery products

Sourdough is used as a tool to improve product quality in bread for centuries. Besides contributing to flavour and structure of the final product, sourdough is known to show health benefits, such as decreasing glycaemic index of bread or increasing mineral bioavailability due to increased phytic acid hydrolysis by sourdough (Katina et al., 2005). Furthermore, the biological production of antimicrobial compounds during sourdough fermentation contributes to prolonging shelf life (Axel et al., 2015).

Some lactic acid bacteria (LAB) and yeast strains are characterized as polyol and/or exopolysaccharide (EPS) producers. In bakery products, naturally produced sugar alcohols can contribute to sweetness and flavour, and EPS act as a bulking agent enhancing dough stability and texture of the final product. This section reveals the sourdough application in traditional bakery products and explains the polyol production by certain LAB and yeast strains. Additionally, the *in-situ* production of EPS is illustrated. A controlled sourdough fermentation with carefully chosen LAB and yeast strains could be a novel technological approach to overcome quality losses in sugar-reduced baked goods.

2.5.1. Traditional application of sourdough in sweet baked goods

The application of sourdough is very common in bread making. In France it is used to produce French Baguette; in Germany sourdough is mainly applied in the production of rye bread. In Italy sourdough is incorporated in a variety of baked goods, such as bread and pizza, but also sweet products like Pandoro, Panettone and Colomba (De Vuyst and Neysens, 2005).

In the 19th century the production process of Pandoro, Panettone or Colomba was established. At this time industrial produced Baker's yeast was not commercially available and the leavening of the product was achieved by the addition of sourdough. Besides the leavening, sourdough contributes to the formation of volatile flavour compounds, which influences the sensorial properties of the product. Traditionally, increasing amounts of sucrose are added to the sourdough itself during the last few back-slopping steps (Gobbetti and Gänzle, 2013). The addition of sucrose during sourdough preparation has two advantages: Firstly, the incorporation of sugar during sourdough-back-slopping will prepare the microbiota slowly for the higher osmotic pressure in the environment caused by sugar, and the microorganisms can adapt faster. Secondly, the

addition of sucrose triggers the production of exopolysaccharides by some lactic acid bacteria, and hence improves the dough properties and the final product quality (Gänzle et al., 2007).

The microbial composition of some traditional sourdoughs of commercially available sweet baked goods has already been investigated.

Colomba, showed higher amounts of LAB than yeasts in the study of Vernocchi et al. (2004). However, the focus in this research was the identification of yeasts in the dough during the manufacture of this baked product. *Saccharomyces cerevisiae* and *Candida milleri* were present, whereas *Candida milleri* dominated in the mother sponge.

The microbiota of the mother sourdoughs for the production of Panettone and Lagaccio, a dry biscuit developed in Genova, Italy, also showed *Saccharomyces cerevisiae* and *Candida milleri* as the two dominant yeasts (Venturi et al., 2012). *Lactobacillus sanfranciscensis* was the dominating LAB strain in both sourdough systems. Additionally, different fermentation conditions, such as temperature and time, during the production process of Panettone and Lagaccio, influenced the ratio of LAB to yeast, being much lower, when lower fermentation temperatures are applied (Venturi et al., 2012).

2.5.2. Lactic acid bacteria and yeasts as cell factories for the *in-situ* polyol production

The demand for organic products and clean label in combination with a healthy diet increased in the last decades. Hence, the incorporation of sourdough containing naturally produced polyols in sugar reduced baked goods is one possibility to ensure product quality and address the consumer's request. Since research in the field of sourdough application for sugar reduction is scarce, this subsection explains the production of different polyols by lactic acid bacteria and yeasts and discusses possibilities to increase the yield of sugar alcohols in sourdough.

The production of polyols by lactic acid bacteria depends on the metabolic pathways of certain bacteria strains. The biological reason for the synthesis of sugar alcohols is a defence mechanism to cope with environmental stress situations, such as osmotic pressure, and to prevent the cell from oxidative harm by binding free reactive oxygen radicals (Chaturvedi et al., 1997; Smirnoff and Cumbes, 1989).

The production of polyols by microorganisms received more and more attention, since the chemical synthesis in an industrial scale is expensive and the product recovery is low

(Vrancken et al., 2010). Furthermore, LAB are known to be suitable cell factories for the biosynthesis of compounds benefiting human health, such as low-calorie sugar alcohols.

In fact, LAB are the most effective mannitol producers compared to other microorganisms. The metabolic pathway for the production of mannitol by LAB is illustrated in Figure 2.3A and 2.3B.

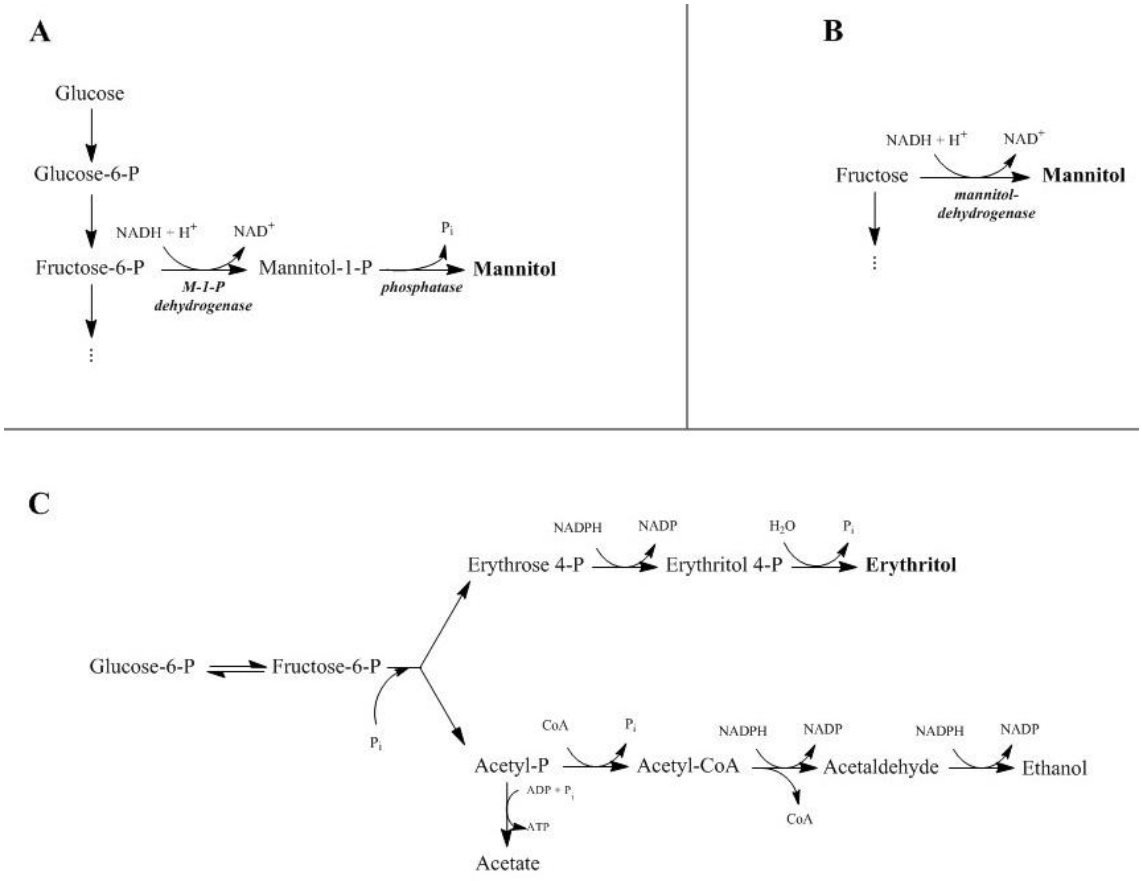


Figure 2.3 Metabolic pathways of polyol production by lactic acid bacteria. Mannitol production by homofermentative (A) and heterofermentative (B) lactic acid bacteria is showed on the top, and erythritol production by *Leuconostoc oenos* (C) is shown on the bottom. M-1-P represents mannitol-1-phosphate

The main intracellular reaction for the synthesis of mannitol is the enzymatic reduction of fructose to mannitol by mannitol-dehydrogenase (Gänzle et al., 2007). The activity of this enzyme is NADH-depended, and the reaction requires fructose as an electron accepting substrate. Mannitol producers are usually heterofermentative (Von Weymarn et al., 2002). However, a few homofermentative LAB, such as *Lactococcus lactis*, are reported to be able to produce this polyol (Neves et al., 2002). Usually, homofermentative LAB follow the glycolysis, also called Embden-Meyerhof–Parnas pathway, for the catabolism of carbohydrates. Only in cases, where homofermentative LAB have a deficient lactate-dehydrogenase, they produce mannitol to regenerate NAD^+. Heterofermentative LAB are the better mannitol producers, since they use a combination of different pathways for carbohydrate metabolism. Following the phosphoketolase pathway, heterofermentative LAB produce lactic acid and ethanol (in anaerobic conditions) from glucose. In the presence of an electron acceptor, such as fructose, NAD^+ is regenerated by the reduction of fructose into mannitol, and an extra ATP can be generated, when acetyl-phosphate is converted to acetate. Hence, in the first place, LAB use the electron acceptor fructose to regenerate more energy in form of ATP.

The conversion of fructose to mannitol by LAB in a sourdough system can be used as a technological approach to produce low calorie sweeteners naturally and reduce the total sugar content in sweet bakery products, while ensuring sweetness and flavour. Mannitol producing strains have to be chosen carefully and the process conditions for a high yield of polyol production have to be optimized.

Studies about mannitol production by LAB reveal the performance of different strains in fructose-rich MRS media. Von Weymarn et al. (2002) investigated the production of mannitol by different strains and concluded that the yield of mannitol achieved depends on the LAB species. *Lactobacillus sanfranciscensis* produced the highest polyol yield (98%), followed by *Leuconostoc mesenteroides* (91%), whereas *Lactobacillus fermentum* only converted 79% of the fructose into mannitol. *Lactobacillus brevis* achieved a yield of 67% in the study by Martinez et al. (1963), while *Leuconostoc citreum* is a very efficient mannitol producer reaching yields between 70-90% (Carvalheiro et al., 2011; Otgonbayar et al., 2011). In the sourdough used for the production of Colomba a significant amount of mannitol was determined, which occurred most likely due to the higher amounts of LAB compared to yeast and their ability to produce mannitol (Vernocchi et al., 2004).

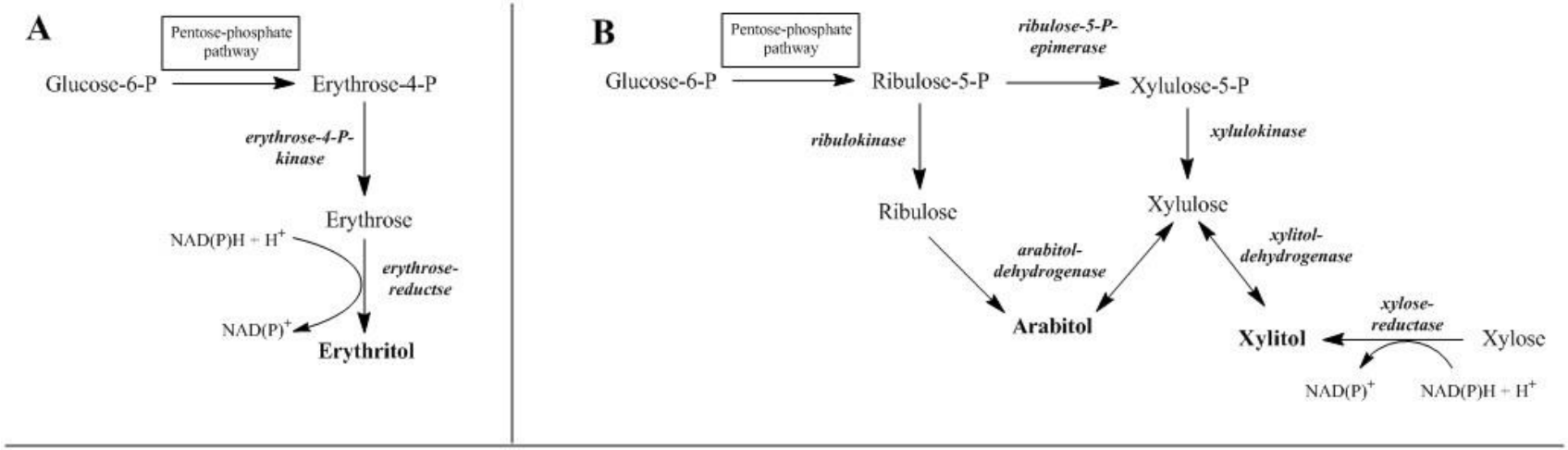
Instead of being reduced to mannitol, small amounts of fructose can be phosphorylated to fructose-6-phosphate in the cell and enter the phosphoketolase pathway resulting in acetic acid, lactic acid, ethanol and CO_2. Furthermore, naturally (uncontrolled) fermentation of barley sourdough showed significant high amounts of mannitol after six times of back-slopping (Harth et al., 2016).

Besides mannitol, the production of erythritol from glucose by different strains of *Leuconostoc oenos* (now known as *Oenococcus oeni*) has been reported by Veiga-Da-Cunha et al. (1993). The erythritol producing strain was isolated from wine and the synthesis of this sugar alcohol took place only under strict anaerobic conditions. The metabolic pathway of the synthesis of erythritol is shown in Figure 2.3C.

The microbiota of most sourdoughs is characterized by a higher population of LAB than yeasts. However, yeasts are also able to produce some polyols. This process is triggered by increased osmotic stress in the cell. Hence, most of the polyol-producing yeasts are osmotolerant.

Erythritol is the best known sugar alcohol produced by yeast fermentation in an industrial scale and the metabolic pathway is shown in Figure 2.4A. This sugar alcohol is commonly used as a sugar replacer, since its laxative threshold is the same as sucrose and fructose (approximately 130 g per day). Furthermore, its relative sweetness is between 0.6 and 0.8 (Ghosh and Sudha, 2012). *Candida magnolia*, *Pichia sorbitophila* and *Yarrowia lipolytica* belong to erythritol-producing yeasts. *Candida magnolia* isolated from honey comb, a high sugar environment, is an osmotolerant yeast and able to produce erythritol yields of up to 41% by fed-batch cultivation (Ryu et al., 2000). *Pichia sorbitophila* is also osmotolerant and releases erythritol at hypoosmotic shock. *Yarrowia lipolytica* synthesises erythritol from glycerol and, interestingly, besides erythritol, this yeast produces mannitol and arabitol additionally (Tomaszewska et al., 2012). In this study, the highest amounts of erythritol, mannitol and arabitol produced were 84 g/L, 27.6 g/L and 9.2 g/L respectively. The metabolic pathways of the production of arabitol is illustrated in Figure 2.4B.

Figure 2.4 Metabolic pathway of erythritol (A) production by yeast. (B) shows the potential synthesis of xylitol and arabitol proposed by Saha et al. (2007)



Besides *Yarrowia lipolytica*, *Saccharomyces sake*, *Asparagillus* spp., *Torulopsis versatilis* and *Torulopsis anomala* are mannitol producing yeasts. Some of these yeasts produce mannitol from glycerol; others synthesise this polyol directly from glucose (Onishi and Suzuki, 1968; Saha and Racine, 2011). It has to be considered that mannitol production occurs only under aerobic conditions. The metabolic pathway of mannitol production by yeast is not yet fully understood.

Some yeasts are able to produce xylitol, which has the same sweetness as sucrose and, thus, would be very suitable as a sugar replacer. *Candida guilliermondii*, *Candida boidinii*, *Candida parapsilosis*, *Candida famata* (also known as *Debaryomyces hansenii*) are xylitol producing yeasts. *Candida famata* is osmotolerant and able to convert xylose to xylitol. This process was investigated in detail by Converti and Domínguez (2001) and describes the involvement of an NADPH-dependent xylose-reductase and a NAD^+-dependent xylitol-dehydrogenase (Figure 2.4B). The production of xylitol by yeast requires aerobic conditions, since the enzyme activity of xylose reductase is oxygen driven. However, aerobic conditions contribute more to an increase in biomass which in turn inhibits the production of metabolites (Sibirny and Voronovsky, 2009). *Candida milleri*, a yeast which is dominating the sourdough during the production of Panettone and Lagaccio, as mentioned above, is able to produce small amounts of xylitol in the presence of xylose (Granstro et al., 2000).

Another sugar alcohol produced by yeasts is arabitol. It has a relative sweetness of about 0.7 and, as the other polyols, a low caloric value (Bhise, 2013). *Zygosaccharomyces rouxii*, *Pichia sorbitophila*, *Candida tropicalis*, *Pachysolen tannophilus*, *Candida shehatae*, *Pichia stipites*, *Torulopsis sonorensis* and *Candida famata* are just a few known arabitol producers (Kayingo et al., 2002; Kordowska-Wiater, 2015). The biosynthesis of arabitol can only be achieved under aerobic conditions. *Candida famata* produces arabitol in the stationary phase and excretes riboflavin at the same time (Sibirny and Voronovsky, 2009). *Candida tropicalis* and *Pachysolen tannophilus* are able to produce arabitol yields of 54% and 36% respectively (Gong et al., 1983). The incorporation of arabitol in sweet baked goods in order to investigate the effect of it on the product quality has not been performed yet.

Paramithiotis et al. (2006) investigated the interactions of *Saccharomyces cerevisiae* and different LAB in wheat sourdough by the determination of metabolites, such as lactic acid, acetic acid, mannitol, glycerol and ethanol. Monocultures showed lower concentration of produced mannitol and acetic acid than co-cultures. The highest amount of mannitol was achieved using a co-culture of *Saccharomyces cerevisiae* and a *Lactobacillus brevis* strain.

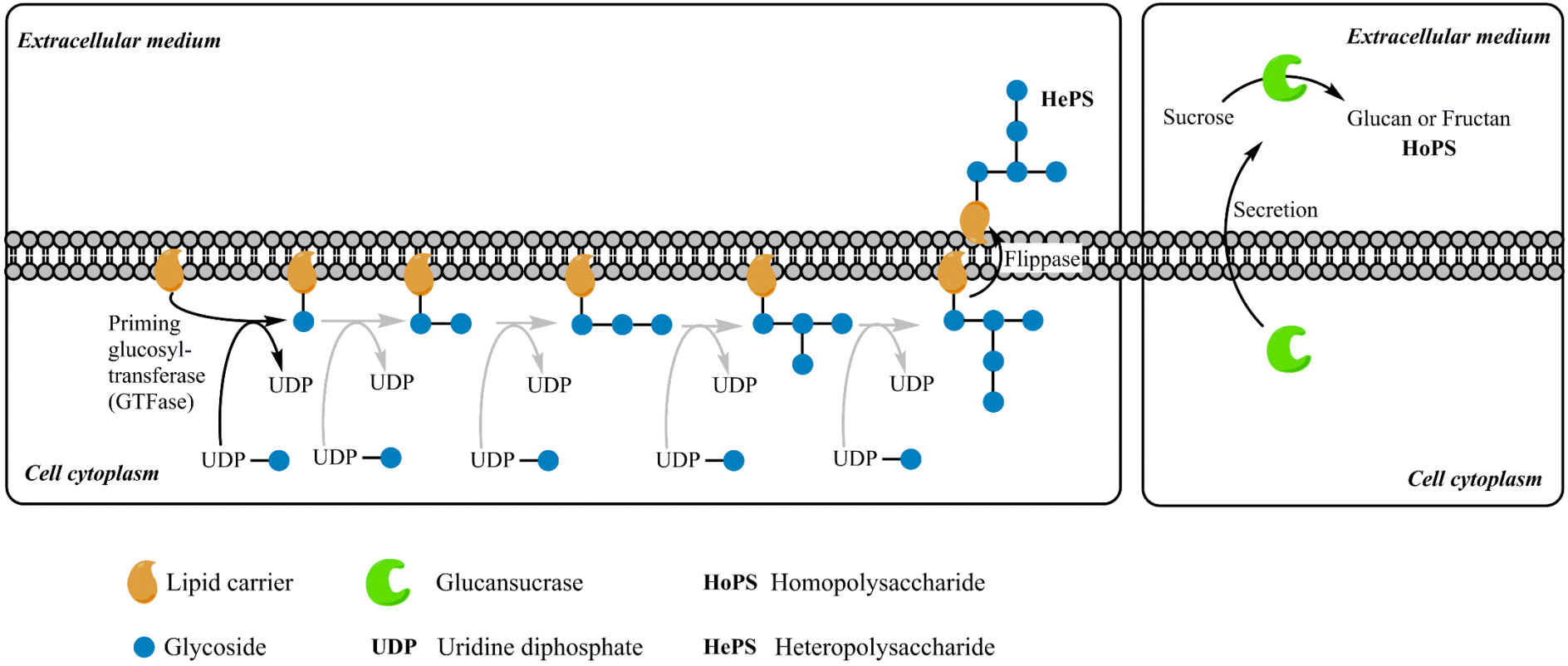
2.5.3. Exopolysaccharides: Molecules which ensure product quality in sugar reduced baked goods

Sweet baked goods which are reduced in sugar often show poorer techno-functional properties compared to sugar rich products. Sugar-reduced cakes, for example, have a low specific volume and a harder crumb texture (Attia et al., 1993). As already mentioned before, long chain carbohydrates are bulking agents and are able to compensate these quality losses in sugar reduced bakery products. Even though they do not increase sweetness, polysaccharides improve techno-functional properties of the product by increasing the dough or batter stability (Setser and Racette, 1992; Galle et al.2011).

Exopolysaccharides (EPS) are long chain sugar molecules, which can be naturally produced by lactic acid bacteria or yeast. There are two groups of EPS, homopolysaccharides and heteropolysaccharides. The synthesis of both types of EPS is illustrated in Figure 2.5. The main difference is the location of the biosynthetic process. Heteropolysaccharides are produced in the cell cytoplasm, while homopolysaccharides are synthesised in the extracellular medium.

Homopolysaccharides are extracellular polysaccharides containing just one type of monosaccharides, such as glucose or fructose in glucans or fructans respectively. The biosynthesis of homopolysaccharides is mediated by glucosyltransferase for glucans and fructosyltransferase for fructans. These are extracellular associated enzymes, which need sucrose as an initial substrate. Thus, these two enzymes are also known as glucansucrase and fructansucrase and can be generalized as glycansucrases (Gobbetti and Gänzle, 2013).

Figure 2.5 The production of homopolysaccharides (HoPS) and heteropolysaccharides (HePS) by microorganisms (Exopolysaccharide production). Modified from Lynch et al. (2018)



Bacterial glycansucrase have two functions: (1) it catalyses the hydrolysis of sucrose into glucose and fructose and (2) it mediates the synthesis of high molecular polysaccharides, such as glucans and fructans. The energy received by the hydrolysis of sucrose into fructose and glucose is essential for the polymerization process. Some homopolysaccharides producing LAB strains were isolated from sourdough (Tieking et al., 2003). Tieking et al. (2003) screened different LABs for their ability to produce EPS. In this study *L. sanfranciscensis*, *L. frumenti* and *L. pontis* were identified as fructan-producers, while one *Lactobacillus* spp. strain produced glucans. Some *L. reuteri* and *W. confuse* strains produced glucans, other synthesised fructans. A cereal related *Leuconostoc citreum* was reported to produce inulin, a fructan characterized by predominantly β-(2→1) linkages, by the expression of inulinase (Tieking et al., 2003). As a conclusion, the LAB strain needs to be carefully chosen, since EPS production is strain depended.

Heteropolysaccharides contain more than one type of monosaccharides. These long chain polysaccharides can contain glucose, fructose, galactose, rhamnose, but also derivates, such as N-acetylglucosamine and N-acetylgalactosamine. The biosynthesis is performed by an intracellular glycosyltransferase (De Vuyst et al., 2001). Different to homopolysaccharides, the production of heteropolysaccharides does not need sucrose as an initial substrate to trigger the polymerization. Since the synthesis of heteropolysaccharides competes with the production of peptidoglucan for cell protection, the yield of heteropolysaccharides is very low. Studies about heteropolysaccharides in sourdough are scarce (Gobbetti and Gänzle, 2013). Galle et al. (2011) investigated the levels of synthesized heteropolysaccharides in wheat and sorghum sourdough fermented with strains of *Lactobacillus casei* and *Lactobacillus buchneri*. According to this study, heteropolysaccharides decrease the resistance of deformation of the dough, which causes an increase in specific volume and a softer crumb in gluten free bread (Arendt et al., 2007). This effect on dough rheology and stability could improve techno-functional properties of sugar-reduced products.

Interestingly, not only LAB synthesise exopolysaccharides, also some yeast strains are known for their potential to produce EPS. There are several yeast genera which are identified as EPS producer, such as *Aureobasidium*, *Bullera*, *Candida*, *Cryptococcus*, *Pichia*, *Phomopsis*, *Exophiala*, *Lipomyces*, *Rhodotorula*, *Sporobolomyces*, *Tremella* and *Trichosporon* (De Baets et al., 2002; Leathers, 2002). A *Cryptococcus laurentii* strain together with its EPS, for example, are patented as a food additive in Russia

(Satyanarayana and Kunze, 2009). It has been reported that EPS produced by this yeast strain is a bioactive compound, which lowers the concentration of cholesterol and triglycerides in blood serum. EPS-producing yeasts were previously isolated from penguin feathers, Arctic soil, lichens and mosses, but also from milk kefir (Satyanarayana and Kunze, 2009). Compared to EPS produced by lactic acid bacteria, yeast-EPS has a very complex structure, constructed by linear mannans which are branched to heteropolysaccharides, such as galacto- and glucooligosaccharides and/or pullulans (Gientka et al., 2015). The composition and construction of the EPS depends on several conditions, such as media composition, pH, temperature and oxygen concentration (Pavlova et al., 2009; Rusinova-Videva et al., 2010). Gientka et al. (2015) reported the optimal process conditions for EPS production by yeast being low temperature and high aeration. Additionally, sucrose should be present in the medium as a carbon source and an inorganic nitrogen source should be added in a ratio of 15:1, carbon source to nitrogen source respectively. Other studies showed the highest EPS production when maltose was present (Gientka et al., 2016).

In general, the yield of EPS produced by yeast is low and, hence, an industrial production is not considered yet. However, some of the exopolysaccharides produced by yeast seem to have antitumor, immunostimulatory and antioxidant activity, whereas others are able to absorb heavy metals and can be used in medicine. For the development of food products, these EPS can be used as thickeners and stabilizers (Gientka et al., 2015).

The incorporation of sourdough fermented by EPS-producing lactic acid bacteria showed a positive effect on the technological properties of baked goods. On the one hand EPS improves dough rheology and facilitates dough handling, on the other hand they give structure to the final product and enhances stability (Decock and Cappelle, 2005). EPS act as bulking agents in the dough system and, thus, could compensate the bulking loss in sugar reduced products. The addition of sourdough rich in EPS could be a novel technological approach to overcome quality loss of the techno-functional properties of sugar-reduced sweet bakery products

2.6. Conclusion and future aspects

Sugar reduction in sweet baked goods is challenging, since sugar interacts with all ingredients and undergoes several reactions during the baking process. Sugar decreases the water activity and influences boiling and freezing points, delays starch gelatinisation and gluten network development and influences yeast activity. Additionally, it promotes emulsification in combination with fat. Depending on the product, sugar contributes to volume, texture, structure, browning, biscuit spreading, microbial shelf life and taste. With the reduction of sugar, these quality parameters change, which affects the final product. The combination of polysaccharides, such as polydextrose, oligofructose or maltodextrin, in combination with high-intensive sweeteners is promising, but needs further investigation and optimisation. The use of sweet bulking agents, such as polyols, is only feasible as partial replacement of sugar. Sweet proteins are another category of potential sugar replacer. Thaumatin is the only sweet protein, which is allowed to be used as a flavour modifier. However, in order to use this protein in bakery products, a higher thermostability would be required. Encapsulation methods might be an approach to increase heat-stability and to ensure the sweetness of the protein. Sourdough technology should be considered as a novel technological approach to overcome quality loss in sugar-reduced bakery products. The presence of strong mannitol producing LAB, such as *Leuconostoc citreum* and *Leuconostoc mesenteroides*, as well as *Lactobacillus sanfranciscensis* and *Lactobacillus brevis* shows very good starting conditions to increase the mannitol concentration in sourdough. Furthermore, the production of exopolysaccharides could contribute to texture improvement of sugar-reduced products. Future studies may consider the performance of a controlled sourdough fermentation with selected LAB and yeast strains to increase the amount of naturally produced polyols and exopolysaccharides. Furthermore, different bioprocessing technologies, such as fed-batch fermentation, or an additional aerobic secondary fermentation for polyol production by yeast might be investigated.

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Chapter 3

UNDERSTANDING THE FUNCTION OF SUGAR IN BURGER BUNS – A FUNDAMENTAL STUDY

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Abstract

The consumption of sugar-reduced bakery products represents a promising way to decrease health problems such as obesity, which can be related to the increased intake of added sugar. One highly consumed food product are burger buns, which can contain up to 12% sugar. This study evaluates the impact of sugar-reduction on the quality of burger buns and their respective doughs from a fundamental perspective. Sucrose was replaced by wheat starch in 1% steps and compared to a control burger bun (10%). A 30%-sugar reduction increased gas formation during fermentation, which resulted in a higher specific volume (3.85 ± 0.08 ml/g) when compared to the full-sugar product (3.52 ± 0.07 ml/g). The gluten network developed faster when no sucrose was added (−66 s). It also became stronger (+11 BU) causing a decrease in dough extensibility. Sugar-reduction led to a lighter crust and higher water activity [0.915 ± 0.006 (full sugar), 0.948 ± 0.004 (no-added sugar)], which shortened shelf life by 6 days. Sugar reduction is highly correlated to dough characteristics, which result in quality changes of the dough as well as product quality parameter. PCA analysis of the data revealed that the addition of 7% sucrose is essential to ensure adequate burger bun quality.

3.1. Introduction

Burger buns are sweet yeast-leavened bread products that are highly appreciated by consumers for their good taste and soft texture. Sucrose is a principal ingredient in sweet baked goods. In particular, in burger buns the amount of sugar ranges generally between 7 and 12%, and can be even higher depending in which country they are sold (Cauvain and Young, 2007). It is well known that increased consumption of added sugar is linked to numerous health problems such as obesity, cardiovascular disease, diabetes and dental caries. According to the WHO, the consumption of added sugar should not exceed 25 g per day to reduce the risk. As a result of the above mentioned conditions the demand for sugar-free or sugar-reduced products is increasing steadily (World Health Organization, 2015).

Bakery is one of the leading sources for added sugar. In yeast-leavened bakery products sugar is an energy source for the baker's yeast. It contributes to a higher CO_2 and ethanol production, which leads to a faster and more efficient fermentation process (Gelinas, 2006). Furthermore, sugar increases the gelatinisation temperature, which then affects volume and appearance of the final product (Bean and Yamazaki, 1978). This increase is caused by the interaction of the starch chains and sugar. Sugar stabilizes the amorphous regions of the starch and, consequently, restrict its flexibility (Kim and Walker, 1992). Additionally, since sugar competes with gluten for water, the protein hydration is restricted, and a dispersion of gluten polymers occurs, which results in less cross-linking of the protein network (Perego et al., 2007).

Regarding the product quality criteria, sugar contributes to the colour formation in baked goods and Maillard reaction, which also influences flavour formation (Davis, 1995). Finding a sucrose substitute is challenging, as the alternative needs to be able to mimic all of its attributes. Scientific literature in sugar-reduction in burger buns is scarce. This study provides a fundamental understanding of the role of sugar in sweet yeast-leavened products, to provide pivotal principals to produce sugar-reduced or even sugar-free baked goods.

3.2. Materials and methods

3.2.1. Materials

Baker's flour (Odlums Group, Dublin, Ireland) with a protein content of 12% and a moisture of 13.5%, tap water, sunflower oil (Musgrave Wholesale Partners, Dublin, Ireland), instant active dried baker's yeast *Saccharomyces cerevisiae* (Puratos, Groot-Bijgaarden, Belgium), salt (Glacia British Salt Limited, Cheshire, UK), wheat gluten (Roquette, Lestrem, France), ascorbic acid (Storefast Solutions, Northfleet, UK), sodium-stearoyl-lactylate (SSL) (Danisco, Copenhagen, Denmark) were used in this study. Sucrose (Siucra, Dublin, Ireland) was replaced by wheat starch (Roquette, Lestrem, France) as a bulking agent. The control burger bun, containing 10% added sucrose, was produced using these ingredients according to the recipe (Table 3.1).

Table 3.1 Recipe for the control burger bun containing 18.8% added sugar based on flour, which is equivalent to 10% added sucrose in the whole recipe

Ingredients	Amount based on flour [%]
Wheat flour	100.0
Water	55.0
Sucrose	18.8
Sunflower oil	6.7
Salt	1.7
Baker's yeast	2.0
SSL	0.5
Ascorbic acid	0.1
Wheat gluten	3.2

3.2.2. Adjustment of the water content

To ensure the same dough consistency for each sucrose level, the water content of the sugar-reduced recipes was adjusted to the control using Farinograph E (Brabender OHG, Duisburg, Germany). The chamber temperature was kept at 30 °C and a mixing speed of 63 rpm was applied. The sucrose content was reduced from 10% to 0% in 1% steps.

3.2.3. Dough analysis

Dough preparation

Instant active dried baker's yeast (*Saccharomyces cerevisiae*) was activated for 10 min in tap water (25 °C). All solid ingredients were mixed for 1 min at minimum speed using Mixer Titanium Major (KM020) (Kenwood, Havant, UK), followed by the addition of oil and activated yeast solution to the solid ingredients, and kneaded for 8 min at speed one. For the evaluation of the viscoelastic properties of the dough and for the imaging of the protein network by confocal laser scanning microscopy, yeast was omitted.

Fermentation quality

The fermentation quality of the dough was determined using Rheofermentometer F3 (Chopin, Villeneuve-la-Garenne, France). 300 g of dough was prepared as described above and placed in the fermentation chamber at 30 °C. A weight of 1500 g was placed on the dough and the dough was fermented for 3 h. The dough development and the gas release were detected, and the time needed to achieve the maximum height (T1), the total volume of the dough reached by carbon dioxide production (V_{tot}), the maximum height of the dough (Hm), and the height of maximum gas formation (Hm′) were evaluated.

Viscoelastic properties of the dough

The viscoelastic properties of the dough were determined using the Rheometer Physica MCR 301 (Anton Paar GmbH, Ostfildern, Germany). Parallel plates' geometries, serrated to prevent slippage, were used. The temperature of the lower plate was set to 30 °C and used in conjunction with a 50 mm diameter upper plate. The linear viscoelastic region was determined by performing an amplitude sweep as described by Hager et al. (2011). Frequency sweeps were conducted using a target strain of 0.01% and a frequency range from 100 to 0.1 Hz. Before each test, the sample rested for 5 minutes to allow equilibration. The viscoelastic properties were determined by the damping factor which represents the ratio between the lost (G″) and stored (G′) deformation energy of the system, tan (G″/G′).

Gluten network development

The influence of sugar on the gluten network development was determined using GlutoPeak (Brabender, Duisburg, Germany). For these measurements, the standard ratio of 50/50 (solid/liquid; w/w) regarding the recipe was used. The torque of the sample was

measured running a constant speed of 2750 min^{-1} and a chamber temperature of 36 °C for 300 s.

Extensibility and resistance to extension

The Extensograph (Brabender, Duisburg, Germany) was used to analyse the extensibility of the dough. Dough pieces of 150 g were moulded and put in the proofing chamber at 30 °C and the extensibility of the dough was measured after proofing for 60 min.

Ultrastructure of the dough

Confocal laser scanning microscopy (CLSM) was used to analyse the gluten network visually. Dough containing 0%, 5% and 10% added sucrose was prepared as described above. The gluten network was dyed by the addition of rhodamine B (Sigma-Aldrich, St Louis, MO, USA), using the method of Jekle and Becker (2011). After mixing the dough it was frozen overnight. A slice of one millimetre thickness was cut off the frozen dough using a scalpel and placed on the slide, covered with a glass cover slip. Dough samples were viewed using Olympus IX81 inverted laser microscope (Olympus, Center Valley, PA) with FluoView FV3256 scanning unit under 20× objective. Proteins were monitored using λ_{exc} = 543 nm and λ_{em} = 590/50 nm.

3.2.4. Quality characteristics of burger buns

Burger bun preparation

Dough (80 g) was moulded and placed on a burger bun tray with a diameter of 105 mm and a height of 15 mm. The buns were fermented at 85% relative humidity and 30 °C in a proofer (KOMA SunRiser, Roermond, The Netherlands) for 1 h. After proofing, the burger buns were baked for 14 min at 200 °C using a deck oven (MIWE condor, Arnstein, Germany), cooled down for 2 h at room temperature and packed in plastic bags. The specific volume, the crumb texture, the crumb structure, and the colour of three buns per batch per sucrose level were analysed 2, 24, 48 and 120 h after baking.

Specific volume

The specific volume of the burger buns was determined using VolScan Profiler (Stable Micro Systems, Godalming, UK).

Crumb hardness and staling

For the determination of the crumb texture, the bottom and the top of the burger buns were cut to a resulting height of 35 mm. The texture was analysed using TA-XT2i texture analyser (Stable Micro Systems, Godalming, UK) as described by Dal Bello et al. (2007).

The rate of staling was evaluated using the following formula:

$$Rate\ of\ staling = \frac{\left(Crumb\ hardness\ (120\ h) - Crumb\ hardness\ (2\ h)\right)}{Crumb\ hardness\ (2\ h)}$$

Crumb structure

The crumb structure was analysed by a C-cell Bread Imaging System (Calibre Control International Ltd., Warrington, UK). The slice area [mm^2] and the area of cells [%] were evaluated.

Crust and crumb colour

The colour of the crumb and crust was measured using Colorimeter CR-400 (Konica Minolta, Osaka, Japan). The CIE L* a* b* colour system was used for the evaluation of the measurements; the L*-value, which shows the lightness, was evaluated.

Water activity and microbial shelf life

The water activity of the crumb was measured with a water activity metre (HygroLab, Rotronic, Bassersdorf, Switzerland). The influence of sugar on the shelf life of burger buns was evaluated using the mould environmental challenge method indicated by Dal Bello et al. (2007). Burger buns were halved horizontally and put on a sterile metal rack. The crumb-side of each half was exposed to the environment for 5 min before they were packed separately in a sterile plastic bag and heat sealed. Two filter pipettes were inserted in each bag to ensure comparable aerobic conditions. The samples were stored in a room with an average temperature of 20 ± 2 °C and the mould growth was monitored for 14 days. The mould growth was visually evaluated and rated as “mould-free”, “<10% mouldy”, “10–24% mouldy”, “25–49% mouldy”, “>50% mouldy”.

Sensory evaluation

Burger buns containing different amounts of added sucrose were evaluated regarding their sweetness-intensity and hedonic, which included appearance, flavour, aroma, texture and sweetness. For the evaluation of the sweetness intensity a sensory panel of 12 people was trained. The crust of the buns was removed to prevent optical influences. The panellists judged the samples on a scale from 0 to 10. The training was conducted by tasting burger buns with 0% sugar addition, as a score of "0" on the scale from 0 to 10, and a burger bun with 10% added sugar as a score of "10" on the scale from 0 to 10. Regarding the sweetness-intensity 0 was "not sweet" and 10 described as "very sweet", whereas the scale for the hedonic ranged from 0 for "extremely dislike" to 10 for "extremely like", with 5 indicating "neither like nor dislike". The sensory evaluation was performed in duplicates on two different days. The evaluated samples were burger buns containing 0–10% sucrose in 2% steps. In addition, the panel was asked for their favourite sample.

3.2.5. Statistical analysis

Dough analysis and burger bun analysis were performed in triplicates. A variance analysis (one-way ANOVA, $p \leq 0.05$, Tukey test) was performed using Minitab 16. Additionally, correlation analysis between all results and the sucrose level was conducted using Microsoft Excel 2010 and PCA analysis was performed using R. For the PCA analysis the sensory evaluation was left out.

3.3. Results

To understand the role of sugar in the burger bun system, the added sucrose content was replaced by wheat starch, and dough attributes as well as burger bun properties were investigated. The sweetness-intensity and other sensory properties were determined by a sensory panel. For a deeper inside a correlation analysis as well as a PCA was performed.

3.3.1. Adjustment of the water content

In this study sugar was reduced from 10 to 0%. The sugar was replaced by wheat starch as a bulking agent. Preliminary studies (data not shown) revealed that wheat starch was the most suitable replacement. Nevertheless, starch has a higher water holding capacity than sugar. Therefore, the water level of the various formulations needed to be adjusted using farinograph (Table 3.2). A total replacement of sucrose by wheat starch required 12.5% more water addition to ensure the same dough consistency of the control recipe.

3.3.2. Dough analysis

Fermentation quality

The characterisation of the dough development and gaseous release during fermentation was performed using the rheofermentometer and included an evaluation of Hm, Hm′, V_{tot} and T1. Dough without any addition of sucrose showed the lowest Hm in the dough development curve (Table 3.2), whereas the highest Hm was achieved by adding 1% sucrose, followed by the addition of 10% sucrose. Furthermore, the longest time needed for the dough to achieve the maximum height (T1) was recorded for the dough, where no sugar was added, while the fastest dough development occurred when 2% sucrose was added. The gas release curve gives the values Hm', and V_{tot}. The curve of dough containing little amounts of added sucrose (0–2%) increased at the beginning of the fermentation, followed by a decrease and a second increase. Dough containing more than 2% added sucrose did not show any decrease of gas release. Hm′ and V_{tot} showed the highest values for dough containing 7% added sucrose. The lowest values were measured, when no sucrose was added.

Table 3.2 Results of dough analysis. V_{tot} is the total volume of the dough reached by CO_2 production, Hm is the maximum dough height after fermentation, T1 is the time needed to achieve Hm and Hm' is the maximum gas formation. PMT stands for peak maximum time of the gluten network development and TM is the torque maximum during the measurement of the gluten network formation

Added sucrose [%]	Water absorption to 530 BU (based on flour) [%]	V_{tot} [ml]	Hm [mm]	T1 [min]	Hm' [mm]	Damping Factor [1]	PMT [s]	TM [BU]	Extensibility after 60 min fermentation [mm]	Resistance to Extension after 60 min fermentation [BU]
0	67.25 ± 1.48	1434 ± 13 (d)	54.8 ± 0.9 (a)	160 ± 6 (a)	70.8 ± 0.9 (c)	0.313 ± 0.002 (d)	94.0 ± 4.2 (f)	55.5 ± 0.7 (a)	104.0 ± 2.8 (f)	475.0 ± 21.2 (ab)
1	66.25 ± 0.07	1868 ± 106 (c)	65.8 ± 4.9 (a)	126 ± 20 (ab)	100.6 ± 9.7 (ab)	0.355 ± 0.003 (cd)	99.5 ± 0.7 (ef)	54.0 ± 0.0 (a)	103.5 ± 2.1 9 (ef)	475.0 ± 21.2 (ab)
2	65.25 ± 0.21	2057± 49 (bc)	57.4 ±3.9 (a)	104 ± 7 (b)	103.0 ± 3.8 (ab)	0.374 ± 0.003 (abc)	103.5 ± 0.7 (def)	52.5 ± 0.7 (ab)	108.0 ± 1.4 (def)	500.0 ± 14.1 (ab)
3	64.10 ± 0.14	2207±51 (abc)	62.2 ± 3.3 (a)	122 ± 11 (ab)	97.3 ± 1.8 (ab)	0.372 ± 0.016 (abc)	105.0 ± 1.4 (cdef)	52.5 ± 0.7 (ab)	104.0 ± 1.4 (ef)	415.0 ± 21.2 (ab)
4	63.01 ± 0.15	2386± 54 (ab)	64.7 ± 2.6 (a)	136 ± 3 (ab)	102.4 ± 0.9 (ab)	0.343 ± 0.016 (cd)	113.5 ± 3.5 (bcde)	50.5 ± 0.7 (bc)	114.0 ± 1.4 (cde)	480.0 ± 28.3 (ab)
5	62.20 ± 0.42	2378 ± 30 (ab)	62.5 ± 6.7 (a)	122 ± 13 (ab)	102.9 ± 0.4 (ab)	0.364 ± 0.012 (bcd)	115.5 ± 3.5 (bcd)	50.0 ± 1.4 (bc)	115.5 ± 9.2 (abcde)	500.0 ± 28.3 (ab)
6	60.86 ± 0.02	2405± 67 (ab)	58.9 ± 0.6 (a)	117 ± 4 (ab)	104.0 ± 1.5 (a)	0.365 ± 0.004 (cd)	120.0 ± 0.0 (bcd)	48.0 ± 0.0 (cd)	120.0 ± 2.8 (abcd)	510.0 ± 14.1 (a)
7	59.75 ± 0.35	2428 ± 148 (a)	60.4 ± 0.4 (a)	115 ± 1 (ab)	104.4 ± 2.5 (a)	0.382 ± 0.016 (abc)	123.5 ± 5.0 (bc)	46.5 ± 0.7 (de)	124.5 ± 3.5 (abc)	455.0 ± 28.3 (ab)
8	59.00 ± 0.71	2184 ± 176 (abc)	58.9 ± 2.4 (a)	137 ± 24 (ab)	99.7 ± 3.8 (ab)	0.402 ± 0.010 (abc)	124.5 ± 6.4 (b)	46.5 ± 0.7 (de)	130.0 ± 0.0 (a)	420.0 ± 28.3 (ab)
9	57.00 ± 1.41	1934 ± 123 (c)	57.7 ± 8.2 (a)	155 ±19 (ab)	89.6 ± 0.2 (b)	0.414 ± 0.029 (ab)	127.0 ± 9.9 (b)	46.0 ± 1.4 (de)	129.0 ± 1.0 (ab)	405.0 ± 28.3 (ab)
10	55.00 ± 0.00	2024 ± 26 (c)	65.0 ± 3.5 (a)	146 ± 8 (ab)	90.8 ± 0.0 (ab)	0.424 ± 0.010 (a)	161.0 ± 7.1 (a)	44.5 ± 0.7 (e)	115.0 ± 0.0 (bcde)	385.0 ± 7.1 (b)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

Viscoelastic properties

Viscoelastic properties of the dough are evaluated by determining the behaviour of G′ and G″ during a frequency sweep. All measurements showed G′ > G″ in the dough system. Consequently, the dough system is more elastic than viscous. Since, the damping factor increased with increasing sucrose content and reached the highest value, when 10% sucrose was added (Table 3.2), the viscous portion increased with the addition of sucrose.

Gluten network development

Figure 3.1 shows the effect of sucrose on the gluten network formation measured under shearing over time in the GlutoPeak. By increasing the sucrose level, the torque force decreased (Table 3.2) indicating a progressive delay in gluten network development.

Extensibility and resistance to extension

The extensibility and the resistance to extension were measured to characterise the dough strength and the gluten network quality after fermentation (Table 3.2). Extensibility increased with increasing sucrose level and reached the highest value, when 8% sucrose was added. Except for doughs containing 6% and 10% added sucrose, the resistance to extension did not show significant differences. Dough containing 6% sucrose resulted in the highest resistance to extension, whereas an addition of 10% sucrose led to the lowest value.

Ultrastructure of the dough

CLSM was applied to visualise the influence of added sucrose on the gluten network of the dough (Figure 3.1). Micrograph (A) shows dough without any addition of sucrose and exhibits an interconnected and compact network of protein filaments (white), whereas a change in the compactness of the network is clearly recognizable by the addition of 5% sucrose in micrograph (B). The addition of 10% sucrose resulted in a fairly weak network, indicated by occasionally occurring protein aggregates (micrograph (C)).

3.3.3. Quality characteristics of burger buns

Specific volume

Since the specific volume is an important attribute of baked goods, the effect of sucrose on the specific volume of burger buns containing different levels of sugar was determined (Table 3.1). In general, a high specific volume with a dense crumb structure is desired for burger buns. In small to moderate amounts of added sucrose (1–4%) the specific volume

increased, followed by a decrease, when higher amounts of sucrose (5–10%) were added. The specific volume of the sucrose-free burger bun was the lowest, whereas the highest value was measured, when 4% sucrose was added.

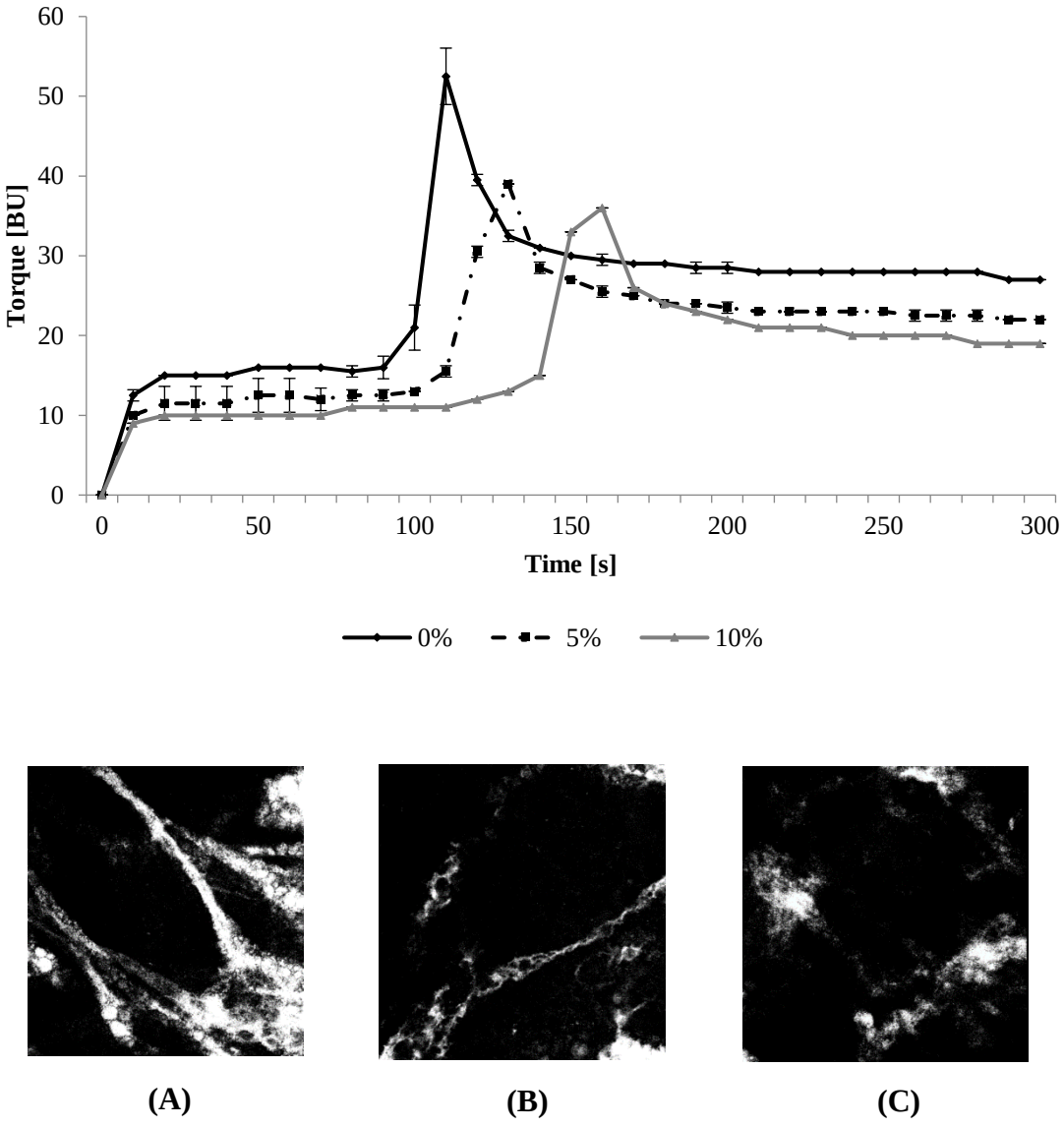


Figure 3.1 Illustration of the gluten network development of recipes containing 0, 5 and 10% added sucrose. The graph shows the mean torque of the recipes (duplicates) during shearing and the standard deviations are shown as error bars. The micrographs show the gluten network (white pattern) taken by a confocal laser scanning microscope of dough with (A) 0% added sucrose, (B) 5% added sucrose and (C) 10% added sucrose

Crumb hardness and staling

The crumb texture is another very important characteristic for bakery products. The changes in crumb hardness over time allow prediction of the staling behaviour of the product. Furthermore, a commercial burger bun is characterized by a soft, but dense crumb. The crumb of the sucrose-free burger bun showed the highest hardness after baking. 1% added sucrose resulted in a significant decrease in crumb hardness, which decreased with the addition of more sucrose up to 5%. The addition of more sugar resulted in a harder crumb. The second highest crumb hardness was measured, when 10% sucrose was added. Regarding the staling rate, burger buns containing sucrose showed a very low staling rate up to 48 h, followed by a significant harder crumb after 120 h. Interestingly, the crumb hardness of buns containing 10% added sucrose did not differ significantly from no-added sucrose buns after 120 h.

Crumb structure

The crumb structure is an important parameter for the evaluation of the burger bun quality. Ideally, the crumb of a commercial burger bun is dense with many small cells and without any holes. The values for the slice area and the area of cells of the burger buns were evaluated (Table 3.3). The crumb structure showed no significant differences between the data after 2 h and after 120 h. Consequently, the analysis after 2 h are described and discussed. Figure 3.2 shows the appearance of the crumb structure. The addition of 4% sucrose caused an increased cell area compared to a no-added burger bun (Table 3.3). The highest area of cells occurred, when 6% sucrose was added. The difference in cell area of buns containing 2–10% added sucrose was not significant.

Crust and crumb colour

Commercial burger buns are characterized by their golden-brown crust-colour. Due to the fact that sugar contributes to browning, the L*-value, as an indicator for lightness, was evaluated. The higher the L*-value, the lighter the analysed product. Regarding the colour of the crumb, the amount of sucrose did not affect the L*-value. In contrast, the sucrose level showed a significant effect on the colour of the crust (Table 3.3). Without added sucrose the burger bun showed the palest crust, whereas the darkest crust occurred, when 8% sucrose was added. This value did not differ significantly from the L*-value of the 4%-sucrose-bun. The effect of sucrose on the crust colour is illustrated in Figure 3.2.

Table 3.3 Results of the quality characteristics of burger buns

Added sucrose [%]	Specific Volume [ml/g]	Crumb Hardness after 2h [N]	Crumb Hardness after 48h [N]	Crumb Hardness after 120h [N]	Slice Area [mm²]	Area of cells [%]	Lightness of the Crust	Water Activity	Shelf Life [d]
0	2.91 ± 0.03 (h)	8.38 ± 0.65 (a)	21.1 ± 1.2 (a)	26.6 ± 1.8 (a)	5462 ± 106 (g)	49.4 ± 0.6 (c)	80.0 ± 1.0 (a)	0.948 ± 0.004 (ab)	4 ± 1 (b)
1	4.16± 0.08 (c)	3.11 ± 0.25 (c)	7.31 ± 0.72 (ef)	12.6 ± 2.4 (efg)	6995 ±134 (b)	51.6 ± 0.8 (b)	74.9 ± 1.9 (b)	0.951 ± 0.003 (a)	7 ± 2 (ab)
2	4.30 ± 0.12 (b)	1.77 ± 0.29 (d)	5.07 ± 0.52 (g)	14.7 ± 2.4 (de)	7108 ± 200 (ab)	53.2 ± 1.7 (a)	70.9 ± 1.4 (c)	0.949 ± 0.002 (a)	7 ± 2 (ab)
3	4.02 ± 0.08 (d)	2.63 ± 0.52 (c)	6.83 ± 0.69 (ef)	12.8 ± 1.9 (ef)	6565 ± 129 (cde)	53.7 ± 0.9 (a)	65.2 ± 1.5 (d)	0.943 ± 0.007 (abc)	5 ± 1 (ab)
4	4.51 ± 0.08 (a)	1.89 ± 0.24 (d)	6.55 ± 0.72 (ef)	10.0 ± 1.4 (g)	7163 ± 145 (ab)	53.8 ± 1.2 (a)	60.8 ± 1.9 (fg)	0.938 ± 0.008 (bc)	6 ± 1 (ab)
5	4.47 ± 0.13 (a)	1.76 ± 0.21 (d)	6.11 ± 0.62 (fg)	11.9 ± 1.1 (fg)	7265 ± 142 (a)	53.7 ± 0.8 (a)	60.7 ±0.9 (fg)	0.938 ± 0.005 (bc)	6 ± 1 (ab)
6	3.92 ± 0.14 (de)	3.39 ± 0.55 (c)	9.55 ± 0.75 (d)	16.3 ± 1.4 (d)	6749 ± 190 (c)	54.3 ± 0.6 (a)	61.4 ± 3.3 (efg)	0.938 ± 0.004 (bc)	5 ± 1 (ab)
7	3.85 ± 0.08 (e)	3.11 ± 0.34 (c)	7.90 ± 0.80 (e)	14.4 ± 1.4 (de)	6653 ± 108 (cd)	53.1 ± 0.6 (a)	62.7 ± 2.5 (def)	0.933 ± 0.004 (cd)	7 ± 2 (ab)
8	3.68 ± 0.07 (f)	3.26 ± 0.34 (c)	9.62 ± 0.97 (d)	19.6 ± 1.8 (c)	6536 ± 104 (de)	53.2 ± 0.7 (a)	58.5 ± 1.5 (g)	0.936 ± 0.005 (c)	8 ± 1 (ab)
9	3.69 ± 0.07 (f)	4.10 ± 0.50 (b)	14.0 ± 1.2 (e)	23.4 ± 1.6 (b)	6398 ± 156 (ef)	53.3 ± 0.9 (a)	62.7 ± 2.1 (def)	0.923 ± 0.007 (de)	8 ± 1 (ab)
10	3.52 ± 0.07 (g)	4.41 ± 0.79 (b)	11.6 ± 1.1 (c)	25.0 ± 1.3 (ab)	6250 ± 106 (f)	53.3 ± 1.1 (a)	65.2 ± 3.4 (de)	0.915 ± 0.006 (e)	10 ± 1 (a)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

Water activity and microbial shelf life

The water activity (a_w) reflects the amount of free water in the system, which has an impact on the shelf life of the product. The addition of sucrose caused a decrease in water activity. The crumb of the burger buns without any added sucrose resulted in a water activity of 0.948 ± 0.004 and differed from the control significantly (Table 3.3). Figure 3.3 reveals the effect of added sucrose on the shelf life of burger buns. Sucrose prolonged shelf life significantly from 4 (no-added-sucrose) to 10 days (control) (Table 3.3).

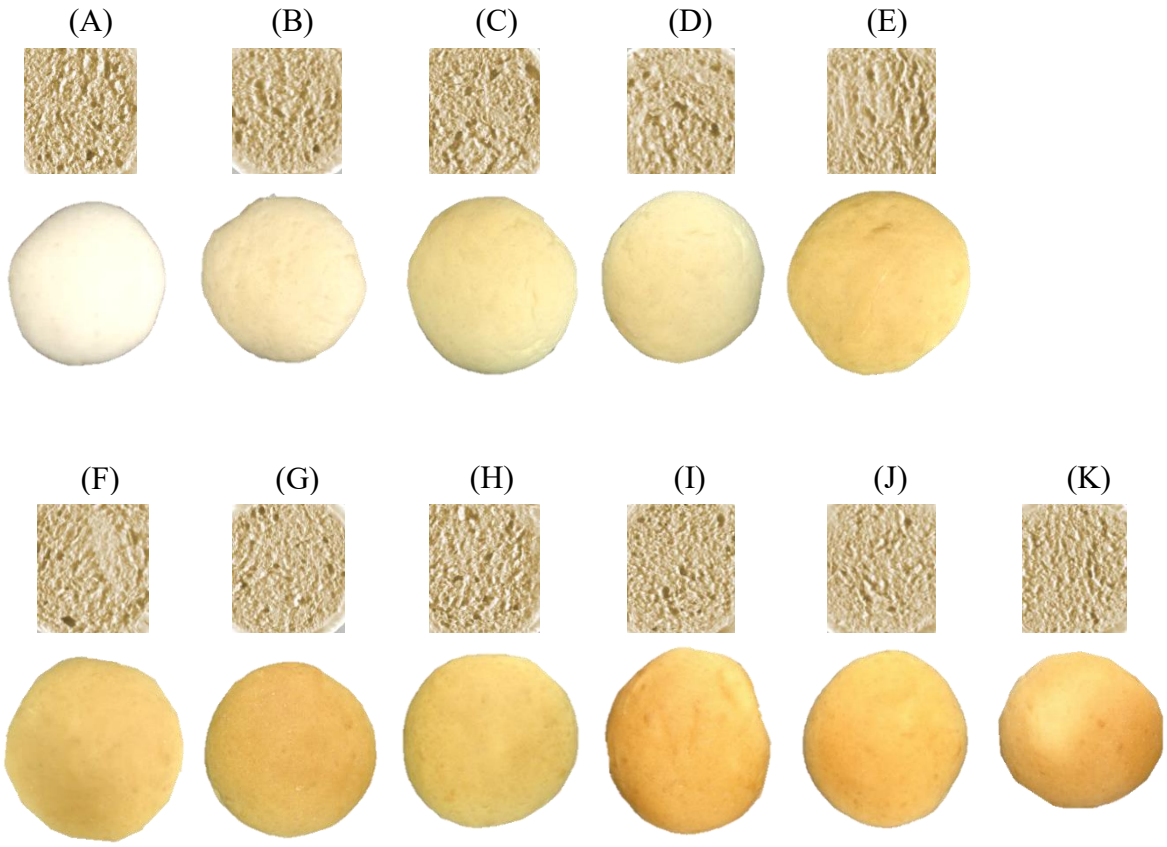


Figure 3.2 Crumb structure (first row) and crust appearance (second row) of burger buns containing 0-10% (A-K)

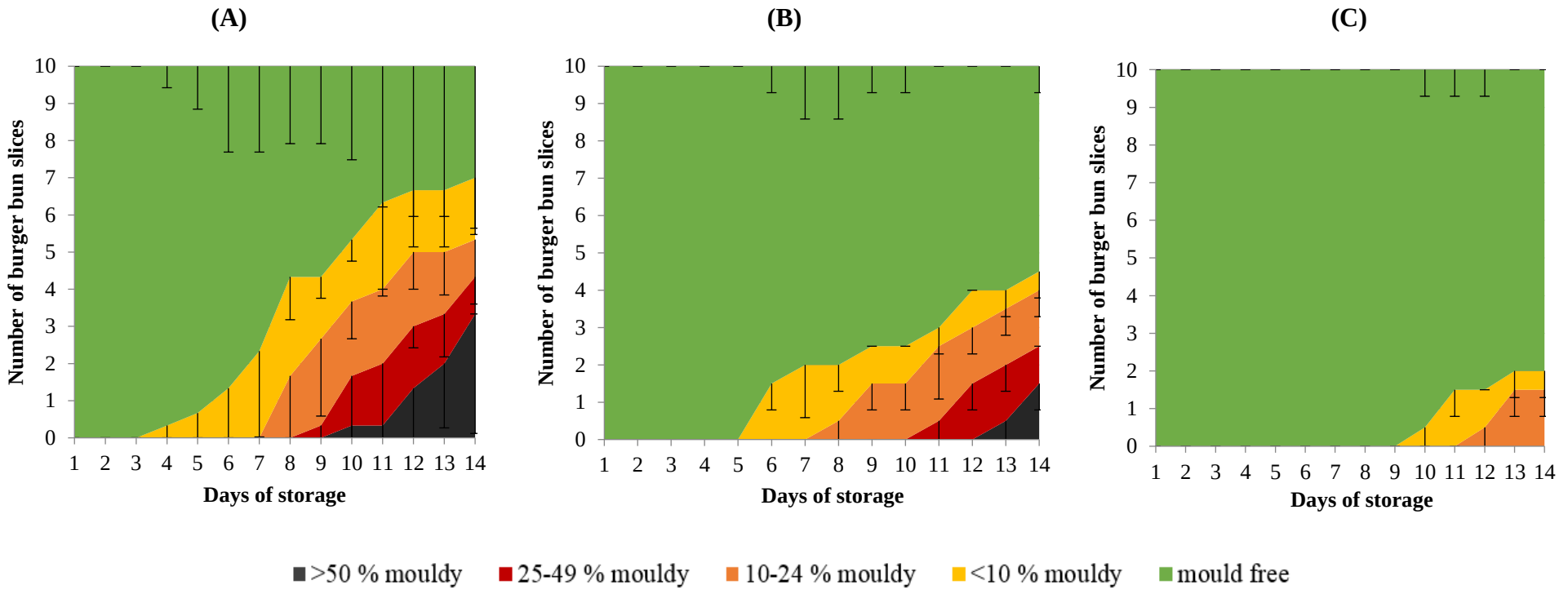


Figure 3.3 Shelf life evaluation of burger buns containing 0% (A), 5% (B) and 10% (C) added sucrose. The number of slices for each mould group ("mould-free", "<10% mouldy", <10-24% mouldy", "<25-49% mouldy" and >50% mouldy") was counted on each day over 14 days. The graph shows mean values with standard deviations as error bars

Sensory evaluation

An investigation of the sweetness-intensity of burger buns containing different amounts of added sucrose was performed. Burger buns without added sucrose showed an average sweetness value of 1.6 ± 1.3 and differed from the other samples significantly ($p \leq 0.05$). The panel judged the sweetness of buns with 2% added sucrose with 3.4 ± 1.6, which differed also significantly from the other samples tasted. Burger buns containing 4 and 6% added sucrose could not be distinguished from each other (4%=5.3 ± 1.4; 6%=6.1 ± 1.2). Additionally, 10% and 8% added sucrose differed significantly from the other samples regarding sweetness intensity, but not from each other. Thus, the panel could distinguish between samples containing little amounts of sucrose, but with the increase of sucrose the perception of sweetness decreased. No significant differences occurred in the hedonic evaluation regarding appearance, flavour, aroma, and texture. The evaluation of the sweetness preference showed that burger buns without any sucrose addition and buns with 10% added sucrose were disliked the most. Regarding this evaluation, buns with 2% (6.0 ± 1.7), 4% (6.0 ± 2.0) or 6% (5.4 ± 2.0) added sucrose did not differ significantly from each other. The most preferred burger bun regarding all attributes contains 4% sucrose (33.33% of the panellists), followed by the addition of 6% sucrose (29.17% of the panellists).

Principal component analysis (PCA)

PCA analysis was performed for all dough characteristics as well as for burger bun characteristics. Dough characteristics resulted in three groups: 0% added sucrose formed one group (Group 1), 1–7% added sucrose belonged to Group 2 and Group 3 included 8%, 9% and 10% added sucrose dough. PCA for burger buns resulted as well in three groups. Group A is represented by 0% added sucrose; Group B is formed by 1–6% added sucrose buns. 7–10% added sucrose buns are defined in Group C.

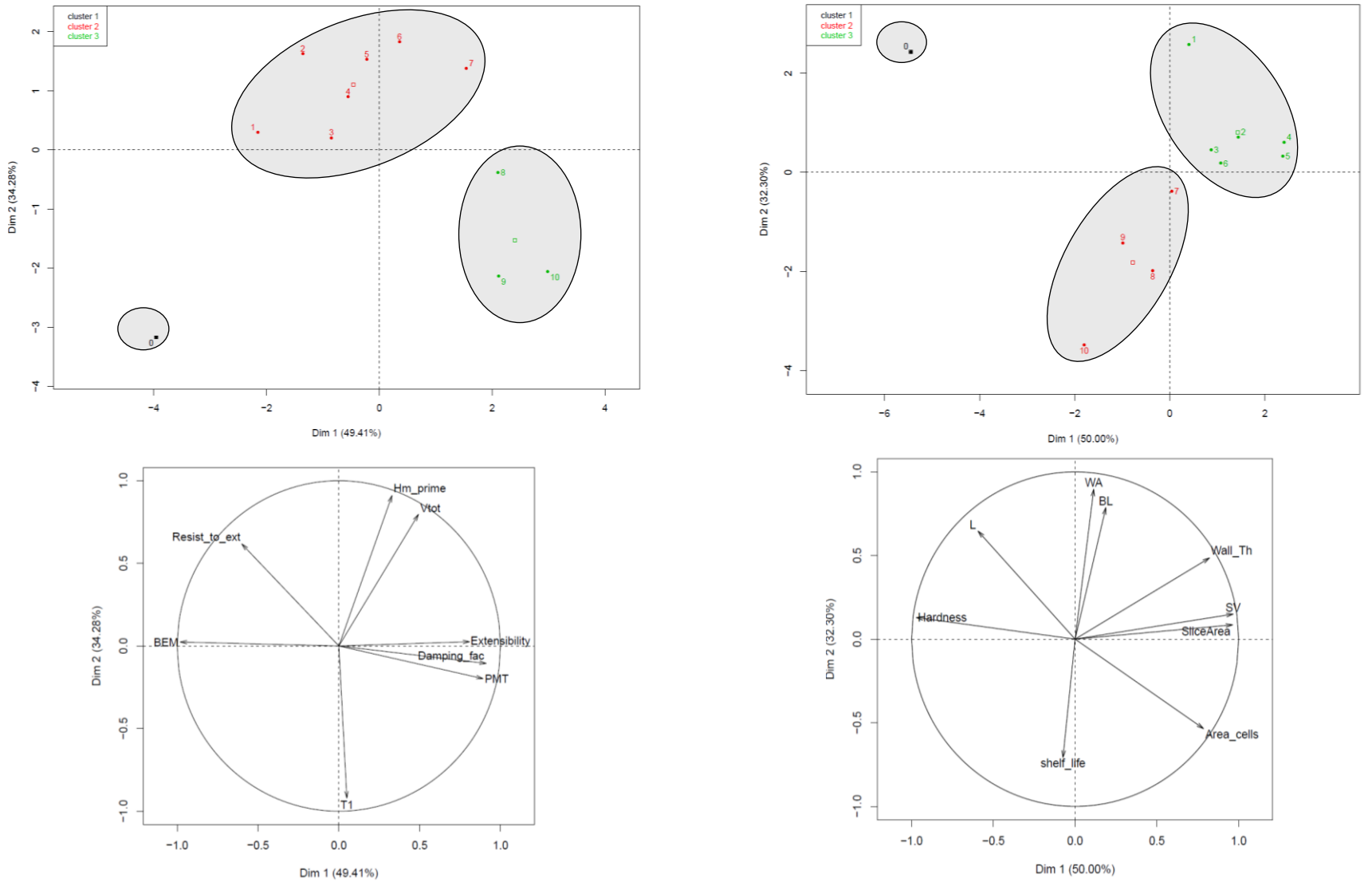


Figure 3.4 Principal component analysis (PCA) of burger bun doughs (left) and burger buns (right). The numbers in the different groups represent the added sugar content.

3.4. Discussion

This study examined the impact of added sucrose on the dough quality, physical properties, shelf life and sensory characteristics of burger buns.

Depending on the added amount, sucrose can increase the fermentation quality as well as decrease it by inhibiting the yeast metabolism. Indeed, on the one hand yeast needs monosaccharides for the growth and the production of CO_2 (Cauvain and Young, 2007). On the other hand, sugar can slow down the yeast's metabolism due to high osmotic stress (Attfield, 1997).

Hm indirectly describes the performance of yeast and the quality of the microstructure of the dough. A high Hm is the result of a dough system characterized by a combination of high gas production and firm microstructure (Huang et al., 2008). Regarding Hm and T1, dough containing different sucrose levels did not differ significantly from each other. Nevertheless, the lowest Hm and the longest T1 occurred in dough with no-added-sucrose. A low Hm and a long T1 could be attributed to a reduced metabolic activity of the yeast. High sucrose levels (9% and 10%) could cause hyperosmolarity, which results in osmotic stress for the yeast negatively influencing the dough development (Hohmann and Mager, 2007).

The gas release curve showed the gas formation during fermentation resulting in Hm' and V_{tot}. Sucrose levels between 5% and 10% are negatively correlated to Hm' ($r = -0.86$, $p \leq 0.05$), which can be explained by the reduction of fermentation activity due to hyperosmolarity in the dough system caused by sucrose (Attfield, 1997). Sucrose concentration of 0–4% correlated positively with V_{tot} ($r = 0.97$, $p \leq 0.01$), but negatively, when 5–10% was added ($r = -0.87$, $p \leq 0.05$). Reduced fermentation led to a decreased CO_2 production, resulting in a lower V_{tot} (Cauvain and Young, 2007).

The results of the viscoelastic properties for the different doughs revealed a positive correlation between sucrose level and damping factor ($r = 0.868$, $p \leq 0.001$). Gluten competes with sugar for water; consequently, a high sugar level reduces the hydration of the protein and delays the protein network development, resulting in an increase of viscous properties of sugar-rich dough (Pareyt et al., 2009).

GlutoPeak measurements showed a positive correlation between the amount of added sucrose and the peak maximum time (PMT) ($r = 0.913$, $p \leq 0.001$) as well as a negative correlation between the amount of added sucrose and the torque maximum (TM) (r =

−0.989, P ≤ 0.001). With increasing sucrose level, a decrease of gluten hydration is observed resulting in a slower gluten network formation (which caused the delayed PMT), and to a weaker gluten network (which is highlighted by the decreased TM). This emphasises the fact that sucrose delayed and weakened the gluten network (Baxter and Hester, 1958). Further evidence for a weaker protein network caused by sugar is shown in the CLSM micrographs (Figure 3.1).

The weak gluten network caused by the addition of sucrose affected also the extensibility and the resistance to extension of the dough. Dough is characterized as strong, if its extensibility is low and its resistance to extension is high at the same time (Bordes et al., 2008). The dough extensibility positively correlated with the sucrose level ($r = 0.831$, $p \leq 0.005$). The resistance to extension correlated negatively with sugar additions of 5–10% ($r = -0.96$, $p \leq 0.005$), which means the dough became weaker (Savitha et al., 2008).

In addition to the effect of sucrose on the dough characteristics, sugar also affected the quality of the burger buns. Due to a combination of high CO_2 production and decreasing resistance to extension in the dough, the highest specific volume was achieved by an addition of 4% sucrose. Correlation analysis exhibited a negative correlation between sucrose levels of 5–10% and the specific volume of the buns ($r = -0.90$, $p \leq 0.05$). The decrease of the specific volume of buns containing more than 5% sucrose could be due to the inhibited yeast activity during fermentation. The results of Hm' (all sucrose levels) during fermentation and the specific volume of the buns correlated positively with each other ($r = 0.840$, $p \leq 0.005$).

The specific volume and the crumb hardness are often related to each other. A positive correlation between sucrose level and crumb hardness occurred, when 5% sugar or more were added ($r = 0.897$, $p \leq 0.05$). A less efficient gas formation (Hm') resulted in a harder burger bun ($r = -0.929$, $p \leq 0.0001$). According to D'Appolonia and Morad (1981) bread staling is mostly characterized by the retrogradation of the starch molecules. Sucrose was replaced by wheat starch in recipes containing less than 10% sucrose. However, the crumb hardness of no-added-sucrose buns and the control did not differ significantly from each other after 120 h.

Sucrose delays starch gelatinisation and protein denaturation during the baking process, which results in an expansion of the gas cells by CO_2 and water vapour, before the dough becomes a set structure (Yamazaki & Kissell, 1978). Sucrose addition of 0–4% in the burger buns correlated positively with the area of cells ($r = 0.929$, $p \leq 0.05$). This

increasing number of cells is most likely the result of more extensible dough in combination with a weak protein network, which allows the dough to rise and avoids a collapse of cells. As expected, the slice area correlated positively with the specific volume ($r = 0.979$, $p \leq 0.0001$) and Hm' ($r = 0.899$, $P \leq 0.0005$).

The formation of a golden crust is influenced by the level of Maillard reaction and caramelisation in which sugars are involved, and has an effect on the lightness of the final product (Esteller et al.,2006; Hashiba, 1982). Burger buns containing 0–3% added sucrose differed significantly in the L*-value, whereas no significant difference in lightness occurred, when 3% or 10% sucrose was added. The L*-value of the crust correlated negatively with the amount of added sucrose up to 7% ($r = -0.888$, $p \leq 0.005$). The addition of 3% sucrose is the minimum amount of sugar to be added to achieve a desired brown crust (Table 3.3).

Sugar also impacts on the water activity of a product and, therefore, the microbial shelf life. Water activity correlated negatively with the amount of added sucrose ($r = -0.927$, $p \leq 0.0001$). Since sugar is known to lower the water activity in a system (Cauvain and Young, 2009), a significant decrease in a_w from 0.948 ± 0.004 in sugar-free burger buns to 0.915 ± 0.006 in buns containing 10% sucrose was expected. The water content had to be adjusted for each sucrose level, to achieve the same dough consistency. Hence, the lower the sucrose content, the higher the amount of wheat starch and the higher the adjusted water in the dough system (Table 3.2). Consequently, the increased water activity could also be a result of the higher amount of added water. However, sucrose is a hygroscopic agent and binds free water, which contributed more likely to the lower water activity of crumbs containing higher amounts of sucrose. Additionally, water activity correlated negatively with PMT ($r = -0.944$, $p \leq 0.0001$), but positively with TM ($r = 0.893$, $p \leq 0.0005$). The more water available in the dough system, the higher and faster the gluten hydration and the stronger the network is. According to Mathlouthi (2001) not all water-activity-depressors extend microbial shelf life.

Shelf life of buns containing 1% added sucrose did not differ significantly from the ones with 9% added sugar (Table 3.3). Sometimes a hygroscopic ingredient, such as sugar, does not bind water strongly enough, which results in mobile water allowing microbial growth (Cauvain and Young, 2007). Nevertheless, a shelf life extension from 4 ± 1 day for buns without added sucrose to 10 ± 1 day for 10% added sucrose buns occurred.

One typical attribute of sugar is its sweet taste. The threshold for sweetness in solution is 6.8 g/L, which is lower than in solid systems (Purves et al., 2001). Regarding sweetness preference, there are four different types of panellists defined: Type I individuals show an inverted U-function with increasing sucrose concentration, whereas Type II individuals prefer sweetness in a monotonic increasing way. People belonging to Type III dislike high concentration of sugar, and show a monotonic decrease with increasing sugar level, and Type IV individuals experience a flat relationship between sweet preference and sugar concentration (Drewnowski, 1987). The sensory evaluation showed that some panellists preferred sweet samples and could be categorized as Type II, whereas, on the other hand other panellists liked non-sweet samples more and belonged to Type III. Considering all individual results together the group could be categorized as Type I.

The overall preference of the burger buns showed that the panellists liked the burger buns containing 4% or 6% sucrose the most.

3.5. Conclusion

The results of this study provide a deep insight of the impact of sucrose on sweet yeast-leavened products. The desired attributes regarding product quality could be achieved by a reduced sucrose addition of 4 to 6%. However, PCA analysis showed that an addition of at least 7% sucrose is necessary to ensure the same burger bun quality considering all attributes. Based on this result, future studies might deal with alternative replacement of sugar by other bulking agents with functional activities. When functional bulking agents are considered as sugar replacer in bakery products, those characterized by low water absorption/water holding capacity are preferred, since high water addition in the recipe will significantly compromise dough rheology and bakery quality parameters.

3.6. Acknowledgement

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Chapter 4

XYLITOL, MANNITOL AND MALTITOL AS POTENTIAL SUCROSE REPLACERS IN BURGER BUNS

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Abstract

Burger buns are a source of added sugar, containing 7-12%, in order to ensure their unique texture and taste. Hence, suitable sugar substitutes for burger buns are urgently needed. This study aimed to elucidate the effect of three different polyols on dough and product quality of burger buns. Xylitol, mannitol and maltitol were incorporated individually in a burger bun system, by replacing added sucrose by 30%, 50% and 100%. Wheat starch was used to compare the impact of polyols with another non-sweet bulking agent. The effects on dough properties as well as on the burger buns themselves were investigated. Compared to sugar-rich doughs, polyols lowered the fermentation quality, resulting in lower dough development (-37 to -81%) and poorer gaseous release (-62 to -87%). Furthermore, a delay in gluten network development (+50 to +161%) and a decrease in extensibility (-14 to -18%) with increasing concentrations were detected. Interestingly, the polyols maltitol and xylitol did not affect the damping factor and pasting properties, whereas mannitol increased the pasting temperature (+15 °C). Moreover, polyols did not influence the viscoelastic properties of the dough. The incorporation of sugar alcohols led to a significant decrease in specific volume (-30 to -48%), and to a harder crumb texture (+135 to +678%). Moreover, the L*-value increased with increasing amount of polyols, resulting in a very pale crust colour. As a conclusion, a reduction of 30% added sucrose by polyols was applicable, whereas mannitol showed the highest acceptance in sensory evaluation. Thus, mannitol was the most suitable sugar replacer amongst the polyols tested.

4.1. Introduction

The number of patients suffering from obesity, diabetes and cardiovascular disease is steadily increasing. Due to the busy life, convenience has become a priority for the consumers and the interest in fast-food increased over the last decades, whereby burgers increased in popularity (Soltanizadeh and Ghiasi-Esfahani, 2014). Surprisingly, burger buns can contain up to 12% added sugar, which contributes to the special texture and flavour (Cauvain and Young, 2007), but also adds to an increase in sugar intake, when consumed.

Sucrose is the most common ingredient used by the food industry to sweeten goods, especially in the bakery sector. It is very unique in its properties and its contribution to texture, sweetness and shelf life (Sahin et al., 2017). Hence, sugar replacement has become a very challenging objective for researchers. Replacing added sucrose by bulking agents with similar properties, such as polyols, is currently under discussion. Polyols, also known as sugar alcohols or polyalcohols, are the hydrogenated form of their analogue carbohydrates. Thereby, the carbonyl group is reduced to a primary or secondary hydroxyl group. Polyols are considered as sugar replacers due to their lower energy value and also due to the fact that they do not contribute to tooth decay, since bacteria are not able to metabolize them (Struck et al., 2014).

Furthermore, Livesey (2003) investigated the influence of polyols on the glycaemic response. Interestingly, polyols interact with macronutrients and reduced postprandial glycaemia, and also inhibit interactions between sugars and fats, which prevents from postprandial insulinaemia. On the other hand, polyols can cause laxative effect and bloating, due to their slow and incomplete absorption in the small intestine (Livesey, 2003). However, the gastrointestinal symptoms depend on several factors such as amount and type of sugar alcohol, and most people could adapt to the symptoms after some days (Bhise, 2013). Polyols seem to be a potential sucrose replacer in sweet bakery products (Bhise and Kaur, 2014; Ghosh and Sudha, 2012; Grembecka, 2015). Martínez-Cervera et al. (2014) compared the impact of different polyols on muffins and concluded that maltitol, isomalt and sorbitol are suitable as sugar replacers due to their similarity in thermosetting attributes to sucrose, resulting in good texture and sensory properties. Furthermore, the effect of polyols and non-digestible oligosaccharides on sponge cakes was investigated by Ronda et al. (2005). That study showed that xylitol and maltitol are the most suitable sugar substitutes amongst all polyols tested. Moreover, the addition of non-digestible oligosaccharides resulted in a bitter aftertaste. Hence, these ingredients are

not suitable as sugar replacers. Sun et al. (2014) investigated the effect of xylitol in different concentrations on bread dough and bread texture. The aim of that study was to assess xylitol as a potential ingredient in yeast leavened wheat doughs. Interestingly, the addition of 5%-10% xylitol showed the optimal specific volume, whereas higher amounts caused poorer dough and bread quality. In spite of several publications on sugar alcohol in baked goods, a deeper insight into the impact of polyols on yeast leavened products is necessary, in order to achieve a successful sugar reduction.

The present work was carried out to analyse the influence of xylitol, mannitol and maltitol, as partial or total sugar replacers, on burger buns and their dough properties. Xylitol and maltitol were chosen due to their suitability as sugar replacers in bakery products, according to Ronda et al. (2005). Although mannitol is used in food products, such as candies or flavoured-coated sweets, due to its ability to extend shelf life and increase flavour, investigations about its effect on baked goods are scarce (Le and Mulderrig, 2001). A 30%, 50% and 100%- sugar-replacement was conducted. In the control samples, added sucrose was replaced by wheat starch as a non-sweet bulking agent, which was tested before (see Chapter 3; Sahin et al. 2017).

4.2. Materials and methods

4.2.1. Materials

The ingredients used were bakers flour (Odlums, Dublin, Ireland), instant active dried baker's yeast *Saccharomyces cerevisiae* (Puratos, Groot-Bijgaarden, Belgium), sugar (Siúcra, Dublin, Ireland), salt (Glacia British Salt Limited, Cheshire, UK), polyols – mannitol (Roquette, Lestrem, France), maltitol (Roquette, Lestrem, France), xylitol (Quay-coop, Cork, Ireland), wheat gluten (Roquette, Lestrem, France), wheat starch (Roquette, Lestrem, France), ascorbic acid (Storefast Solutions, Northfleet, UK) and sunflower oil (Musgrave Wholesale Partners, Dublin, Ireland), Sodium Stearoyl Lactate (SSL) (Danisco Grindstead Co., Copenhagen, Denmark) and tap water (25 °C).

4.2.2. Adjustment of water content

The replacement of sucrose by different polyols can change the dough consistency. The water content of each recipe was adjusted to the dough consistency of the control full-sugar burger bun (10% sucrose) using Farinograph E (Brabender OHG, Duisburg, Germany). The chamber temperature was kept at 30 °C.

4.2.3. Burger bun preparation

Doughs and burger buns were produced as previously described in Chapter 3 (Sahin et al., 2017). Based on their control recipe, added sucrose, 10% based on the whole recipe, was partially or totally replaced by xylitol, mannitol or maltitol (w/w) (Table 4.1). Burger buns containing 10%, 7%, 5% and 0% added sucrose were used as controls. In these control samples, wheat starch was used as a bulking agent. After baking, the buns were placed on a wire rack and cooled at room temperature for 1 hour. Samples were stored in plastic bags at room temperature for further analysis.

Table 4.1 Recipes of the evaluated burger buns. The values are given in % based on flour. The water content for each recipe was adjusted to a full-sugar burger bun containing 18.8% sucrose based on flour, which is 10% based on the whole recipe

Ingredients	**Control recipes [%][a]**	**Polyols containing recipes [%][b]**
Wheat flour	100.00	100.00
Water	Adjusted water	Adjusted water
Sucrose	A) 18.80 B) 13.16 C) 9.40 D) 0.00	a) 13.16 b) 9.40 c) 0.00
Wheat starch	A) 0.00 B) 5.64 C) 9.40 D) 18.80	0.00
Polyol	0.00	a) 5.64 b) 9.40 c) 18.80
Sunflower oil	6.70	6.70
Salt	1.70	1.70
Baker's yeast	2.00	2.00
SSL	0.50	0.50
Ascorbic acid	0.10	0.10
Wheat gluten	3.20	3.20

[a] Composition of the control recipes, whereas A) is the full-sugar burger bun recipe, B) is a 3% sugar replacement by wheat starch, C) is a 5% sugar substitution by wheat starch and D) is a full-sugar reduction by wheat starch

[b] Recipes used for the sucrose replacement by polyols, whereas a) is a 3% replacement, b) is a 5% sugar-reduction and c) is a total sugar substitution by sugar alcohols

4.2.4. Dough properties

Fermentation behaviour

Fermentation quality was determined using a Rheofermentometer (Chopin, Villeneuve-la-Garenne Cedex, France), which measures dough development according to the production and retention of carbon dioxide during fermentation. The dough (300 g) was placed into the fermentation chamber with a 1500 g cylindrical weight placed on top and fermented at 30 °C for 180 min. The fermentation quality of the dough was expressed by several parameters such as, the volume of CO_2 production (V_{tot}), the maximum height of dough (Hm), maximum height of gaseous release (Hm') as well as time required for dough to achieve this height (T1).

Extensibility and resistance to extension

The Extensograph (Brabender, Duisburg, Germany) was used to analyse the extensibility and the resistance of extension of the dough. Therefore, dough pieces of 150 g were moulded and placed in the proofing chamber at 30 °C. After a proofing time for 60 min the measurement was conducted and the extensibility and the resistance to extension were evaluated.

Pasting properties

Starch pasting properties were determined using Rapid Visco Analyser (RVA) (Newport Scientific, Warriewood, Australia). The analysed samples contained 3 g of solids and 25 g of liquids. Hence, a total of 3 g (on a basis of 14% moisture) dry ingredients of the recipe were weight into a RVA aluminium sample canister, sunflower oil was added and filled up with distilled water to reach 25 g of liquids. To initialize the sample measurement, a special plastic stirrer was placed in the torque measuring arm and the canister was pushed into the hot block of the RVA. The temperature profile used was heating to 95 °C (6.3 °C/s), holding at 95 °C for 162 s and cooling to 50 °C (5.1 °C/s), followed by a hold at 50 °C for 120 s. A rotation speed of 160 rpm of the stirrer was set throughout the measurement.

Viscoelastic properties

Viscoelastic properties of the dough were determined by performing oscillation measurements using the Rheometer Physica MCR 301 (Anton Paar GmbH, Ostfildern, Germany). Parallel plates' geometries, serrated to prevent slippage, were used. The temperature of the lower plate was set to 30 °C and used in conjunction with a 50 mm

diameter upper plate. The linear viscoelastic region was determined by performing an amplitude sweep as described by Hager et al. (2011) (results not shown). Frequency sweeps were conducted using a target strain of 0.01% and a frequency range from 100 to 0.1 Hz. Before each test, the sample rested for 5 min to allow equilibration. The viscoelastic properties were determined by the damping factor which represents the tangent of the ratio between the lost (G″) and stored (G′) deformation energy of the system, tan (G″/G′).

Gluten network formation

The gluten network formation was determined by using GlutoPeak (Brabender, Duisburg, Germany). For these measurements, the standard ratio of 50/50 (solid/liquid; w/w) regarding the recipe was used. The torque of the sample was recorded running a constant speed of 2750 min^{-1} and a chamber temperature of 36 °C for 600 s. The torque maximum (TM) and the peak maximum time (PMT) were evaluated.

4.2.5. Burger bun characteristics

Three burger buns from each batch were analysed 2 h after baking for specific volume, colour, crumb structure, texture (TPA) and water activity (a_w). Texture profile analysis (TPA) was also carried out after 24 h, 48 h and 120 h to determine the staling rate.

Specific volume

The specific volume [ml/g] of the buns was measured by a 3D laser scan using a VolScan Profiler 300 (Stable Micro Systems, Godalming, UK).

Crust colour

The crust colour of the buns was determined using a colorimeter (Minolta CR-331, Konica Minolta Holdings Inc., Osaka, Japan). The L*-values for crust were recorded. L* indicates the lightness of the sample, the lower the L*-value the darker the sample and the higher L* the lighter the sample colour (Sullivan et al., 2010).

Crumb structure

The crumb structure of the buns was determined using imaging analysis with the C-cell Bread Imaging System (Calibre Control International Ltd., Warrington, UK). Samples were prepared by cutting the top and bottom of the bun to an end height of 35 mm using a bread slicer. Image analysis parameters measured were slice area, number of cells, number of holes and area of cells [%].

Crumb texture and staling

Crumb texture was determined after storage times of 1 h, 24 h, 48 h and 120 h using a TA-XT2i texture analyser (Stable Micro Systems, Godalming, UK). The system was equipped with a 25 kg load cell and a 20 mm cylindrical probe. Measurements were performed under the following settings: test speed of 5 mm/s, post-test speed of 10.0 mm/s, force of 0.05 N and a 5 s waiting time between first and second compression. A two-compression test was used to investigate the changes in crumb hardness as well as in cohesiveness, springiness, chewiness and resilience. Since no significant difference in cohesiveness, springiness, chewiness or resilience was recognised only the results of crumb hardness are reported and discussed. Samples were prepared using the same manner for crumb structure. The rate of staling was evaluated using the formula demonstrated in Chapter 3.

Water activity and microbial shelf life

Water activity (a_w) was measured using material from the centre of the crumb, with a water activity meter (HygroLab Rotronic, Bassersdorf, Switzerland). The effect of commercially available polyols on the shelf life of burger buns was evaluated by using the mould environmental challenge method indicated by Dal Bello et al. (2007). Burger buns were halved horizontally, placed on a sterile metal rack and the crumb-side of each half was exposed to the environment for 5 min. Finally, the buns were packed separately in a sterile plastic bag and heat sealed. The samples were stored in a room with an average temperature of 20 ± 2 °C, after inserting two filter tips in each bag to ensure aerobic conditions. The mould growth was monitored for 14 days. Shelf life was express in days after the first visual mould growth appeared.

Sensory evaluation

For the evaluation of sensory properties of the burger buns, a trained panel of 11 people (6 female and 5 male, age: 25-32) was chosen to investigate the intensity of crumb hardness (touching and biting), of aroma and flavour intensity as well as of sweetness. Crumb hardness (touching) was defined as the resistance to crumb pressure on the finger, whereas crumb hardness (bite) was described as the force required by first bite through the sample with the molar. Flavour evaluation was defined as the degree of perceived intensity of overall flavour, after chewing the sample. The aroma was evaluated as the perceived first impression of odour intensity. For this descriptive sensory analysis, the panel was trained 6 hours weekly over 6 months prior to participation. Therefore, samples

with different hardness levels (based on texture analyser results) defined on a scale from 0 (very soft; 5% sugar burger bun) to 10 (very hard; 10% xylitol burger bun), aroma intensities (using vanilla as an example aroma) from a scale from 0 (not perceptible; 0% vanilla aroma solution) to 10 (very strong aroma; 2% vanilla aroma solution), flavour intensity (same as for aroma) and sweetness intensity using solutions with different sucrose concentrations, defining sweetness on a scale from 0 (not perceptible; 0.5% sugar solution) to 10 (very sweet; 10% sugar solution). Furthermore, the sensory panel collected descriptors to define the overall flavour and aroma. Typical descriptors collected were: cereals, roasted grains, sweetness, earthy. All training sessions and sensory analysis were performed in a sensory panel room at 21 ± 1 °C. Burger bun samples were presented one at a time in order to avoid the panel to compare the samples with each other. The panellists were asked to evaluate the intensity on a scale from 0 to 10, whereas "0" is "not perceptive at all", and "10" is "very intense". The total amount of samples evaluated was 13. Hence, two sessions with 6 and 7 samples were held and repeated once. Instead of performing an ANOVA, a PCA (Principal Component Analysis) taking all results into account was conducted.

All experiments were performed in compliance with the relevant national laws in Ireland and the institutional guidelines of University College Cork. Consent was obtained for any experimentation with human subjects. All participants received written information about the study before giving their informed consent.

Ultrastructure of polyols and their effect on the crumb structure

In order to investigate the influence of different commercial polyols on the crumb structure, scanning electron microscopy (SEM) was used. Burger buns were cut into small cubes and freeze-dried (SP Scientific, Warminster, USA). Double-sided carbon tape was used to immobilize the freeze-dried bun samples as well as the polyols on an aluminium stub. The samples were coated with a layer of 25 nm of sputtered palladium-gold, followed by the observation of the particles in a field emission scanning electron microscope with a working distance of 8 mm. Images were taken at an accelerating voltage of 5 kV and using SEM Control User Interface software, Version 5.21 (JEOL Technics Ltd., Tokyo, Japan).

Particle size of sucrose and polyols

The particle size of sucrose, xylitol, mannitol and maltitol was determined by laser diffraction using a dry feed cell (Malvern Mastersizer 3000, Instruments Ltd., UK).

Therefore, the sample was dispersed in air using a pressure of 1.5 bar, and the measurement started, when an obscuration of 5-7% was achieved, with a refractive index of 1.51. The values Dx10, Dx50 and Dx90 of each sample were evaluated, whereas Dx10 represents the particle size 10% of the analysed particles is smaller than, Dx50 stands for the particle size 50% of the particles are smaller than and Dx90 is the particle size 90% of the analysed particles are smaller than.

4.2.6. Statistical analysis

Dough and burger bun analysis were performed in triplicates. A variance analysis (one-way ANOVA, $p \leq 0.05$, Tukey test) was performed using Minitab 17. Additionally, correlation analysis between all results was conducted using Microsoft Excel 2010 and PCA analysis of the sensory attributes was performed using R 3.0.1.

4.3. Results

In order to understand the effect of polyols and to study their potential as sugar replacers in a burger bun system, added sucrose was partially or totally replaced (w/w) by xylitol, maltitol and mannitol. Dough attributes were evaluated and burger bun characteristics as well as sensory properties were investigated.

4.3.1. Adjustment of the water content

The water content of each formulation was adjusted to ensure an equivalent dough consistency (Table 4.2)

Farinograph-measurements of the control full-sugar burger bun dough reached a dough torque of 530 BU. Among the sugar-replacers investigated in the present research work, polyols absorbed less water than wheat starch to achieve the dough consistence (530 BU) of the full-sugar control. However, the adjusted water content for all formulations containing polyols did not differ significantly from the full-sugar control recipe (55.30 ± 0.54% based on flour) containing no sugar replacer.

4.3.2. Dough properties

Fermentation behaviour

The rheofermentometer evaluates the dough behaviour during proofing by measuring gas production over time, gas retention and the effect on dough development, providing information about yeast activity and the rate of fermentation. The effect of polyols on the total volume (V_{tot}) after fermentation, maximum height of the dough (Hm) during fermentation, the time needed to achieve the maximum height (T1) and the height of maximum gas formation (Hm') are also summarized in Table 4.2.

The highest total volume was achieved, when 7% sugar and 3% wheat starch were added (2434 ± 105 ml). The highest V_{tot} amongst the polyol containing doughs occurred, when 5% mannitol was added (1780 ± 71 ml), whereas the addition of 10% xylitol resulted in the lowest V_{tot} (278 ± 3 ml). A replacement of 3% sugar by any polyol showed the same V_{tot} as dough containing 10% wheat starch instead of sugar (0% sugar control) (1433 ± 9 ml).

Doughs containing polyols showed a lower Hm value than samples without any added sugar alcohols. Hm decreased with increasing amount of polyol, whereas the addition of xylitol resulted in the lowest maximum dough height (12.5±1.3 mm). Burger bun dough

containing 10% sugar (full-sugar control) showed the highest values (65.0 ± 2.0 mm), while the highest Hm value amongst polyol-rich doughs was achieved by a sugar replacement of 3% by maltitol (58.0 ± 2.0 mm).

T1 indicated the time the dough needs to develop to its maximum height. The fastest development was measured for the full-sugar control dough (145 ± 6 min), while doughs containing 10% sugar alcohols showed the longest fermentation time of 180 min. A 3% sugar replacement by xylitol demonstrated results closest to the full-sugar control dough. The highest gas formation was achieved, when 7% sugar and 3% wheat starch was incorporated (104.3 ± 1.8 mm), followed by a 5% sugar 5% wheat starch containing dough (102.9 ± 0.3 mm). A total replacement of sugar by polyols caused the lowest gas formation during fermentation, with xylitol showing the lowest value (12.2 ± 0.4 mm).

In conclusion, considering all quality parameters, a partially replacement of sugar by polyols resulted in similar results as dough containing no sugar, while a total replacement caused a significantly lower V_{tot}, Hm and Hm', and a higher T1 value than the control doughs.

Extensibility and resistance to extension

Sugar replacement by wheat starch showed a decrease in extensibility (Table 4.2). The incorporation of polyols instead of wheat starch showed the same effect. The more sugar was replaced, the lower the extensibility was, regardless which bulking agent was used. Nevertheless, the lowest extensibility was measured, when 10% maltitol was used (93.5 ± 2.4 mm), while the full-sugar control dough showed values of 144.8 ± 1.7 mm.

The highest resistance to extension occurred in doughs containing 10% mannitol (883.8 ± 22.1), whereas the lowest value was determined for doughs containing just 3% of mannitol and 7% of sugar (381.7 ± 2.9). Interestingly, the addition level of xylitol or maltitol had no significant effect on the resistance of extension.

Table 4.2 Results of dough analysis. V_{tot} is the total volume of the dough reached by CO_2 production, Hm is the maximum dough height after fermentation, T1 is the time needed to achieve Hm and Hm' is the maximum gas formation. PMT stands for peak maximum time of the gluten network development and TM is the torque maximum during the measurement of the gluten network formation

Added sucrose [%]	Water (based on flour) [%]	V_{tot} [ml]	Hm [mm]	T1 [min]	Hm' [mm]	Damping Factor [1]	PMT [s]	TM [BU]
0	66.26 ± 1.03 (a)	1433 ± 9 (def)	55.0 ± 1.0 (cde)	160 ± 5 (ab)	70.8± 0.6 (de)	0.316 ± 0.005 (e)	94.3 ± 3.06 (e)	55.7 ± 0.6 (a)
5	62.49 ± 0.51 (b)	2377 ± 22 (a)	63.0 ± 5.0 (ab)	121 ± 9 (cd)	102.9 ± 0.3 (a)	0.369 ± 0.009 (d)	115.3 ± 2.5 (de)	50.0 ± 1.0 (ab)
7	59.45 ± 0.66 (c)	2434 ± 105 (a)	60.0 ± 0.3 (abc)	115 ± 1 (d)	104.3 ± 1.8 (a)	0.390 ± 0.014 (cd)	123.7 ± 3.5 (de)	46.3 ± 0.6 (bc)
10	55.30 ± 0.54 (def)	2024 ± 19 (b)	65.0 ± 2.0 (a)	145 ± 6 (bcd)	90.7± 0.2 (b)	0.419 ± 0.009 (ab)	163.0 ± 13.5 (de)	43.7 ± 3.1 (bcd)
Xylitol content [%]								
3	56.36 ± 0.95 (de)	1563 ± 55 (cde)	56.0 ± 0.6 (cde)	150 ± 6 (abc)	76.8 ± 2.7 (de)	0.416 ± 0.010 (ab)	158.7 ± 7.2 (de)	40.7 ± 2.5 (cde)
5	55.27 ± 0.05 (def)	1286 ± 24 (f)	54.3 ± 4.1 (cde)	171 ± 5 (ab)	64.8 ± 3.1 (e)	0.414 ± 0.016 (abc)	185.0 ± 5.2 (bcd)	36.7 ± 0.6 (efg)
10	53.72 ± 0.05 (f)	278 ± 3 (h)	12.5 ± 1.3 (h)	180 ± 0 (a)	12.2 ± 0.4 (g)	0.404 ± 0.015 (bc)	245.0 ± 9.9 (b)	33.3 ± 2.1 (g)
Mannitol content [%]								
3	56.31 ± 0.53 (de)	1450 ± 9 (def)	56.0 ± 1.4 (cde)	162 ± 14 (ab)	71.1 ± 0.9 (de)	0.402 ± 0.010 (bc)	166.0 ± 18.0 (bcde)	41.3 ± 2.1 (cde)
5	55.32 ± 0.24 (def)	1405 ± 77 (ef)	52.0 ± 1.1 (de)	159 ± 18 (ab)	69.3 ± 1.1 (de)	0.413 ± 0.014 (abc)	176.7 ± 23.4 (bcd)	38.3 ± 4.0 (defg)
10	54.81 ± 0.16 (ef)	687 ± 7 (g)	41.0 ± 0.8 (f)	180 ± 0 (a)	34.7 ± 0.7 (f)	0.422 ± 0.017 (ab)	425.5 ± 58.7 (a)	34.0 ± 1.0 (fg)
Maltitol content [%]								
3	56.50 ± 0.01 (de)	1641 ± 220 (cd)	58.0 ± 2.0 (bcd)	178 ±1 (a)	77.4 ± 8.5 (cd)	0.431 ± 0.012 (a)	165.0 ± 9.2 (cde)	39.7 ± 1.2 (cdef)
5	56.91 ± 0.14 (d)	1780 ± 71 (c)	49.9 ± 3.5 (e)	169 ± 13 (ab)	80.7 ± 3.0 (c)	0.407 ± 0.011 (bc)	186.7 ± 11.9 (bcd)	39.3 ± 1.5 (defg)
10	54.86 ± 0.07 (ef)	464 ± 11 (gh)	29.2 ± 1.2 (g)	180 ± 0 (a)	19.0 ± 0.4 (g)	0.424 ± 0.011 (ab)	243.0 ± 20.3 (bc)	37.0 ± 1.0 (defg)

Table 4.2 continued

Added sucrose [%]	Extensibility 60 min [mm]	Resistance to Extension 60 min	Peak Viscosity [cP]	Final Viscosity [cP]	Pasting Temperature [°C]	Breakdown Viscosity [cP]
0	96.0 ± 2.8 (d)	475.2 ± 9.9 (defg)	1209.3 ± 16.1 (a)	1647.7 ± 24.8 (a)	63.1 ± 1.3 (b)	450.7 ± 12.1 (a)
5	115.0 ± 4.6 (ab)	499.8 ± 15.1 (cdefg)	865.3 ± 78.8 (b)	1225.0 ± 73.7 (b)	63.6 ± 0.0 (b)	324.0 ± 8.0 (b)
7	124.8 ± 1.8 (a)	455.3 ± 23.4 (defg)	826.7 ± 13.3 (b)	1165.3 ± 35.5 (b)	64.4 ± 1.7 (b)	332.7 ± 49.7 (b)
10	114.8 ± 1.7 (ab)	385.2 ± 3.8 (g)	677.0 ± 22.5 (c)	968.3 ± 73.9 (c)	66.65 ± 1.6 (b)	236.7 ± 7.4 (c)
Xylitol content [%]						
3	119.3 ± 4.4 (ab)	575.0 ± 54.5 (bcd)	620.0 ± 10.1 (c)	930.3 ± 11.4 (c)	66.6 ± 1.6 (b)	207.7 ± 5.1 (c)
5	114.2 ± 5.6 (ab)	624.0 ± 56.8 (bc)	622.0 ± 16.6 (c)	940.0 ± 24.6 (c)	64.5 ± 1.7 (b)	228.3 ± 46.5 (c)
10	97.8 ± 11.1 (cd)	540.0 ± 23.5 (cde)	667.3 ± 57.8 (c)	974.3 ± 77.7 (c)	62.7 ±0.1 (b)	256.3 ± 66.5 (bc)
Mannitol content [%]						
3	111.0 ± 8.3 (bc)	381.7 ± 2.9 (fg)	624.7 ± 12.1 (c)	938.0 ± 21.4 (c)	82.0 ± 0.6 (a)	215.7 ± 7.6 (c)
5	120.7 ± 7.2 (ab)	691.3 ± 50.7 (b)	651.0 ± 39.1 (c)	969.0 ± 48.5 (c)	82.4 ± 0.6 (a)	236.3 ± 28.3 (c)
10	98.5 ± 7.5 (cd)	883.8 ± 22.1 (a)	602.3 ± 59.5 (c)	913.7 ± 67.5 (c)	82.7 ± 0.5 (a)	197.3 ±20.8 (c)
Maltitol content [%]						
3	117.8 ± 7.2 (bc)	520.0 ± 33.7 (efg)	621.3 ± 20.6 (c)	940.3 ± 26.95 (c)	65.7 ± 1.7 (b)	207.0 ± 10.2 (c)
5	113.0 ± 3.2 (ab)	508.3 ± 55.9 (cdef)	658.7 ± 49.8 (c)	975.7 ± 67.4 (c)	65.3 ± 1.1 (b)	221.0 ± 17.8 (c)
10	93.5 ± 2.4 (d)	497.5 ± 38.6 (defg)	624.0 ± 10.6 (c)	929.3 ± 22.9 (c)	65.3 ± 1.1 (b)	208.3 ± 5.5 (c)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

Pasting properties

Pasting properties can be influenced by functional ingredients, especially bulking agents, due to their interaction with free water. The results in Table 4.2 show an influence of peak viscosity amongst samples containing wheat starch as a bulking agent. The higher the sugar replacement by wheat starch, the higher the peak viscosity was. The incorporation of different polyols instead of wheat starch showed no significant differences in peak viscosity compared to the full-sugar recipe (677.0 ± 22.5 cP), regardless of the amount added. Hence, polyols showed the same effect on peak viscosity as sugar did. The results of the final viscosity behaved in the same way as the peak viscosity. However, mannitol caused an increase in pasting temperature, whereas xylitol and mannitol showed no significant differences compared to all control samples, regardless the amount of replacement.

Sugar replacement by wheat starch caused an increase in the breakdown from 236.7 ± 7.4 cP (10% sugar control) to 450.7 ± 12.1 cP (0% sugar-10% wheat starch). The incorporation of sugar alcohols resulted in the same value in breakdown as the full- sugar burger bun recipe did. The amount of polyols in the system did not affect this value significantly.

Viscoelastic properties

The damping factor is an indicator for the proportion of viscous and elastic parts in the dough system. The replacement of sugar by wheat starch showed an increase in elastic parts and has already been discussed in Chapter 3 (Sahin et al., 2017). Sugar replacement by sugar alcohols revealed no significant differences in viscoelastic properties, illustrated by the damping factor, regardless of whether it is a partially replacement or a total one (Table 4.2)

The highest damping factor was measured, when sugar was totally replaced by maltitol, whereas the lowest value occurred in dough containing 10% wheat starch and no added sugar.

Gluten network formation

The PMT is the duration the gluten network needs to develop. The fastest development was determined, when sugar was totally replaced by wheat starch (0% sugar control) (94.3±3.06 min), whereas the addition of 10% mannitol caused the highest delay in development (425.5±58.7 min), followed by samples containing 10% xylitol (245±9.9

min) and 10% maltitol (243.0 ± 20.3 min). Hence, the total replacement of sugar by polyols furthered the delay of gluten network formation, while a partial replacement demonstrated the same results as 10% sugar did (full-sugar control). The TM represents the strength of the gluten network. Amongst the controls, the more sugar was incorporated the weaker was the gluten network (0% sugar: 55.7 ± 0.6 BU; 10% sugar 43.7 ± 3.1 BU). Polyols weakened the network even more. The lowest TM was determined, when 10% xylitol (33.3 ± 2.1 BU) and 10% mannitol (34.0 ± 1.0 BU) were added, closely followed by 10% maltitol (37.0 ± 1.0 BU).

4.3.3. Burger bun characteristics

Specific volume

The incorporation of polyols caused a decrease in specific volume, not just, when compared with a full-sugar burger bun containing 10% added sugar (3.53 ± 0.04 ml/g), but also, when compared with burger buns containing wheat starch instead of polyols (controls) (Table 4.3).

Figure 4.1 illustrates the appearance of the buns, also indicating the volume. The higher the amount of added sugar alcohols the lower the specific volume, with 10% added xylitol causing the lowest specific volume (1.84 ± 0.04 ml/g). A 3% sugar replacement by xylitol caused a significant decrease in specific volume compared to its control containing 7% sugar and 3% wheat starch (3.83 ± 0.05 ml/g). Hence, polyols influenced the specific volume immensely.



Figure 4.1 Appearance and crumb structure of controls (Sahin et al., 2017) , and polyol containing burger buns. Samples containing 3% polyols, also contain 7% added sucrose; whereas 5% sugar replacement by polyols includes 5% added sucrose. A total replacement by polyols is labelled with 10% polyol. The first row shows buns containing xylitol, with increasing amounts of xylitol from left to right. In the second row buns with mannitol are illustrated and the third row demonstrated the effect of maltitol

Table 4.3 Results of the quality characteristics of burger buns

Control sucrose content [%]	Specific Volume [ml/g]	Crumb Hardness [N]	Crumb Hardness after 24h [N]	Crumb Hardness after 48h [N]	Crumb Hardness after 120h [N]	Staling rate
0	2.90 ± 0.03 (de)	8.41 ± 0.63 (cde)	15.33 ± 0.70 (cd)	21.19 ± 0.87 (cd)	26.63 ± 1.64 (de)	2.18 ± 0.21 (ef)
5	4.49 ± 0.09 (a)	1.74 ± 0.19 (g)	2.95 ± 0.52 (g)	6.13 ± 0.59 (f)	11.94 ± 1.00 (f)	5.93 ± 1.00 (a)
7	3.83 ± 0.05 (b)	3.10 ± 0.33 (fg)	6.34 ± 0.73 (fg)	7.79 ± 0.73 (f)	14.27 ± 1.30 (f)	3.66 ± 0.78 (bcd)
10	3.53 ± 0.04 (c)	4.41 ± 0.75 (f)	8.10 ± 0.90 (ef)	11.54 ± 1.17 (ef)	24.56 ± 1.56 (e)	4.69 ± 1.07 (ab)
Xylitol content [%]						
3	3.01 ± 0.09 (d)	8.63 ± 0.90 (cde)	15.11 ± 1.64 (cd)	20.08 ± 2.69 (cd)	36.34 ± 4.33 (c)	3.23 ± 0.54 (cde)
5	2.83 ± 0.09 (e)	9.03 ± 1.00 (cd)	15.80 ± 1.00 (cd)	21.47 ± 3.65 (cd)	35.47 ± 4.76 (cd)	2.96 ± 0.68 (cdef)
10	1.84 ± 0.04 (i)	34.32 ± 3.46 (a)	83.00 ± 11.34 (a)	83.01 ± 9.02 (a)	122.52 ± 17.53 (a)	2.59 ± 0.52 (def)
Mannitol content [%]						
3	2.82 ± 0.06 (e)	7.83 ± 0.88 (de)	11.63 ± 2.07 (de)	23.59 ± 2.39 (c)	37.10 ± 2.69 (c)	3.78 ± 0.53 (bc)
5	2.67 ± 0.07 (f)	9.88 ± 1.5 (cd)	17.72 ± 1.40 (c)	23.48 ± 3.92 (c)	40.23 ± 3.77 (c)	3.13 ± 0.55 (cde)
10	2.47 ± 0.04 (g)	10.37 ± 0.87 (c)	21.36 ± 3.05 (cd)	21.36 ± 3.05 (cd)	29.79 ± 2.89 (de)	1.88 ± 0.31 (fg)
Maltitol content [%]						
3	2.82 ± 0.20 (e)	8.32 ± 1.70 (cde)	11.72 ± 2.72 (de)	22.84 ± 6.43 (cd)	33.55 ± 5.66 (cde)	3.16 ± 0.91 (cde)
5	2.94 ± 0.16 (de)	6.75 ± 1.04 (e)	12.93 ± 1.47 (cde)	16.46 ± 1.95 (de)	32.91 ± 4.33 (cde)	3.97 ± 1.04 (bc)
10	2.09 ± 0.04 (h)	17.10 ± 1.18 (b)	23.22 ± 3.62 (b)	25.14 ± 1.90 (b)	30.55 ± 2.13 (b)	0.79 ± 0.19 (g)

Table 4.3 continued

Control sucrose content [%]	Slice Area [mm²]	Area of cells [%]	Number of holes	Lightness of the Crust	Water Activity	Shelf life [days]
0	5461 ± 101 (hi)	49.4 ± 0.5 (b)	0.05 ± 0.12 (e)	79.9 ± 0.9 (b)	0.945 ± 0.004 (a)	4.5 ± 0.8 (c)
5	7269 ± 142 (a)	53.6 ± 0.7 (a)	0.45 ± 0.72 (de)	60.8 ± 1.0 (g)	0.940 ± 0.004 (ab)	6.0 ± 0.0 (bc)
7	6650 ± 111 (b)	53.0 ± 0.6 (a)	0.57 ± 0.69 (cde)	62.8 ± 2.3 (fg)	0.930 ± 0.004 (bc)	7.5 ± 0.7 (abc)
10	6235 ± 93 (cde)	53.3 ± 1.1 (a)	0.50 ± 0.52 (de)	65.0 ± 2.9 (def)	0.918 ± 0.005 (cd)	10.5 ± 0.7 (a)
Xylitol content [%]						
3	6502 ± 306 (bc)	49.5 ± 0.9 (b)	0.96 ± 0.87 (cde)	63.0 ± 3.2 (fg)	0.915 ± 0.012 (de)	10.0 ± 1.7 (a)
5	6102 ± 263 (def)	48.3 ± 0.7 (bc)	1.56 ± 0.58 (bcde)	64.9 ± 3.8 (ef)	0.905 ± 0.006 (ef)	9.3 ± 1.5 (ab)
10	5374 ± 73 (i)	44.8 ± 0.3 (e)	3.96 ± 0.96 (a)	77.7 ± 1.2 (b)	0.891 ± 0.007 (g)	8.7 ± 0.6 (ab)
Mannitol content [%]						
3	5907 ± 237 (fg)	48.6 ± 0.8 (bc)	2.12 ± 0.73 (bc)	67.3 ± 3.3 (d)	0.899 ± 0.004 (fg)	9.3 ± 1.2 (ab)
5	5745 ± 158 (gh)	49.2 ± 0.6 (b)	1.76 ± 1.14 (bcd)	71.6 ± 3.9 (c)	0.901 ± 0.005 (fg)	10.3 ± 1.5 (a)
10	5272 ± 150 (i)	47.3 ± 0.4 (cd)	2.93 ± 1.26 (ab)	83.6 ± 0.9 (a)	0.916 ± 0.004 (de)	7.7 ± 0.6 (abc)
Maltitol content [%]						
3	6046 ± 207 (efg)	48.2 ± 1.1 (bc)	1.12 ± 1.06 (cde)	66.0 ± 2.5 (de)	0.915 ± 0.006 (de)	8.7 ± 0.6 (ab)
5	6355 ± 183 (bcd)	48.7 ± 0.9 (b)	1.03 ± 0.76 (cde)	66.6 ± 4.0 (de)	0.906 ± 0.012 (def)	8.3 ± 0.6 (ab)
10	5356 ± 274 (i)	46.5 ± 0.9 (d)	3.84 ± 2.12 (a)	79.2 ± 1.6 (b)	0.905 ± 0.006 (def)	7.7 ± 0.4 (abc)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower-case letter are not significantly different ($P<0.05$).

Crust colour

The crust colour of baked products is an important characteristic as it is the one that is firstly perceived by the consumer and it affects the acceptability of the product. As shown in Table 4.3 a sugar replacement of 3% sugar by polyols did not cause any significant difference compared to a full-sugar burger bun (65.0 ± 2.9). The incorporation of 5% mannitol resulted in a significant paler crust colour, but still gave an adequate browning. A total replacement of sugar by xylitol and maltitol resulted in the same lightness as the no-added-sugar control burger bun (79.9 ± 0.9), while mannitol caused even a higher L*-value (83.6 ± 0.9).

Crumb structure

The visual attributes of the crumb are an important characteristic of bread quality. The crumb structure of bread, formed as a result of gas production by yeast and the action of heat on the bread dough, determines the sensorial quality as well as storage and staling properties. The slice area and the area of cells in a bread slice give an indication of the size of CO_2 cells captured during proofing and hold in the system during baking, but moreover, it expresses the density of the burger bun crumb. Figure 4.1 shows the differences in crumb structure.

The slice area of burger buns containing xylitol or mannitol decreased with the amount of added sugar alcohols (Table 4.3). Interestingly, the replacement of 5% added sucrose by maltitol showed a positive effect, meaning an increase in slice area. In general, a total replacement by any of the tested polyols resulted in the same slice area achieved, when no sugar was added. The area of cells, as a percentage of the total slice area, decreased significantly by the addition of polyols and reduction of sugar. The full-sugar burger bun showed the highest area of cells (53.3 ± 1.1%). A replacement of only 3% of added sugar by any type of polyol resulted in an area of cells comparable to a burger bun containing no added sugar (49.4 ± 0.5%). The number of holes significantly increased in the 10% polyol burger buns. Mannitol caused already holes, when used in small amounts (3% mannitol: 2.17 ± 0.73).

Crumb texture and staling rate

The evaluation of the crumb texture after 1 hours revealed significant changes in crumb hardness. A softer crumb of burger buns was determined in buns without added polyols (controls) (Table 4.3).

Burger buns containing sugar alcohols showed the same hardness as burger buns without any added sucrose, whereas a total sugar replacement by xylitol or maltitol caused the hardest crumbs (xylitol: 34.32 ± 3.46 N, maltitol: 17.10 ± 1.18 N).

The replacement of sugar by polyols resulted in a lower staling rate, whereas a total replacement by maltitol showed the lowest staling rate (0.79 ± 0.19).

Water activity and microbial shelf life

Water activity and shelf life are often linked to each other. The amount of free water in the system is reflected by the a_w-value. Free water is available for microorganisms to grow and thus impacts on the shelf life of food products.

Table 4.3 demonstrates the water activity of all formulations. Generally, the addition of polyols reduced the water activity in the burger buns. Maltitol, regardless of the amount added, ensured the water activity of a 10% sucrose burger bun (0.918 ± 0.005). The highest water activity was achieved, when sucrose was totally replaced by wheat starch (0.945 ± 0.004), whereas the lowest water activity was caused by the addition of 10% xylitol (0.891 ± 0.007) and 3% mannitol (0.899 ± 0.004). Interestingly, in contrast to xylitol and maltitol, the water activity of buns containing mannitol increased with increasing concentration.

The results of the shelf life study are also shown in Table 4.3. The incorporation of sugar alcohols caused an extension of shelf life compared to a no-added-sugar burger bun (4.5 ± 0.8 days) and did not differ significantly from the full-sugar burger bun control (10.5 ± 0.7 days). A 5% sugar replacement by mannitol (10.3 ± 1.5 days) demonstrated similar results to burger buns containing 10% sugar, which resulted in the longest shelf life. Maltitol showed the shortest shelf life among all polyol rich buns.

Sensory properties

The results of the sensory evaluation are demonstrated in Table 4.4 and showed that the hardest crumb structure occurred, when sugar was totally replaced by maltitol, while the softest crumb was determined for the 5% sugar control burger bun. Interestingly, the addition of polyols showed a decrease in aroma intensity with increasing addition level. Furthermore, the 0% sugar control burger buns were evaluated as the least sweet samples and also showed low intensity-scores in flavour. Sugar replacement of 3% and 5% by xylitol resulted in the sweetest samples. PCA analysis was used to group samples showing similar sensorial intensity-attributes evaluated (Figure 4.2). The results showed that the

control samples containing sucrose and wheat starch formed one group. Moreover, the total replacement of sugar by maltitol and xylitol represented the second group. All other samples, together with the full-sugar burger bun and also the 10% mannitol bun, formed the third group. The addition of 5% maltitol was the closest to the full-sugar burger bun control regarding intensity of hardness, aroma, flavour and sweetness.

Table 4.4 Sensory evaluation of the intensity of hardness (touching and bite), aroma, flavour and sweetness Each value is given as the average value ± standard deviation

Control sucrose content [%]	Hardness touching	Hardness bite	Intensity Aroma	Intensity Flavour	Intensity Sweetness
0	4.77±2.40	4.80±2.14	5.23±2.12	3.68±2.33	0.75±0.67
5	2.84±1.61	3.30±1.99	5.09±2.04	3.86±2.25	3.77±2.12
7	2.89±1.51	3.61±2.24	5.09±1.97	3.53±1.62	3.32±1.53
10	4.45±1.76	4.32±2.27	5.50±2.06	5.73±1.98	5.66±1.74
Xylitol content [%]					
3	6.86±2.17	5.16±2.09	6.18±1.65	6.93±1.60	7.93±1.92
5	7.48±1.74	5.34±2.19	5.80±2.05	6.95±2.04	7.93±1.75
10	9.32±0.82	7.07±2.42	4.25±2.21	6.91±1.83	7.59±1.64
Mannitol content [%]					
3	5.70±1.87	4.95±1.73	6.27±1.46	7.07±1.64	7.89±1.66
5	6.57±1.66	4.48±2.10	6.55±1.77	6.25±1.52	6.09±2.16
10	6.55±2.14	5.95±2.20	5.93±1.84	5.48±2.10	5.41±2.48
Maltitol content [%]					
3	4.66±2.11	3.93±2.16	5.91±1.87	5.98±2.23	7.30±2.19
5	4.75±1.81	3.64±1.99	5.39±1.77	5.25±1.95	6.18±2.06
10	9.95±0.21	9.59±0.96	4.34±2.27	5.50±2.54	6.50±2.63

Ultrastructure of polyols and their effect on the crumb structure

The micrographs taken using SEM are shown in Figure 4.3. Firstly, the raw ingredients a) sucrose, b) xylitol, c) mannitol and d) maltitol used in this study were compared. The shape and size of xylitol is very similar to sucrose's. Maltitol has a broader variation range of particle sizes, whereas the biggest particles are approximately half the size as sucrose or xylitol. The particles of mannitol are conspicuously different from any other polyol or sugar analysed. Its shape is more elongated, and its particle size is much smaller than xylitol, maltitol or sucrose. Micrographs e)-i) show the effect of no-added-sugar (e), 10% sucrose (f), 10% xylitol (g), 10% mannitol (h) and 10% maltitol (i) on the crumb structure of burger buns. Xylitol, mannitol and maltitol affected the crumb the same way as sucrose did, forming a layer covering the crumb. Comparing the layers formed by different sugar alcohols, maltitol seemed to develop a weaker film, since just parts of the crumb were covered, and disruptions were recognized. Interestingly, the crumb of buns containing 10% mannitol showed particles, similar to the raw ingredient.

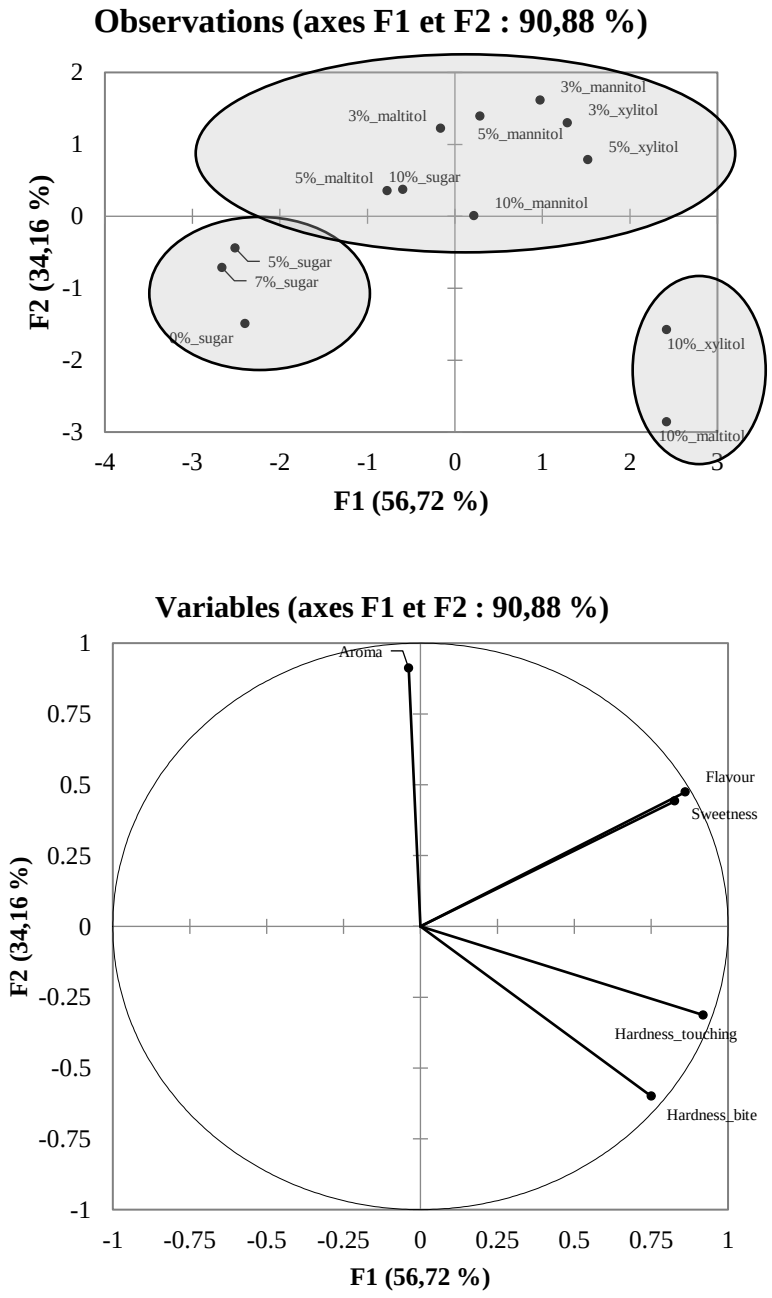


Figure 4.2 Principal component analysis (PCA) of the sensory evaluation of burger buns containing polyols as well as the controls with wheat starch as a bulking agent

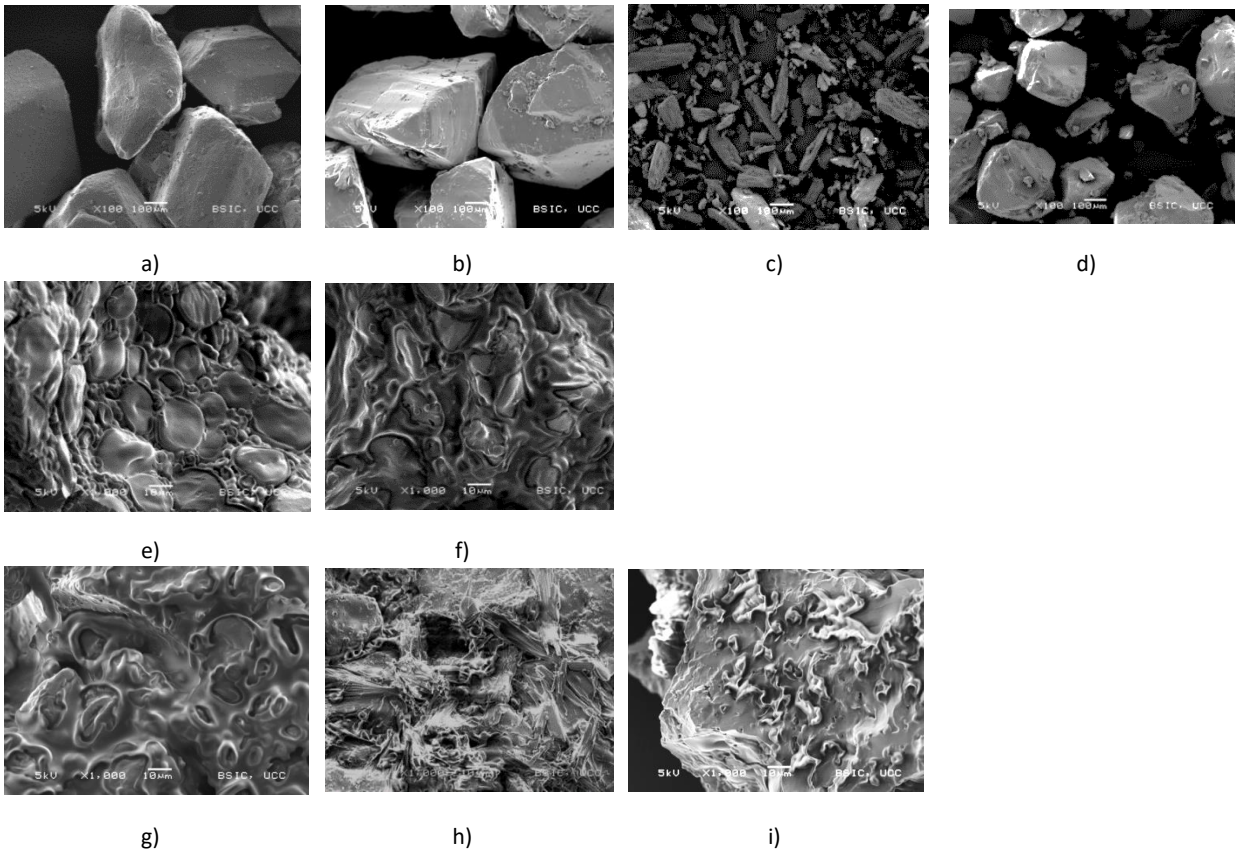
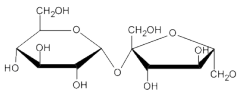
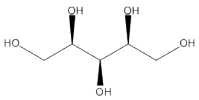
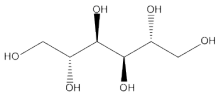
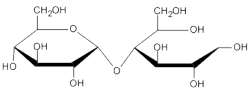


Figure 4.3 Scanning electron micrographs. a) sucrose (x100), b) xylitol (x100), c) mannitol (x100), d) maltitol (x100), e) crumb with 0% sucrose (x1000), f) crumb with 10% sucrose (x1000), g) crumb with 10% xylitol (x1000), h) crumb with 10% mannitol (x1000), i) crumb with 10% maltitol (x1000)

Particle sizes

The particle size distribution of sucrose, xylitol, mannitol and maltitol is shown in Table 4.5. Mannitol showed the smallest particles, having a Dx50 value of 60.2 ± 0.7 μm. The biggest particles were found in sucrose (Dx50 = 276.0 ± 0.0 μm), followed by xylitol (Dx50 = 228.8 ± 2.6 μm).

Table 4.5 Chemical structure, relative sweetness and particle size of sucrose, xylitol, mannitol and maltitol

	Sucrose	**Xylitol**	**Mannitol**	**Maltitol**
Chemical structure				
Relative sweetness [%] (Saulo, 2005)	100	100	50-70	75
Particle size				
Dx10	139.5±0.7	70.6±1.5	11.4±0.2	46.1±1.3
Dx50	276.0±0.0	228.8±2.6	60.2±0.7	199.0±7.5
Dx90	482.0±0.0	450.8±1.3	212.3±0.6	431.0±5.6

4.4. Discussion

This study investigated the effect of three different commercially available polyols as potential sugar replacers on the dough characteristics, physicochemical properties and shelf life of burger buns as well as sensory attributes.

The incorporation of polyols as sugar replacers in a burger bun system had a significant impact on the fermentation behaviour of the yeast. Lower total volume, smaller maximum dough height, longer fermentation time and lower gas formation were observed for dough samples containing polyols. The low gas formation occurred due to the fact that polyols cannot be fermented by yeast. Their lack of the aldehyde group is the reason for polyols being non-reducing and non-fermentable by yeast (Hough et al., 1979).

Canh et al. (1975) investigated the transport of acyclic polyols in *Saccharomyces cerevisiae*. This study showed that acyclic polyols like xylitol and mannitol are transported by simple diffusion into the cell, but they are not metabolized by the yeast. Hence, doughs containing 10% polyols resulted in a fairly poor dough development and gaseous release during fermentation. Correlation analysis demonstrated a positive correlation between V_{tot} and Hm' ($r > 0.98$, $P \leq 0.03$) regardless which polyol was incorporated. Furthermore, the addition of xylitol with increasing amounts correlated negatively with the dough extensibility ($r = -0.99$, $P \leq 0.02$) and the damping factor ($r = -0.99$, $P \leq 0.02$) of the burger bun dough. In general, the decreased extensibility might be the result of a weaker gluten network. This decrease might be also responsible for the lower specific volume and harder crumb (Mariotti and Alamprese, 2012). The dough resistance increased probably due to the formation of polyol-gluten-complexes. These complexes can be formed through ionic interactions and hydrogen bonds between polyols and gluten (Gharaie et al.,2015).

Sugar replacement by polyols showed no significant differences in damping factor, and, hence, ensured the viscoelastic proportions in the dough. Mariotti and Alamprese (2012) investigated the impact of sucrose and fructose on the viscoelastic properties of croissant-like-dough. Interestingly, they discovered that sucrose and fructose increased the viscous characteristics of the dough, whereas sugar-free doughs were more elastic.

The results of the pasting properties presented an increase in peak viscosity and final viscosity, when sugar was replaced by wheat starch. Presumably, the incorporation of more starch offers higher potential of swelling and gelatinization during the RVA

measurement, resulting in a generally more viscous system. Furthermore, sugar competes with starch for water resulting in a limitation for granules to swell.

This mechanism could also be an explanation for a lower peak and final viscosity of the full-sugar control sample (Tomasik et al, 1995). Even lower values in peak and final viscosity occurred, when sugar was partially replaced by polyols. There are three potential explanations for this observation: Firstly, the structural flexibility of the polyols, especially the acyclic ones, and their poor structural complexity could cause a lack of ordering and results in a decrease of viscosity (Tomasik et al., 1995). Secondly, most polyols are smaller molecules than sucrose. Hence, they succeed more in the interaction with water, while competing with starch for it. This could lead to a lower rate of hydration and swelling of the starch molecules, which causes a decrease in viscosity (Tomasik et al., 1995). Thirdly, an interaction between polyol and starch could have taken place and affect the viscosity. These interactions are described to result in closely packed swollen starch granules, which can resist the heat and shearing for a longer period of time (Sun et al., 2014).

The increase of breakdown viscosity, when sugar was replaced by wheat starch showed that sugar contributed to the resistance and stability of dough against heat and shear force (Newport, 1995; Zaidulet al., 2007). Polyols showed the same attributes towards breakdown viscosity as sucrose did. This could be due to the fact that polyols, or sucrose, interact with starch and stabilize the granules towards heat and shearing, as already mentioned before. The pasting temperature indicates the temperature required for gelatinization of the starch granules. Mannitol was the only polyol, which increased the pasting temperature significantly compared to sucrose, xylitol or maltitol. In contrast to sucrose, xylitol and maltitol, mannitol rather crystalizes and is only moderately soluble (Whistler and BeMiller, 1997), which can be also seen in the ultrastructure of the crumb containing 10% mannitol (Figure 4.3h). Thus, insoluble mannitol could, hypothetically, stabilize the starch granules and delay the starch swelling process (Hartel and Hasenhuettl, 2013). Hence, higher energy in the form of heat, here pasting temperature, is needed. Mannitol particles are the smallest amongst all polyols and sugar used in this study. As already mentioned before, the small particle size could have contributed to a higher interaction between mannitol and starch or protein, resulting in an increased pasting temperature.

As sugar, polyols also delayed and weakened the gluten network development resulting in higher PMT and lower TM values. Xylitol and maltitol are hygroscopic agents. Thus, they compete with gluten for water, resulting in a delayed and incomplete protein network formation (Pareyt et al., 2009). Mannitol is non-hygroscopic and moderately soluble. Nevertheless, it has the same effect on the gluten network as insoluble fibres, like cellulose, resulting in hydrophobic interactions with gluten and hence delaying the network formation (Chen et al., 1988). Furthermore, mannitol showed the smallest particle size, which could lead to a higher interaction between mannitol and gluten (Tomasik et al., 1995).

The specific volume of the burger buns reflected the impact of sugar and the different polyols on the yeast activity. Increasing amounts of added xylitol or maltitol resulted in a positive correlation between V_{tot} and specific volume of the burger buns ($r>0.99$, $P\leq0.05$). The addition of mannitol showed a higher specific volume than buns containing xylitol or maltitol. Since mannitol is just moderately soluble, it is assumed that the osmotic stress of the system was lower and hence the yeast produced more CO_2 compared to dough containing xylitol or maltitol. In general, specific volume is linked to the crumb hardness. However, in this study, there was no significant correlation. Some polyols have a plasticizing effect on gluten, when added in greater quantities, leading to increasing crumb hardness (Zhou et al., 2016). This is likely the reason for the remarkable firm crumb containing 10% of xylitol and maltitol.

The evaluation of the crumb structure showed quality differences in the slice area, the area of cells and holes in the crumb. The area of cells gives an indication of the size of the CO_2 bubbles captured during proofing and depends mainly on the CO_2 production (Hager et al., 2012). Less CO_2 was produced, when sucrose was replaced, due to lower amounts of substrate for the yeast metabolism, since polyols are non-fermentable (Hough et al., 1979). The number of holes in the crumb increased with increasing amount of sugar alcohols. Holes in bread could be the result of a weak gluten matrix. Thus, CO_2 cells could have aggregated and formed holes (Sullivan et al., 2010). These results coincide with studies conducted by Sun et al. (2014).

During baking sucrose is inverted to its reducing sugars, fructose and glucose, which react with amino acids by the Maillard reaction and provide bread crust with its characteristic brown colour. The replacement of added sucrose by polyols caused a paler crust. Firstly, the amount of reducing sugars, like fructose and glucose, in the system decreased, leading

to less Maillard reaction (Struck et al., 2014). Secondly, polyols lack reactive aldehyde groups and as a result do not participate in Maillard browning (Ghosh and Sudha, 2012).

Polyols showed an effect on the water activity of burger buns. Xylitol and maltitol seemed to bind free water stronger than sucrose, resulting in a lower a_w-value. Xylitol depressed the water activity more than maltitol did, most likely, due to its lower molecular weight and higher hygroscopicity (Ghosh and Sudha, 2012; Saulo, 2005).

As already mentioned, mannitol behaved totally different from the other sugar alcohols tested. The more mannitol was added, the higher the water activity was. Mannitol is non-hygroscopic; hence it did not interact with water to a high extent. This led to a higher a_w-value due to more free available water in the system. Furthermore, mannitol is moderately soluble, which limited the yield of water-activity-lowering agent in the system. Water activity is often linked to microbial shelf life. However, the incorporation of all three polyols showed no significant difference in the time the first mould growth was observed. Due to the fact that polyols lack an aldehyde group, which makes them non-fermentable by yeast/fungi and also resistant to bacterial degradation, an extension of shelf life was expected (Hough et al., 1979). Even if the results are statistically not significant different, it has to be noted that sugar prevented burger buns more effective from microbial growth than polyols did.

Sensory evaluation revealed a positive correlation between flavour and sweetness ($r=0.92$, $P=0.05$) considering all burger buns tested. Maltitol and mannitol have a lower relative sweetness, which decreased the flavour of the product. Nevertheless, PCA analysis grouped burger buns containing 3% or even 5% polyols in the same group as the control regarding sweetness and flavour. Hence, 50% sugar replacement by polyols could be considered, if the texture was improved. A daily intake of more than 0.37 g/kg body weight for males and 0.42 g/kg body weight for females xylitol (Oku and Nakamura, 2007), or 20 g of mannitol (Ninni et al., 2000), or more than 92 g maltitol (Ruskone-Fourmestraux et al., 2003), would cause laxative effect. Thus, the daily consumption of up to three burger buns containing 3% or 5% of one of these polyols would not cause diarrhoea.

4.5. Conclusion

This study revealed the impact of xylitol, mannitol and maltitol on the burger bun system. The incorporation of the polyols decreased the fermentability of the dough and, hence, caused a poorer product quality, meaning a reduction in specific volume and an increase in crumb hardness, but also a lighter crust colour and shorter shelf life. Furthermore, the three tested sugar alcohols delayed the gluten network formation and weakened it significantly, which in turn decreased the extensibility of the dough. Nevertheless, xylitol and maltitol are ideal sucrose replacers in relation to the pasting properties of the dough.

Sensory evaluation demonstrated a higher flavour, aroma and sweetness intensities compared to samples containing wheat starch as a sugar replacer. If xylitol, mannitol or maltitol are considered as sugar replacers, a partial replacement of maximum 50% of the sugar content is recommended, whereas mannitol showed the best overall results. Furthermore, the addition of functional ingredients should be considered, such as softening agents to improve the crumb texture, preservatives or antifungal agents to ensure shelf life or stabilizer, which prevent the formation of holes in the crumb and to ensure the number of cells.

Moreover, naturally produced polyols by fermentation with lactic acid bacteria could influence the product differently due to its *in-situ* production and might be another potential strategy in the development of sugar reduced bakery products.

4.6. Acknowledgement

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Chapter 5

LEUCONOSTOC CITREUM TR116: *IN-SITU* PRODUCTION OF MANNITOL IN SOURDOUGH AND ITS APPLICATION TO REDUCE SUGAR IN BURGER BUNS

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Abstract

A marketing study revealed that commercially available burger buns can contain up to 10% (w/w) of added sugar. In order to reduce sugar and maintaining the product quality at the same time, functional ingredients and alternative sweetening agents have to be incorporated. In this study, the sourdough lactic acid bacteria *Leuconostoc citreum* TR116, selected for its ability to produce high amounts of mannitol, was used to produce wheat sourdough and its biochemical characteristics (cell count, pH, TTA, sugar- and acid profile, as well as mannitol production) were monitored over 48 h. The so produced sourdough was then incorporated, as a functional ingredient, into a sugar reduced burger bun system and the quality characteristics of the dough and the final product were determined. Sourdough incorporation counteract the negative effects of sugar reduction on dough properties and resulted in the same viscoelastic properties (0.423±0.008) and gluten-network-development (PMT: 160±12.6s; TM: 44.0±2.6 BU) as the full-sugar control dough. Furthermore, the investigation of specific volume, crumb hardness and chewiness revealed no significant differences between sugar reduced sourdough burger buns and its control. It is noteworthy that sourdough contributed to browning reaction resulting in darker crumb and crust colour (−8.2%; −9.6%) and it extended microbial shelf life of the burger buns significantly (+3.5days). Sensory evaluation showed no significant differences in sweetness and sourness between sugar reduced buns containing sourdough and the full-sugar control. In conclusion, the incorporation of mannitol-rich sourdough fermented by *Leuconostoc citreum* TR116 represents a novel technological approach in the field of sugar reduction and showed high potential as a functional ingredient to ameliorate the losses of important quality parameters. Especially sourdough containing higher amounts of mannitol and lower amounts of lactate improved significantly the dough and burger bun quality.

5.1. Introduction

The increasing cases of childhood obesity, type two diabetes and cardiovascular disease, as well as the debate about the introduction of sugar tax in some European countries are the main reasons for the growing demand of sugar reduced products. Consumers in Europe eat in average 50 g added sugar per day, which is double the amount recommended by the WHO (Azaïs-Braesco et al., 2017; WHO, 2015). According to a recently published study, in the US sweet bakery products are the second main source of added sugar, after sweetened beverages, whereas 14.7%/ 11.4%/ 10.4% of the total added sugar are consumed in form of sweet baked goods by children/ adolescents/ adults respectively (Bailey et al., 2018). Furthermore, the baking industry in the UK is advised by the government to reduce the total sugar content by 20% by 2020. Hence, companies are urgently looking for a solution, such as the use of functional ingredients or the implementation of novel technologies, in order to adhere to the new government recommendations ensuring, at the same time, good quality product for consumers. A marketing study revealed that commercially available burger buns can contain up to 8 g of added sugar per unit, which can equate to 10% (w/w) of the whole product. Basically, the consumption of one burger bun covers one third of the recommended daily sugar intake.

Reducing sugar in burger buns is challenging, since it influences important quality parameters, such as volume, texture and taste (Sahin et al., 2017). In order to maintain these attributes, functional ingredients need to be incorporated into the system. In a previous study, the effect of different commercially available polyols on the dough and product quality of sugar reduced and no-added sugar burger buns was investigated (Sahin et al., 2018), leading to the conclusion that mannitol showed the most promising results regarding sensory properties; however, the incorporation of other functional ingredients, such as softening agents, stabilizer or preservatives would be needed to ensure to match the physiochemical properties of the commercial buns.

In order to combine the positive effect of mannitol, such as a sugar replacer, and the need for functional ingredients, to mimic the rheology properties exerted by sucrose in wheat dough systems, naturally produced mannitol by sourdough lactic acid bacteria (LAB) can be considered as a key to reduce sugar in baked goods. Sourdough studies reveal an improvement of bread quality (Arendt et al., 2007; Axel et al., 2015; Robert et al., 2006) and an enhancement of flavour and aroma (Montanari et al., 2014; Salim-ur-Rehman et al., 2006). Several studies and reviews about mannitol production by LAB in laboratory

media are published and revealed different production yields depending on the microbial strain and the fermentation conditions (Carvalheiro et al., 2011; Gobbetti et al., 2005; Otgonbayar et al., 2011; Saha and Racine, 2011; Wisselink et al., 2002). Among all mannitol producing LAB, *Leuconostoc* spp. can achieve a reduction of fructose to mannitol of up to 95% (Otgonbayar et al., 2011). This study investigates the potential of sourdough as a functional ingredient in sugar reduced burger buns representing a novel technological approach. Therefore, the performance of *Leuconostoc citreum* TR116, in wheat sourdough systems, with and without added fructose, was analysed by evaluating its biochemical traits such as microbial growth, pH, total titratable acids (TTA), sugar profile, mannitol production and acid profile over 48 h in 6 h intervals. Furthermore, *Leuconostoc citreum* TR116 sourdoughs were incorporated in a sugar reduced burger bun system and the effects on dough properties and burger bun quality were investigated.

5.2. Materials and methods

Sourdough fermentation by using the LAB strain *Leuconostoc citreum* TR116 was performed to reduce mannitol. Two different sourdough formulations were tested, one with the addition of fructose and one without, and pH, TTA, as well as microbial cell count, sugar-, polyol and acid-profile were determined every 6 h over a period of 48 h. Furthermore, the sourdoughs with the optimized fermentation time, regarding to polyol yield, were applied in two different concentrations in a sugar reduced burger bun system.

5.2.1. Raw materials

Sourdough production was carried out by using wheat flour (bakers flour, Odlums Group, Dublin, Ireland), fructose (Sigma-Aldrich, Gillingham, UK), the LAB strain *Leuconostoc citreum* TR116 and sterile tap water. Burger buns were produced using wheat flour (bakers flour, Odlums, Dublin, Ireland), instant active dried baker's yeast *Saccharomyces cerevisiae* (Puratos, Groot-Bijgaarden, Belgium), sucrose (Siúcra, Dublin, Ireland), salt (Glacia British Salt Limited, Cheshire, UK), wheat gluten (Roquette, Lestrem, France), wheat starch (Roquette, Lestrem,France), ascorbic acid (Storefast Solutions, Northfleet, UK)and sunflower oil (Musgrave Wholesale Partners, Dublin, Ireland), Sodium Stearoyl Lactate (SSL) (Danisco Grindstead Co., Copenhagen, Denmark) and tap water (25 °C).

5.2.2. *Leuconostoc citreum* TR116, medium and growth conditions

The LAB strain *Leuconostoc citreum* TR116 used for sourdough fermentation trials, has been previously isolated from yellow pea sourdough and belong to the culture collection of the Department of Biological Sciences, Cork Institute of Technology, Ireland. The strain was stored in a 40% glycerol stock at −80 °C and routinely cultivated on de Man-Rogosa-Sharpe (MRS) agar (Sigma-Aldrich, Gillingham, UK), supplemented with 0.05 g/L bromocresol green (Sigma-Aldrich, Gillingham, UK). Incubation was conducted anaerobically at 30 °C for 48 h.

5.2.3. Sourdough fermentation

Prior to sourdough production, a cell suspension of the strain TR116 was prepared. Therefore, single colonies were pre-inoculated in 10 mL MRS-broth at 30 °C for 24 h, and subcultured (1%) in 10 mL MRS broth at 30 °C for 16 h. Afterwards, cells were harvest by centrifugation (4500 rpm, 15 min, 4 °C) and washed in 10 mL sterile tap water, followed by another centrifugation step (same settings) and resuspension in sterile tap water. Five-hundred grams of sourdough was produced by the addition of the strain

TR116 at a density of 7.0 log CFU/g dough with a dough yield (DY) of 200. Two different sourdoughs were produced. Sourdough SD contained 50% wheat flour, 50% sterile tap water and the starter culture, whereas in sourdough SD_{FRU} 10% of wheat flour was replaced by fructose to trigger mannitol production, resulting in a composition of 40% wheat flour, 10% fructose and 50% sterile tap water. Ingredients were mixed using a Kenwood Major Titanium KM 020 mixer (Kenwood, Havant, UK) at speed 1 for one minute, followed by speed 2 for one minute. Sourdough samples were transferred in sterile stomacher bags and airtightly sealed. Analysis of microbial growth, pH, total titratable acidity (TTA) and sugar-, mannitol-and acid profile and quantification were performed every 6 h over a fermentation period of 48 h starting with time point 0. The pH, TTA and microbial growth were determined directly after sample collection, while the determination of sugars, mannitol and acids required freeze-drying before analysis. All fermentations were performed in triplicates.

Microbial growth and acidification of the sourdough

Microbial growth was determined for each sample taken by homogenising 1 g of sourdough sample in 10 mL of sterile ringer solution using a vortexer and the addition of sterile glass beats. Serial dilution was performed and the enumeration of TR116 was carried out by plating on MRS agar supplemented with 0.05 g/L bromocresol green (Sigma-Aldrich, Gillingham, UK) after anaerobic incubation for 48 h at 30 °C. LAB cell count, pH and total titratable acids (TTA) were determined every 6 h, starting with time point 0 and ending with 48 h. The identity of starter cultures was verified by the morphology and metabolites of the strain (Wolter et al., 2014). TTA and pH values of the fermented sourdoughs were measured using standard procedures (Arbeitergemeinschaft Getreideforschung e.V., 1994). Additionally, the difference in log cfu/g sourdough between the initial cell count and the value reached after 48 h of fermentation (Δlog), the maximum growth rate (μ_{max}), the difference in pH value between time 0 and 48h (ΔpH), the maximum acidification rate (ν_{max}) as well as the changes in TTA between 0h and 48h (ΔTTA) were determined.

Sugar-, mannitol- and acid quantification of the sourdoughs

For the analysis of sugars (glucose, fructose, sucrose and maltose), mannitol, as well as lactic acid and acetic acid, freeze-dried sourdough samples were extracted in Milli-Q water, clarified with 2.5% (w/v) Carrez I and 2.5% (w/v) Carrez II and syringed filtered (pore size of 0.2µm). The clarification of the extract for acid analysis was performed by

the addition of 2.5% (w/v) 70%-perchloric acid. The quantification of glucose (1–50 mmol/L), fructose (1–100 mmol/L), sucrose (1–50 mmol/L) and maltose (1–50 mmol/L), as well as mannitol (1–100 mmol/L), lactic acid (1–20 mmol/L) and acetic acid (1–20 mmol/L) was conducted by using an Agilent 1260 high performance liquid chromatography system equipped with a refractive index detector (RID) and an ultra violet diode array detector (UV/DAD). Standard calibration curves were determined with 5 different concentrations per compound, and measured at the beginning and at the end of a sample set. The concentration of sugars and mannitol was determined over the RID (40 °C) by elution of the extract from a RezexTM RPM-Monosaccharide Pb^{+}-column (300×7.8mm, Phenomenex, California, USA) at 80 °C, equipped with a guard column (Carbo-Pb, 4×3.0mm, Phenomenex, California, USA), using MiliQ-water at a flow rate of 0.6 mL/min and an injection volume of 20 µL.

Lactic acid and acetic acid concentrations in the sourdough samples were analysed by using a Hi-Plex H column (300×7.7mm, 8mm, Agilent, Cork, Ireland) at 65 °C, equipped with a guard column (50×7.7 mm, 8 mm, Agilent, Cork, Ireland) and setting the UV/DAD detector to 210 nm. 0.005 M sulphuric acid was applied as an eluent with a flow rate of 0.5 mL/min. The injection volume of the sample was 20 µL. The extraction was conducted twice for each sample. The identified concentration was given in g/100 g based on dry matter of the freeze-dried sourdough by considering its moisture content (results of moisture content not shown) and the average of 6 values (2 extractions for 3 batches) was determined. Additionally, the fermentation quotient (lactic acid/acetic acid) was calculated.

5.2.4. Sourdough application in sugar reduced burger buns

Two different sourdoughs, SD and SD_{FRU}, were incorporated in a sugar reduced burger bun system in order to investigate the effect of naturally *in-situ* produced mannitol on the quality of burger buns and their dough properties. Each sourdough was added in two different concentrations, 5% and 10% based on flour.

Dough properties

Several dough analyses, such as gas production during fermentation, changes in the proportion of viscous and elastic properties, extensibility of the dough, the impact of sourdough on the gluten network formation and changes in pasting properties, were performed.

Burger bun dough preparation

Sourdoughs were applied in the burger bun recipe containing 5% (w/w) added sucrose. The recipes of all formulations are shown in Table 5.1. All doughs were produced as previously described in Chapter 3 (Sahin et al., 2017). Flour and water were replaced by 5% (w/w) or 10% (w/w) sourdough respectively.

Table 5.1 Recipes of control burger buns and buns with sourdough incorporation in two different concentrations (5% and 10%). SD represents the sourdough with very low amounts of mannitol, whereas SD_{FRU} contains mannitol produced during fermentation

	Full-sugar control	**Sugar reduced control**	**5% Sourdough (SD or SD_{FRU})**	**10% Sourdough (SD or SD_{FRU})**
Wheat flour	100	100	95	90
Wheat starch	-	9.4	9.4	9.4
Sourdough (solid part)	-	-	5	10
Sourdough (liquid part)	-	-	5	10
Water	55	62.2	57.2	52.2
Sugar	18.8	9.4	9.4	9.4
Salt	1.7	1.7	1.7	1.7
Sunflower oil	6.7	6.7	6.7	6.7
Baker's yeast	2.0	2.0	2.0	2.0
Wheat gluten	3.2	3.2	3.2	3.2
SSL	0.5	0.5	0.5	0.5
Ascorbic acid	0.1	0.1	0.1	0.1

Dough development and gaseous release during proofing

The determination of the fermentation quality was conducted by using a Rheofermentometer (Chopin, Villeneuve-la-Garenne Cedex, France). Three-hundred grams of burger bun dough was placed in a fermentation chamber, a cylindrical weight of 1500 g was put on top of the dough and fermentation was performed at 30 °C for 180 min. The fermentation quality was investigated by the evaluation of several parameters such as, the volume of CO_2 production (V_{tot}), the maximum height of dough (Hm), the maximum height of gaseous release (Hm') and the time required to achieve Hm (T1).

Viscoelastic properties of the burger bun dough

A Rheometer Physica MCR301 (Anton Paar GmbH, Ostfildern, Germany) was used in order to investigate the changes in viscous and elastic proportions of burger bun dough containing different sourdoughs, as well as various sourdough concentrations. For the determination of the viscoelastic properties, the addition of yeast to the burger bun doughs was omitted (Lynch et al., 2009). An oscillating mode was used in combination with parallel plate geometry (50 mm diameter), serrated to prevent slippage. The temperature of the lower plate was set to 30 °C and first an amplitude sweep was performed, as described by Hager et al. (2011) to determine the linear viscoelastic region (data not shown) and to set the target strain for the frequency sweep. Frequency sweep was performed as described in Chapter 3 using a constant target strain of 0.01% and a frequency range from 100 to 0.1 Hz (Sahin et al., 2017) . The damping factor as an expression of the proportion of viscous and elastic parts in the burger bun dough system was evaluated.

Extensibility and resistance to extension

The extensibility and the resistance to extension were determined by using the Extensograph (Brabender, Duisburg, Germany). 150 g dough was moulded and placed in the proofing chamber at 30 °C for 60 min, followed by the measurement and the evaluation of the extensibility and the resistance of extension.

Gluten network formation

For the investigation of the impact of sourdough on the gluten network formation GlutoPeak (Brabender, Duisburg, Germany) was used. Therefore, a standard ratio of 50/50 (solid/liquid) of the recipe was weight in, considering the dough yield (200) of the sourdough (50% solid, 50% liquid). The chamber temperature was set to 36 °C and torque was recorded over a period of 600 s. The torque maximum (TM) and the peak maximum time (PMT) were evaluated.

Pasting properties

Starch pasting properties were analysed using Rapid Visco Analyser (RVA) (Newport Scientific, Warriewood, Australia). Samples containing 3 g of solids and 25 g of liquids, considering the sourdough as 50% liquid and 50% solid, were prepared. Therefore, 3 g solid ingredients (on a basis of 14% moisture in total) of the recipe were weight into a RVA aluminium sample canister, and the liquid ingredients sunflower oil and 50%

sourdough part were added, followed by the inclusion of distilled water up to a total volume of 25 g liquids. The test was conducted as described by (Horstmann et al., 2017).

Burger bun quality

The quality of sugar reduced burger buns containing sourdough compared to 10% added sugar burger bun (full-sugar control) and a 5% added sugar burger bun without sourdough addition (reduced-sugar control) was investigated by the evaluation of several properties, such as specific volume, texture, crumb (ultra-) structure, colour, water activity and microbial shelf as well as sensory characteristics. Three burger buns per batch were analysed 1 h after baking. Additionally, texture profile analysis (TPA) was carried out after 24 h, 48 h and 120 h to determine the staling rate.

Burger bun preparation

Burger bun dough was scaled to 80 g pieces, moulded, placed on a burger bun tray and baked as previously described in Chapter 3 (Sahin et al., 2017). After baking, the buns cooled down on a rack for one hour at room temperature and packed in plastic bags.

Specific volume

The determination of the specific volume [ml/g] of the burger buns was conducted by using a 3D laser scan using a VolScan Profiler 300 (Stable Micro Systems, Godalming, UK).

Crumb structure and ultrastructure

The crumb structure of the buns was determined using imaging analysis with the C-cell Bread Imaging System (Calibre Control International Ltd., Warrington, UK). Samples were prepared by cutting the bottom and the top of the burger buns to a resulting height of 35mm using a bread slicer. The area of cells [%] was evaluated.

The effect of sourdoughs on the ultrastructure of the crumb was investigated by using scanning electron microscopy (SEM). Burger buns were prepared as described in Chapter 3 (Sahin et al., 2018), coated with a layer of 25 mm of sputtered palladium-gold and observed using SEM with a working distance of 8 mm. Micrographs were taken at an accelerating voltage of 5 kV and using SEM Control User Interface software, Version 5.21 (JEOL Technics Ltd., Tokyo, Japan).

Crumb texture

TA-XT2i texture analyser (Stable Micro Systems, Godalming, UK) was used to investigate the effect of sourdough on texture properties of sugar reduced burger buns.

Therefore, the system was equipped with a 25 kg load cell and a cylindrical probe (35 mm) was chosen. Burger buns were prepared using the same manner for crumb structure. The test was performed with a test speed of 5 mm/s, a post-test speed of 10 mm/s, a force of 0.05 N and a 5 s waiting time between the first and second compression. Burger buns were analysed after 1 h, 24 h, 48 h and 120 h, and the crumb hardness, cohesiveness, springiness, chewiness and resilience were evaluated, and the staling rate was calculated using the formula mentioned in Chapter 3.

Crumb and crust colour

The colour of the burger buns was determined using a colorimeter (Minolta CR-331, Konica Minolta Holdings Inc., Osaka, Japan). The L*-value of the crumb and crust were evaluated.

Water activity and microbial shelf life

The determination of the water activity of the crumb was performed by using a water activity meter (HygroLab, Rotronic, Bassersdorf, Switzerland). The influence of sourdough on the shelf life of sugar reduced burger buns was evaluated by using the mould environmental challenge method indicated by (Dal Bello et al., 2007). Sahin et al. (2017) described the preparation of the buns for microbial shelf life (Chapter 3).

Sensory evaluation

The impact of sourdough on the sensory properties of sugar reduced burger buns was investigated by performing an intensity evaluation. A trained panel of 10 people (5 female and 5 male, age: 24–32) was chosen to determine the intensity in crumb hardness (bite), aroma, flavour, sourness and sweetness of the buns. The training of the chosen panel took place 6 h weekly over a period of 6months prior the evaluation. The procedure of the training is described in Chapter 4 (Sahin et al., 2018). The training for sourness intensity was conducted by lactic acid solution to define the scale “0” (0.01% lactic acid solution) to “10” (1% lactic acid solution). The panel collected descriptors to define the overall flavour and aroma in order to evaluate the intensity. Flavour and aroma descriptors collected are: cereals, roasted grains, sweetness, sourness (more specific: lactic acid), musty, earthy. The training as well as the sensory evaluation were conducted in a sensory panel room at 21 ± 1 °C. Sensory evaluation was performed in a duplicate.

5.2.5. Statistical analysis

All analyses were performed in triplicates. A variance analysis (one-way ANOVA, $p \leq 0.05$, Tukey test) was performed using Minitab 17. Furthermore, correlation analysis was conducted using Microsoft Excel 2016.

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5.3. Results

In order to investigate the effect of sourdough as a potential sugar replacer in sugar reduced burger buns, the characterisation of two sourdoughs was performed, followed by the application and the determination of several dough properties and product quality parameters.

5.3.1. Sourdough fermentation

Two different sourdoughs were fermented for 48 h. Sampling took place every 6 h and microbial growth as well as acidification (pH and TTA) were determined. Furthermore, sourdough samples were freeze-dried for the quantification of sugars and metabolites. In both sourdoughs a cell density of 7 log cfu/g sourdough was determined before fermentation (time point 0), which complied with the inoculation. An increase of cell density in the first 6 h of fermentation (+1.45 ± 0.43 log cfu/g in SD and +1.82 ± 0.11 log cfu/g in SD_{FRU}) was observed, followed by a further growth in SD up to 9.40 ± 0.21 log cfu/g and up to 9.21 ± 0.09 log cfu/g in SD_{FRU}. After 12 h of fermentation a stationary phase of growth occurred, which lasted until 24 h in SD and until 36 h in SD_{FRU}, followed by the decline phase of growth. SD_{FRU} showed a significant higher Δlog (2.05 ± 0.08) than SD (1.68 ± 0.04) due to a more pronounced death phase in SD (−1.80 log cfu/g) resulting in a significant lower cell count compared to SD_{FRU} after 48 h. However, the maximum growth rate μ_{max} during the exponential phase of growth of both sourdoughs did not differ significantly from each other (SD: 0.66 ± 0.07; SD_{FRU}: 0.72 ± 0.03).

The acidification of the sourdoughs is represented by the pH and TTA and shown in Table 5.2.

Both sourdoughs had a starting pH value of 5.5 and, also demonstrated the same end value of 4.16. Furthermore, the maximum acidification rate was 0.14 ± 0.01 pH/h in both sourdoughs and, hence, did not differ significantly from each other. Before fermentation, the TTA equalled in both sourdoughs. After fermentation, the value increased by 16.80 ± 1.44 mL of 0.1 M NaOH in SD_{FRU}, whereas the increase in TTA in SD was significantly lower (+9.53 ± 0.64 mL of 0.1 M NaOH). This difference is reflected by the higher ΔTTA in SD_{FRU}.

Table 5.2 . The fermentation quotient is the quotient of the amounts of lactic acid and acetic acid. TTA is presented as mL 0.1 M NaOH, and pH values of sourdough is shown. Absent molecules are marked as "not detected" (n.d.)

Fermentation time [h]		Fermentation quotient	pH	TTA [ml 0.1 M NaOH]
0	SD	-	5.50±0.06 (a)	2.82±0.21 (j)
	SD_{FRU}	-	5.54±0.16 (a)	2.78±0.13 (j)
6	SD	-	5.27±0.06 (b)	4.85±0.16 (i)
	SD_{FRU}	4.33	5.36±0.04 (b)	4.58±0.48 (i)
12	SD	21.20	4.44±0.10 (cd)	9.25±0.89 (h)
	SD_{FRU}	5.19	4.50±0.05 (c)	10.80±0.73 (g)
18	SD	25.64	4.24±0.03 (ef)	10.97±0.40 (g)
	SD_{FRU}	3.86	4.33±0.02 (de)	12.73±1.07 (e)
24	SD	28.30	4.18±0.03 (f)	11.98±0.16 (fg)
	SD_{FRU}	3.00	4.27±0.04 (ef)	15.17±0.43 (d)
30	SD	28.80	4.20±0.05 (f)	11.47±0.27 (efg)
	SD_{FRU}	3.15	4.21±0.04 (ef)	17.00±0.59 (c)
36	SD	27.73	4.18±0.05 (f)	11.77±0.25 (efg)
	SD_{FRU}	3.04	4.20±0.06 (ef)	17.38±0.46 (bc)
42	SD	24.08	4.17±0.06 (f)	11.98±0.54 (efg)
	SD_{FRU}	3.35	4.15±0.03 (f)	18.28±0.44 (b)
48	SD	22.08	4.16±0.05 (f)	12.35±0.59 (ef)
	SD_{FRU}	4.27	4.16±0.05 (f)	19.58±1.31 (a)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

The sugar and acid profiles during fermentation are demonstrated in Table 5.3. The same amount of sucrose was present in both sourdoughs at the beginning of the fermentation (0 h), whereas after 6 h of fermentation no sucrose was detected anymore. Furthermore, the concentration of maltose in both sourdoughs did not differ at time point 0. During fermentation the amounts of maltose decreased after 6 h, followed by an increase, resulting in a final maltose concentration of 4.95 ± 0.34 g/ 100g dry matter (DM) in SD and 2.62 ± 0.08 g/100g DM in SD_{FRU}. The glucose quantities in both sourdoughs were very low at the beginning of fermentation.

Table 5.3 Quantities of sugars (sucrose, maltose, glucose, fructose), mannitol and acids (lactic acid and acetic acid) in sourdough SD and sourdough SD_{FRU} in gram per 100g sourdough based on dry matter (DM) during fermentation. Absent molecules are marked as "not detected" (n.d.)

Fermentation time [h]		Sucrose [g/100gDM]	Maltose [g/100gDM]	Glucose [g/100gDM]	Fructose [g/100gDM]	Mannitol [g/100gDM]	Lactic acid [g/100gDM]	Acetic acid [g/100gDM]
0	SD	0.32 ± 0.06 (a)	2.51 ± 0.36 (fg)	0.33 ± 0.08 (j)	< 0.19 (ij)	0.20 ± 0.03 (f)	< 0.10 (i)	n.d.
	SD_{FRU}	0.21 ± 0.16 (b)	2.24 ± 0.62 (fghi)	< 0.37 (j)	11.54 ± 0.25 (a)	< 0.36 (ef)	< 0.09 (i)	< 0.06 (g)
6	SD	n.d.	2.77 ± 0.25 (defg)	0.71 ± 0.04 (i)	< 0.19 (j)	0.55 ± 0.11 (ef)	0.80 ± 0.21 (g)	n.d.
	SD_{FRU}	n.d.	2.21 ± 0.16 (ghi)	1.14 ± 0.04 (de)	10.31 ± 0.43 (b)	0.82 ± 0.12 (ef)	0.39 ± 0.06 (h)	0.09 ± 0.02 (f)
12	SD	n.d.	2.49 ± 0.19 (fg)	0.72 ± 0.05 (hi)	< 0.19 (j)	0.73 ± 0.04 (ef)	2.12 ± 0.07 (c)	0.10 ± 0.02 (f)
	SD_{FRU}	n.d.	1.67 ± 0.28 (i)	0.79 ± 0.01 (hi)	4.77 ± 0.23 (c)	3.44 ± 0.38 (d)	1.35 ± 0.02 (f)	0.26 ± 0.03 (e)
18	SD	n.d.	3.21 ± 0.28 (de)	1.03 ± 0.01 (efg)	< 0.19 (j)	0.83 ± 0.03 (ef)	2.82 ± 0.14 (b)	0.11 ± 0.02 (f)
	SD_{FRU}	n.d.	1.81 ± 0.30 (hi)	0.87 ± 0.02 (ghi)	2.03 ± 0.07 (d)	6.14 ± 0.75 (c)	1.39 ± 0.07 (ef)	0.36 ± 0.01 (d)
24	SD	n.d.	4.55 ± 0.17 (ab)	0.92 ± 0.03 (fgh)	< 0.19 (j)	0.90 ± 0.06 (e)	2.83 ± 0.13 (b)	0.10 ± 0.01 (f)
	SD_{FRU}	n.d.	2.46 ± 0.15 (fg)	1.12 ± 0.06 (de)	1.43 ± 0.16 (e)	8.41 ± 0.63 (b)	1.32 ± 0.03 (f)	0.44 ± 0.05 (c)
30	SD	n.d.	4.11 ± 0.61 (abc)	1.50 ± 0.14 (abc)	< 0.19 (j)	0.98 ± 0.12 (ef)	2.88 ± 0.12 (ab)	0.10 ± 0.02 (f)
	SD_{FRU}	n.d.	2.86 ± 0.35 (def)	1.30 ± 0.05 (cd)	0.78 ± 0.08 (f)	9.08 ± 0.26 (ab)	1.45 ± 0.04 (ef)	0.46 ± 0.02 (bc)
36	SD	n.d.	4.12 ± 0.33 (b)	1.67 ± 0.09 (a)	< 0.19 (j)	0.94 ± 0.08 (e)	3.05 ± 0.17 (a)	0.11 ± 0.01 (f)
	SD_{FRU}	n.d.	2.40 ± 0.18 (fgh)	1.09 ± 0.03 (ef)	0.40 ± 0.05 (ghi)	9.08 ± 0.18 (ab)	1.58 ± 0.05 (de)	0.52 ± 0.06 (a)
42	SD	n.d.	4.01 ± 0.42 (b)	1.68 ± 0.24 (a)	< 0.19 (j)	0.92 ± 0.09 (e)	2.89 ± 0.10 (ab)	0.12 ± 0.02 (f)
	SD_{FRU}	n.d.	3.34 ± 0.09 (cd)	1.39 ± 0.04 (bc)	0.50 ± 0.04 (g)	9.69 ± 0.42 (a)	1.64 ± 0.07 (d)	0.49 ± 0.02 (abc)
48	SD	n.d.	4.95 ± 0.34 (a)	1.69 ± 0.11 (a)	< 0.19 (j)	0.91 ± 0.01 (e)	2.87 ± 0.08 (ab)	0.13 ± 0.02 (f)
	SD_{FRU}	n.d.	2.62 ± 0.08 (efg)	1.52 ± 0.22 (ab)	0.44 ± 0.06 (gh)	9.63 ± 0.25 (a)	1.70 ± 0.05 (d)	0.52 ± 0.03 (ab)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

However, the amount of glucose increased after 6 h. During fermentation the amounts of glucose increased and decreased slightly over time, resulting in final concentrations of 1.69 ± 0.11 g/100g DM in SD and 1.52 ± 0.22 g/100g DM in SD_{FRU} after 48 h. In both sourdoughs the amounts of fructose differed in starting concentration (0.16 ± 0.04 g/100g DM in SD, 11.54 ± 0.25 g/100g DM in SD_{FRU}). Nevertheless, fructose quantities decreased over time resulting in a final concentration lower than 0.5 g/ 100g DM in both sourdoughs. Mannitol was produced in both sourdoughs. The production of this polyol stopped after 18 h in SD, whereas in SD_{FRU} the highest amount of mannitol was reached after 30 h. SD_{FRU} contained the ten-fold amount of mannitol compared to SD at the end of fermentation. Regarding the acids, citric acid, malic acid and succinic acid were detected in small amounts (lower than 1 mmol/L) in both sourdoughs (data not shown), whereas the concentration of lactic acid and acidic increased during fermentation. Lactic acid was detected after 6 h and increased over time, resulting in a final concentration of 2.87 ± 0.08 g/100g DM in SD and 1.70 ± 0.05 g/100g DM in SD_{FRU}. Acetic acid occurred after 12 h in SD and after 6 h in SD_{FRU} and the amount also increased over time. The end concentration of acetic acid in SD_{FRU} (0.52 ± 0.03 g/100g DM) was 4 times higher than in SD (0.13 ± 0.02g/100 g DM). The fermentation quotient showed significant higher values in SD, which reached between 4.1- and 9.4-fold the amount of SD_{FRU} over time (Table 5.2).

5.3.2. Sourdough application in sugar reduced burger buns

In order to investigate the potential of both sourdoughs as a tool to reduce added sugar in burger buns, SD and SD_{FRU} were applied in a 50% (w/w) sugar reduced burger bun formulation by wheat starch (5% (w/w) added sucrose) in two concentrations (5% and 10% of flour and water). Based on the results of the kinetic study above, a fermentation time of 30 h was chosen to achieve the highest mannitol production. The sourdoughs were freshly applied and their effect on dough quality (Table 5.4) and burger bun properties (Table 5.5) was evaluated.

Effect of sourdoughs on dough properties

The impact of two different sourdoughs in two different concentrations on a burger bun dough was investigated. Therefore, the dough development and gas production during dough fermentation, the extensibility and the resistance to extension, the effect on gluten network formation, as well as the impact of the sourdoughs on the pasting properties of the burger bun dough system, were evaluated.

Fermentation quality

The application of both sourdoughs, regardless the type and amount, did not affect the maximum dough height Hm significantly, neither did it influence the time required to reach this height (T1), or the volume of CO_2 production (V_{tot}) compared to both controls (Table 5.4). On the other hand, the incorporation of sourdough revealed a significantly higher Hm' value than the full-sugar control (increase of 15.9–22.8%), but significantly lower Hm' than the sugar reduced control (decrease of 24.7–29%). Moreover, Hm' of the burger bun doughs containing SD or SD_{FRU} did not differ from each other (between 105.0 ± 2.8 mm and 111.3 ± 2.9 mm).

Viscoelastic properties

The viscoelastic properties of burger bun doughs were determined by oscillation and are demonstrated in Table 5.4. The damping factor of the reduced sugar burger bun control with 5% (w/w) added sucrose (0.365 ± 0.011) differed significantly from the damping factor of the full-sugar control dough with 10% (w/w) added sucrose (0.423 ± 0.008). The incorporation of sourdough caused the same viscoelastic dough properties as the full-sugar control dough regardless the type or amount of sourdough added.

Extensibility and resistance to extension

The extensibility and the resistance of extension are main characteristics of doughs and reflect the microstructural properties as well as the consistency of the dough. These parameters were determined to evaluate the influence of sourdough on the strength of the dough (Table 5.4). No significant differences in extensibility of the doughs with sourdough compared to the controls occurred. On the other hand, a significant increase in resistance of extension by the incorporation of 5% sourdough was determined. The addition of 10% SD resulted in the same resistance of extension as the full-sugar control dough.

Gluten network development

The impact of two different types of sourdough in two different concentrations on the gluten network formation was determined by the evaluation of the torque maximum (TM) and the peak maximum time (PMT) and are demonstrated in Table 5.4. The highest TM occurred in the control recipe containing 5% (w/w) added sucrose (49.8 ± 1.0 BU), whereas the lowest TM was detected in recipes containing 10% SD (35.2 ± 2.2 BU) and 10% SD_{FRU} (33.5 ± 2.7 BU). The addition of 5% SD or 5% SD_{FRU} showed the same torque maximum as the full-sugar control recipe did (44.0 ± 2.6 BU). PMT shows how

fast the gluten network develops. The fasted formation was detected in the sugar reduced control (114.8 ± 2.4 s), while the longest development occurred, when 10% SD_{FRU} was incorporated. The addition of 5% sourdough caused the same PMT as the full-sugar control (160.0 ± 12.6 s).

Pasting properties

In order to investigate the impact of SD and SD_{FRU} on the starch gelatinization of sugar reduced burger bun dough, peak viscosity, breakdown, final viscosity and pasting temperature of these systems were determined by using RVA. The peak viscosity is the viscosity at which the starch swells to its maximum capacity. The results of the pasting properties are shown in Table 5.4. The sugar reduced control resulted in a significant higher peak viscosity than the full-sugar control (865.3 ± 78.8 cP). The addition of sourdough to the sugar reduced recipe showed the same peak viscosity as 10% added sucrose control. Breakdown is an indication of the stability of the system towards heat and sheering. The highest breakdown occurred in the 5% (w/w) added sugar control (324.0 ± 8.0 cP), whereas the full-sugar control showed the lowest stability (236.7 ± 7.4 cP). The addition of sourdough caused a lower stability than the sugar reduced control, but it withstood heat and sheering more than the full-sugar control, whereas the incorporation of 10% SD_{FRU} showed the same breakdown as the 10% (w/w) added sugar control system. The final viscosity represents the viscosity of the system after gelatinization and cooling. The addition of 10% SD, 5% SD_{FRU} and 10% SD_{FRU} resulted in the same final viscosity as the full-sugar control (968.3 ± 73.9 cP), which was significantly lower than the final viscosity of the sugar reduced control (1225.0 ± 73.7 cP). No significant differences in pasting temperature were determined.

Table 5.4 Dough properties of burger buns containing SD or SD_{FRU} compared with the full-sugar- and sugar reduced burger bun controls. Hm represents the maximum dough height, Hm' is the maximum height of gaseous release, T1 reflects the time needed to reach Hm, Vtot is the volume of CO_2 production, PMT illustrates the peak maximum time during the gluten network development and TM is the torque maximum reached

		Hm [mm]	Hm' [mm]	T1 [min]	V_{tot} [ml]	Damping factor	Extensibility [mm]	Resistance to extension [BU]
Control	**5% sucrose**	62.3 ± 3.7 (a)	147.8 ± 5.0 (a)	126.9 ± 9.2 (a)	2349.4 ± 25.5 (a)	0.364 ± 0.012 (b)	110.5 ± 3.5 (ab)	475.0 ± 21.2 (bc)
	10% sucrose	65.3 ± 4.5 (a)	90.6 ± 0.6 (c)	102.8 ± 0.3 (a)	2004.4 ± 14.2 (a)	0.423 ± 0.008 (a)	115.0 ± 0.0 (ab)	382.5 ± 3.5 (d)
SD	**5%**	62.8 ± 9.0 (a)	105.0 ± 2.8 (b)	161.3 ± 26.5 (a)	2337.0 ± 64.7 (a)	0.408 ± 0.011 (a)	116.0 ± 3.9 (a)	605.2 ± 30.5 (a)
	10%	58.4 ± 8.6 (a)	109.2 ± 9.0 (b)	100.5 ± 28.0 (a)	2517.7 ± 260.6 (a)	0.413 ± 0.016 (a)	104.7 ± 2.4 (b)	428.3 ± 19.4 (cd)
SD_{FRU}	**5%**	66.0 ± 5.2 (a)	105.1 ± 4.1 (b)	149.0 ± 27.0 (a)	2305.3 ± 121.0 (a)	0.399 ± 0.014 (ab)	114.2 ± 7.3 (a)	616.7 ± 20.7 (a)
	10%	55.6 ± 5.5 (a)	111.3 ± 2.9 (b)	113.5 ± 16.7 (a)	2313.0 ± 68.6 (a)	0.407 ± 0.011 (a)	114.3 ± 1.6 (a)	475.0 ± 25.9 (b)

Table 5.4 continued

		PMT [s]	TM [BU]	Peak viscosity [cP]	Breakdown [cP]	Final viscosity [cP]	Pasting temperature [°C]
Control	**5% sucrose**	114.8± 2.4 (d)	49.8 ± 1.0 (a)	865.3 ± 78.8 (a)	324.0 ± 8.0 (a)	1225.0 ± 73.7 (a)	63.6 ± 0.0 (a)
	10% sucrose	160.0 ± 12.6 (c)	44.0 ± 2.6 (b)	677.0 ± 22.5 (cd)	236.7 ± 7.4 (d)	968.3 ± 73.9 (c)	66.7 ± 1.6 (a)
SD	**5%**	158.3 ± 4.1 (c)	40.2 ± 2.4 (b)	727.0 ± 18.1 (b)	271.5 ± 5.8 (b)	1112.3 ± 18.1 (ab)	64.2 ± 1.5 (a)
	10%	186.0 ± 4.7 (b)	35.2 ± 2.2 (c)	716.5 ± 6.0 (bc)	264.5 ± 5.5 (bc)	1046.0 ± 91.4 (bc)	65.9 ± 1.7 (a)
SD_{FRU}	**5%**	158.8 ± 5.4 (c)	40.3 ± 2.3 (b)	715.4 ± 18.8 (bc)	269.6 ± 12.3 (b)	1079.2 ± 14.8 (bc)	65.0 ± 1.3 (a)
	10%	212.3 ± 15.5 (a)	33.5 ± 2.7 (c)	677.0 ± 8.7 (d)	253.8 ± 6.7 (cd)	1026.8 ± 14.2 (bc)	64.9 ± 1.4 (a)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

Impact of sourdoughs on burger bun quality

The effect of both sourdoughs on burger bun characteristics as well as their sensory attributes was investigated.

Specific volume

The highest specific volume was determined in the sugar reduced control containing 5% (w/w) added sucrose (4.47 ± 0.09 ml/g), whereas the lowest value occurred, when 10% SD_{FRU} (3.27 ± 0.06 ml/g) was incorporated (Table 5.5). The 5% (w/w) sucrose control differed significantly from the full-sugar burger bun (3.51 ± 0.07 ml/g), indicating less yeast inhibition with lower amounts of added sucrose. However, the full-sugar recipe did not show any significant differences to burger buns containing sourdough, regardless the type or amount of added sourdough. The incorporation of 10% SD_{FRU} showed the closest results to the 10% (w/w) added sugar control.

Crumb structure and ultrastructure

The most noteworthy parameter of the crumb structure was the area of cells. The biggest area of cells was determined, when SD (55.1 ± 0.8%–55.7 ± 1.9%) was incorporated into the burger bun system, whereas the full-sugar burger bun (53.2 ± 0.9%) and the addition of SD_{FRU} (52.5 ± 1.1%–53.4 ± 1.6%) resulted in the smallest area of cells Table 5.5. The effect of the sourdough incorporation on the crumb ultrastructure compared with a full-sugar control and a no-added sugar sample is illustrated in Figure 5.1. The incorporation of sourdough showed a significant change in crumb structure in form of a “film” which coated the starch granules. This coating was visible in full-sugar burger buns, but did not appear in buns without added sucrose.

Crumb texture

The crumb texture was measured using a texture profile analyser. The results are demonstrated in Table 5.5. No significant differences in cohesiveness, springiness or resilience amongst all samples were detected (data not shown). However, the addition of sourdough influenced the crumb hardness and chewiness of burger buns, as well as their staling rate. The addition of 10% SD_{FRU} caused the hardest crumb (5.24 ± 0.60 N) and showed no significant differences to the full-sugar control (4.39 ± 0.79 N). The softest crumb occurred in the reduced sugar burger bun control (1.73 ± 0.16 N). Furthermore, differences in chewiness between the controls and the buns containing sourdough were determined. Buns containing 10% SD_{FRU} showed the highest chewiness value (3.29 ± 0.44) and, again, did not differ significantly from the full-sugar control (2.68 ± 0.57).

Table 5.5 Burger bun properties and sensory characteristics of buns containing SD or SD_{FRU} compared to 10% added sugar and sugar reduced burger bun controls. TPA stands for "texture profile analyser"

		Specific volume [ml/g]	Crumb Hardness (TPA) [N]	Chewiness	Staling rate	Area of cells [%]	L*-value crust	L*-value crumb
Control	**5% sucrose**	4.47 ± 0.09 (a)	1.73 ± 0.16 (c)	1.24 ± 0.13 (d)	5.94 ± 1.00 (a)	53.7 ± 0.8 (bc)	60.7 ± 0.9 (b)	78.6 ± 1.3 (a)
	10% sucrose	3.51 ± 0.07 (bcd)	4.39 ± 0.79 (a)	2.68 ± 0.57 (ab)	4.58 ± 0.92 (b)	53.2 ± 0.9 (c)	65.1 ± 3.3 (a)	78.22 ± 2.0 (a)
SD	**5%**	3.73 ± 0.36 (b)	3.04 ± 0.73 (b)	2.05 ± 0.49 (c)	5.07 ± 0.82 (ab)	55.7 ± 1.9 (a)	54.9 ± 2.0 (d)	74.2 ± 3.4 (b)
	10%	3.62 ± 0.24 (bc)	3.23 ± 0.39 (b)	2.32 ± 0.47 (bc)	5.18 ± 1.34 (ab)	55.1 ± 0.8 (ab)	56.8 ± 2.6 (c)	71.9 ± 3.0 (cd)
SD_{FRU}	**5%**	3.40 ± 0.34 (cd)	3.45 ± 0.62 (b)	2.31 ± 0.38 (bc)	5.39 ± 0.58 (ab)	53.4 ± 1.6 (bc)	55.2 ± 2.1 (d)	73.0 ± 2.7 (bc)
	10%	3.27 ± 0.06 (d)	5.24 ± 0.60 (a)	3.29 ± 0.44 (a)	4.42 ± 0.67 (b)	52.5 ± 1.1 (c)	59.4 ± 1.0 (b)	70.69 ± 1.6 (d)

Table 5.5 continued

		Water activity	Shelf life [d]	Sweetness	Sourness	Aroma	Flavour	Hardness (bite)
Control	**5% sucrose**	0.937 ± 0.005 (b)	6.5 ± 0.7 (c)	3.67±1.21 (a)	2.67±1.89 (a)	6.67±2.22 (ab)	6.44±1.40 (ab)	4.28±1.87 (a)
	10% sucrose	0.914 ± 0.006 (c)	10.0 ± 0.0 (a)	6.00±1.26 (a)	2.17±2.62 (a)	7.28±1.44 (a)	6.78±1.18 (a)	4.50±1.94 (a)
SD	**5%**	0.947 ± 0.003 (a)	7.0 ± 0.0 (c)	4.33±1.63 (a)	1.44±1.36 (a)	4.33±2.19 (b)	4.78±1.30 (ab)	3.83±1.50 (a)
	10%	0.951 ± 0.002 (a)	10.0 ± 0.0 (a)	4.50±1.22 (a)	1.94±1.59 (a)	4.94±1.78 (ab)	4.89±1.71 (ab)	4.06±1.47 (a)
SD_{FRU}	**5%**	0.950 ± 0.007 (a)	7.5 ± 0.7 (bc)	4.50±2.26 (a)	1.78±1.66 (a)	5.00±2.05 (ab)	4.56±1.31 (b)	4.11±1.27 (a)
	10%	0.955 ± 0.006 (a)	9.0 ± 0.0 (ab)	5.00±1.67 (a)	2.39±2.06 (a)	5.39±2.53 (ab)	5.89±1.62 (ab)	4.00±1.00 (a)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

The less chewy crumb occurred in the sugar reduced control (1.24 ± 0.13). The staling rate indicates the retrogradation of the starch in the product. The highest staling rate was measured in the sugar reduced control burger bun (5.94 ± 1.00), which did not differ significantly from buns containing 5% SD (5.07 ± 0.82), 10% SD (5.18 ± 1.34) or 5% SD_{FRU} (5.39 ± 0.58). The full-sugar burger bun (4.58 ± 0.92) showed together with buns accommodating 10% SD_{FRU} (4.42 ± 0.67) the lowest staling rates.

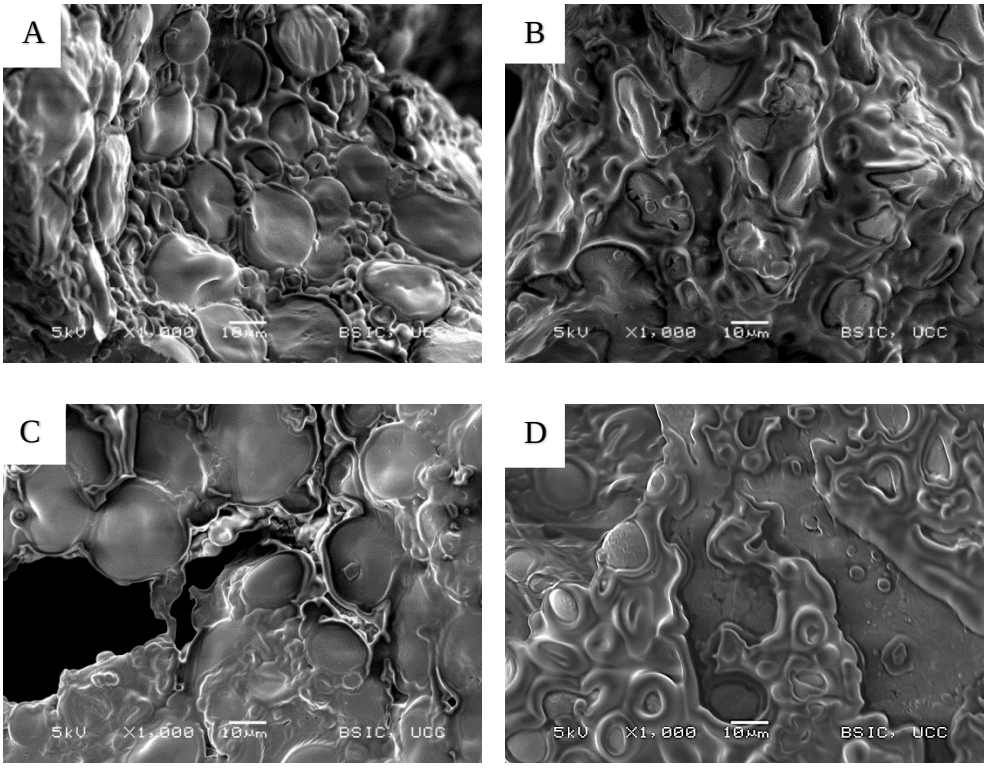


Figure 5.1 Micrograph of burger bun crumb taken by a scanning electron microscope (SEM). (A) no added sucrose (x1000)*, (B) 10% added sucrose (full-sugar control) (x1000)*, (C) 10% SD, (D) 10% SD_{FRU}.

** micrograph from Chapter 1 (Sahin et al., 2017)*

Lightness of crumb and crust

The darkest crust colour appeared, when 5% SD (54.9 ± 2.2) or 5% SD_{FRU} (55.2 ± 2.1) was incorporated. The full-sugar control bun showed the palest crust amongst all samples (65.1 ± 3.3). The addition of 10% SD_{FRU} (59.4 ± 1.0) showed the same lightness value as the sugar reduced burger bun control (60.7 ± 0.9). Furthermore, sourdough influenced the lightness of the crumb. The more sourdough was incorporated into the system the darker was the crumb Table 5.5.

Water activity and microbial shelf life

The water activity (a_w) is an important parameter which reflects the amount of free water available in the system. The lowest water activity was measured in the full-sugar control bun (0.914 ± 0.006), followed by the sugar reduced control (0.937 ± 0.005). The incorporation of sourdough, regardless the type and amount, increased the a_w-value significantly compared to the controls (Table 5.5). The shelf life profile is shown in Figure 5.2. The longest shelf life was detected in burger buns containing 10% (w/w) added sucrose (10.0 ± 0.0 days), 10% SD (10.0 ± 0.0 days) or 10% SD_{FRU} (9.0 ± 0.0 days). The shortest shelf life was determined in the sugar reduced control (6.5 ± 0.7 days) and in buns with 5% SD (7.0 ± 0.0 days) or 5% SD_{FRU} (7.5 ± 0.7 days) (Table 5.5).

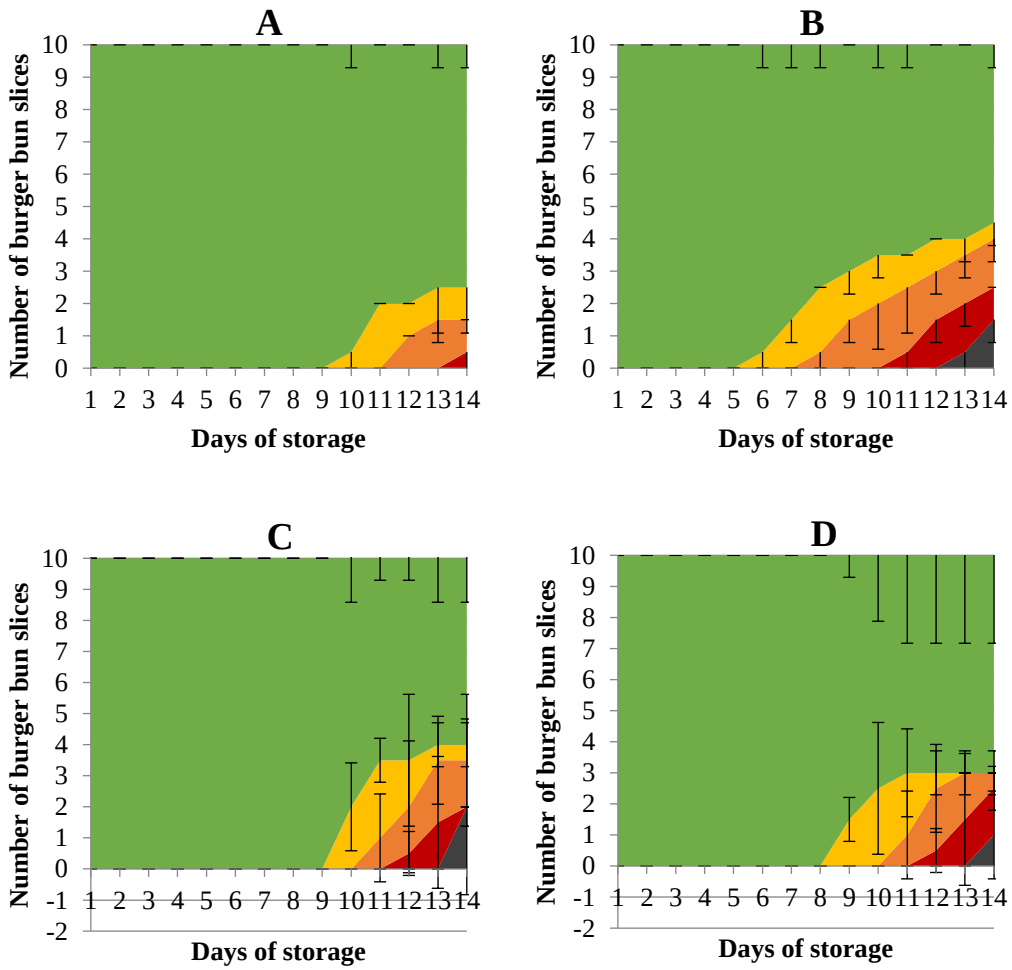


Figure 5.2 Shelf life profile of burger buns containing (A) 10% added sucrose (full sugar control), (B) 5% added sucrose (sugar-reduced control), (C) 10% SD and (D) 10% SD_{FRU}. The number of burger bun halves for each mould group ("mould-free", "<10% mouldy", < 10-24% mouldy", "<25-49% mouldy" and >50% mouldy") was counted on each day over 14 days. The graph shows mean values with standard deviations as error bars

Sensorial properties

Sensory characteristics of a product are one of the most important parameters for consumer acceptance. The incorporation of sourdough revealed no significant differences in hardness amongst all samples. Furthermore, although all samples did not differ significantly from each other regarding sweetness intensity, absolute values indicated an amelioration of sweetness by sourdough addition. Regarding flavour and aroma, the full-sugar control showed significant higher intensities than the sugar reduced control. Burger buns containing SD_{FRU}, regardless of the amount added, showed no significant differences in aroma compared to the controls. Furthermore, the incorporation of 10% SD or SD_{FRU} caused the same flavour intensity as the full-sugar control (Table 5.5).

5.4. Discussion

The application of sourdough in baked goods illustrates a longstanding tradition to enhance flavour and textural properties of the endproduct. Previously, sourdough was never considered as a functional ingredient to reduce sugar in sweet baked goods.

The results of this study showed high potential of sourdough fermentation by the mannitol producing strain *Leuconostoc citreum* TR116 for the production of sugar reduced burger buns. Before the incorporation of sourdough to sugar reduced burger buns, the suitable fermentation time was investigated, evaluating the microbial growth, acidification and quantifying sugars, mannitol, lactate and acetate.

The microbial growth in SD and SD_{FRU} over time did not differ significantly from each other, except for time point 48 h, where the cells of SD entered the death phase. The starting point of the stationary phase of microbial growth after 12 h of fermentation occurred, most likely, due to growth inhibition by the acidy environment (pH 4.5) (Hemme and Foucaud-Scheunemann, 2004). The added fructose in SD_{FRU} served as an additional fermentable substrate, which prolonged the microbial stationary phase of growth compared to SD (Wisselink et al., 2002). Furthermore, heterofermentative LAB, such as *Leuconostoc citreum* TR116, preferably produce acetate in the presence of fructose, which results in the regeneration of one extra ATP (Saha and Racine, 2011). This provides additional energy for the cell and, hence, could contribute to the extended survival (Gänzle, 2015).

During fermentation, the pH dropped from 5.5 (initial pH) to 4.2 (pH after 48 h fermentation) in both sourdoughs, due to the production of organic acids, such as lactate and acetate. Although both sourdoughs had the same ν_{max} and pH profile, the TTA values of SD and SD_{FRU} showed significant differences. These differences are most likely related to the buffering capacity of the produced acids, mainly acetic acid. After 48 h of the fermentation the starting point of titration was in both sourdoughs a pH of 4.2. The titration was conducted towards pH 8.5, while mainly the buffer range of acetic acid (3.75–5.75) compared to lactic acid (2.86–4.86) was traversed (Chang, 2005). Considering that the concentration of acetic acid in SD_{FRU} is four-fold of the amount determined in SD, a higher TTA occurred in SD_{FRU}.

Due to the presence of little amounts of fructans, which got broken down into fructose during fermentation (Escrivá and Martínez-Anaya, 2000), small concentrations of mannitol and acetate occurred in sourdough with no added fructose. It is noteworthy that

the increased concentration of acetate during fermentation correlated positively with the amounts of mannitol detected in both sourdoughs (SD: r=0.89, $P < 0.005$; SD_{FRU}: r=0.98, $P < 0.001$). Hereby, fructose acts as an electron acceptor and gets enzymatically reduced to mannitol by a mannitol-dehydrogenase, while regenerating the co-factor NAD^+. Acetate is formed from acetyl-phosphate by an acetate kinase (Gänzle, 2015). Evidently, acetate production is connected to fructose reduction to mannitol. The reduction of fructose to mannitol in SD_{FRU} showed a yield of 86.8%. It has been reported that the yield of mannitol production by *Leuconostoc* species depends on the species and cultivation conditions, such as medium composition, temperature and pH, and can vary between 26% and 90% (Carvalheiro et al., 2011; Erten, 1998; Otgonbayar et al., 2011; Saha and Racine, 2011; Von Weymarn et al., 2002). Taking into account that sourdough is a complex system, a yield of 86.8% can be considered as high.

Furthermore, the production of other metabolites, such as lactate and acetate, also depend on environmental conditions the microorganism is exposed to and on the strain itself. Hence, after 48 h fermentation a lower concentration of metabolites in SD_{FRU} (1:0.34:0.15 (mannitol:lactate:acetate) in mole) than theoretical possible (1:0.5:0.5 (mannitol:lactate:acetate) in mole), occurred (Saha and Racine, 2011). Lower amounts of acetate were determined either due to a potential lack of ADP, which is needed to convert acetyl-phosphate into acetate, or, more likely, due to stress in form of a low extracellular pH, since pH is the most important factor, which influences microbial growth and metabolite production (Pimentel et al., 1994). At the same time mannitol production is still implemented, most likely, due to the fact that mannitol, like other polyols, protect the living cell by, for example, ensuring no structure changes of membrane lipids and other proteins at low water activity, which in turn can also be responsible for the longer stationary phase of microbial growth (Wisselink et al., 2002).

The sugar profile indicates an increase in maltose and glucose during the first 24 h in both sourdoughs, followed by a fluctuation or no change in the concentrations. The increase can be explained by the starch degradation by amylases in the flour during fermentation, resulting in maltose and glucose molecules, also reflected by the increase in glucose concentration during the stationary phase of microbial growth (after 18 h). The followed fluctuation occurred due to a decrease in maltose content. This reduction of maltose concentration is putatively due to, firstly, a further breakdown of maltose into glucose by β-amylases in the flour and, secondly, the metabolisation of maltose by the strain.

The application of both sourdoughs in a burger bun system showed different influence on dough and burger bun quality. The evaluation of the burger bun dough fermentation showed a decrease in maximum height of gaseous release (Hm') due to sourdough application. The incorporation of sourdough is known to change the dough structure due to hydrolysis of starch and modification in gluten network by natural acidification (Arendt et al., 2007). A decreased Hm', when sourdough was incorporated into the system, occurred, most likely, because the dough network became weaker with sourdough addition. The decrease of elastic parts of the dough, represented by an increase of the damping factor, also proofed the weakening effect of sourdough on the burger bun dough. Hence, the entrapment of the gas cells in the dough system was less efficient and resulted in lower dough rise during proofing.

Furthermore, the prolonged PMT and lower TM of the gluten network, when sourdough was incorporated, reinforce this theory of a weak dough. The acidification of the dough causes a positive net charge and enhances the protein solubility, which contributes to an unfolding of the gluten. This change in tertiary structure leads to the exposure of hydrophobic groups, which do not form new bonds due to strong intermolecular interactions (Galal et al., 1978; Takeda et al., 2001; Wehrle et al., 1997). The entangled protein network, in turn, increases the resistance to elongated extension (Masi et al., 2001).

Furthermore, the micrographs (Figure 5.1) of the burger bun crumbs showed clearly a film coating the starch granules, when 10% sugar (control) or 10% sourdough was incorporated into a sugar reduced system. This “film” represents, most likely, the fully hydrated and weakened gluten network in a more viscous dough system (Amend and Belitz, 1991; Bache and Donald, 1998).

Seemingly, the addition of sourdough did not just weaken the gluten network, it also inhibited starch gelatinization due to starch degradation reflected by a decrease in peak and final viscosity, as well as the breakdown, during pasting (Bertolini et al., 2000). Furthermore, lactate has been discovered to increases the solubility of amylopectin and, hence, decreases the viscosity of the dough system (Shandera and Jackson, 1996).

The weakening of the gluten network and the enhanced degradation of starch resulted in a lower specific volume of the burger buns, and, thus, a denser and harder crumb structure, which is connected to the increased chewiness. Usually, sourdough is known to increase specific volume in a bread system (Corsetti et al., 2008). Due to the presence of 5% (w/w)

added sucrose in the system, the dough structure is additionally weakened, since sugar itself inhibits the gluten network development (see Chapter 3; Sahin et al., 2017). Hence, the weak protein network of the dough, as well as the inhibited structure formation of starch during baking could cause the loss of produced CO_2.

The addition of sourdough, especially 10% SD_{FRU}, delayed staling of the burger bun. Proteases in the sourdough contribute in an increase of free water in the system and, thus, enhanced the activity of alpha amylases (Arendt et al., 2007) and increased the water activity. The microbial shelf life is often correlated to the water activity. A low water activity is known to result in a longer microbial shelf life than products with a high a_w value. However, water activity did not correlate with microbial shelf life. The more sourdough was incorporated the longer was the shelf life. As a heterofermentative LAB strain *Leuconostoc citreum* TR116 produces organic acids and potentially ethanol (Choi et al., 2012; Corona et al., 2016), which can act as antimicrobial compounds. Hence, these antimicrobial compounds incorporated into the burger bun system in form of sourdough prolonged microbial shelf life (Choi et al., 2012).

Furthermore, the degradation of protein into amino acids contributed to browning reaction and resulted in a darker crust and crumb (Martins et al., 2000). Free amino acids also contribute to taste, flavour and aroma of the final product. Interestingly, sensory evaluation of sourdough burger buns compared to the full-sugar control showed no significant differences in hardness (bite), sweetness and sourness. It has been reported that some amino acids contribute to sweetness, sourness, bitterness or saltiness (Kato et al., 1989). Furthermore, amongst all sourdough burger buns, the incorporation of SD_{FRU} resulted in the highest sweetness. SD_{FRU} contained a higher amount of mannitol, which has a sweetness of 50–70% relative to sucrose. Chapter 4 demonstrated that sugar replacement by commercial mannitol showed, regardless the amount, the same sensorial properties as a 10% (w/w) added sugar control (Sahin et al., 2018).

5.5. Conclusion

A reduction of specific volume, a denser crumb structure, a prolonged microbial shelf life, a brown crust colour and sweetness are typical burger bun attributes, and difficult to maintain during sugar reduction. The incorporation of sourdough fermented by *Leuconostoc citreum* TR116, as a high mannitol producer, showed high potential to ameliorate the losses of the named quality characteristics. Especially sourdough containing higher amounts of mannitol and lower amounts of lactate benefited the dough properties and burger bun quality. Hence, the incorporation of 10% SD_{FRU} into a sugar reduced burger bun is promising and highly recommended. An increase in mannitol production could be achieved by setting up a fed-batch sourdough fermentation, incorporating more fructose into the system over time.

5.6. Acknowledgement

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Chapter 6

SOURDOUGH TECHNOLOGY AS A NOVEL APPROACH TO OVERCOME QUALITY LOSSES IN SUGAR-REDUCED CAKES

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Abstract

Sugar reduction in sweet baked goods is one of the most popular trends on the food market. However, reducing sugar without lowering the product quality with respect to sweetness, texture and microbial shelf life is challenging. Sugar alcohols are one group of sugar replacers which maintain the bulk and contribute to sweetness. Nevertheless, alternative approaches, particularly those that are seen as 'clean label', are highly demanded. Hence, the natural, *in-situ* production of mannitol in a sourdough system was performed and its potential as a functional ingredient to improve the product quality of a sugar-reduced cake was investigated. A full sugar cake (C1), a 50% sugar-reduced cake with wheat starch (C2) or with commercially available mannitol (C3) were considered as controls. The substitution of sugar by wheat starch or mannitol caused lower specific volume (-15.5%; -10.7%), a harder crumb (+17.1 N; +4.4 N), less browning, shorter microbial shelf life (-5.7 days; -4.3 days) and poorer sensory properties. The incorporation of sourdough in C2 improved the pasting properties by decreasing the peak viscosity. Moreover, it resulted in specific volume similar to the full-sugar control, contributed to significantly softer crumb (-8.6 N) and increased browning compared to C2. Sensory evaluation revealed an increased sweetness perception (+93%), aroma (+30%) and flavour (+25.5%) by the incorporation of sourdough. In conclusion, the addition of sourdough in amounts lower than 10% can be considered a useful tool to improve specific volume, crumb structure, colour, taste and flavour, as well as shelf life of sugar-reduced cakes.

6.1. Introduction

In 2015 the World Health Organization (WHO) published a guideline regarding the consumption of sugar as part of the "global action plan for the prevention and control of non-communicable diseases 2013-2020". This guideline advises the reduction of the daily intake of free sugars to 10% of the total daily energy intake in order to reduce the risk of overweight, obesity and tooth decay (World Health Organization, 2015). As a reaction to the concerns about public health, in 2018, the UK government introduced a sugar tax on sugar-sweetened beverages and advised the baking industry to reduce the sugar content of their sweet baked goods by 20% by 2020 (Tedstone et al., 2017). Added sugar is one of the major ingredients in cakes, which can contain between 27% and 45% sugar, and plays a crucial role in the contribution to taste, texture and microbial shelf life. During cake preparation, sugar is known to influence structure by delaying starch swelling and increasing the thermal stability of proteins in aqueous solution. Sugar also delays structure setting resulting in higher specific volumes (Wilderjans et al., 2013). Furthermore, compared to sugar alternatives, sucrose has a high sweetness and, hence, contributes to sweetness, aroma and flavour. With the reduction of added sugar, researchers face a number of challenges to overcome the associated quality loss. Hence, sugar substitutes as well as novel technological approaches are required.

Frye and Setser (1991) determined the effect of different bulking agents and combinations of these as sugar replacements in a cake system and optimized the structure of reduced-calorie cakes. Nevertheless, sensory quality was significantly reduced due to a bitter, astringent aftertaste and a dry crumb texture. A stepwise substitution of added sucrose by polydextrose was investigated by Hicsasmaz et al. (2003), demonstrating promising results for the partial replacement up to 25% of added sugar. Furthermore, other sugar alternatives, such as glucose syrups high in maltotrioses and maltotetraoses, were used to successfully decrease the calorie content of high-ratio-cakes (Kweon et al., 2012).

As potential substitutes for sugar, polyols have shown promising results. However, the specific volume and the level of Maillard browning was found to decrease which was associated with reduced product quality (Ronda et al., 2005). Although sugar alcohols are a popular sugar replacement, due to their ability to contribute to bulk and sweetness, the incorporation of industrially produced polyols is relatively expensive and from a consumer perspective, may not be seen as 'clean label' (Bhatt et al., 2013; Ghindea et al., 2010).

As an alternative, Sahin et al., (2018) recently investigated the *in-situ* production of mannitol by lactic acid bacteria (LAB) in a sourdough system for the reduction of sugar in burger buns (see Chapter 5). The incorporation of sourdough with naturally produced mannitol in a burger bun system compensated the quality deficit in structure, colour and taste caused by a sugar reduction by 50%. Sourdough technology is used to ameliorate product quality and to enhance aroma and flavour (Arendt et al., 2007; Thiele et al., 2002). It also plays a major role in the production of traditional Italian sweet leavened cakes, such as Pandoro or Panettone, mainly as a natural leavening agent (Signori, 2004).

In this study the potential of sourdough as a functional ingredient to enhance the techno-functional and sensory properties of sugar-reduced cakes was investigated. The LAB strain *Leuconostoc citreum* TR116, isolated from yellow pea sourdough, was used for the natural, *in-situ* production of mannitol in a sourdough system. This sourdough was incorporated in a 50% sugar-reduced cake formulation and the batter properties in addition to cake quality characteristics were examined.

6.2. Material and methods

Sugar reduction of a high-ratio-cake containing 30% added sugar (control 1, C1) was conducted by replacing 50% of the added sugar by wheat starch (control 2, C2) on the one hand, and by commercially available mannitol as an alternative sugar replacer (control 3, C3) on the other hand.

Sourdough technology was used in order to ameliorate quality characteristics of sugar-reduced cakes. Therefore, controlled sourdough fermentation using the mannitol producing lactic acid bacteria (LAB) strain *Leuconostoc citreum* TR116 was conducted and incorporated in C2. Two different sourdoughs were tested, one with triggered mannitol production by the addition of fructose (SD_{FRU}) and one without the addition of fructose (SD).

6.2.1. Raw materials

For sourdough preparation baker's flour (Odlums Group, Dublin, Ireland), sterile tap water and fructose (Sigma-Aldrich, Gillingham, UK) were used. Furthermore, *Leuconostoc citreum* TR116 isolated from yellow pea sourdough and part of the culture collection of the Department of Biological Sciences, Cork Institute of Technology, was used for controlled fermentation. The cultivation and storage conditions were reported previously in Chapter 5 (Sahin et al., 2018).

Cakes were prepared using baker's flour (Odlums Group, Dublin, Ireland) with a protein content of 11% and a moisture of 13%, sucrose (Siucra, Dublin, Ireland), sunflower oil (Musgrave Wholesale Partners, Dublin, Ireland), liquid whole egg (Servis' Oeuf, Eragny-sur-Epte, France), salt (Glacia British Salt Limited, Cheshire, UK), tap water and baking powder (Valeo Foods, Dublin, Ireland). Sucrose was replaced by 50% with wheat starch (Roquette, Lestrem, France) or mannitol (Roquette, Lestrem, France).

6.2.2. Sourdough fermentation

Sourdough technology as a potential tool to ameliorate the quality characteristics of cakes reduced by 50% added sugar by wheat starch was investigated. Therefore, the mannitol producing LAB strain *Leuconostoc citreum* TR116 was used for a controlled fermentation of wheat flour. *Leuconostoc citreum* TR116 is proven to produce naturally mannitol by a mannitol-dehydrogenase, when fructose is present (see Chapter 5; Sahin et al., 2018).

The preparation of the inoculum and the cell harvest procedure of *Leuconostoc citreum* TR116 for sourdough fermentation were performed as reported previously (Sahin et al., 2018). A cell density of log 7 CFU/g sourdough was chosen for the preparation of two different sourdoughs with dough yields of 200. The sourdough preparation is outlined as a flow chart illustrated by Figure 6.1, showing the differences of both sourdoughs. One sourdough SD was produced by mixing baker's flour (Odlums Group, Dublin, Ireland) with sterile tap water in the same ratio (50:50, w/w) and the starter culture was added. The other sourdough SD_{FRU} contained 40% baker's flour, 10% fructose, 50% sterile tap water and the starter culture. Fructose was added in SD_{FRU} to trigger mannitol production by the LAB strain TR116. The ingredients were mixed by using a Kenwood Major Titanium KM 020 mixer (Kenwood, Havant, UK) set to speed 1 for one minute. After this first mixing step, another mixing followed at speed 2 for one minute. The sourdoughs were sealed airtightly in stomacher bags and incubated at 30 °C anaerobically. A fermentation time of 30 h was chosen, since a previous kinetic study in Chapter 5 showed the highest mannitol concentration in SD_{FRU} at this time point (Sahin et al., 2018).

In order to ensure the same sourdough quality of all replicates, the pH, the total titratable acids (TTA) and cell count were determined (data not shown). The fermentations were performed in triplicates.

6.2.3. Effect of sugar substitution on pasting properties

Due to high amounts of fat and sugar in a high-ratio cake, a simplified model system was established in order to investigate the effect of sugar, wheat starch, mannitol and sourdough (SD and SD_{FRU}), on the pasting properties. In the simplified model system, the full-sugar control (C1) was represented by a mixture of 70% baker's flour and 30% sucrose. Control 2 (C2) was modified to 70% baker's flour, 15% sugar and 15% wheat starch, while control 3 (C3) included 70% baker's flour, 15% sugar and 15% mannitol. Sourdough was incorporated in C2 deducing the amount of water and flour depending on the amount of sourdough added (5%, 10% or 20%) in order to ensure the same ratio of solids and liquids in the system. Three g solid ingredients (on a basis of 14% moisture in total) of the simplified formulations were weight into a RVA aluminium sample canister, and the liquid ingredients were added, followed by the incorporation of distilled water up to a total volume of 25 g liquids. The test was conducted as described by Horstmann et al. (2017) using a Rapid Visco Analyser (RVA) (Newport Scientific, Warriewood, Australia).

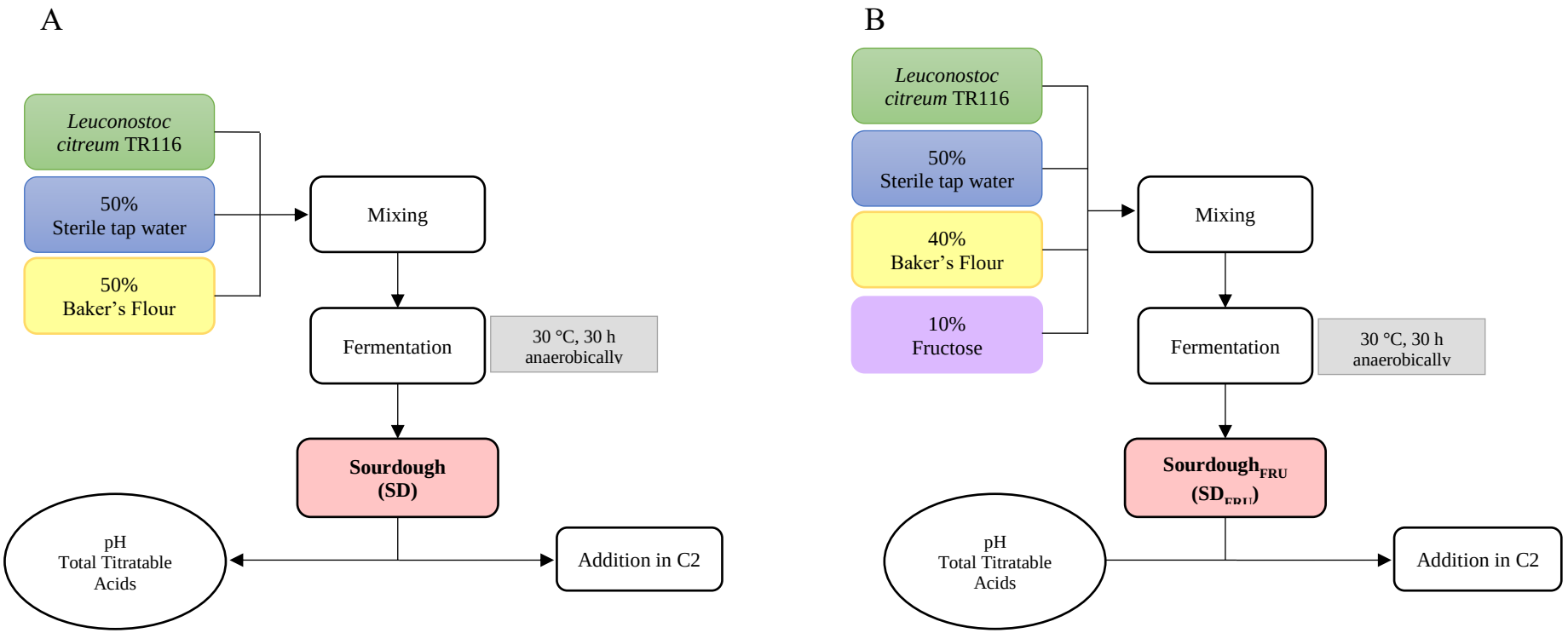


Figure 6.1 Preparation procedure of two different sourdoughs. A) Production of sourdough without fructose addition (SD); B) preparation of sourdough with fructose addition before fermentation in order to trigger mannitol production (SD_{FRU}). C2 represents the cake formulation in which 50% of added sucrose was replaced by wheat starch

6.2.4. Adjustment of the water content

The water content was adjusted, when sucrose was replaced by a bulking agent, such as wheat starch or mannitol, to ensure the same batter consistency in each cake. Therefore, Farinograph E (Brabender GmbH & Co KG, Duisburg, Germany) with a chamber temperature of 30 °C was used. The consistency of each cake batter was adjusted by the addition of water in order to reach the target torque of the batter of C1 (40 BU).

6.2.5. Cake batter properties

Cake batter preparation

The cake batters were prepared by premixing all dry ingredients shown in Table 6.1 using minimum mixing speed of a Kenwood Chef classic (Kenwood, Havant, UK) with a whisk mixing attachment for one minute, followed by the addition of the liquid ingredients. After mixing the batter at speed 1 for one minute, a second mixing step at highest speed for 3 minutes was performed.

pH and total titratable acids (TTA) of cake batters

The pH and TTA of the batters were measured applying the standard methods, after homogenisation in solution (Moritz Schäfer GmbH &Co., 1994). 0.1 M NaOH was used for the titration to a pH of 8.5.

Batter changes during baking: Micro-baking

Micro-baking is an expression for a performed temperature ramp on a batter to simulate the batter properties during baking using a Rheometer Physica MCR 301 (Anton Paar GmbH, Ostfildern, Germany). Parallel plates' geometries were used, whereas the upper plate had a diameter of 50 mm. The method was conducted as reported before by Schirmer et al. (2012). The complex modulus (G*) as well as the temperature (Ti) at the inflection point of the curve were determined.

Table 6.1 Recipes of the full-sugar control cake (C1) and cakes reduced by 50% of the added sucrose content with wheat starch (C2) or mannitol (C3) in per cent based on flour. Three different concentrations of sourdough without fructose (SD) or sourdough with fructose (SD_{FRU}) were incorporated in the C2-formulation

Ingredients	C1	C2	C3	C2 + 5% Sourdough (SD or SD_{FRU})	C2 + 10% Sourdough (SD or SD_{FRU})	C2 + 20% Sourdough (SD or SD_{FRU})
Baker's Flour	100	100	100	95.00	90.00	80.00
Sucrose	124.00	62.00	62.00	62.00	62.00	62.00
Wheat starch	-	62.00	-	62.00	62.00	62.00
Mannitol	-	-	62.00	-	-	-
Sourdough (solid part)	-	-	-	5.00	10.00	20.00
Sourdough (liquid part)	-	-	-	5.00	10.00	20.00
Vegetable Oil	65.00	65.00	65.00	65.00	65.00	65.00
Whole Egg	85.00	85.00	85.00	85.00	85.00	85.00
Salt	1.00	1.00	1.00	1.00	1.00	1.00
Water	35.00	45.00	35.00	40.00	35.00	15.00
Baking powder	3.51	3.51	3.51	3.51	3.51	3.51

6.2.6. Cake quality characteristics

Cake preparation

Two hundred and fifty grams of cake batter were placed in a pan (height: 58.14 mm; top length: 149.62 mm; bottom length: 138.46 mm; top wideness: 85.16 mm, bottom wideness: 75.22 mm). The cakes were baked in a deck oven (MIWE condo, Arnstein, Germany) preheated to 170 °C for 45 min. For further analyses the cakes were cooled down for 2 h, packed in plastic bags and stored at room temperature.

Specific volume

The specific volume of the cakes was determined by using VolScan Profiler (Stable Micro Systems, Godalming, UK).

Crumb hardness and staling

For the determination of the crumb texture, the cakes were sliced into 25 mm thick slices. The TA-XT2i texture analyser (Stable Micro Systems, Godalming, UK) was used as described by Dal Bello et al. (2007) and the crumb hardness after 2 h was evaluated. Furthermore, the crumb hardness after 120 h was measured in order to determine the staling rate by using the formula explained in Chapter 3.

Imaging analysis of the crumb structure

The crumb structure was analysed by a C-cell Bread Imaging System (Calibre Control International Ltd., Warrington, UK). The number of cells and the cell elongation were evaluated.

Crust and crumb colour

The colour of the crumb and crust was measured by using Colorimeter CR-400 (Konica Minolta, Osaka, Japan). The CIE L* a* b* colour system was used for the evaluation of the measurements and the colour difference compared to the full-sugar control cake (C1) was calculated using the Scofield equation:

$$\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$$

Water activity and microbial shelf life

The water activity of the crumbs was measured with a water activity meter (HygroLab, Rotronic, Bassersdorf, Switzerland). Microbial shelf life of the different cakes was evaluated by using the mould environmental challenge method introduced by Dal Bello et al. (2007). Therefore, the cakes were sliced into 25 mm thick slices and each side was exposed to the environment for 5 min before they were packed separately in a sterile plastic bag and heat sealed. Two filter pipettes were inserted in each bag to ensure comparable aerobic conditions. The samples were stored in a room with an average temperature of 20 ± 2 °C and the mould growth was monitored for 21 days. The mould growth was visually evaluated and rated as “mould free”, “<10% mouldy”, “10-24% mouldy”, “25-49% mouldy”, “>50% mouldy”.

Ultrastructure of cake crumb

The ultrastructure of the cake crumb was investigated using scanning electron microscopy (SEM) in order to evaluate the structure of the full-sugar control (C1) and structural

changes caused by the reduction of sugar by wheat starch (C2) or mannitol (C3) as well as by the incorporation of sourdoughs in C2. Therefore, cakes were cut into cubes, freeze dried (SP Scientific, Warminster, USA) and immobilized on an aluminium stub by using double-sided carbon tape. Afterwards, sputter-coating technique using palladium-gold (25 nm coat thickness) was applied and samples were observed in a field emission scanning electron microscope with a working distance of 8 mm. SEM Control User Interface software, Version 5.21 (JEOL Technics Ltd., Tokyo, Japan), was used for taking images at an accelerating voltage of 5 kV.

Determination of the concentration of sugars, mannitol and acids in the cakes

The quantification of glucose (1-100 mmol/l), fructose (1-100 mmol/l), sucrose (1-100 mmol/l) and maltose (1-100 mmol/l) as well as mannitol (1-50 mmol/l), lactate (1-50 mmol/l) and acetate (1-50 mmol/l) in the cakes was carried out by using an Agilent 1260 high performance liquid chromatography system.

Freeze dried crumbs of the cake samples were grinded, and sugars, mannitol and acids were extracted in NaN_3 (50 ppm) solution by shaking for 20 min on an orbital shaker followed by centrifugation (3,000 rpm, 10 min).

For the determination of glucose, fructose, mannitol, lactate and acetate, the supernatant was filtered through a syringe driven filter (pore size 0.2 µm) and transferred into HPLC vials. In order to quantify sucrose and maltose the supernatant was diluted by acetonitrile (Merck KGaA, Darmstadt, Germany) in a ratio 40:60 (supernatant:acetonitrile) before filtration and transfer.

Acids were quantified by using a Hi-Plex H column (300 x 7.7 mm, 8 mm, Agilent, Cork, Ireland) at 60 °C, equipped with a guard column (50 x 7.7 mm, 8 mm, Agilent, Cork, Ireland). The detection of the compounds was carried out at 210 nm (DAD detector). As a mobile phase 0.005 M sulphuric acid at a flow rate of 0.5 ml/min was applied.

The quantification of glucose, fructose and mannitol was carried out using a refractive index detector (RID) (40 °C) by the elution of the extract from a Sugar-Pak I column (300 x 6.5 mm, Waters, Massachusetts, USA) at 80 °C, set up with a guard column Sugar-Pak II, "Guard-Pak" (Waters, Massachusetts, USA). As an eluent 0.0001 M CaEDTA at a flow rate of 0.5 ml/min was used, and a sample volume of 20 µl was chosen.

For the determination of sucrose and maltose a High Performance Carbohydrate column (4.6 mm x 250 mm, Waters, Massachusetts, USA) at 40 °C with a guard column "Guard-

Pak" (Waters, Massachusetts, USA) was used. As a mobile phase 75:25 (acetonitrile:MiliQ water) with a flow rate of 1 ml/min was applied.

Extraction was repeated twice and the concentration was calculated based on dry matter of the freeze dried cake samples and is illustrated as an average value out of six values.

Sensory evaluation

The impact of a 50% reduction of added sucrose by wheat starch (C2) as well as mannitol (C3) on sensory characteristics such as sweetness, sourness and crumb hardness (bite) of a high-ratio-cake was investigated. Additionally, the effect of two sourdoughs incorporated in C2 on these descriptors was determined. Therefore, a chosen sensory panel of 10 people (5 females and 5 males, age: 24-32) was trained 6 hours weekly over a period of 6 months prior the evaluation. The definition of the descriptors and the procedure of the training were previously described in Chapter 4 (Sahin et al., 2018) and Chapter 5. The training as well as the sensory evaluation were conducted in a sensory panel room at 21 ± 1 °C. The intensity was judged on a scale between 0 (not perceived at all) and 10 (highest intensity). Sensory evaluation was performed in a duplicate on two different days.

6.2.7. Statistical analysis

All analyses were performed in triplicates, if not stated differently in the method. A variance analysis (one-way ANOVA, $p \leq 0.05$, Tukey test) was performed using Minitab 17.

6.3. Results

The effect of sourdoughs on batter and product quality parameters of cakes reduced by 50% added sugar by wheat starch was evaluated. The results were compared to the full-sugar cake/batter (C1), cakes/batters reduced by 50% added sugar with wheat starch (C2) and cakes/batters reduced by 50% added sugar with mannitol (C3).

6.3.1. Adjustment of the water content

In order to ensure the same consistency of the cake batters, the water content of each recipe was adjusted to a torque of 40 BU (Table 6.2). The full-sugar control batter (C1) had a water content of 8.80%. The sugar replacement by wheat starch resulted in a water level of 10.70 ± 0.40% (C2-batters). Sugar substitution by commercially available mannitol did not change the consistency of the cake batter compared to C1 (8.85 ± 0.08% water). When sourdough was incorporated, less water was needed to reach the target torque. The higher the level of sourdough addition the lower the added water in the recipe was.

6.3.2. Effect of sugar and sugar replacers on pasting properties

The investigation of changes in pasting behaviour of the different samples included the evaluation of the peak viscosity, breakdown, the final viscosity, illustrated in Figure 6.2, and the pasting temperature (Table 6.2). Sugar replacement by wheat starch led to an increase in peak viscosity by 687.2 cP and in breakdown by 315.2 cP as well as in final viscosity by 626.0 cP compared to C1. The pasting temperature decreased by 2.4 °C. A 50%-sugar-reduction by commercially available mannitol resulted in a significantly lower peak viscosity (-367.5 cP) compared to C1. Furthermore, the breakdown value was lowered by 193.5 cP and the final viscosity was significantly reduced (-194.3 cP). Additionally, the pasting temperature increased significantly by 27.6 °C.

The incorporation of sourdough in C2 induced a decrease in peak viscosity resulting in values, which did not differ significantly from C1. Furthermore, the final viscosity was reduced by sourdough addition and the pasting temperature increased to values between 89.3 °C and 90.8 °C, which did not differ significantly from C3. It is noteworthy that the incorporation of sourdough led always to the same effect regardless of the type and the amounts added.

Table 6.2 Effect of the reduction of added sucrose on starch gelatinisation and properties of cake batter including wheat starch or mannitol as sugar replacers, and the impact of sourdough without (SD) and with fructose (SD_{FRU}) during heating. C1 represents the full-sugar control cake with 30% added sucrose, C2 shows the results of cakes reduced in added sugar by 50% with wheat starch and C3 demonstrates cakes with sugar substitution of 50% by commercially available mannitol

	Water addition [%] (based on recipe)	**Pasting temperature [°C]**	**pH**	**Total titratable acids [ml 0.1 M NaOH]**	**Temperature at inflection point (T_i) [°C]**	**Complex modulus G* at inflection point [P·s]**
C1	8.80 ± 0.00 (c)	63.7 ± 1.7 (c)	7.27 ± 0.08 (abc)	1.70 ± 0.06 (d)	73.0 ± 0.7 (a)	112.8 ± 17.6 (d)
C2	10.70 ± 0.40 (a)	61.3 ± 0.6 (d)	7.33 ± 0.09 (ab)	1.70 ± 0.13 (d)	63.3 ± 3.1 (b)	420.3 ± 47.3 (c)
C3	8.85 ± 0.08 (c)	91.3 ± 1.0 (a)	7.35 ± 0.08 (a)	1.52 ± 0.09 (e)	70.6 ± 0.7 (a)	133.7 ± 8.7 (d)
C2+5% SD	9.73 ± 0.09 (b)	89.3 ± 0.5 (b)	7.23 ± 0.04 (cd)	1.82 ± 0.10 (cd)	51.5 ± 4.3 (c)	526.3 ± 28.3 (ab)
C2+10% SD	8.51 ± 0.09 (c)	89.8 ± 0.5 (ab)	7.14 ± 0.03 (de)	1.68 ± 0.11 (de)	54.4 ± 0.0 (c)	602.7 ± 77.8 (a)
C2+20% SD	6.08 ± 0.07 (d)	90.8 ± 1.1 (ab)	7.10 ± 0.03 (ef)	1.89 ± 0.07 (c)	56.0 ± 1.4 (c)	515.0 ± 28.8 (abc)
C2+5% SD_{FRU}	9.67 ± 0.07 (b)	89.7 ± 0.1 (ab)	7.03 ± 0.04 (f)	2.18 ± 0.11 (b)	54.8 ± 0.7 (c)	452.7 ± 16.1 (bc)
C2+10% SD_{FRU}	8.63 ± 0.06 (c)	90.1 ± 0.4 (ab)	7.05 ± 0.07 (f)	2.28 ± 0.13 (b)	54.8 ± 0.7 (c)	456.0 ± 6.2 (bc)
C2+20% SD_{FRU}	6.00 ± 0.09 (d)	90.7 ± 0.5 (ab)	6.80 ± 0.05 (g)	2.91 ± 0.13 (a)	55.6 ± 2.1 (c)	476.7 ± 12.3 (bc)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

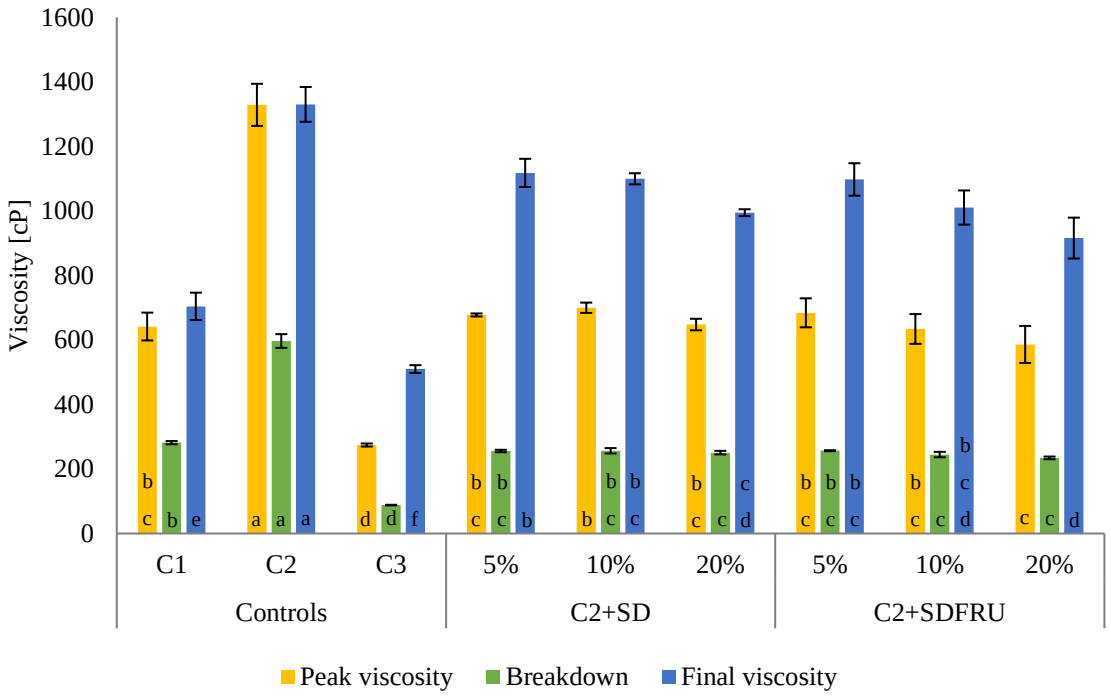


Figure 6.2 Peak viscosity, breakdown viscosity and final viscosity of the simplified model systems. C1 represents the full-sugar control, C2 shows the values for the system reduced in sugar by 50% with wheat starch and C3 illustrates the viscosities of the system reduced in sugar by 50% with ingredient mannitol. SD represents sourdough which was prepared without the addition of fructose, whereas SD_{FRU} is the sourdough with addition of fructose. No significant difference occurred between values in the same column with the same lower case letter ($P < 0.05$)

6.3.3. Cake batter properties

pH and TTA of cake batters

The pH of cake batters including wheat starch as a sugar replacement showed no significant difference compared to the C1 (7.27 ± 0.08), neither did the pH of cakes with mannitol (7.35 ± 0.08) as a sweet bulking agent. The incorporation of sourdough decreased the pH significantly, especially the addition of 20% SD_{FRU} (6.80 ± 0.05).

The TTA was not affected by the sugar replacement with wheat starch. However, the TTA of batters with commercially available mannitol (1.52 ± 0.09 ml 0.1 M NaOH) decreased significantly. Interestingly, the addition of SD did not increase the TTA significantly, but SD_{FRU} did. The incorporation of 20% SD_{FRU} caused an increase in TTA

by 1.21 ml 0.1 M NaOH compared to the full-sugar control batter (1.70 ± 0.06 ml 0.1 M NaOH).

Structure changes during heating

The investigation of structure changes during baking was conducted by micro-baking using a rheometer. The temperature Ti necessary to cause an inflection point in complex modulus G* of the batter, as well as G* at the inflection point were determined (Table 6.2). G* indicates the batter stiffness. The higher G* the stiffer and higher in elastic parts the cake batter is.

Ti of C1 batters was 73.0 ± 0.7 °C showing no significant differences to batter reduced in sugar by 40%. A 50%-sugar reduction by wheat starch was significantly lower in Ti (63.3 ± 3.1) than C1 (73.0 ± 0.7 °C), C3 did not differ significantly from C1. The incorporation of sourdough, regardless type and amounts, caused a further decrease in Ti (between 51.5°C and 56.0 °C).

Commercially available mannitol (133.7 ± 8.7 P·s) as a replacement resulted in the same batter stiffness as C1 (112.8 ± 17.6 P·s). The addition of sourdough to a 50%-sugar-reduced high-ratio-cake increased the complex modulus at the inflection point. Interestingly, the incorporation of SD led to a higher G* than SD_{FRU} regardless of the amounts added.

6.3.4. Cake quality characteristics

Specific volume

The specific volume is one of the main quality parameter of bakery products. Figure 6.3 demonstrates the results of the specific volume for all samples evaluated. A reduction of sugar by wheat starch caused a significant decrease in specific volume by 0.31 ml/g compared to C1. C3 resulted in a specific volume of 1.77 ± 0.02 ml/g, which was significantly smaller (-0.21 ml/g) than C1 and did not differ from C2. Hence, sugar substitution led to a decrease in specific volume. The loss in specific volume is also illustrated by Figure 6.4.

The incorporation of sourdough induced different results depending on the type of sourdough added. SD caused a lower specific volume compared to C1 (-0.38 ml/g to -0.46 ml/g) and C2 (-0.07ml/g to -0.15 ml/g). On the other hand, SD_{FRU} increased the specific volume significantly, compared to C2. Interestingly, the addition of 5% SD_{FRU}

increased the specific volume to 1.82 ± 0.06 ml/g and did not differ significantly from the full sugar control cake (1.98 ± 0.10 ml/g). It is noteworthy that the more sourdough was added the lower the specific volume was.

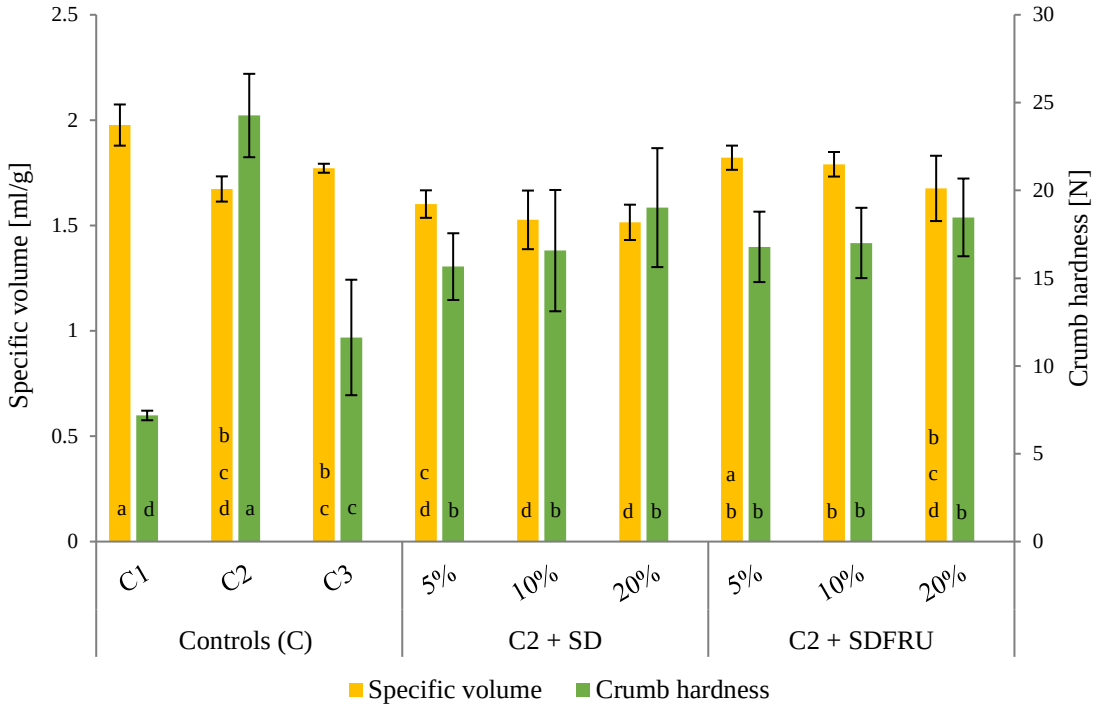


Figure 6.3 Specific volume and crumb hardness of the different cakes produced. Full-sugar control cake (C1), the 50% sugar-reduced cake with wheat starch (C2) and the 50% sugar-reduced cake by commercially available mannitol (C3) are illustrated in the control section, followed by values of sugar-reduced cakes (C2) including sourdough without fructose addition (SD) in three different concentrations (5%, 10% and 20%) as well as values of cakes with sourdough produced with fructose addition (SD_{FRU}). No significant difference occurred between values in the same column with the same lower case letter ($P < 0.05$)

Crumb hardness and staling rate

Another significant parameter for the determination of cake characteristics is the hardness of the crumb, which is presented in Figure 6.3. The softest crumb was achieved in C1 by the addition of 30% sucrose (7.18 ± 0.27 N), whereas C2 resulted in the hardest crumb (24.26 ± 2.37 N). The replacement by commercially available mannitol increased the crumb hardness slightly (+4.44 N), still significantly, compared to C1. The incorporation of sourdough caused a softening effect compared to C2 (-8.60 N). Generally, lower amounts of sourdough resulted in a softer crumb texture. Nevertheless, the differences amongst the sourdough samples (type of sourdough and incorporated amounts) were not significant.

The staling rate showed no significant differences between the samples, except for cakes with ingredient mannitol, which resulted in the highest rate of 1.84 ± 0.87, almost four-fold the rate of C1.

Crumb structure

The crumb structure is another essential quality criteria and is characterised by different parameters, such as the number of cells and the average cell elongation (Table 6.3).

A reduction in added sugar by wheat starch induced a decrease in number of cells (C1: 3207 ± 82; C2: 2448 ± 121). Commercially available mannitol as a sugar replacer caused the same number of cells (3128 ± 200) as C1. The incorporation of sourdough, generally, decreased the amounts of cells in the crumb. However, the addition of 5% SD_{FRU} led to an increase in cell number compared to C2, which is also demonstrated in Figure 6.4.

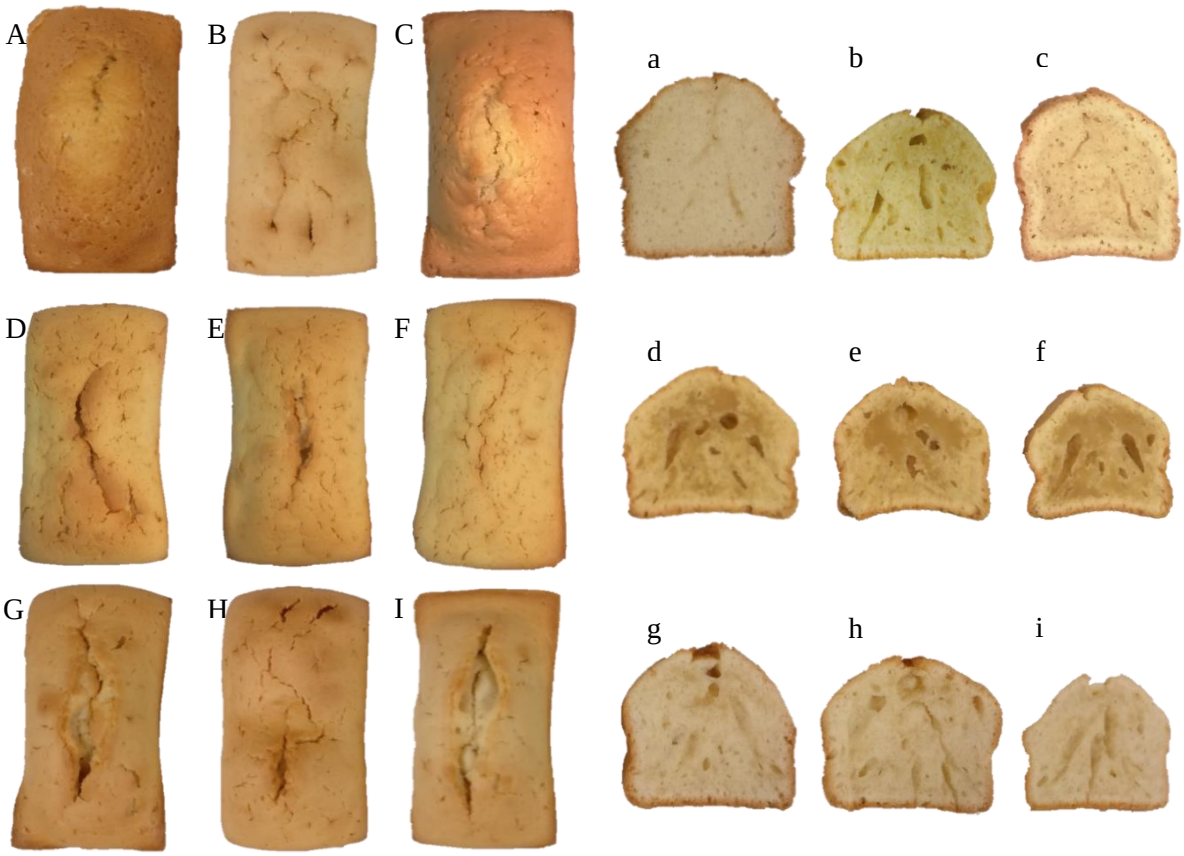


Figure 6.4 Appearance of the crust and the crumb of high-ratio-cakes. In the first row (A) and (a) represent the full-sugar control cake; (B) and (b) show the 50% sugar-reduced cake with wheat starch; (C) and (c) illustrate the 50% sugar-reduced cake with mannitol. In the second row (D) and (d) demonstrate sugar-reduced cakes with the addition of 5% sourdough produced without the addition of fructose (SD), (E) and (e) the addition of 10% SD and (F) and (f) the addition of 20% SD. In the last row (G) and (g) presents sugar-reduced cakes with the incorporation of 5% sourdough produced with the addition of fructose (SD_{FRU}), (H) and (h) with 10% added SD_{FRU} and (I) and (i) with 20% added SD_{FRU}

The average cell elongation is defined as the ratio of the diameter in horizontal (x) and vertical (y) direction, hence, an elongation of 1.00 would represent an ideal round shaped cell. C1 showed the closest value to round shaped cells (1.42 ± 0.01), while a sugar reduction caused an increase in average cell elongation (Figure 6.4). The addition of sourdough did not change the elongation significantly compared to C2, yet resulted in higher values than C1.

Crust and crumb colour

The colour of the crust and the crumb is an important parameter to evaluate the appearance of cakes. Sugar is involved in Maillard reaction and, hence, influences the browning of the final product. The ΔE-value reflects the differences in colour compared to C1, considering L*, a* and b* values. The higher ΔE the greater the colour difference to C1 is. The values are given in Table 6.3 and Figure 6.4 illustrates the visual differences in crust and crumb colour.

Sugar reduction by wheat starch (C2) resulted in the highest ΔE-value of all crusts (19.75 ± 6.29), and, hence, was the most different compared to C1. The lowest ΔE-value (crust) was determined in cakes containing commercially available mannitol (C3) (8.97 ± 5.17), 5% SD (11.82 ± 4.33), or 10% SD_{FRU} (11.89 ± 4.33).

Regarding the differences in crumb colour, C3 showed the lowest ΔE-value (5.74 ± 3.22), followed by C2 (6.98 ± 3.74). The addition of 5% SD_{FRU} (7.47 ± 4.03) and 10% SD_{FRU} (7.93 ± 4.04) resulted in ΔE-values which did not differ significantly from C2 and C3. The incorporation of SD or 20% SD_{FRU} caused the highest ΔE-values and thus differed from the crumb of C1 the most.

Water activity and microbial shelf life

The water activity (a_w) demonstrates the amount of free water available for hydration in a system. C2 (0.927 ± 0.005) as well as C3 (0.908 ± 0.006) resulted in an increase in a_w-value compared to C1 (0.883 ± 0.003) (Table 6.3). The incorporation of sourdough in C2 did not change the water activity significantly. Nevertheless, the highest a_w-value was determined in cake crumbs including 10% SD (0.929 ± 0.011) and 20% SD_{FRU} (0.929 ± 0.009).

Table 6.3 Properties of cakes reduced in added sucrose by wheat starch/mannitol and the effect of sourdough without (SD) and with fructose (SD_{FRU}) on the cakes. C1 is the full-sugar control cake, C2 illustrates the reduction of added sugar by 50% with wheat starch and C3 represents cakes reduced in added sugar by 50% with commercially available mannitol

	Staling rate	Number of cells	Average cell elongation	ΔE (crust)	ΔE (crumb)	Water activity	Microbial shelf life (first day of mould)
C1	0.47 ± 0.07 (b)	3207 ± 82 (a)	1.42 ± 0.01 (b)	0.00±0.00 (d)	0.00±0.00 (c)	0.883 ± 0.003 (c)	11.0 ± 1.0 (a)
C2	0.54 ± 0.20 (b)	2448 ± 121 (de)	1.49 ± 0.02 (a)	19.75 ± 6.29 (a)	6.98 ± 3.74 (b)	0.927 ± 0.005 (a)	5.3 ± 0.6 (c)
C3	1.84 ± 0.87 (a)	3128 ± 200 (ab)	1.49 ± 0.02 (a)	8.97 ± 5.17 (c)	5.74 ± 3.22 (b)	0.908 ± 0.006 (b)	6.7 ± 0.6 (bc)
C2+5% SD	0.96 ± 0.44 (b)	2245 ± 110 (ef)	1.49 ± 0.03 (a)	11.82 ± 4.33 (bc)	13.36 ± 4.97 (a)	0.926 ± 0.007 (a)	6.0 ± 0.0 (bc)
C2+10% SD	0.59 ± 0.36 (b)	1978 ± 319 (f)	1.50 ± 0.03 (a)	14.10 ± 4.69 (b)	14.84 ± 6.44 (a)	0.929 ± 0.011 (a)	6.7 ± 0.6 (bc)
C2+20% SD	0.47 ± 0.39 (b)	1942 ± 79 (f)	1.49 ± 0.03 (a)	13.23 ± 5.10 (b)	17.82 ± 5.42 (a)	0.927 ± 0.006 (a)	6.7 ± 0.6 (bc)
C2+5% SD_{FRU}	0.78 ± 0.29 (b)	2831 ± 222 (bc)	1.52 ± 0.02 (a)	14.64 ± 5.63 (b)	7.47 ± 4.03 (b)	0.927 ± 0.006 (a)	6.0 ± 0.0 (bc)
C2+10% SD_{FRU}	0.66 ± 0.18 (b)	2623 ± 146 (cd)	1.50 ± 0.03 (a)	11.89 ± 4.33 (bc)	7.93 ± 4.04 (b)	0.928 ± 0.005 (a)	6.7 ± 0.6 (bc)
C2+20% SD_{FRU}	0.51 ± 0.19 (b)	2247 ± 429 (ef)	1.49 ± 0.03 (a)	14.61 ± 5.69 (b)	13.45 ± 5.95 (a)	0.929 ± 0.009 (a)	7.3 ± 1.2 (b)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

The longest microbial shelf life was determined in C1 (11.0 ± 1.0 days), while C2 resulted in the shortest shelf life (first mould growth after 5.3 ± 0.6 days). C3 showed the first mould growth after 6.7 ± 0.6 days.

The addition of sourdough to C2 prolonged shelf life significantly. An extension of at least one day was determined for cakes containing 10% sourdough or more, regardless which sourdough (SD or SD_{FRU}) was incorporated. The addition of 20% SD_{FRU} induced the longest microbial shelf life among all sugar-reduced cakes (7.3 ± 1.2 days).

Ultrastructure of the cake crumb

The ultrastructure of the cake crumbs was investigated in order to determine effects of sugar replacers, such as wheat starch and mannitol, as well as sourdough as a functional ingredient on the crumb structure (Figure 6.5). C1 is characterized by a plain surface, a perfect alignment of structure giving molecules, such as starch and proteins. The air cells are roundly shaped with smooth edges. C2 resulted in a denser structure illustrated by the smaller cells, which occurred like cracks on the surface. Sugar substitution by commercially available mannitol caused significant differences in crumb structure. Long spike-shaped particles were observed, cells were randomly distributed and the cell size occurred to be smaller than in C1. The incorporation of sourdough led to significant structure changes with increasing amounts added. With higher levels of sourdough in the cake system, the cells became shaped and precise. Furthermore, the surface became plainer and edges became smoother.

Sugar, mannitol and acid concentrations in the cakes

The concentrations of sugars, such as sucrose, maltose, glucose and fructose, as well as of mannitol and the acids lactate and acetate in the final cakes are shown in Table 6.4. As expected, the highest sucrose level was determined in C1 (30.16 ± 1.21 g/100g DM). The reduction of 50% added sucrose by either wheat starch or mannitol resulted in sucrose concentration between 11.86 ± 2.37 g/100g DM and 15.62 ± 1.74 g/100g DM and did not differ significantly from each other. No maltose was detected in C1, C2 or C3, while the addition of sourdough led to a slight increase. Nevertheless, the concentrations determined were below the detection limit of 0.10 g/100g DM. Moreover, glucose was only detected in cakes with sourdough incorporation. The addition of 10% SD resulted in the highest glucose concentration (0.76 ± 0.25 g/100g DM), whereas the lowest amounts occurred in cakes with 20% SD_{FRU}. The levels of fructose determined in the cakes were

either not detectable or below the detection limit of 0.04 g/100g DM; only the addition of 20% SD_{FRU} led to a detectable fructose content of 0.05 ± 0.01 g /100g DM.

The substitution of added sucrose by commercially available mannitol resulted in a mannitol concentration of 14.95 ± 0.46 g/100g DM. The addition of sourdough without fructose (SD) resulted in no mannitol detection. On the contrary, the addition of SD_{FRU} induced the highest concentration of mannitol among cakes including SD_{FRU} with 20% SD_{FRU} showing the highest naturally produced mannitol amounts (0.70 ± 0.04 g/100g DM). No acids were detected in all control cakes. The incorporation of SD resulted in higher amounts of lactic acid compared to SD_{FRU} with 20% SD showing the highest concentrations (0.102 ± 0.001 g/100g DM).

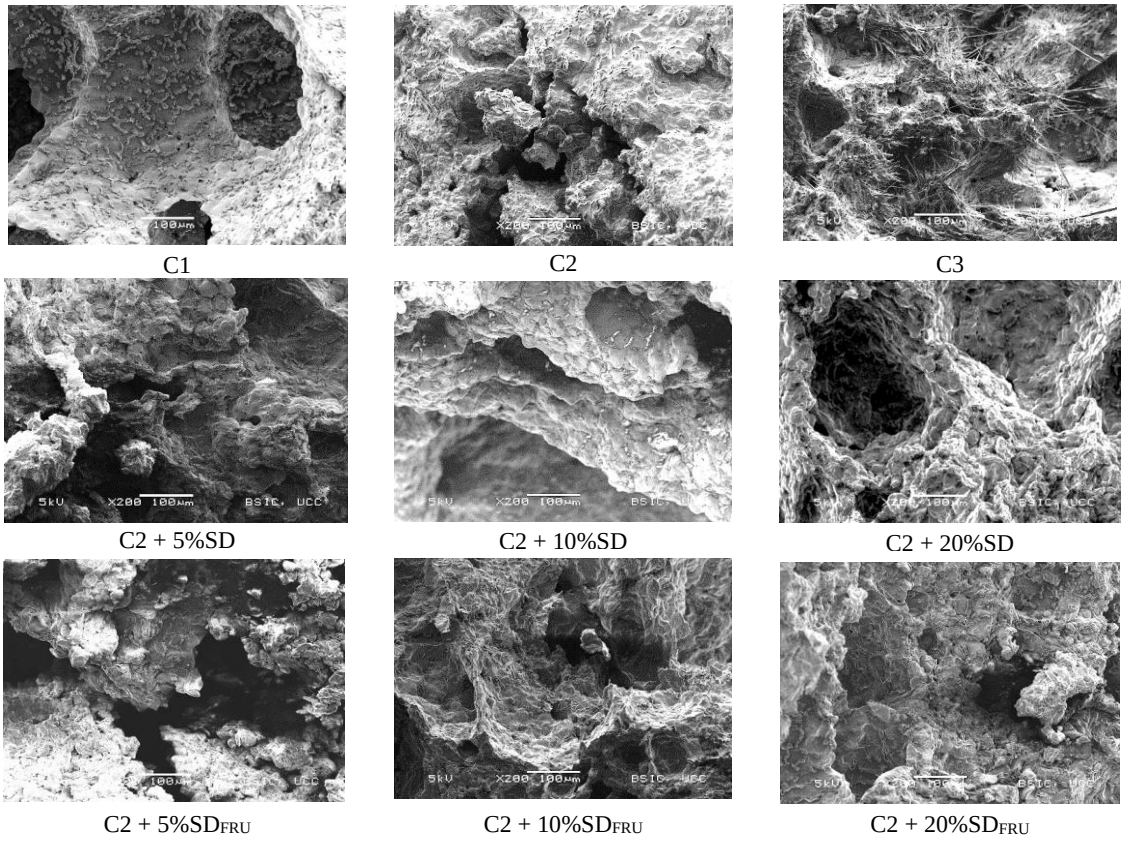


Figure 6.5 Micrographs of cake crumbs taken by using a scanning electron microscope (SEM) with a magnification of x200. In the first row C1 (the crumb of a full-sugar control cake), C2 (the crumb of a 50% sugar-reduced cake with wheat starch) and C3 (the crumb of a 50% sugar-reduced cake with mannitol) are presented. The second row illustrates the crumb structure of sugar-reduced cakes (C2) with the addition of sourdough produced without fructose addition (SD) with increasing amounts from left to right. The third row demonstrates the crumb structure of sugar reduced cakes (C2) with the addition of sourdough produced with the addition of fructose (SD_{FRU}) with increasing amounts from left to right

Table 6.4 Concentration of sugars, mannitol as well as lactate and acetate in the produced cakes. C1 represents the full-sugar control cake with 30% added sucrose, C2 shows the results of cakes reduced in added sugar by 50% with wheat starch and C3 demonstrates cakes with sugar substitution of 50% by commercially available mannitol. SD represents sourdough without the addition of fructose, whereas SD_{FRU} is sourdough with the addition of fructose. The concentrations are based on 100g dry matter (DM). Absent molecules are marked as "not detected" (n.d.)

	Sucrose [g/100gDM]	**Maltose [g/100gDM]**	**Glucose [g/100gDM]**	**Fructose [g/100gDM]**	**Total sugar [g/100gDM]**	**Mannitol [g/100gDM]**	**Lactic acid [g/100gDM]**	**Acetic acid [g/100gDM]**
C1	30.16±1.21 (a)	n.d.	n.d.	n.d.	30.16	n.d	n.d.	n.d.
C2	12.42±1.55 (b)	n.d.	n.d.	n.d.	12.42	n.d	n.d.	n.d.
C3	11.86±2.37 (b)	n.d.	n.d.	n.d.	11.86	14.95±0.46 (a)	n.d.	n.d.
C2+5% SD	12.78±1.09 (b)	<0.10 (a)	n.d.	n.d.	12.78	n.d.	0.029±0.001 (c)	<0.015 (e)
C2+10% SD	12.48±2.10 (b)	<0.10 (a)	0.76±0.25 (a)	n.d.	13.24	n.d.	0.052±0.002 (b)	0.014±0.002 (d)
C2+20% SD	13.68±2.47 (b)	<0.10 (a)	0.43±0.04 (bc)	n.d.	14.11	n.d.	0.102±0.001 (a)	0.014±0.001 (d)
C2+5% SD_{FRU}	13.50±2.56 (b)	<0.10 (a)	0.57±0.17 (ab)	<0.04 (b)	14.07	0.16±0.01 (d)	<0.026 (d)	0.019±0.002 (c)
C2+10% SD_{FRU}	15.62±1.74 (b)	<0.10 (a)	0.59±0.08 (ab)	<0.04 (b)	16.21	0.33±0.01 (c)	0.027±0.001 (c)	0.040±0.001 (b)
C2+20% SD_{FRU}	14.64±2.08 (b)	<0.10 (a)	0.30±0.06 (c)	0.05±0.01 (a)	14.99	0.70±0.04 (b)	0.052±0.001 (b)	0.086±0.002 (a)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

On the contrary, levels of acetate were higher in cakes including SD_{FRU} than SD. The incorporation of 20% SD_{FRU} resulted in the highest acetate concentration (0.086 ± 0.002 g/100g DM).

Sensory characteristics

A trained panel was set up for the evaluation of sensory attributes. Sweetness, sourness, aroma, flavour and crumb hardness (bite) were determined on a scale between 0 (least intense) and 10 (very intense). Figure 6.6 illustrates the sensory profile of all evaluated cakes. C1 showed the highest sweetness value (8.20 ± 1.23), whereas C2 was rated as the least sweet cake (3.00 ± 0.82). C3 resulted in a sweetness value of 5.60 ± 0.97. All three controls differed in sweetness significantly from each other ($P \leq 0.05$).

Furthermore, the incorporation of sourdough led to significant differences among samples with different type of sourdough. Interestingly, SD did not increase the sweetness of the cakes significantly, whereas the incorporation of SD_{FRU} caused an increase in sweetness close to C1 ($P \leq 0.05$). The more SD_{FRU} was added, the higher the sweetness perception was.

Since sourdough was added, the trained panel was asked to evaluate the intensity of sourness of the cakes. A significant increase in sourness was perceived, when 20% sourdough was added (+1.60 (SD), +1.40 (SD_{FRU})). Apart from these samples, no significant differences in sourness were detected ($P \leq 0.05$).

The aroma intensity decreased with decreasing amounts of added sucrose with C1 being the most aromatic sample (5.80 ± 0.79) and the C2 being the least aromatic one (4.00 ± 1.05). The replacement by mannitol (C3) induced significant differences in aroma intensity compared to C1 (-1.6).

The incorporation of SD caused the same increase in aroma intensity regardless of the amount added. On the other hand, the addition level of SD_{FRU} played a significant role in aroma intensity. 10% SD_{FRU} caused the highest aroma intensity among all cakes containing sourdough (5.80 ± 1.40) and was not significant different to the full-sugar control ($P \leq 0.05$).

Flavour intensity evaluation showed the same trend as aroma intensity. Sugar replacement by wheat starch caused a decrease in flavour intensity (-36%) and did not differ significantly from cakes including ingredient mannitol as a supplement. Sourdough incorporation caused in increase in flavour with increasing amounts added. The highest

flavour intensity in sugar-reduced cakes was achieved by the addition of 10% SD_{FRU} (6.50 ± 1.27) and 20% SD_{FRU} (6.50 ± 1.08).

C1 showed the softest crumb texture (1.40 ± 0.52), while the crumb of C2 was judged as the hardest 6.70 ± 0.82. C3 showed a crumb hardness intensity of 3.40 ± 0.52. The addition of sourdough to C2 resulted in softer crumbs compared to C2 without sourdough. Nevertheless, the more sourdough was applied the harder the crumb became. Furthermore, the sensory panel evaluated cake samples with SD_{FRU} generally as harder than samples with SD.

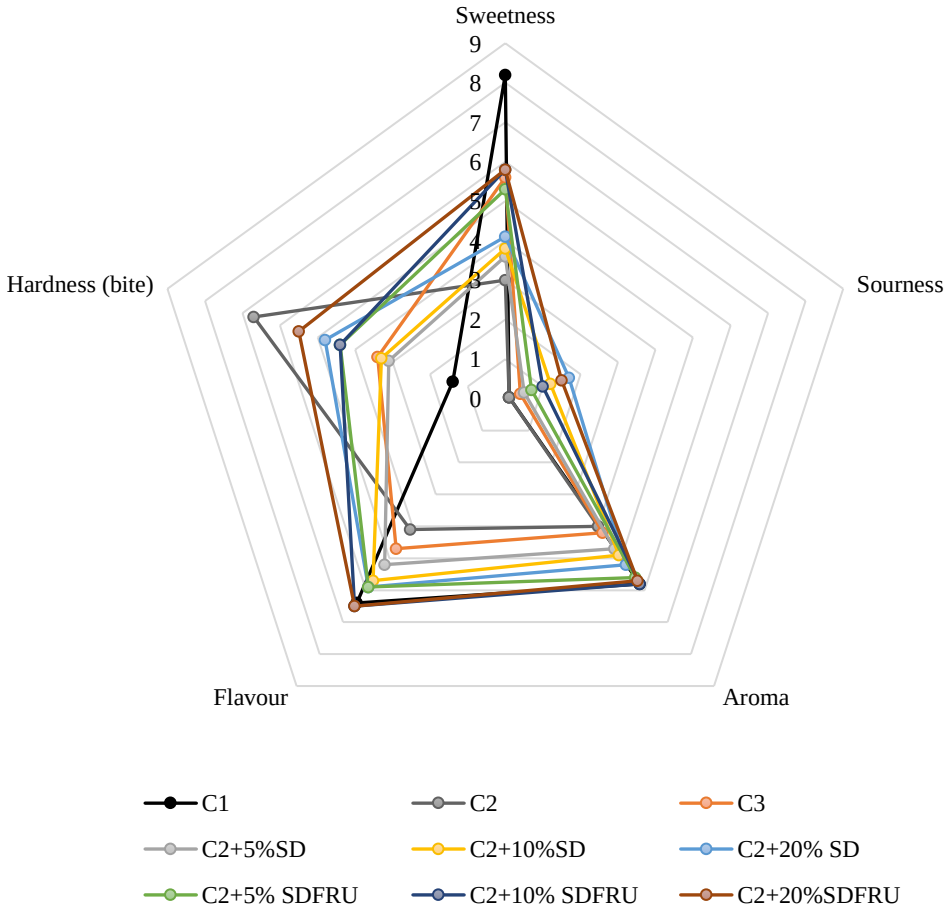


Figure 6.6 Sensory evaluation of the sweetness, sourness, aroma, flavour and hardness (bite) of the full-sugar control cake (C1), the 50% sugar-reduced cake with wheat starch (C2), the 50% sugar-reduced cake with ingredient mannitol (C3) and sugar-reduced cakes with wheat starch (C2) including three different levels of sourdough (5%, 10%, 20%) without fructose addition (SD) and with fructose addition (SD_{FRU}). The chart shows the average values which had a confidence interval of ≤ 1.11 ($\alpha = 0.05$, $n = 10$)

6.4. Discussion

This study reveals, firstly, the crucial function of sugar in a cake system by replacing added sucrose with wheat starch or mannitol and, secondly, the effect of sourdough as a functional ingredient in sugar-reduced cakes in order to improve product quality.

Cakes in which sugar was replaced with wheat starch showed a decrease in specific volume. The increasing amounts of wheat starch instead of sucrose rose the degree of starch swelling reflected by a higher peak viscosity, but also resulted in a more fragile system illustrated by the higher breakdown viscosity (Tester and Morrison, 1990), which is due to more amylose leaching out of the granules. Moreover, a reduction of sugar in the system caused an earlier structure setting of the cake during baking, determined by a lower Ti, which proofed that sugar delays the structure setting.

Sugar delays starch gelatinisation, which provides the system more time for the expansion of CO_2 and water vapour in the cells and results in a higher specific volume (Yamazaki and Kissell, 1978). The accentuated expansion of gas cells and delayed structure setting could be the reason for the plain surface of the crumb and the round shaped cells observed in the ultrastructure (Figure 6.5). Furthermore, the substitution of sugar by wheat starch, a long polysaccharide, could cause interactions of starch and proteins as well as competition for water, resulting in incomplete network development during heating and, thus, results in a weaker protein network (Hicsasmaz et al., 2003; Ronda et al., 2005; Stauffer and Beech, 1990). This could lead to a lower resistance of the cell walls, which consequently collapsed, illustrated by the lower number of cells and a higher cell elongation.

Additionally, the crumb hardness increased, when wheat starch was used as a sugar replacement, firstly, due to the denser crumb structure (Figure 6.4.), but also due to the fact that more incorporated starch resulted in a higher degree of starch gelatinisation and realignment during cooling, illustrated by the higher final viscosity during pasting. Moreover, the increase in starch gelatinisation is proven by the increased G*, demonstrating a stiffer structure of the cakes with high amounts of wheat starch (Donovan, 1979; Schirmer et al., 2012).

The water activity increased with the addition of wheat starch, which occurred most likely due to the increased water incorporation to ensure the batter consistency of 40 BU. As expected, higher amounts of free water in the system caused a faster microbial growth.

Furthermore, sugar reduction led to an increase in ΔE-value of the crust indicating an increase in colour changes. These colour changes are due to lower amount of reducing sugars which are involved in Maillard reaction (Hashiba, 1982).

Mannitol as a sugar replacer did not ameliorate the specific volume compared to wheat starch, which is, most likely, due to its interactions with the starch and proteins. Since it showed the same G* and Ti as C1, mannitol influenced the structure setting in the same way as sugar did. Nevertheless, it is only moderately soluble and more likely present in its crystalline state, which is also proven by the spiky particles observed in the crumb (Figure 6.2) (Whistler and BeMiller, 1997). This could hinder water molecules to enter the starch granules resulting in a lower extent of starch swelling, reflected by the significant lower peak and final viscosity during pasting. Furthermore, mannitol could interfere with the realignment of the starch molecules during cooling leading to a lower final viscosity (Hartel and Hasenhuettl, 2013). Moderately soluble ingredients, such as mannitol, could cause hydrophobic interactions with proteins, such as ovalbumin of the added whole egg or gluten of the flour, causing higher flexibility of the tertiary structure and, thus, faster protein denaturation on the top surface of the cake resulting in early crust formation (Wilderjans et al. 2010). Thus, an early structure setting, especially of the crust, is expected, causing the limitation in cake rise and a horizontal expansion of gas cells, reflected by the higher cell elongation.

The significant higher staling rate of these cakes occurred, most likely, due to the non-hygroscopic character of mannitol resulting in poor water binding and faster staling (Ronda et al., 2005). Furthermore, this poor water binding character is, putatively, the reason for the higher water activity and shorter microbial shelf life compared to the full-sugar control (Whistler and BeMiller, 1997). It has to be noted that mannitol does not undergo Maillard browning due to the lack of free aldehyde groups, which are essential for this reaction, and, thus, caused a lighter crust colour (Ghosh and Sudha, 2012). Although sugar substitution by mannitol caused a lighter crust, the ΔE value was the lowest indicating the least changes in colour compared to C1. This is due to the slightly increase in a* value (data not shown), which could have occurred due to the insolubility of mannitol and its reaction during baking.

The incorporation of sourdough caused, generally, a reduction in specific volume, which is, most likely, due to an increase in acidity and its effect on the functional ingredients in the cake system. The incorporation of organic acids in the system promotes protein

denaturation by the disruption of either the hydrogen bonds between the polar R-groups or the salt bridges (Tanford, 1968), and caused a higher stiffness, G*, of the batter during baking.

Furthermore, it is known that organic acids have a weakening effect on protein and starch structure (Galal et al., 1978; Takeda et al., 2001). Lactic acid is known to promote the solubility of amylopectin, reflected by the lower peak viscosity and final viscosity of this study (Shandera and Jackson, 1996). *Leuconostoc citreum* TR116 is a heterofermentative lactic acid bacterium, which is able to produce, besides lactic acid, acetic acid, when fructose is present. An earlier study has shown that lactic acid production was lower, when fructose was present, like in SD_{FRU} (see Chapter 5) (Sahin et al., 2018). This could explain why SD, which is higher in lactic acid, caused significantly lower specific volumes. Moreover, the TTA of the batters including SD_{FRU} was higher than all other batters. Hence, the higher buffering capacity could decrease the weakening effect of the organic acids and result in higher specific volume.

Sourdough functioned as a softening agent, due to delay of starch gelatinisation and interactions with starch, during the realignment of amylose and amylopectin (Bertolini et al., 2000), reflected by the lower final viscosity compared to C2 without sourdough addition. Interestingly, a softer crumb texture was achieved, although the number of cells decreased, putatively, due to the collapse of air cells during baking, as a result of a weak batter stability, as earlier discussed.

Sourdough incorporation resulted in a higher ΔE-value, representing a high degree in colour changes compared to C1. This is most likely due to a higher degree of Maillard reaction caused by the increased amount of amino acids, which are obtained from the wheat gluten in the flour during sourdough fermentation, and also the incorporation of little amounts of glucose present in the sourdough system (Martins et al., 2000).

The addition of sourdough as a functional ingredient increase microbial shelf life of cakes, demonstrated by an extension of one to two days compared to C2 without sourdough addition. *Leuconostoc citreum* TR116 produces organic acids, such as lactic acid and acetic acid (Choi et al., 2012). These metabolites are known for their antimicrobial performance and, thus, with increasing amounts of sourdough the degree of preservation rose.

Interestingly, the incorporation of SD_{FRU} increased the sweetness of the sugar-reduced cakes. Naturally produced mannitol by *Leuconostoc citreum* TR116, which has a relative

sweetness of 50-70% (Saulo, 2005), was present in SD_{FRU}, and contributed to sweetness. Glucose and maltose are increasing in a sourdough system by fermentation. Nevertheless, the amounts of residual glucose, fructose and maltose in the final product are below the recognition threshold and hence, did not contribute directly to the increase in sweetness perception (Belitz et al., 2009). Yet, the presence of these simple sugars could, potentially, lead to synergistic sensory sensations.

Furthermore, the increase in flavour and aroma is, putatively, due to the increasing amounts of free amino acids, which are known to contribute to these sensory attributes (Kato et al., 1989). It has been shown previously that sweetness and flavour correlate positively, and that sourdough contributes to flavour and enhances sweetness perception (see Chapter 5; Sahin et al., 2018). Although the sourness in cakes with sourdough increased slightly, the scores are considered as low and, thus, the sourness did not play a significant factor in terms of off-flavour. None of the cakes reached the softness of the control cake. Nevertheless, a decrease in hardness perception could be achieved by the addition of sourdough. This is due to the increasing acidity, which stimulates saliva production and increase the chewiness of the product (Ekström et al., 2012).

6.5. Conclusion

A high specific volume, dense and soft crumb structure, low staling rate, prolonged microbial shelf life and a sweet taste are desirable attributes of commercial cake products. These typical cake properties are difficult to maintain, when sugar is reduced. The application of sourdough, using *Leuconostoc citreum* TR116 as a single culture, increased the specific volume, softened the crumb and reduced the staling rate of a 50% sugar-reduced cake. In particular, sourdough in which mannitol was produced, further improved additional quality parameters of sugar-reduced cakes, such as microbial shelf life and sweet taste. Residual fructose in the final products were below the detection limit. This can be explained by the high conversion yield of 86.7% of fructose to mannitol by *Leuconostoc citreum* TR116 (see Chapter 5; Sahin et al., 2018).

Furthermore, the incorporation of sourdough increased the total sugar content on average only by maximum 1% (based on dry matter) due to carry-over of residual sugars. Thus, the aim of a 50% reduction in sugar was maintained. The total sugar content of the full-sugar control was approximately 30.1% (based on dry matter), while the sugar-reduced cakes incorporating sourdough had an average sugar content of approximately 14.2% (based on dry matter).

The natural production of mannitol in a sourdough system showed high potential as a sugar replacer compared to commercially available mannitol. Indeed, cakes produced with sourdough incorporation showed a higher specific volume, lower staling rate and comparable microbial shelf life.

In conclusion, this study demonstrates that sourdough technology, exploiting specific mannitol-producing strains, can be used as a novel technological approach to reduce added sugar in cakes by up to 50%. The most promising results in terms of taste and texture were obtained with the addition of 5% SD_{FRU}. In addition, from a consumer perspective, the sugar content of cake was reduced from 8.4 to 4.2 g per slice (28 g). Such an achievement in sugar reduction can contribute to a reduced sugar intake by consumers, which is in line with the WHO-guideline regarding sugar consumption. Furthermore, the novel technological approach in this study demonstrates a natural means in which sugar can be reduced in baked goods, while simultaneously maintaining product quality attributes such as texture and taste. Thus, such an approach could potentially be applied for sugar reduction in other baked goods.

6.6. Acknowledgement

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Chapter 7

THE INCORPORATION OF SOURDOUGH IN SUGAR-REDUCED BISCUITS: A PROMISING STRATEGY TO IMPROVE TECHNO-FUNCTIONAL AND SENSORY PROPERTIES

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Abstract

The demand for sugar-reduced, low-sugar, or even sugar-free food products is increasing. Sweet bakery products are the second main source of added sugar after sugary drinks. The reduction of sugar causes a loss of product quality, since sugar contributes to structure and flavour. The most common sugar replacers on the market are polyols, which act as sweet bulking agents. However, alternatives, which can be considered as 'clean-label' are in high demand. Sourdough technology was applied using the bi-functional lactic acid bacteria strain *Leuconostoc citreum* TR116. This strain is able to produce mannitol and/or exopolysaccharides to compensate structure loss in sugar reduced products. As controls, the full-sugar biscuit (C1) and biscuits reduced in added sugar by 75% by wheat starch (C2) or commercially available mannitol (C3) were considered. Wheat starch created a lower biscuit firmness (-10.7 N), while mannitol increased the hardness significantly (+12.9 N). Both sugar replacers caused less biscuit spreading and a poorer sensory profile. The addition of sourdough (5% or 10%) in C2 improved the viscoelastic properties, dough stickiness and dough hardness, as well as biscuit firmness. Furthermore, it contributed to colour (lowered the ΔE-value) and increased sweetness and flavour intensity (+140%; +139%). The predicted glycemic index (pGI) of C2-biscuits (73.5) were lower than C3-biscuits (80.8). Sourdough did not influence the release of reducing sugars during digestion. In conclusion, 10% sourdough incorporation represents a useful tool to overcome quality loss caused by the reduction of sugar by improving texture, taste and flavour.

7.1. Introduction

Sugar is one of the main factors contributing to several health issues, such as type-2-diabetes, cardiovascular disease and childhood obesity. The increasing number of people suffering from non-communicable diseases was recognized by the World Health Organization (WHO). Consequently, in 2015 the WHO published a global action plan, which advised consumers to reduce their daily intake of free-sugars to 10% based on their total energy intake (World Health Organization, 2015). The UK government reacted with the introduction of a taxation on sugary drinks and advised the bakery industry to reduce the sugar content in their products by 20% by 2020.

Biscuits contain 10-30% sugar, which influences the techno-functional properties as well as taste and flavour of the product (Wilderjans et al., 2013). Added sugar contributes to biscuit spreading caused by the decrease of dough viscosity during baking, and influences the time of structure setting (Vetter, 1984; Miller et al., 1996; Sumnu and Sahin, 2008; Pareyt et al., 2009; Sman and Renzetti, 2018). Moreover, sugar increases the biscuit hardness by its recrystallization during cooling, and it undergoes Maillard reaction, which enhances colour and flavour (Pareyt et al., 2009).

Reducing sugar in biscuits without compromising the product quality is challenging. Gallagher et al. (2003) substituted up to 30% of added sucrose with raftilose, an fructooligosaccharide (inulin derivate). Raftilose caused a lower biscuit hardness and a darker biscuit colour. The total replacement of added sugar by maltodextrin led to an increase in biscuit spreading and hardness, and reduced the sweetness of the biscuit significantly (Pourmohammadi et al., 2017).

Zoulias et al. (2000) replaced the total amount of added sugar by different polyols. The biscuits showed less spreading and were softer than the full-sugar control. Sugar replacement by erythritol showed the same trend: less spreading and lower biscuit firmness (Laguna et al., 2013).

Since the substitution of sugar by bulking agents either changed the techno-functional characteristics, such as colour, hardness and spreading, or reduced the taste and flavour of biscuits, alternative approaches for the reduction of added sugar are needed.

The natural *in-situ* production of mannitol in a sourdough system, using the lactic acid bacteria strain *Leuconostoc citreum* TR116, was used as a novel approach to overcome quality loss in sugar-reduced burger buns. Techno-functional properties as well as taste

and flavour intensity improved and a 50% sugar reduction was feasible (Sahin et al., 2018).

In this study sourdough was, firstly, produced by using the LAB strain *Leuconostoc citreum* TR116. This strain is a bi-functional strain and able to produce mannitol triggered by the addition of fructose, and also exopolysaccharides in the presence of sucrose. Three different sourdoughs were produced and characterised (pH, TTA, microbial cell count): One without any trigger (SD), another one which was rich in mannitol (SD_{FRU}), and a third one which showed mannitol and EPS (SD_{FS}) after fermentation. Secondly, the produced sourdoughs were applied individually in two concentrations (5% and 10%) in a low-sugar biscuit (sugar content ≤ 5%) to improve techno-functional properties as well as sensory characteristics.

7.2. Material and methods

The reduction of sugar in biscuits containing 20% added sucrose (control 1, C1) was conducted by the replacement of 75% sugar by wheat starch (control 2, C2) or commercially available mannitol (control 3, C3). In order to improve the techno-functional properties and the sensory characteristics of low-sugar biscuits (C2), sourdoughs were incorporated individually in two different concentrations (5% and 10% based on flour) by replacing the wheat flour and water in the recipe (Table 7.1).

Table 7.1 Biscuit recipes. C1 is the full sugar control, C2 represents the sugar reduced recipe containing 5% added sucrose and 15% wheat starch, C3 shows the formulation of the sugar reduced biscuit containing 5% added sucrose and 15% commercially available mannitol. Two different concentrations of sourdough without fructose or sucrose (SD), with fructose (SD_{FRU}) or with fructose and sucrose (SD_{FS}) were incorporated in C2

Ingredients	**C1**	**C2**	**C3**	**C2 + 5% Sourdough (SD or SD_{FRU} or SD_{FS})**	**C2 + 10% Sourdough (SD or SD_{FRU} or SD_{FS})**
Biscuit Flour	49.5	49.5	49.5	47.1	44.7
Sucrose	20.0	5	5	5	5
Wheat starch	-	15	-	15	15
Mannitol	-	-	15	-	-
Sourdough (solid part)	-	-	-	2.4	4.8
Sourdough (liquid part)	-	-	-	2.4	4.8
Shortening	19.8	19.8	19.8	19.8	19.8
Sodium Stearoyl Lactylate (SSL)	0.3	0.3	0.3	0.3	0.3
Salt	0.4	0.4	0.4	0.4	0.4
Water	9.9	15.6	13.8	13.2	10.8
Baking powder	0.3	0.3	0.3	0.3	0.3

7.2.1. Raw materials

Sourdoughs were prepared using baker's flour (Odlums Group, Dublin, Ireland), sterile tab water, fructose (Sigma-Aldrich, Gillingham, UK) and sucrose (Sigma-Aldrich, Gillingham, UK). *Leuconostoc citreum* TR116, isolated from yellow pea sourdough, belongs to the culture collection of the Department of Biological Sciences, Cork Institute of Technology, and was used for a controlled single strain fermentation. The conditions for cultivation and storage were chosen as previously reported in Chapter 5 (Sahin et al., 2018).

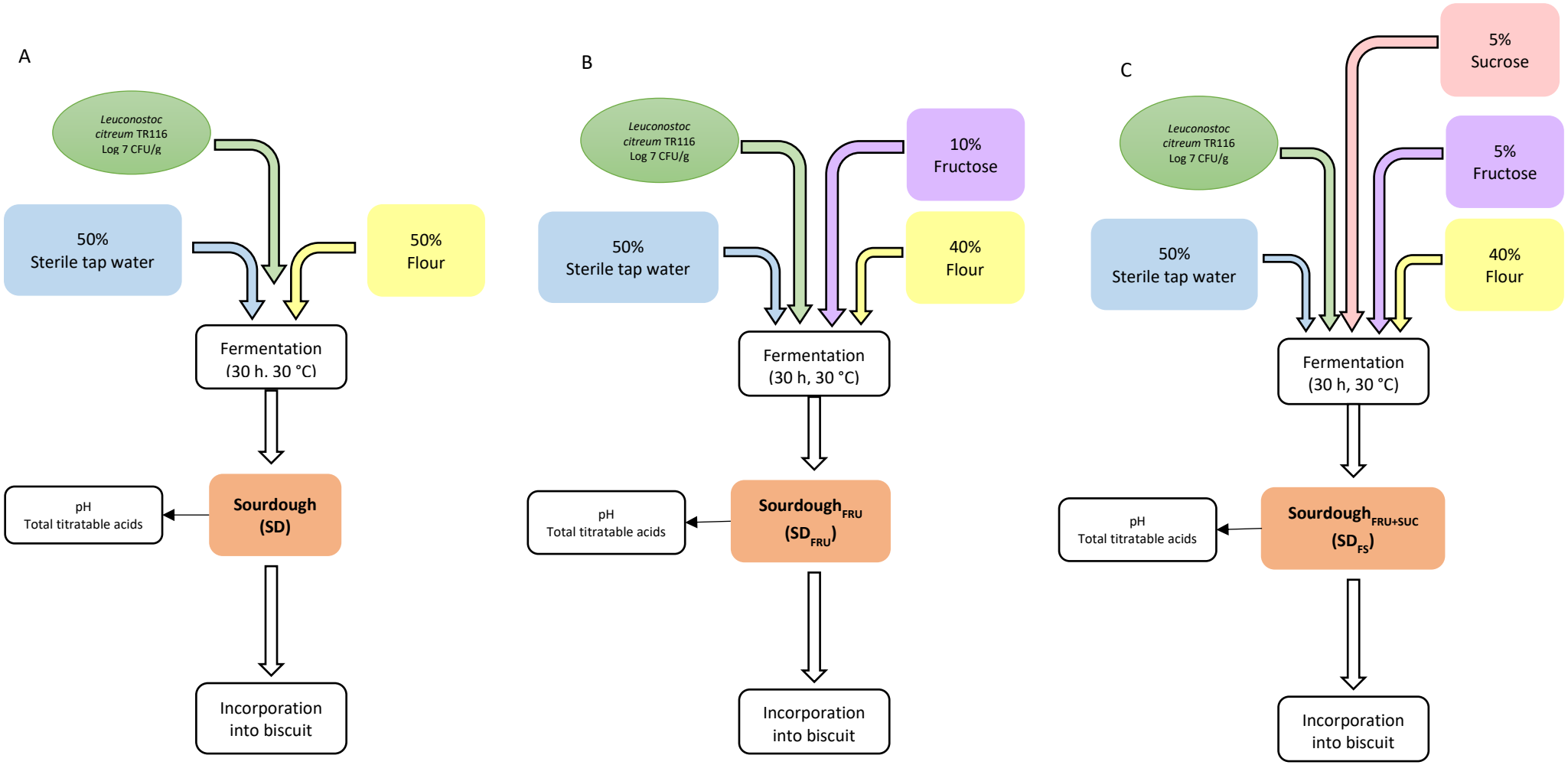
Biscuits were produced using biscuit flour (Odlums Group, Dublin, Ireland) with a protein content of 8% and a moisture of 14%, sucrose (Siucra, Dublin, Ireland), shortening (Stork, Sussex, UK), sodium stearoyl lactylate (SSL) (Danisco, Copenhagen, Denmark), salt (Glacia British Salt Limited, Cheshire, UK), baking powder (Valeo Foods, Dublin, Ireland) and tap water. 75% of the added sucrose was replaced by wheat starch (Roquette, Lestrem, France) or mannitol (Roquette, Lestrem, France).

7.2.2. Sourdough preparation

The EPS and mannitol producing LAB strain *Leuconostoc citreum* TR116 was used for a controlled single strain fermentation of wheat flour. The preparation of the inoculum and the cell harvest procedure of *Leuconostoc citreum* TR116 for sourdough fermentation were performed as reported previously in Chapter 5 (Sahin et al, 2018).

Three different sourdoughs were produced, among which, one sourdough (SD) contained only wheat flour (50%) and sterile tap water (50%). The second sourdough (SD_{FRU}) was a mixture of wheat flour (40%), sterile tap water (50%) and fructose (10%) in order to trigger mannitol production, and the third sourdough (SD_{FS}) contained wheat flour (40%), sterile tap water (50%), fructose (5%) and sucrose (5%) to initiate mannitol and EPS production. A scheme which illustrates the production of all three sourdoughs is shown in Figure 7.1.

Figure 7.1 Sourdough preparation scheme. A represents the production of SD (50% flour and 50% sterile tap water); B shows the production of SD_{FRU} (40% flour, 10% fructose and 50% sterile tap water); C illustrates the production of SD_{FS} (40% flour, 5% fructose, 5% sucrose and 50% sterile tap water)



All sourdoughs were produced by mixing the solid ingredients with sterile tap water and the LAB strain *Leuconostoc citreum* TR116 with a density of log 7 cfu/g sourdough. The ratio between solids and liquids was always 50:50 to ensure a dough yield of 200. The mixing procedure was conducted by using Kenwood Major Titanium KM 020 mixer (Kenwood, Havant, UK) set to speed 1 for one minute, followed by a second mixing step at speed 2 for one minute. Sourdoughs were packed in sterile stomacher bags, sealed airtightly and incubated at 30 °C for 30 h to achieve the highest mannitol concentration in sourdoughs spiked with fructose (see Chapter 5; Sahin et al., 2018).

The pH, the total titratable acids (TTA) and cell count after fermentation were determined to ensure a constant sourdough quality of all replicates. All fermentations were performed in triplicates.

7.2.3. Characterisation of the different sourdoughs

Determination of pH, total titratable acids (TTA) and microbial cell count

The pH, TTA and microbial cell count of all sourdough (fermentation time of 30 h) were determined as described in Chapter 5 (Sahin et al., 2018).

Apparent Viscosity of the different sourdoughs

In order to determine differences in the sourdough consistencies after fermentation, the apparent viscosity at a shear rate of 10 s^{-1} of each sourdough was determined using a Rheometer Physica MCR 301 (Anton Paar GmbH, Ostfildern, Germany), since the sourdoughs showed shear thinning behaviour as non-Newtonian fluids. The sourdough was equilibrated during a resting time of two minutes prior to the test. A flow ramp increasing the shear rate from 0 s^{-1} to 100 s^{-1} in one minute, followed by constant shearing at 100 s^{-1} and hold for another minute was performed. For the measurement a parallel plate system was used with an upper plate of 50 mm in diameter. The temperature of the lower plate was set to 20 °C. The apparent viscosity was determined as a triplicate for each sourdough.

Quantitative determination of produced exopolysaccharides (EPS)

The amount of exopolysaccharides (EPS) in the sourdoughs was determined by, firstly, the extraction of the polysaccharides from the sourdoughs, followed by a dialysis and a freeze-drying step. For the extraction, 50 g of sourdough was mixed with 100 ml of sterile dH_2O and homogenized by shaking. The dissolved dough was centrifuged (4 °C; 1000

rpm; 20 minutes) and the supernatant was weight out. Afterwards, the supernatant was precipitated by adding double the amount of 96% ethanol and the sample was stored at 4 °C for 20 h, followed by centrifugation (4 °C; 12,000 rpm; 20 minutes). The supernatant was discarded, and the precipitate was resolved in 50 ml sterile dH_2O and placed in the shaking incubator (20 °C, 160 rpm, 20 h). The solution was transferred into a dialysis tube (25 cm strips, 20 mm, 12-14 kDa, Sigma-Aldrich, Gillingham, UK) and a dialysis was performed in dH_2O at 4 °C overnight. After dialysis, the solution was freeze-dried, and the yield [%] of polysaccharides with a higher molecular weight than 14 kDa was determined.

7.2.4. Biscuit dough analysis

Water adjustment

The substitution of sugar by wheat starch or mannitol changed the consistency of the dough. In order to maintain the consistency of the biscuit dough, Farinograph E (Brabender OHG, Duisburg, Germany) was used to adjust the water content of each recipe to the torque of the full-sugar control dough (240 BU). The chamber temperature was set to 30 °C and the mixing speed was 63 min^{-1}.

Biscuit dough preparation

The biscuit dough was prepared by mixing all ingredients with a K-beater attachment for 3 minutes at speed 1 using a Kenwood chef mixer (Kenwood Ltd, New Hampshire, UK). After mixing, the biscuit dough was covered with cling film and allowed to rest for 20 minutes. 75% of sugar was substituted either by wheat starch (C2) or mannitol (C3) in order to produce a low-sugar biscuit. Additionally, all three different sourdoughs were incorporated in a low-sugar biscuit (C2) in two different concentrations (5% and 10%) respectively. The biscuit formulations are presented in Table 7.1.

Dough hardness

The dough hardness was measured by applying a compression test using TA-XT2i texture analyser (Stable Micro Systems, Godalming, UK). 110 g of dough was placed in a test cell as part of the dough preparation set and a flattening plunger was used to distribute the dough evenly in the cell. Afterwards, an aeration plunger was applied to eliminate air from the dough. The measurement was carried out using a cylindrical probe with a diameter of 6 mm and a test speed of 3 mm/sec. The test was performed for a distance of 20 mm. The hardness was evaluated in Newton [N].

Dough stickiness

The TA-XT2i texture analyser (Stable Micro Systems, Godalming, UK) with a Chen-Hosney dough stickiness rig probe in combination with an acrylic glass cylinder probe (25 mm diameter) was used to measure the dough stickiness. The test speed was 0.5 mm/sec and the return distance was set to 4 mm. The stickiness value was evaluated in Newton [N].

Viscoelastic properties of biscuit dough

The differences in rheological properties of the biscuit doughs were determined by performing oscillation measurements using a Rheometer Physica MCR 301 (Anton Paar GmbH, Ostfildern, Germany). The same geometries of the probes were used as mentioned before for the determination of the viscosity of the sourdough. However, in order to prevent slippage, both plates, upper and lower, were serrated. The temperature was set to 20 °C and an amplitude sweep was conducted to determine the linear viscoelastic region (data not shown), which allowed to set the target strain for the frequency sweep. A constant target strain of 0.01% was chosen during the frequency sweep with a frequency range from 100 to 0.1 Hz. The damping factor, as a measure for the proportion of elastic and viscous parts of the biscuit doughs, was evaluated.

7.2.5. Biscuit quality characteristics

Biscuit baking procedure

After the dough resting time, the biscuit dough was sheeted to 3 mm thickness using a laminator (Rondo, Chessington, UK), followed by the cutting using a biscuit cutter with a diameter of 70 mm. The cut biscuit dough was placed on a baking tray and backed for 12 min at 220 °C upper and lower heat using a deck oven (MIWE condo, Arnstein, Germany). After baking, the biscuits were cooled down at room temperature for one hour.

Diameter

In order to determine the spreading or shrinking of biscuits during baking, the diameter of the biscuits was measured using a calliper. For each batch ten biscuits were analysed.

Moisture content and water activity

The moisture of the biscuits was measured gravimetrically by using the air oven method (AACC Method 44-15 A). Therefore, biscuits were firstly ground using a food processor

(Kenwood Ltd, New Hampshire, UK) at speed 1 for 30 seconds, and the moisture content of the ground biscuits was analysed.

The water activity of the biscuits was determined by using water activity meter (HygroLab, Rotronic, Bassersdorf, Switzerland). Therefore, 8 g of ground biscuit sample was used. Three biscuits per batch were measured.

Biscuit firmness

Biscuit firmness was evaluated by performing a snap test using a three-point bend rig on a TA-XT2i texture analyser (Stable Micro Systems, Godalming, UK). One biscuit was placed on 2 parallel supports, which were set 40 mm apart. A rounded blade snapped the biscuits with a test speed of 3 mm/sec. The force [N] required to snap the biscuit was evaluated. Ten biscuits per batch were measured.

Colour

The colour of the biscuits was measured using Colorimeter CR-400 (Konica Minolta, Osaka, Japan). The evaluation of colour changes compared to C1 was conducted by the calculation of ΔE using the Scofield equation:

$$\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$$

Protein content

The protein content was calculated by the determination of the total nitrogen content in the biscuit samples using the Kjeldahl method with Kjeltec (Foss, Hilleroed, Denmark). As a conversion factor 5.7 was used (WHO/FAO, 2003).

Sugar, mannitol and acid concentrations

Biscuit samples were ground and the extraction of glucose, fructose, sucrose and maltose, as well as mannitol, lactate and acetate in NaN_3 (50 ppm) solution was carried out by shaking for 20 min on an orbital shaker followed by centrifugation (3,000 rpm, 10 min). The supernatant was filtered using a syringe driven filter with a pore size of 0.2 μm and transferred into a HPLC vial. The quantification of sucrose and maltose required a dilution with acetonitrile (Merck KGaA, Darmstadt, Germany) in a ratio 40:60 (supernatant: acetonitrile) before filtration and transfer.

The quantification of sugars, mannitol and acids was carried out using Agilent 1260 high performance liquid chromatography system.

Acetate (1-50 mmol/l) and lactate (1-50 mmol/l) were quantified by using a Hi-Plex H column (300 x 7.7 mm, 8 mm, Agilent, Cork, Ireland) at 60 °C, set up with a guard column (50 x 7.7 mm, 8 mm, Agilent, Cork, Ireland). The compounds were detected at 210 nm (DAD detector) using 0.005 M sulphuric acid as a mobile phase at a flow rate of 0.5 ml/min.

Glucose (1-100 mmol/l), fructose (1-100 mmol/l) and mannitol (1-50 mmol/l) were quantified using a refractive index detector (RID) (40 °C) by the elution of the extract from a Sugar-Pak I column (300 x 6.5 mm, Waters, Massachusetts, USA) at 80 °C, equipped with a guard column Sugar-Pak II, "Guard-Pak" (Waters, Massachusetts, USA). 0.0001 M CaEDTA at a flow rate of 0.5 ml/min was used as an eluent.

The concentrations of sucrose (1-100 mmol/l) and maltose (1-100 mmol/l) were determined using a RID (40 °C) in combination with a High Performance Carbohydrate column (4.6 mm x 250 mm, Waters, Massachusetts, USA) at 40 °C and a guard column "Guard-Pak" (Waters, Massachusetts, USA). The elution was carried out with an acetonitrile solution (75%) and a flow rate of 1 ml/min.

Extraction of one sample was conducted in duplicate and the concentration was calculated based on 100g biscuit.

Ultrastructure of the biscuits

The ultrastructure of biscuits was determined using scanning electron microscopy (SEM). Ground biscuits were freeze-dried (SP Scientific, Warminster, USA) and immobilized on aluminium stubs, followed by sputter-coating using palladium-gold (25 nm coat thickness). Samples were observed in a field emission scanning electron microscope using a working distance of 8mm. The ultrastructure was observed with a magnification of x500 and images were taken using SEM Control User Interface software, Version 5.21 (JEOL Technics Ltd., Tokyo, Japan) at an accelerating voltage of 5kV.

7.2.6. *In-vitro* digestions of biscuits and the determination of a predicted glycemic index (pGI)

An *in-vitro* digestion method used by Hager et al. (2013) was applied in order to simulate starch digestion by a multi-enzymatic treatment of the biscuit sample in a dialysis tubing system. Due to the high sucrose concentration in the samples the enzymatic treatment was slightly modified by the addition of invertase.

The biscuit samples were ground using a food processor (Kenwood Ltd, New Hampshire, UK) at speed 1 for 10 seconds. Four grams of the ground biscuit were homogenized in 20 ml sodium potassium phosphate buffer (0.2 M, pH 6.9). The pH was adjusted to 1.5 using 8 M HCl, 5 mL pepsin solution (115 U/ml) (EC 3.4.23.1, 674 U/ml solid, Sigma-Aldrich, Gillingham, UK) was added and the sample was incubated in a water bath (37 °C) for 30 min.

After incubation, the pH was readjusted to 6.9 using 6 M NaOH and 1 ml of porcine pancreatic α-amylase solution (110 U/ml) (EC 3.2.1.1, 22 U/ml solid, Sigma-Aldrich, Gillingham, UK) as well as 500 µl of invertase solution (510.7 U/ml) (EC 3.2.1.24, 200-300 U/mg solid, Sigma-Aldrich, Gillingham, UK) were added.

The volume was adjusted to 50 ml using sodium potassium phosphate buffer, the samples were transferred into dialysis tubes (25 cm strips, 20 mm, 12-14 kDa, Sigma-Aldrich, Gillingham, UK) and 5 glass beads (Sigma-Aldrich, Gillingham, UK) were added. Each tube was placed in a beaker containing 450 ml sodium potassium phosphate buffer and the samples was incubated in a water bath (37 °C) for 300 min.

The tubes in the beakers were turned every 15 min to mimic the peristaltic of the human intestine, and sampling took place every 30 min as reported by Hager et al. (2013).

The concentration of reducing sugars released from the dialysis tube into the buffer solution was determined by using the 3,5-dinitrosalicylic acid (DNS) method as described by Hager et al. (2013). After the incubation at 110 °C for 15 min in a dry heating block, the samples were put on ice to stop the reaction. 1 ml dH_2O was added and the absorbance at 546 nm was measured.

The release of reducing sugars (RSR) over time was calculated by using the following equation:

$$RSR\ [\%] = \frac{A_{sample} \times 500 \times 0.95}{A_{maltose} \times C_{carbs}} \times 100$$ (Hager et al., 2013),

whereas A_{sample} is the absorbance of the sample at 546 nm; 500 is the total volume [ml]; 0.95 is the conversion factor of maltose from starch; $A_{maltose}$ is the absorbance of maltose as the main reducing sugar during starch degradation; C_{carbs} is the total amounts of carbohydrates [mg] including starch and sugars in 4 g of biscuit sample. The total starch content was determined using commercially available assay kits (Megazyme International, Ireland Ltd., Wicklow, Ireland).

The release of reducing sugars was plotted over time and the areas under the curves (AUC) were calculated using integration. The hydrolysis index (HI) was related to the full-sugar control (C1) and determined using the following formula:

$$HI = \frac{AUC_{sample}}{AUC_{control}} \times 100$$

The predicted glycemic index (pGI) was calculated using the equation:

$$pGI=39.71+0.549xHI,$$

which is based on an *in-vivo-in-vitro* correlation of GI-values of different starch based food products, such as biscuits (Goni et al., 1997; Wolter et al., 2014).

7.2.7. Sensory evaluation

Sensory properties of the biscuits were determined by a trained sensory panel of 10 people (5 females and 5 males, age: 24-32) which were briefed 6 hours per week over 6 months prior the tasting. The descriptors were defined as previously reported in Chapter 4 and Chapter 5. As an additional parameter the "dry mouth-feel" during chewing was evaluated. The room in which the training and the final evaluations were conducted had a room temperature of 21±1 °C. The intensity of each sample was graded individually on a scale between 0 (not perceived at all) and 10 (highest intensity). Tasting of the biscuit samples was performed in duplicates on two different days. All controls (C1, C2 and C3) as well as biscuits containing 10% of SD, SD_{FRU} or SD_{FS} respectively were chosen for the sensory.

7.2.8. Statistical analysis

All experiments were performed in triplicates. Therefore, a variance analysis (one-way ANOVA, $p \leq 0.05$, Tukey test) was performed using Minitab 17. Correlation analysis was conducted using Microsoft Excel 2016.

7.3. Results

The characterisation of different sourdoughs fermented by the mannitol and EPS producing strain *Leuconostoc citreum* TR116 included the determination of the pH, TTA and microbial cell count before and after fermentation (30 h) as well as the viscosity and the quantity of produced exopolysaccharides. These sourdoughs were incorporated in a low-sugar biscuit formulation and dough properties as well as biscuit characteristics were analysed.

7.3.1. Sourdough characteristics

pH, TTA and microbial cell count

For the characterisation of the sourdoughs, pH, TTA and microbial cell count were determined (Table 7.2). The pH of the different sourdoughs did not differ from each other and showed values between 5.41 (SD_{FS}) and 5.47 (SD, SD_{FRU}) before fermentation and between 4.18 (SD) and 4.20 (SD_{FRU}, SD_{FS}) after 30 h of fermentation. The amount of total titratable acids was determined by the addition of 0.1 M NaOH with a target pH of 8.5. The TTA of the sourdoughs were the same before fermentation. After 30 h of fermentation, SD_{FRU} and SD_{FS} showed the highest TTA values (SD_{FRU} = 16.90 ± 0.18 ml; SD_{FS} = 16.75 ± 0.18 ml), while the TTA of SD was only 11.43 ± 0.10 ml. The microbial cell count at time point 0 of the fermentation resulted in log 7 cfu/g sourdough, as desired and controlled by the calculated inoculation level. The cell count, after 30 h fermentation, was the same in all different sourdoughs (log 9 cfu/g sourdough).

Viscosity

The apparent viscosity of all three sourdoughs showed significant differences among each other (Table 7.2). The highest apparent viscosity was determined in SD (1.23 ± 0.05 Pa·s), followed by SD_{FS}, in which 10% of the flour was replaced by 5% fructose and 5% sucrose (0.70 ± 0.06 Pa·s). The lowest apparent viscosity was recorded in SD_{FRU}, in which 10% flour was replaced by fructose (0.40 ± 0.06 Pa·s).

Table 7.2 Characterisation of the different sourdoughs before and after fermentation (30 h). SD represents the sourdough produced by 50% wheat flour and 50% water; in SD_{FRU} mannitol production was triggered by the replacement of 10% flour by fructose; in SD_{FS} mannitol production as well as exopolysaccharide (EPS) production was triggered by the replacement of 10% flour by 5% fructose and 5% sucrose. TTA reflects the total titratable acids

	pH		**TTA [ml 0.1 M NaOH]**		**Cell count [log cfu/g]**		**Apparent viscosity at 10^{-1} [Pa·s]**	**EPS [g/kg]**
	0 h	**30 h**	**0 h**	**30 h**	**0 h**	**30 h**	**30 h**	**30h**
SD	5.47±0.04 (a)	4.18±0.02 (a)	2.73±0.08 (a)	11.43±0.10 (b)	7.22±0.09 (a)	9.25±0.01 (b)	1.23±0.05 (a)	8.84±0.24 (a)
SD_{FRU}	5.47±0.06 (a)	4.20±0.03 (a)	2.67±0.07 (a)	16.90±0.18 (a)	7.11±0.03 (a)	9.22±0.03 (b)	0.40±0.06 (c)	7.81±0.19 (b)
SD_{FS}	5.41±0.03 (a)	4.20±0.02 (a)	2.77±0.13 (a)	16.75±0.18 (a)	7.12±0.03 (a)	9.38±0.06 (a)	0.70±0.06 (b)	8.52±0.36 (a)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

Produced exopolysaccharides during sourdough fermentation

The extraction of polysaccharides resulted in significant differences between SD_{FRU} and the other two sourdoughs (Table 7.2). SD_{FRU} showed 7.81 ± 0.19 g/kg of polysaccharides having a higher molecular mass than 14 kDa, while SD (8.84 ± 0.24 g/kg) and SD_{FS} (8.52 ± 0.36 g/kg) included significant higher amounts. Since in both sourdoughs, SD_{FRU} and SD_{FS}, 10% of the flour was replaced by monosaccharides, SD_{FRU} represents the negative EPS control (EPS(-)), while EPS-production was triggered by sucrose in SD_{FS} (EPS(+)). The determination of the difference resulted in an average EPS production of 0.71 ± 0.41 g/kg in SD_{FS}.

7.3.2. Dough characteristics

Water adjustment

The water content of each recipe was adjusted to the consistency of the full-sugar control recipe (water content of 9.9%) in order to maintain the biscuit dough consistency (Table 7.1). The sugar replacement with wheat starch resulted in a higher water level (15.6%). Commercially available mannitol as a sugar substitution also caused an increase in water addition to 13.8%. The incorporation of 5% sourdough in C2 resulted in a water addition of 13.2%, while the added water level required for recipes with 10% sourdough was only 10.8%.

Dough hardness

Changes in dough hardness reflect quality changes of the biscuits during production caused, for example, by the supplementation of functional ingredients, such as sugar. The supplementation of added sucrose with a bulking agent resulted in an increase in dough hardness (Table 7.3), while commercially available mannitol caused the hardest dough (2.71 ± 0.26 N). The incorporation of sourdough in C2 decreased the dough hardness. It is noteworthy that the addition of 10% sourdough, regardless which type, showed no significant differences compared to the dough hardness of C1 (1.44 ± 0.28 N). The incorporation of SD_{FRU} caused the same dough hardness as determined for C1, regardless the amount added.

Table 7.3 Characterisation of biscuit dough and biscuits. C1 represents the full-sugar control, in C2 75% of the sugar in C1 was replaced by wheat starch, and C3 includes commercially available mannitol as a sugar replacer instead of wheat starch. Three different types of sourdough, SD (without fructose or sucrose addition), SD_{FRU} (10% flour replacement by fructose) and SD_{FS} (10% flour replacement by 5% fructose and 5% sucrose), were incorporated in C2 in two different concentrations (5% and 10%)

	Dough properties			**Biscuit properties**						
	Hardness [N]	**Stickiness [N]**	**Damping factor**	**Diameter [mm]**	**Moisture [%]**	**Firmness [N]**	**ΔE-value**	**Water activity**	**Total starch [%]**	**Protein content [%]**
C1	1.44 ± 0.28 (e)	0.34 ± 0.03 (b)	0.394 ± 0.009 (a)	71.79 ± 0.72 (a)	2.69 ± 0.11 (f)	33.20 ± 3.52 (cd)	0.00±0.00 (f)	0.218±0.034 (f)	39.73 ± 0.18 (b)	5.68±0.12 (abc)
C2	1.85 ± 0.17 (bc)	0.29 ± 0.04 (c)	0.285 ± 0.003 (d)	69.32 ± 0.17 (c)	9.28 ± 0.38 (a)	22.50 ± 1.73 (e)	8.64 ± 1.83 (a)	0.696±0.004 (a)	51.07 ± 5.57 (a)	5.00±0.03 (d)
C3	2.71 ± 0.26 (a)	0.29 ± 0.04 (c)	0.317 ± 0.005 (bc)	69.42 ± 0.21 (c)	6.31 ± 0.19 (e)	46.13 ± 2.88 (a)	3.44 ± 1.51 (e)	0.490±0.017 (e)	39.01 ± 4.79 (b)	5.20±0.03 (bcd)
C2+5% SD	1.78 ± 0.14 (cd)	0.29 ± 0.02 (c)	0.289 ± 0.011 (d)	70.10 ±0.47 (b)	6.83 ± 0.51 (d)	34.34 ± 2.68 (bcd)	7.24 ± 2.15 (bc)	0.599±0.034 (cd)	53.95 ± 2.05 (a)	5.05±0.04 (d)
C2+10% SD	1.62 ± 0.19 (de)	0.27 ± 0.03 (c)	0.323 ± 0.016 (b)	70.13 ± 0.45 (b)	6.67 ± 0.29 (de)	37.25 ± 5.23 (bc)	5.13 ± 2.56 (d)	0.568±0.054 (d)	54.88 ± 3.59 (a)	5.67±0.49 (abc)
C2+5% SD_{FRU}	1.54 ± 0.20 (e)	0.42 ± 0.06 (a)	0.293 ± 0.017 (d)	70.13 ± 0.51 (b)	7.46 ± 0.30 (c)	34.58 ± 3.21 (bcd)	7.43 ± 1.49 (b)	0.646±0.022 (b)	51.37 ± 2.52 (a)	5.14±0.14 (cd)
C2+10% SD_{FRU}	1.50 ± 0.23 (e)	0.37 ± 0.05 (ab)	0.322 ± 0.019 (b)	69.98 ± 0.41 (b)	8.01 ± 0.56 (b)	30.39 ± 3.23 (d)	6.61 ± 2.93 (bc)	0.638±0.007 (bc)	56.12 ± 5.46 (a)	5.75±0.24 (ab)
C2+5% SD_{FS}	2.03 ± 0.19 (b)	0.32 ± 0.01 (b)	0.299 ± 0.004 (cd)	69.89 ± 0.47 (b)	7.54 ± 0.27 (bc)	32.05 ± 2.89 (d)	6.10 ± 3.02 (cd)	0.656±0.007 (b)	52.58± 0.67 (a)	5.35±0.14 (bcd)
C2+10% SD_{FS}	1.45 ± 0.16 (e)	0.33 ± 0.01 (b)	0.329 ± 0.009 (b)	70.05 ± 0.31 (b)	7.68 ± 0.25 (bc)	37.76 ± 4.17 (b)	3.47 ± 1.52 (e)	0.600±0.011 (cd)	50.53 ± 1.41 (a)	5.97±0.21 (a)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

Dough stickiness

Another important dough quality parameter is the dough stickiness. Dough stickiness can influence the spreading of the biscuit during baking. The substitution of added sucrose by wheat starch or mannitol decreased the dough stickiness significantly (Table 7.3). The addition of SD to C2 did not influence the dough stickiness in any way. On the contrary, SD_{FRU} increased the stickiness from 0.29 ± 0.04 N (C2) to 0.42 ± 0.06 N and caused even higher values than the full-sugar control (C1). The incorporation of SD_{FS} also increased the dough stickiness and resulted in the same values as C1.

Damping factor

The damping factor is a parameter which can attain values between 0 and 1 and reflects the proportions of viscous and elastic parts in a dough system. The higher the damping factor the higher the viscous proportion in the system. Among all biscuit doughs, the full-sugar control (C1) showed the highest damping factor (0.394 ± 0.009) and differed significantly from all sugar reduced samples (Table 7.3). The replacement of added sucrose by wheat starch (C2) decreased the damping factor to 0.285 ± 0.003. Mannitol as a sugar substitute resulted in a higher damping factor than wheat starch, but did not reach the values of C1. The addition of sourdough to C2 increased the damping factor, especially the incorporation of 10% sourdough, regardless the type.

7.3.3. Biscuit quality

Biscuits are characterised by different quality parameters, such as diameter, moisture, firmness, colour, water activity and sensory attributes. Furthermore, the effect of sugar reduction and of the incorporation of sourdough on the rate of sugar release during digestion was investigated by the determination of a predicted *in-vitro* glycemic index.

Diameter

The biscuit diameter expresses the ability of the biscuit dough to spread or shrink during baking. C1 showed the largest diameter (71.79 ± 0.72 mm), while C2 (69.32 ± 0.17 mm) and C3 (69.42 ± 0.21 mm) were significantly smaller. Sourdough incorporation increased the diameter of C2 by up to 0.81 mm, yet, did not reach the dimensions of the full-sugar control (Table 7.3).

Moisture content and water activity

The moisture content can influence sensory attributes and the water activity of a product. Full-sugar biscuits had the lowest moisture content (2.69 ± 0.11%), while C2 showed the

highest value (9.28 ± 0.38%). The incorporation of SD_{FRU} and SD_{FS} in a low-sugar biscuit (C2) increased the moisture content of biscuits to a higher extent than SD did (Table 7.3). Among all sourdough applications, the addition of 10% SD_{FRU} showed the highest moisture (8.01 ± 0.56%), while 10% SD resulted in the lowest value (6.67 ± 0.29%).

The lowest water activity occurred in the full-sugar control biscuits (C1) (0.218 ± 0.034), while C2 showed the highest a_w-value (0.696 ± 0.004). Commercially available mannitol as a sugar replacer (C3) resulted in an a_w-value of 0.490 ± 0.017. The incorporation of sourdough in C2 decreased the water activity, whereas SD_{FRU} caused the highest values among the sourdoughs and SD the lowest (Table 7.3). A relation between moisture content and water activity was investigated and a positive correlation between these two parameters was determined ($r = 0.97$, $p \leq 0.001$)

According to Abellana et al., (2001), *Penicillium* spp. and *Aspergillus* spp. require a minimum water activity of 0.85 in order to grow on baked products. Hence, the microbial shelf life has not been further investigated.

Biscuit firmness

The full-sugar control biscuit showed a firmness of 33.20 ± 3.52 N. Interestingly, the substitution with wheat starch softened the biscuit (22.50 ± 1.73 N), whereas mannitol as a sugar replacement caused a significant increase in biscuit hardness (46.13 ± 2.88 N). The incorporation of sourdough in C2 increased the biscuit firmness and showed values close to C1. 5% sourdough addition resulted in the same biscuit firmness as C1, while 10% sourdough caused even higher values (Table 7.3). Interestingly, SD_{FRU} caused lower hardness values when 10% sourdough (30.39 ± 3.23 N) was added compared to the addition of 5% (34.58 ± 3.21 N).

Colour

One important biscuit characteristic regarding appearance is the colour. Figure 7.2 demonstrates the differences in colour of the biscuits. Compared to C1, the biggest colour difference occurred in C2 (8.64 ± 1.83), while C3 (3.44 ± 1.51) showed the closest results to C1 (Table 7.3). The incorporation of sourdough reduced the colour differences compared to C1, especially the addition of 10%. Among all sourdough biscuits, the addition of 10% SD_{FS} resulted in a ΔE-value of 3.47 ± 1.52, and showed, besides C3, the least changes in colour compared to C1.

Protein content

The amount of proteins in the biscuit can contribute to the final biscuit quality. The total protein contents of the biscuits are demonstrated in Table 7.3. Sugar substitution by wheat starch or commercially available mannitol decreased the total amount of proteins in the system significantly (C1 = 5.68 ± 0.12; C2 = 5.00 ± 0.03%; C3 = 5.20 ± 0.03). The addition of 5% sourdough to C2 did not change the protein content, regardless the type of sourdough. However, the incorporation of 10% sourdough increased the total protein content in the biscuit (between 5.67% and 5.97%), and resulted in the same protein content as determined in C1.

Sugar and acid profile

The determination of the sugar profile is illustrated in Table 7.4 and revealed that there was no glucose in the biscuits. Fructose quantities were relatively low with a maximum amount of 0.10 g/100g in low-sugar biscuits containing 10% SD_{FS}.

The sucrose concentrations in the final biscuits reflect the reduction of added sugar by at least 75%. Maltose was not detected in the control biscuits. However, biscuits containing sourdough showed maltose concentration between 0.93 and 1.08 g/100g. The total amount of mono- and disaccharides in sugar reduced biscuits showed values between 4.15 g/100g (C3) and 5.24 g/100g (C2+10% SD).

The amounts of mannitol detected in the biscuits were the highest in C3 (16.81 ± 0.50 g/100g) in which 15% added sucrose was replaced by commercially available mannitol. Biscuits containing SD showed mannitol concentrations below the detection limit of 0.04 g/100g. On the contrary, the incorporation of SD_{FRU} and SD_{FS} increased the concentration of mannitol in the biscuits significantly, resulting in 0.48 ± 0.07 g/100g in biscuits with 10% SD_{FRU} and 0.41 ± 0.01 g/100g in biscuits with 10% SD_{FS}.

The evaluation of lactate and acetate in the biscuits showed the presence of lactic acid in all biscuits, while the highest amounts were detected in biscuits including 10% SD (0.112 ± 0.006 g/100g), followed by 10% SD_{FRU} (0.076 ± 0.001 g/100g) and 10% SD_{FS} (0.076 ± 0.002 g/100g). Acetic acid did not occur in the control biscuits C1, C2 or C3. The lowest amounts of acetate were detected in biscuits with 5% sourdough, whereas the addition of 10% sourdough resulted in the highest concentrations of acetate, with SD_{FS} causing the highest amounts (0.204 ± 0.025 g/100g), followed by SD_{FRU} (0.195 ± 0.030 g/100g).

Table 7.4 Sugar and acid concentrations of the full-sugar control biscuit (C1), low-sugar biscuit with wheat starch as a sugar replacer (C2), low-sugar biscuit with commercially available mannitol as a sugar replacer (C3), and biscuits including sourdough prepared without fructose or sucrose addition (SD), with 10% fructose (SD_{FRU}) or 5% fructose and 5% sucrose (SD_{FS}). The abbreviation "n.d." stands for "not detected".

	Glucose [g/100g]	**Fructose [g/100g]**	**Sucrose [g/100g]**	**Maltose [g/100g]**	**Total sugars [g/100g]**	**Mannitol [g/100g]**	**Lactic acid [g/100g]**	**Acetic acid [g/100g]**
C1	n.d.	0.08 ± 0.02 (bc)	18.72 ± 0.15 (a)	n.d.	18.80 ± 0.14 (a)	n.d.	<0.02 (e)	n.d.
C2	n.d.	0.04 ± 0.00 (e)	4.28 ± 0.08 (b)	n.d.	4.32 ± 0.08 (c)	n.d.	<0.02 (e)	n.d
C3	n.d.	n.d.	4.15 ± 0.11 (b)	n.d.	4.15 ± 0.11 (c)	16.81 ± 0.50 (a)	<0.02 (e)	n.d
C2+5% SD	<0.04	0.04 ± 0.01 (de)	4.40 ± 0.11 (b)	0.96 ± 0.04 (ab)	5.16 ± 0.13 (b)	<0.04 (d)	0.053±0.005 (c)	n.d.
C2+10% SD	<0.04	0.06 ± 0.01 (cd)	4.32 ± 0.19 (b)	1.08 ± 0.10 (a)	5.24 ± 0.14 (b)	<0.04 (d)	0.112±0.006 (a)	0.041±0.012 (c)
C2+5% SD_{FRU}	<0.04	0.04 ± 0.01 (de)	4.36 ± 0.18 (b)	0.93 ± 0.02 (b)	5.14 ± 0.21 (b)	0.23 ± 0.04 (bcd)	0.043±0.004 (d)	0.167±0.015 (b)
C2+10% SD_{FRU}	<0.04	0.09 ± 0.01 (ab)	4.31 ± 0.11 (b)	1.04 ± 0.08 (ab)	5.22 ± 0.13 (b)	0.48 ± 0.07 (b)	0.076±0.001 (b)	0.195±0.030 (ab)
C2+5% SD_{FS}	<0.04	0.07 ± 0.02 (cd)	4.29 ± 0.07 (b)	0.96 ± 0.04 (ab)	5.12 ± 0.05 (b)	0.19 ± 0.01 (bcd)	0.047±0.006 (cd)	0.169±0.018 (ab)
C2+10% SD_{FS}	<0.04	0.10 ± 0.01 (a)	4.25 ± 0.10 (b)	1.05 ± 0.08 (ab)	5.18 ± 0.13 (b)	0.41 ± 0.01 (bc)	0.076±0.002 (b)	0.204±0.025 (a)

Each value is given as the average value ± standard deviation.

Values in one column followed by the same lower case letter are not significantly different ($P<0.05$).

Ultrastructure of biscuits

Scanning electron microscopy was used to take images of the ultrastructure of the biscuits in order to investigate structural changes, which influence the physicochemical properties. The micrographs in Figure 7.2 clearly show an increasing amount of intact starch granules in C2 and in all the sourdough-biscuits. Additionally, sugar reduction by commercially available mannitol (C3) caused a compact structure with less starch molecules which are embedded in the biscuit and very tightly connected. The addition of sourdough caused the development of a film-like structure covering the shape of the starch granules, which is different from the coating occurred in C3.

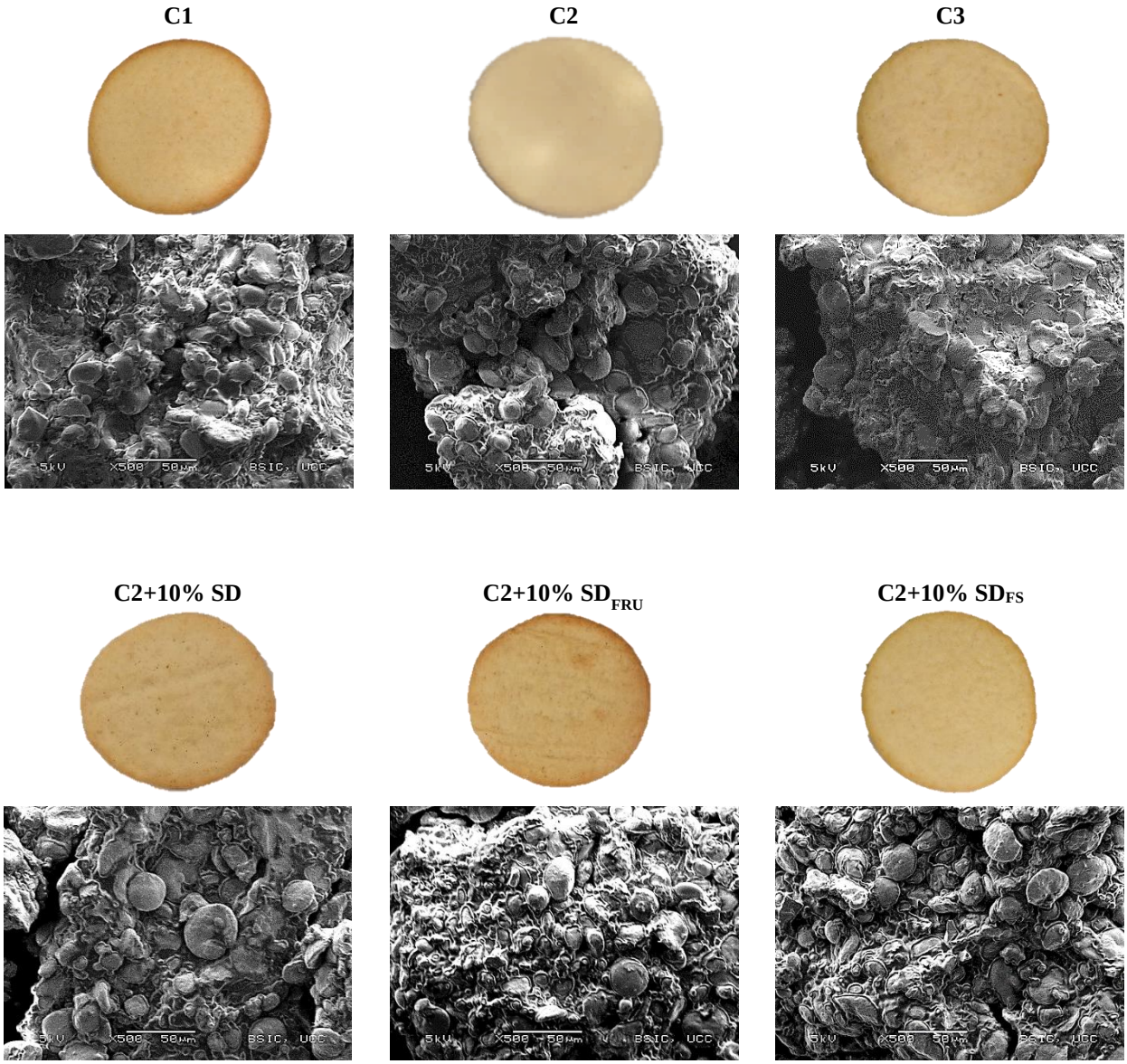


Figure 7.2 Appearance of biscuits and their ultrastructure analysed by scanning electron microscopy (SEM). C1=full-sugar control; C2=75% sugar reduction by wheat starch; C3= 75% sugar replacement by mannitol; The effect of different sourdoughs, SD (without fructose or sucrose addition), SD_{FRU} (10% flour replacement by fructose) and SD_{FS} (10% flour replacement by 5% fructose and 5% sucrose) on the crumb structure of a low-sugar biscuit (C2) is illustrated in the second row

7.3.4. Predicted glycemic index

The release of reducing sugar during a simulated digestion of the biscuits over five hours was the base of the calculation of the predicted glycemic index (pGI) obtained by starch and sucrose hydrolysis. This method is comparing the pGI-values to a reference product, the full-sugar biscuit (C1); thus, C1 has a pGI of 100 (Figure 7.3). The pGI-values of all sugar-reduced biscuits showed significant lower values than C1. Among all control biscuits, C3 showed the highest pGI (80.8 ± 1.6) and C2 the lowest pGI (73.5 ± 2.4). The incorporation of sourdough, regardless the amount and type of sourdough, did not influence the pGI of C2.

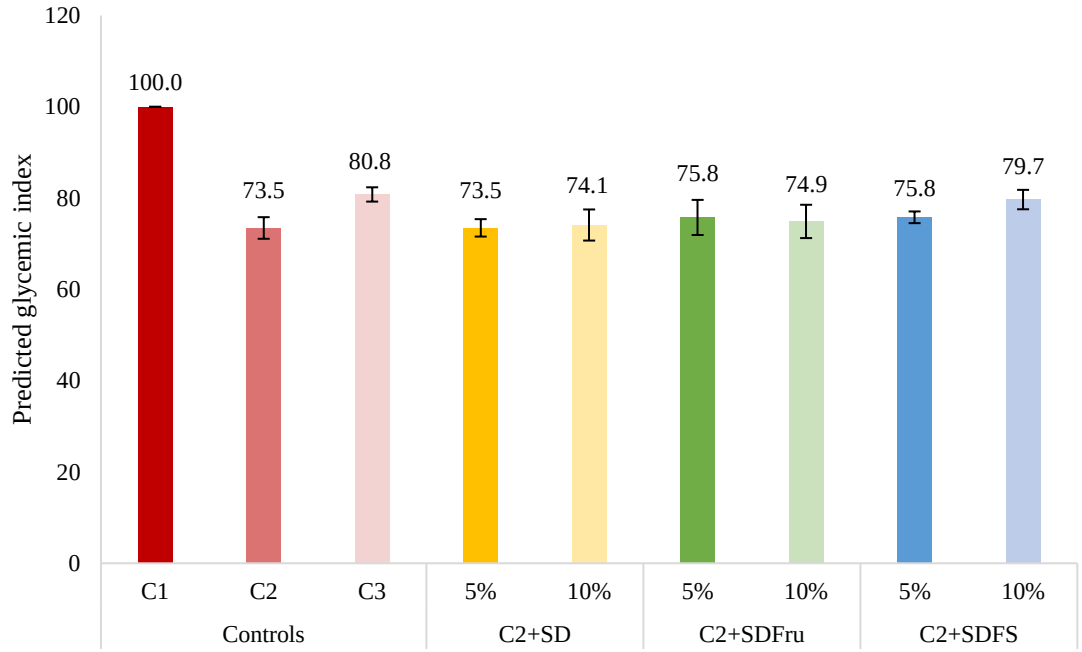


Figure 7.3 Predicted glycemic index of biscuits. C1=full-sugar control; C2=75% sugar reduction by wheat starch; C3= 75% sugar replacement by mannitol. The effect of the incorporation of different sourdoughs, SD (without fructose or sucrose addition), SD_{FRU} (10% flour replacement by fructose) and SD_{FS} (10% flour replacement by 5% fructose and 5% sucrose), in C2 on the release of reducing sugars during digestion is demonstrated

7.3.5. Sensory attributes

The sensory evaluation of the biscuits was performed in order to investigate the changes in texture, taste, aroma, flavour as well as mouth-feel by the addition of sourdough (Figure 7.4). C1 was evaluated as the hardest (7.94 ± 1.80) and the sweetest (9.04 ± 1.22) biscuit with the highest flavour (7.31 ± 1.34) and aroma (6.38 ± 2.02) intensity compared to all biscuits evaluated. Biscuits in which 75% added sugar was replaced by wheat starch (C2)

showed the lowest hardness (4.29 ± 2.77) and sweetness (2.05 ± 1.18) intensity resulting in low intensity scores in flavour and aroma. C3 was judged sweeter than C2. However, regarding flavour and aroma, C3 resulted in the same low intensity scores as C2.

The incorporation of sourdough, especially SD_{FRU} and $SD_{FS,}$ in a low-sugar biscuit (C2) increased the hardness (+2.67) and sweetness (+2.87). Besides increasing the sweetness intensity, sourdough incorporation resulted in a higher sourness. An increase from 0.64 ± 0.22 (C2) to 2.20 ± 1.75 (C2+10% SD_{FS}) was determined. The application of sourdough increased flavour and aroma of C2. SD_{FRU} achieved the highest flavour intensity (5.67 ± 1.72) and aroma intensity (5.08 ± 2.22) among all sourdough-biscuits. Furthermore, all sourdoughs decreased the dry mouth-feel during chewing. Considering sweetness, flavour and aroma, low-sugar biscuits including 10% SD_{FRU} showed the closest results to the full-sugar control.

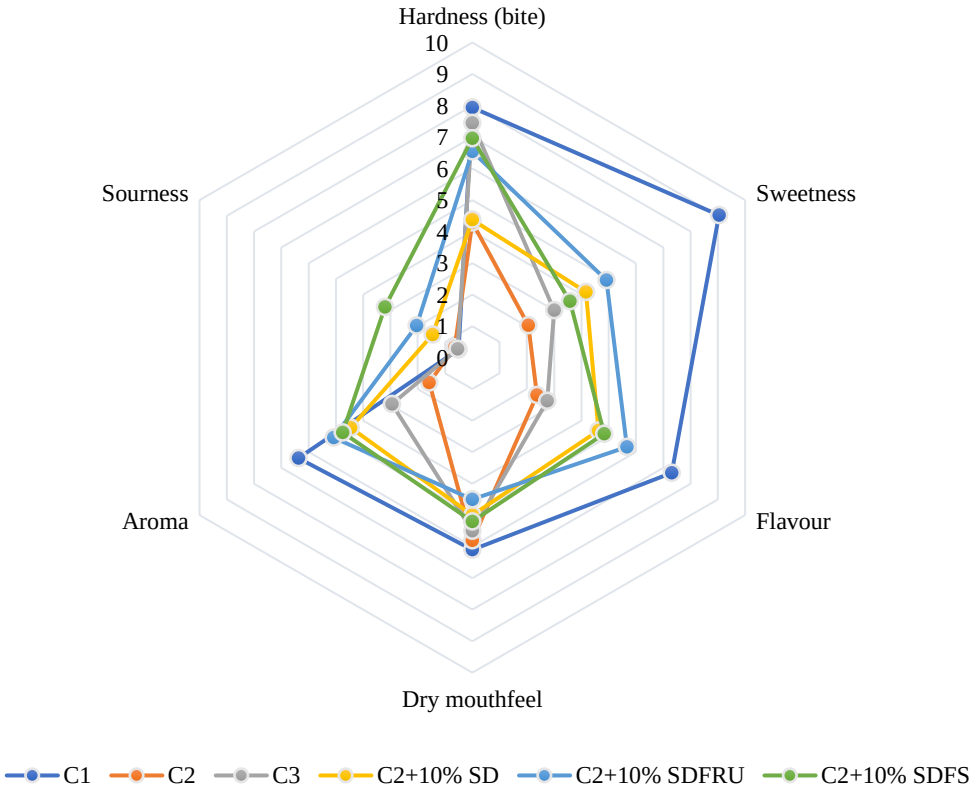


Figure 7.4 Sensory evaluation of the control biscuits with C1 being the full-sugar control, C2 standing for the replacement by wheat starch and C3 reflecting the substitution with commercially available mannitol. The effect of the incorporation of different sourdoughs, SD (without fructose or sucrose addition), SD_{FRU} (10% flour replacement by fructose) and SD_{FS} (10% flour replacement by 5% fructose and 5% sucrose), in C2 on the sensory properties is also illustrated. The chart shows the average values which had a confidence interval of ≤ 1.77 ($\alpha = 0.05$, $n = 10$)

7.4. Discussion

This study reveals the effect of sugar replacement on biscuit dough and biscuit quality, and, additionally, illustrates the potential of three different wheat sourdoughs as functional ingredients to overcome quality loss in sugar reduced biscuits. All sourdoughs were prepared by a controlled single strain fermentation using the mannitol and exopolysaccharide producing LAB strain *Leuconostoc citreum* TR116.

The investigation of sourdough properties revealed significant differences in total titratable acids (TTA) after fermentation, due to changes in the acid profile of the sourdoughs. *Leuconostoc citreum* TR116 produced acetic acid, when mannitol production is triggered, in order to regenerate ATP. The incorporation of SD_{FRU} or SD_{FS} in the biscuit showed higher concentration of acetate and lower amounts of lactate. During titration of the sourdough to from pH 4.20 to 8.50, the pH in which acetate has the highest buffering capacity was traversed (pH 3.75-5.75). The buffering range of lactate, on the contrary is between pH 2.86 and 4.86 (Chang, 2005). Hence, high TTA values occurred in sourdough, in which mannitol production was triggered.

Furthermore, all three sourdoughs differed significantly in apparent viscosity. SD showed the highest apparent viscosity due to the higher amount of flour, which caused a higher water absorption by starch and protein as humectants. The replacement of 10% flour by sugar reduced the apparent viscosity of the sourdough. Interestingly, significant differences in apparent viscosity between SD_{FRU} and SD_{FS} occurred. SD_{FS} included 5% sucrose, which triggers EPS-production. The enzyme glucansucrase is responsible for the synthesis of EPS by *Leuconostoc citreum* TR116. Glucansucrase is expressed in the cell and secreted into the extracellular medium. At first energy is regenerated by the cleavage of sucrose into glucose and fructose. This energy is used to mediate the synthesis of dextrans (Lynch et al., 2018). The produced EPS increased the apparent viscosity of the sourdough significantly. Since 10% flour was replaced in SD_{FRU} and SD_{FS}, SD_{FRU} represents the negative control. Evidently, besides EPS other carbohydrates originated from the flour were remained in the dialysis tube reflected by the apparent viscosity of SD. However, the presence of 0.71 g/kg EPS in SD_{FS} significantly influenced the sourdough rheology.

The replacement of sugar by wheat starch increased the elastic portion of the dough reflected by the decrease in damping factor, which results in a stiffer dough and, consequently, an increased dough hardness. The increase of elastic proportion of the

dough decreased the stickiness of the biscuit dough and could have caused biscuit shrinking instead of spreading during baking (Pareyt et al., 2009). Laguna et al. (2013) determined a higher dough resistance, when the amount of added sugar was reduced, which counteracted the spreading process. Wheat starch as a sugar replacer caused a lower snap firmness. This softening effect occurred, putatively, due to the higher moisture content and free water (higher a_w-value) in the biscuit. To ensure the consistency of C1 a higher water level needed to be added in C2, since wheat starch binds more water than sugar (Handa et al., 2012). Wheat starch as a sugar substitute caused the highest colour difference compared to C1, because of the lower level of Maillard browning reaction (Martins et al., 2000). The protein content in C2 is significantly lower compared to the full-sugar control, which led to lower amounts of amino acids and consequently less Maillard reaction (Martins et al., 2000; Sahin et al., 2017).

Mannitol also increased the elastic parts in the biscuit dough, which contributed to dough hardness and decreased dough stickiness. These rheological properties occurred, putatively, due to the solubility character of mannitol. This polyol is only moderately soluble, which does not change with increasing temperature. The low solubility could cause a higher biscuit hardness due to, firstly, its action as an insoluble fibre, interacting with starch and protein molecules and, thereby, causing a more compact structure (Laguna et al., 2014). Secondly, the insolubility restricted biscuit spreading and resulted in smaller biscuit diameter and, thus, a thicker biscuit, which contributed to the hardening (Zoulias et al., 2000). Furthermore, the melting point (165-175 °C) of mannitol is traversed during balking, which could cause the very dense and compact biscuit structure, illustrated in Figure 2, resulting in harder biscuits. Sugar replacement by commercially available mannitol led to the least changes in colour compared to the full-sugar control (C1). This occurred most likely due to the increased level of caramelisation due to the lower moisture content and lower water activity (Soria and Villamiel, 2012).

The incorporation of sourdough in a low-sugar biscuit (C2) decreased the dough hardness, increased the viscous portion and the stickiness of the dough, especially, when 10% SD_{FRU} or 10% SD_{FS} was applied. In these sourdoughs higher amounts of acetic acid were detected, resulting in a higher total acid concentration. *Leuconostoc citreum* TR116 is a heterofermentative lactic acid bacterium, which possesses the gen for the expression of the enzyme mannitol-dehydrogenase (MDH), which reduces fructose to mannitol. In the presence of fructose, *Leuconostoc citreum* TR116 produces acetate to regenerate one extra ATP, which provides energy for the cell and prolongs survival (Saha and Racine,

2011; Gänzle, 2015). A previous study showed a positive correlation of mannitol and acetate concentration in sourdough spiked with fructose (see Chapter 5; Sahin et al., 2018).

The acidification of the biscuit dough could initiate an unfolding of the proteins and the hydrolysis of the amorphous regions of the starch, which leads to a less compact structure of the biscuit dough reflected by a higher stickiness and lower dough hardness (Arendt et al., 2007; Wang and Copeland, 2015; Sahin et al., 2018).

Sourdough application ameliorated the spreading process of a low-sugar biscuit significantly, which is presumably due to the increase in viscous proportions of the dough. Furthermore, the incorporation of acids and proteolytic enzymes increases the solubility of flour compounds and proteins, which contributed to the rheological changes of C2 by sourdough (Galal et al., 1978; Wehrle et al., 1997; Arendt et al., 2007).

Although the incorporation of sourdough softened the biscuit dough and increased the viscous portion of it, the application resulted in an increased biscuit firmness, which did not differ from the full-sugar control. It has to be noted that the level of water addition was the same in the sourdough biscuits and C2. With the addition of sourdough, acids, partially hydrolysed starch, denatured protein (gluten) or proteolyzed protein subunits, low amounts of single sugars and mannitol are included.

The inclusion of acids in the biscuit dough could promote starch hydrolysis and could change the flour-protein conformation. This contributes to the release of bound water and promotes different protein interactions (Wang and Copeland, 2015; Arendt et al., 2007). This in turn contributes to the formation of bonds with carbohydrates, denatured protein or protein subunits, and/or free amino acids from the sourdough, or with fat in the biscuit dough (hydrophobic interactions). The formation of new bonds between the molecules decreases the amount of bond water, which causes a higher moisture loss during baking. This is reflected by the lower moisture and a_w-value of sourdough-biscuits, which leads to a higher biscuit hardness. Furthermore, Wang and Copeland (2015) reported an accelerated retrogradation of acidified starch, which could cause a higher biscuit hardness. Another factor which needs to be considered regarding biscuit hardness, is the incorporation of small amounts of sugars (up to 0.92%) and mannitol (up to 0.48%) produced during fermentation. Monosaccharides recrystallize during baking, which promotes biscuit hardness, and mannitol could contribute to hardness due to its low solubility, as discussed above (Mamat et al., 2010; Laguna et al., 2014).

Sourdough addition decreased the degree of colour difference, which is most likely due to the increasing amount of free amino acids and sugars, which contribute to Maillard reaction (Martins et al., 2000).

Sugar reduction affected the predicted glycemic index differently. The replacement of sugar by wheat starch showed a lower pGI value than the biscuits containing mannitol as a sugar substitute. In this biscuit the amount of gelatinized starch plays the key role. The incorporation of 15% wheat starch resulted in less hydration and gelatinization of the starch granules compared to the degree of gelatinisation in C3 (15% mannitol). Starch is a bigger molecule than mannitol and, thus, requires more water for hydration. Figure 2 shows the increasing amount of intact starch granules in biscuits, when wheat starch was used as a sugar replacer. Gelatinised starch is more susceptible for the enzymatic cleavage of the α-(1,4)-glycosidic bonds in amylose than native starch (Slaughter et al., 2001; Sluimer, 2005). Hence, biscuits with mannitol as a sugar replacer were higher in pGI than C2. The incorporation of sourdough did not affect the pGI, since it did not influence the degree of gelatinisation (Figure 7.2).

The addition of sourdough improved sensory properties of the biscuits. The increased hardness due to intermolecular changes in the biscuit during baking was significantly noticeable in the tasting. All low-sugar biscuits achieved lower intensity-scores regarding sweetness compared to the full-sugar control. However, the increase in sweetness-intensity caused by sourdough addition occurred, putatively, due to the presence of small amounts of mannitol, which has a sweetness value of 50-70% relative to sucrose (Saulo, 2005). Furthermore, the increase in flavour and aroma due to sourdough addition can be explained by the incorporation of acids and free amino acids, which also contributed to Maillard reaction and, in turn, to the synthesis of flavour compounds (Kato et al., 1989; Martins et al., 2000).

7.5. Conclusion

Sugar is one of the main ingredients in biscuits and contributes to techno-functional properties as well as taste. Short dough biscuits are mainly characterised by a certain hardness and texture, colour and sweetness. An extreme reduction of sugar by 75% to achieve low-sugar biscuits caused significant changes of these properties. The incorporation of sourdough in a low-sugar biscuit recipe contributed to biscuit spreading, overcame the biscuit softening and increased biscuit colour. Furthermore, sourdough, in particular, SD_{FRU} enhanced sensory attributes by increasing the sweetness intensity as well as flavour and aroma, and it decreased the dry-mouthfeel during chewing.

Commercially available mannitol is also suitable as a sugar replacer in biscuit regarding biscuit properties. However, these biscuits contain 16.8 % mannitol, which allows a consumption of maximum 119 g biscuits per day without triggering laxative effect, based on a tolerance of 20 g per day (Saulo, 2005). Sourdough-biscuits contain maximum 0.5% mannitol which allows a consumption of 4 kg low-sugar biscuits per day. Furthermore, sourdough-biscuits caused a lower predicted glycemic index.

This study reveals the potential of sourdough technology as a natural approach to reduce high amounts of added sugar and produce low-sugar biscuits. The achievement can contribute to the reduction of consumers' daily sugar intake, which was strongly advised by the WHO. Further improvement of texture, flavour and taste could be accomplished by the use of other bulking agents instead of wheat starch, while it is recommended to incorporate SD_{FRU}. An alternative option would be a higher production of EPS by the addition of higher amount sucrose at the beginning of sourdough fermentation.

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Chapter 8

GENERAL DISCUSSION

Sugar reduction became one of the most popular trends on the food market, since consumers' awareness of the contribution of sugar to non-communicable diseases increased.

Bakery is one of the leading sources of added sugar (Guallar-Castillón et al., 2013). It contributes to sweetness, flavour and aroma, but also influences techno-functional properties of the final product, such as volume, texture, structure and colour (Frazier, 2009; Struck et al., 2014). The reduction of sugar in sweet baked products is very challenging, since sugar interacts with all ingredients and influences product quality significantly. Its high affinity to water decreases the water activity of the product and contributes to water retention (Cauvain and Young, 2006). Furthermore, is competes with starch and proteins (gluten) for water and, hence, elevates the gelatinisation temperature, weakens the gluten network and delays its development (Cauvain and Young, 2006; Baxter & Hester, 1958; Wilderjans et al., 2013). Sugar influences the yeast activity; moderate amounts promote CO_2-production, while too high amounts inhibit the fermentation process due to osmotic stress (Attfield, 1997). Additionally, it can act as an emulsifier in products with a high fat content (Clemens et al., 2016; Pareyt and Delcour, 2008).

In cakes, sugar ensures the equal distribution of gas cells and stabilises the batter, which results in a high specific volume (Clemens et al., 2016). Furthermore, sugar contributes to biscuit spreading and hardening due to its hygroscopicity and recrystallization (Vetter, 1984; Sumnu and Sahin, 2008; Pareyt et al., 2009). In sweet-yeast-leavened products, for example burger buns, sugar causes a dense but soft crumb texture (Amendola and Rees, 2003; Sahin et al., 2017). Common strategies for sugar reduction include its substitution by a combination of bulking agent and sweetener, or by sweet bulking agents, such as polyols. Some tested formulations are promising, but always involve the use of artificial sweeteners, or polyols. Products containing more than 10% polyols need to be labelled as "excessive consumption can cause laxative effect", which deters consumers from buying these products (Regulation (EU) No 1169/2011, 2011).

The first part of this thesis highlights the unique properties of sugars and its influence on the product quality of burger buns, followed by the investigation of the effect of commercially available polyols, such as xylitol, maltitol and mannitol, as sugar replacers.

Burger buns contain 7-12% sugar, which causes the typical attributes, such as dense crumb structure, soft and brown crust, sweetness and long microbial shelf life (Cauvain

and Young, 2007). Research about the role of sugar in burger buns or other sweet-yeast-leavened bakery products is scarce. Reducing the sugar content by wheat starch, as a non-sweet bulking agent, led to significant changes in all these attributes. Hence, a fundamental understanding of the impact of sugar on the product quality explained by its influence on macromolecules, such as starch and proteins, during the baking process is essential. In burger buns the leavening is caused by yeast. Sucrose is broken down by an extracellular invertase into glucose and fructose, and glucose provides the energy to maintain the yeast metabolism and growth (Zhang et al., 2010). During dough fermentation, yeast produces CO_2, which contributes to the specific volume of the final product (Gelinas, 2006). However, sugar concentrations higher than 4% resulted in a linear decrease of specific volume and, concomitant, in an increase in crumb hardness. High sugar concentration increased the osmotic pressure in the system which stressed the yeast cells and reduced the fermentation activity and, hence, the CO_2 production (Attfield, 1997).

Sugar is hygroscopic and has a high affinity to water. Consequently, it competes with gluten for water (Baxter and Hester, 1958; Van Steertegem et al., 2014). Thus, the gluten network development required longer time in the presence of high sugar concentrations and the network formed was weaker. The evaluation of GlutoPeak measurement, resistance to extension and extensibility, as well as visual observation of the network by confocal laser scanning microscopy (CLSM) is strong evidence for the effect of sugar on the gluten network. Additionally, the hygroscopicity of sugar decreased the water activity of the burger buns and caused a prolonged microbial shelf life. It has to be noted that the adjustment of the water content could also be the reason for the lower water activity.

The formation of a desired golden crust in bakery products occurs due to Maillard browning reaction and caramelisation. In both reaction, sugar plays the key role. Millard browning is the result of the interaction between reducing sugars and amino acids under certain pH and temperature conditions (Hashiba, 1982). The extent of the reaction increases with increasing amounts of added sugar (Esteller et al., 2006). During Maillard browning, flavour compounds are formed which influenced the sensory attributes of burger buns. A trained sensory panel was able to clearly notice the differences in sweetness, when sugar was replaced.

Hence, sugar plays an important role in the production of burger buns, contributing to techno-functional properties as well as to sweetness and flavour. The fundamental

understanding of the role of sugar revealed that a simple reduction of sugar in bakery products without the addition of functional ingredients is not feasible. Thus, sugar substitution with functional ingredients, which provide bulk as well as sweetness and flavour, is required.

Polyols, also known as sugar alcohols, are sweet bulking agents, which can be considered as sugar replacers. Xylitol and maltitol are suitable sugar substitutes in bakery products (Ronda et al., 2005), and mannitol is used in food products, such as candies and flavoured-coated sweets (Le and Mulderrig, 2001). The effect of these three polyols as partial or total sugar replacements in burger bun was investigated. Xylitol, maltitol and mannitol showed a significant impact on the fermentation behaviour of the yeast. The CO_2 production during proofing was significantly lower, which resulted in smaller specific volumes. Acyclic polyols, such as xylitol and mannitol, are not metabolised by *Saccharomyces cerevisiae* (Canh et al., 1975). Polyols might diffuse into the yeast cell, but do not contribute to CO_2 production (Hough et al., 1979). This, in turn, led to a lower specific volume and harder crumb texture.

Polyols interact with other macromolecules in the dough system. It has been shown that xylitol, maltitol and, especially, mannitol weakened the gluten network and delayed its development. Sugar alcohols can interact with gluten, form polyol-gluten-complexes through ionic interactions and hydrogen bonds, and, hence, restrict gluten network formation. Moreover, xylitol and maltitol are, as sugar, hygroscopic agents and compete with gluten for water, which led to the weakened network formation (Pareyt et al., 2009a). Mannitol, on the contrary, is non-hygroscopic and only moderately soluble. This polyol can act as an insoluble fibre resulting in hydrophobic interactions with gluten and delayed the network development (Chen et al., 1988). Furthermore, its small particle size favours these interactions (Tomasik et al., 1995). These effects on the gluten network resulted in a lower dough extensibility caused by polyols. This, in turn, results in a denser and firmer crumb texture. The amount of holes increased with increasing amount of sugar alcohols in the system, which is also related to the weak gluten network.

Sugar and polyols also competed with starch for water, which influenced the pasting properties of the burger bun system. Sugar replacement by polyols resulted in lower peak and final viscosity, which could have three reasons: firstly, the structural flexibility of the polyols, especially the acyclic ones, and their poor structural complexity could cause a lack of ordering and results in a decrease of viscosity (Tomasik et al., 1995). Secondly,

most polyols are smaller molecules than sucrose. Hence, they succeed more in the interaction with water, while competing with starch for it. This could lead to a lower rate of hydration and swelling of the starch molecules, which causes a decrease in viscosity (Tomasik et al., 1995). Thirdly, an interaction between polyol and starch could have taken place to affect the viscosity. These interactions are described to result in closely packed swollen starch granules, which can resist the heat and shearing for a longer period of time (Sun et al., 2014).

Polyols did not contribute to browning reaction due to their lack of a reactive aldehyde group and thus, caused a paler crust colour (Ghosh and Sudha, 2012). Furthermore, the hygroscopicity of xylitol and maltitol lowered the water activity of the product (Ghosh and Sudha, 2012; Ninni et al., 2000). Mannitol, on the contrary, resulted in a higher a_w-value due to its non-hygroscopicity and low solubility. Although the incorporation of polyols as sugar replacers lowered the water activity, sugar was more effective in prolonging microbial shelf life. Furthermore, all sugar alcohols contributed to sweetness intensity of the burger buns, but could not replace sucrose.

A total replacement of sugar by polyols was not feasible. Polyols had a negative impact on burger bun properties, such as fermentation properties, specific volume, crumb hardness, colour and flavour, but contribute to sweetness. A 50%- sugar replacement could be considered on condition that the texture will be improved by other functional ingredients and considering the laxative effect of polyols and the tolerance levels of the human body. Among all polyols tested, mannitol showed the most promising results taking techno-functional properties and sensory evaluation into account.

The replacement of sugar by commercially available polyols emphasised the challenges occurring during sugar reduction. Thus, this research thesis introduces a novel technological approach to overcome quality loss in sugar reduced products: The use of sourdough technology.

Some lactic acid bacteria are able to produce naturally mannitol. For the production of mannitol-rich wheat sourdough, the heterofermentative LAB strain *Leuconostoc citreum* TR116, isolated from yellow pea sourdough, was used. *Leuconostoc citreum* TR116 possesses the gen for the synthesis of the enzyme mannitol-dehydrogenase (MDH) which converts fructose into mannitol by the regeneration of the co-factor NAD^+. The production of acetate by an acetate kinase is linked to the mannitol production and regenerates a ATP (Gänzle, 2015). In order to trigger mannitol production during

sourdough fermentation, 10% of the flour was replaced by fructose, and a control sourdough without fructose was also produced. Before sourdough was incorporated in sugar reduced burger buns, the suitable fermentation time was determined, taking the microbial growth, acidification and the concentrations of mannitol, sugars and acids into account.

Sourdough fermentation showed higher TTA in mannitol-rich sourdough, which is attributed to the increasing amounts of acetate and the fact that acetate shows the highest buffering capacity at pH 3.75-5.75, the pH range which is traversed during titration from pH 4.2 to 8.5 (Chang, 2005). During sourdough fermentation a fructose-mannitol conversion-yield of 87% was achieved. Taking into account that sourdough is a complex system, this yield can be considered as high. Furthermore, a lower concentration of metabolites, such as mannitol, acetate and lactate, than theoretical possible occurred, which is putatively due to either a lack of ADP or/and increased stress in form of low extracellular pH (Pimentel et al., 1994). The microbial growth showed a longer stationary phase in mannitol-rich sourdough compared to the control sourdough, which occurred most likely due to higher concentrations of mannitol. The biological reason for polyol production by LAB is a defence mechanism in order to cope with environmental stress situations, such as osmotic pressure, and to prevent the cell from oxidative harm by binding free reactive oxygen radicals (Chaturvedi et al., 1997; Smirnoff and Cumbes, 1989).

The highest amount of produced mannitol occurred after 30 h of sourdough fermentation. Thus, this fermentation time was chosen for the addition of sourdough to the burger buns. The incorporation of both sourdoughs individually in a burger bun system weakened the gluten network and delayed its formation, as sugar does. These changes in dough properties occurred due to acidification (Arendt et al., 2007). Acidification causes a positive net charge of the proteins and enhances their solubility, which contributes to an unfolding. This change in tertiary structure leads to the exposure of hydrophobic groups which do not form new bonds due to strong intermolecular interactions (Galal et al., 1978; Takeda et al., 2001; Wehrle et al., 1997). These strong intermolecular interactions, in turn, contributed to the increased resistance to elongated dough extension (Masi et al., 2001). Furthermore, sourdough addition caused an inhibition of starch gelatinisation due to an increasing amount of degraded starch molecules (Bertolini et al., 2000). Additionally, lactate increased the solubility of amylopectin and decreased the viscosity during pasting (Shandera and Jackson, 1996).

The weakened gluten network and delayed structure formation of starch during baking led to a lower specific volume and a harder and chewier crumb very similar to the full-sugar control bun. Although sourdough increased the water activity of the burger buns, the microbial shelf life was prolonged. As a heterofermentative LAB *Leuconostoc citreum* TR116 produces organic acids and potentially ethanol, which can act as antimicrobial compounds (Choi et al., 2012; Corona et al., 2016). Sourdough incorporation increased the degree of browning reaction due to the incorporation of free amino acids and, putatively, due to pH changes (Martins et al., 2000). During Maillard browning, aroma compounds are formed which increased the flavour and aroma intensity of sourdough burger buns. The addition of 10% mannitol-rich sourdough increased significantly the sweetness intensity of the buns, since mannitol contributed with its sweetness of 50-70% (relative to sucrose) to the overall sweetness intensity. Hence, the incorporation of 10% mannitol-rich sourdough into a sugar reduced burger bun is promising and highly recommended.

Since the addition of mannitol-rich sourdough fermented by the LAB strain *Leuconostoc citreum* TR116 compensated the quality loss in sugar reduced burger buns and showed promising potential as a functional ingredient, the effect of this sourdough on cakes and biscuits was investigated.

In a cake system, a 50%-replacement of sugar by wheat starch, as a non-sweet bulking agent caused a lower specific volume and a denser and harder crumb texture. These changes in the burger bun properties occurred due to a weaker batter stability. High amounts of starch compete with protein for water and results in a weaker network (Hicsasmaz et al., 2003; Ronda et al., 2005; Stauffer and Beech, 1990). Furthermore, the incorporation of starch resulted in a higher degree of starch gelatinisation and realignment during cooling. The lower amounts of sugar also caused an earlier structure setting. Mannitol as a sweet-bulking agent also caused a lower specific volume. The crumb occurred to be harder than the full-sugar control, but softer than cakes with wheat starch as a sugar replacer, which is most likely due to the physicochemical properties of mannitol. Its low solubility could cause hydrophobic interactions with proteins, such as ovalbumin of the added whole eff or gluten of the flour, leading to a high flexibility of the tertiary structure and, thus, faster protein denaturation on the cake surface, resulting in early crust formation (Wilderjans et al., 2010). Furthermore, mannitol increased the staling rate, which is, putatively, due to its non-hygroscopic character (Ronda et al.,

2005). Less bond water in the system led to an increased a_w-value and a shorter microbial shelf life (Whistler and BeMiller, 1997).

The incorporation of mannitol-rich sourdough improved the structure and sensory attributes of the cakes more than sourdough without natural production of mannitol. The addition led to a softer crumb texture caused by the delay of starch gelatinisation and interaction with starch during realignment of amylose and amylopectin (lower final viscosity during pasting procedure) (Bertolini et al., 2000). The specific volume did not improve by sourdough without mannitol. With sourdough addition, organic acids, such as lactate and acetate, are present. Organic acids promote protein denaturation by the disruption of either the hydrogen bonds between the polar R-groups or the salt bridges, leading to a higher batter stiffness which counteracts the increase in volume (Tanford, 1968; Galal et al., 1978). Mannitol-rich sourdough caused an increase in specific volume compared to sourdough without natural mannitol production due to its higher buffering capacity. Hence, the differences in the ratio of acetate and lactate influences the specific volume. Sourdough addition improved the colour formation in cakes due to a higher degree of Maillard reaction, and it also prolonged microbial shelf life by the incorporation of organic acids (Martins et al., 2000). The sensory evaluation showed an increase in sweetness and flavour, when mannitol-rich sourdough was added. The small amount of simple sugars potentially caused a synergistic sensory sensation. Furthermore, the increase in flavour and aroma is putatively due to the presents of free amino acids which are known to contribute to sensory characteristics (Kato et al., 1989). The addition of 5% mannitol-rich sourdough is recommended to improve techno-functional and sensory attributes of 50%-sugar-reduced cakes. The addition of batter stabilizers, such as hydrocolloids, can be considered to further improve the texture.

As already mentioned before, *Leuconostoc citreum* TR116 is a multifunctional strain which is able to produce mannitol, but also exopolysaccharides, when sucrose as a trigger is present during sourdough fermentation. The main functions of sugar in biscuits is the spreading process during baking, as well as, the hardening effect due to recrystallization at high temperatures and the contribution to sweetness and flavour.

The replacement of 75% sugar by wheat starch, as a non-sweet bulking agent, caused biscuit shrinkage instead of spreading. Shrinkage occurred due to the higher resistance of the dough, reflected by the lower damping factor, which counteracts the spreading process (Laguna et al., 2013; Pareyt et al., 2009b). Moreover, sugar reduction by wheat starch

decreased the biscuit hardness significantly due to the higher moisture content and a_w-value. Additionally, wheat starch as a sugar substitute caused a higher colour change; a result of less Maillard reaction and caramelisation. Commercially available mannitol as a sugar replacer increased the biscuit hardness significantly. The low solubility of mannitol could cause a higher biscuit hardness due to, firstly, its action as an insoluble fibre, interacting with starch and protein molecules and, thereby, causing a more compact structure (Laguna et al., 2014). Secondly, the insolubility restricted biscuit spreading and resulted in smaller biscuit diameter and, thus, a thicker biscuit, which contributed to the hardening (Zoulias et al., 2000). The decrease in damping factor of the biscuit dough could also contribute to less spreading. The moisture as well as the a_w-value of biscuits with commercially available mannitol as a sugar replacer were significantly lower, which favours caramelisation, and resulted in the least changes in colour compared to the full-sugar control (Soria and Villamiel, 2012).

In order to overcome quality loss occurring in biscuits due to sugar reduction, sourdough technology using the multifunctional strain *Leuconostoc citreum* TR116 was used to increase the sweetness perception and improve biscuit properties, such as spreading, hardness and colour. Besides mannitol-rich and no-mannitol sourdoughs, a third sourdough was produced in which mannitol production as well as EPS synthesis was triggered. The amount of EPS produced was 0.71 g/kg, which can be conserved as low; however, the EPS increased the sourdough viscosity significantly.

The addition of sourdough, especially mannitol-rich sourdough, decreased dough hardness and increased stickiness as well as the viscous proportion in the biscuit dough. These changes in dough properties occurred due to the acidification, which initiates the unfolding of the proteins and the hydrolysis of the amorphous regions of the starch (Arendt et al., 2007; Wang et al., 2015). This, in turn, ameliorated the spreading process of the biscuits. Furthermore, the incorporated acids and proteolytic enzymes in the sourdough increased the solubility of flour compounds and enhanced spreading (Galal et al., 1978; Wehrle et al., 1997). The acidification by sourdough addition promoted starch hydrolysis and changes in protein confirmation, which contributes to the liberation of bond water. The formation of new interactions between molecules, such as carbohydrates, denatured protein or protein subunits from the sourdough, or with fat in the biscuit dough, occurred. Due to high temperatures during baking, the moisture loss was higher, reflected by the lower biscuit moisture and lower a_w-value, which could have caused the increased biscuit hardness. Moreover, the incorporation of acids with the sourdough, putatively,

accelerated the retrogradation of starch and, additionally, monosaccharides and mannitol present in the sourdough recrystallize during baking and also contribute to biscuit hardening (Mamat et al., 2010; Wang and Copeland, 2012). Sourdough addition in biscuit also promoted browning reaction due to the increased amount of free amino acids and the changes in pH (Martins et al., 2000). As in burger buns and biscuits, mannitol-rich sourdough increased the sweetness and flavour intensity of the biscuits due to the presence of small amounts of mannitol, which has a sweetness of 50-70% relative to sweetness. Furthermore, the incorporation of acids and free amino acids contribute to Maillard browning, which initiates the formation of flavour compounds.

Sugar in a product is known to influence the glycemic index of a product. A predicted glycemic index (pGI) of biscuits reduced in added sugar by wheat starch or commercially available mannitol was determined, and the effect of sourdough addition on this predicted value was evaluated.

The replacement by wheat starch showed a lower pGI value than the biscuits with mannitol as a sugar substitute. In this scenario the amount of gelatinized starch plays the key role. Starch is a bigger molecule than mannitol and, thus, requires more water for hydration. Hence, a high number of starch granules in biscuits with wheat starch as a sugar replacer were still intact and not easily susceptible for the enzymatic cleavage of the α-(1,4)-glycosidic bonds in amylose (Slaughter et al., 2001; Sluimer, 2005). Thus, sugar substitution by high amounts of wheat starch decreases the pGI more than commercially available mannitol. Sourdough was added in the formulation, in which sugar was replaced by wheat starch. It did not affect the pGI, since it did not influence the degree of gelatinisation.

Conclusively, sugar is a vital ingredient in sweet bakery products. Researchers face challenges during sugar reduction. Polyols show a high potential as partial sugar replacers, but it has to be noted that they effect the human gut in an unhealthy way by causing laxative effect. Sourdough technology showed promising potential to overcome quality loss occurring in sugar reduced products. Thereby, the lactic acid bacteria strain needs to be carefully chosen, and the fermentation conditions to achieve high mannitol conversion yields needs to be optimised. *Leuconostoc citreum* TR116 as a high mannitol producing strain showed promising results.

For future research, a co-fermentation using either other LAB strains additionally, or even a suitable yeast strain, should be considered. Furthermore, the incorporation of stabilisers, such as hydrocolloids, in the baked product could further improve product quality.

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APPENDIX

Publications

Sahin, A.W., Axel, C. and Arendt, E.K., Understanding the function of sugar in burger buns– A fundamental study. *European Food Research and Technology* (2017) 243: 1905-1915. 2)

Sahin, A. W., Axel, C., Zannini, E. and Arendt, E. K., Xylitol, mannitol and maltitol as potential sucrose replacers in burger buns. *Food & Function* (2018)., 9(4), 2201-2212.

Sahin, A. W., Rice, T., Zannini, E., Axel, C., Coffey, A., Lynch, K. M. and Arendt, E. K. *Leuconostoc citreum TR116*: *In-situ* production of mannitol in sourdough and its application to reduce sugar in burger buns. *International Journal of Food Microbiology.* (2019), 302: 80-89.

Sahin, A. W., Rice, T., Zannini, E., Lynch, K. M., Coffey, A. and Arendt, E. K., Sourdough technology as a novel approach to overcome quality losses in sugar-reduced cakes. *Food & Function.* (2019), in press

Sahin, A. W., Rice, T., Zannini, E., Lynch, K. M., Coffey, A. and Arendt, E. K., The incorporation of sourdough in sugar-reduced biscuits: A promising strategy to improve techno-functional and sensory properties. *European Food Research and Technology*. (2019), in press

S**ahin, A. W., Zannini, E., Coffey, A. and Arendt, E. K.,** Sugar reduction in bakery products: Current strategies and sourdough technology as a potential novel approach. *Food Research International*. (2019), in press

Awards

Travel award for the abstract submission, Sahin, A.W.; Rice, T.; Zannini, E.; Axel, C.; Coffey A.; Lynch, K.M. and Arendt, E.K. (2018). Sourdough as a tool for sugar reduction in burger buns. *AACC International Annual Meeting Cereals & Grains 18,*

Best research paper of the Food Science Department (UCC) in 2018 (Internal paper competition), Sahin, A. W., Rice, T., Zannini, E., Axel, C., Coffey, A., Lynch, K. M. and Arendt, E. K. *Leuconostoc citreum TR116*: *In-situ* production of mannitol in sourdough and its application to reduce sugar in burger buns. *International Journal of Food Microbiology.* (2018)

Oral presentations

Sahin, A.W.; Arendt, E.K. (2016). Fundamental studies on the reduction of sugar in burger buns. *15th European Young Cereal Scientists and Technologists Workshop, Bergamo, Italy, April 2016*

Sahin, A.W.; Axel, C.; Zannini, E.; and Arendt, E.K. (2017). Xylitol, mannitol and maltitol as potential sucrose replacers in burger buns. *46th Annual Food Science and Technology Conference, Dublin, Ireland, December 2017*

Sahin, A.W.; Rice, T.; Zannini, E.; Axel, C.; Coffey A.; Lynch, K.M. and Arendt, E.K. (2018). *Leuconostoc citreum* TR116: *In-situ* production of mannitol in sourdough and its application to reduce sugar in burger buns. *7th International Symposium on Sourdough, Cork, Ireland, June 2018*

Sahin, A.W.; Rice, T.; Zannini, E.; Axel, C.; Coffey A.; Lynch, K.M. and Arendt, E.K. (2018). Sourdough as a tool for sugar reduction in burger buns. *AACC International Annual Meeting Cereals & Grains 18, London, England, October 2018*

Poster presentations

Sahin, A.W., Arendt, E.K. (2015). Fundamental studies on the reduction of sugar in burger buns. *44th Annual Food Research Conference, Fermoy, Ireland, December 2015*

Rice, T., Axel, C., Sahin, A.W., Lynch, K.M., Benz, C., Arendt E.K. and Coffey, A. (2016). Gluten free sourdough lactic acid bacteria- Isolation and Characterisation of polyol and EPS producing strains. *4th international symposium on gluten-free cereal products and beverages, Cork, Ireland, October 2016*

Rice, T., Axel, C., Sahin, A.W., Lynch, K.M., Heitmann, M., Arendt, E.K., Benz, C., and Coffey, A. (2017). Characterisation of mannitol producing lactic acid bacteria from sourdough fermentations and application of *Leuconostoc citreum* TR116 for sugar reduction of food and beverage products. *12th international symposium on lactic acid bacteria, Egmond aan Zee, the Netherlands, August 2017*

Rice, T., Axel, C., Sahin, A.W., Lynch, K.M., Arendt, E.K. and Coffey, A. (2017). Identification of carbohydrate transport and utilisation pathways in *Leuconostoc citreum* TR116 and gene expression analysis. *12th international symposium on lactic acid bacteria, Egmond aan Zee, the Netherlands, August 2017*

Rice, T., Axel, C., Sahin, A.W., Lynch, K.M., Heitmann, M., Arendt, E.K., Benz, C., and Coffey, A. (2017). Characterisation of mannitol producing lactic acid bacteria and application of *Leuconostoc citreum* TR116 for the reduction of sugar in foods and beverages. *46th Annual Food Science and Technology Conference, Dublin, Ireland, December 2017*

Sahin, A.W.; Axel, C.; Zannini, E.; and Arendt, E.K. (2018). Replacement of added sucrose in burger buns: Are commercially available polyols suitable? *AACC International Annual Meeting Cereals & Grains 18, London, England, October 2018*