# Possible sugar substitutes to reduce sugar in bread

- Without affecting taste and quality

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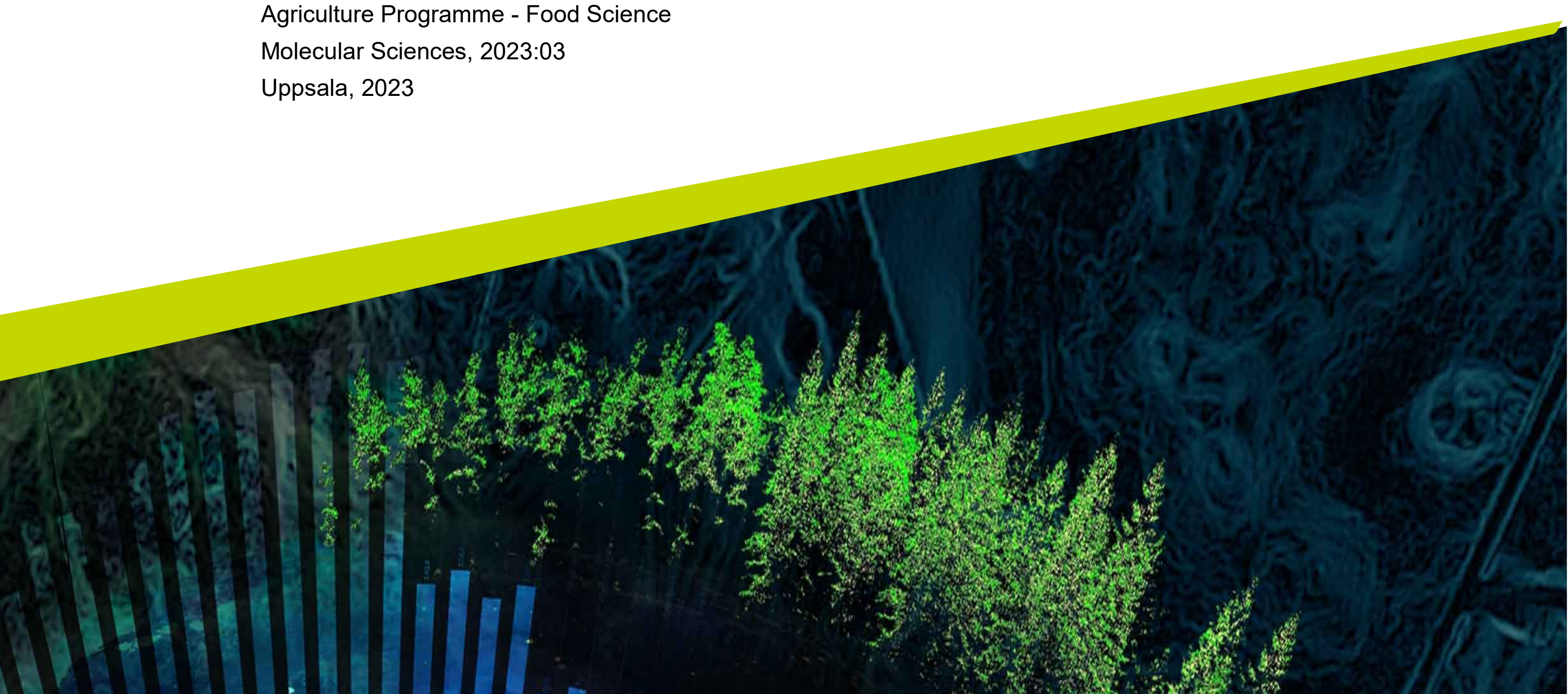

# Possible sugar substitutes to reduce sugar in bread - without affecting taste and quality

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# Abstract

The World Health Organization (WHO) is recommending a reduction of added sugars in food to decrease non-communicable diseases. This study was made in a collaboration with the bread company Pågen, who wants to reduce the added sugar, sucrose, to meet the recommendations from WHO and the consumer's wishes for less sugar in bread. The purpose of this thesis was to investigate inulin and glucoamylase, separately, as sugar substitutes in three different bread products from Pågen and reducing the sugar content by 20-30% without affecting the quality. This was done by using instrumental and sensory measurements to examine e.g., the texture, volume, crumb structure, pH and acid content, as well as the taste of the bread samples with different concentrations of inulin and glucoamylase. The results show that within the products there were significant differences in texture when replacing sugar with inulin or glucoamylase. There was also great variation in texture and taste between the products. In this study alterations in texture and taste were noticed when using inulin or glucoamylase as sugar substitutes while reducing the sugar content by 20-30%. Due to the complex matrix in bread and the significant role of sugars in bread it is difficult to find sugar substitutes that don't change the quality or the taste. Additional research may be needed to find sugar replacers that have the same characteristics and taste as sucrose has in bread.

*Keywords:* bread, sugar substitute, sugar reduction, inulin, oligofructose, amyloglucosidase, glucoamylase, dough, wheat dough, clean label

# Sammanfattning

Världshälsoorganisationen (WHO) rekommenderar en minskning av tillsatt socker i livsmedel för att kunna reducera icke-smittsamma sjukdomar. Denna studie gjordes i ett samarbete med brödföretaget Pågen som vill sänka mängden tillsatt socker i sina bröd för att kunna möta rekommendationen från WHO och konsumenters önskemål om bröd som innehåller mindre mängd socker. Syftet med denna masteruppsats var att undersöka inulin och glukoamylas separat som sockerersättare i tre olika brödprodukter från Pågen och att sänka mängden socker med 20-30% utan att påverka brödens kvalité. Instrumentella och sensoriska mätningar utfördes för att undersöka t ex. textur, volym, pH och syrahalt, samt smak i de olika proverna med olika koncentrationer av inulin eller glukoamylas. Resultaten visar att det var signifikanta skillnader i textur inom de olika produkterna. Det fanns också stor variation mellan produkterna gällande smak och textur. I denna studie påvisades förändringar i både textur och smak vid användning av antingen inulin eller glukoamylas som sockerersättare och minskad mängd socker. På grund av den komplexa matrisen och sockrets betydande roll i bröd är det svårt att hitta sockerersättare som varken förändrar kvalitén eller smak. Ytterligare forskning kan behövas för att kunna hitta de sockerersättningar som har samma egenskaper och smak som sackaros har i bröd.



*Nyckelord:* bröd, sockersubstitut, sockerreduktion, inulin, oligofruktos, amyloglukosidas, glukoamylas, deg, vetedeg, clean label

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# 1. Introduction

Today noncommunicable diseases (NCDs) are increasing globally and are the leading causes of death. A diet with high sugar intake has been shown to be associated with increasing NCDs and obesity. The consumption of sugars has increased for the last 20 years and is used in many food products (Sahin et al. 2019). World Health Organization (WHO) is recommending a reduction of sugar to less than 10% of total energy and further reducing it to 5% of total energy to enhance beneficial effects (World Health Organization 2015). Today bread is a staple food all over the world and a traditional craft that comes in many different shapes and varieties (Goesaert et al. 2005). The sugar content of bread varies widely and consumer awareness of eating a healthier diet has increased. To meet public health recommendations and consumer demand, the baking industry has the desire to reduce the sugar content in order to get a more sustainable and healthier bread (Müller et al. 2021). Sugar is involved in various chemical reactions in bread that provide important functions in the baking process such as browning, taste, and retention of moisture. Therefore, it is a challenging task for the baking industry to reduce the sugar content while maintaining the same quality of the same product (Trinh et al. 2015; Müller et al. 2021).

## 1.1 Aim

The aim of the thesis was to reduce added sugars in bread by 20-30% without changing the bread quality. Glucoamylase and inulin were applied in this trial to replace sugar in three different bread products from the bread company Pågen. The study aimed to investigate the future possibilities to find sugar replacers in bread without affecting the quality and maintaining a clean label ingredient list.

*Purpose and Objectives*

Inulin and glucoamylase were chosen to be investigated if they could replace the sugar in bread with 20% or 30% less sugar. The purpose of this thesis was to provide knowledge of the different substitutes and if any alterations in taste and texture occurred compared to bread with no sugar reduction. The objectives were the following:

- Study the effect of two different types of inulin; inulin A and inulin B, replacing the added sugar content by the ratio 1:1 in 20% and 30% sugar reduced bread.
- Study the effect of the addition of glucoamylase and aroma in different concentrations based on flour basis in 20% and 30% sugar reduced bread.
- Analyze the bread trials by instrumental measurements and sensory evaluation to investigate if any of the substitutes can be a potential sugar replacer not affecting the texture and taste compared to bread with no sugar reduction.

# 2. Background

## 2.1 Bread

The common ingredients in bread are wheat flour, water, yeast, sourdough, and sometimes additional components such as sugar, fat, and enzymes. Throughout the bread-making process, complex chemical, biochemical and physical transformations take place that are affected by the various components present in the bread (Goesaert et al. 2005). The interaction between the raw ingredients, their quality and quantities, and the bread-making process are important aspects that can affect the quality of the bread. This makes bread a complex matrix with several factors that must be considered when changing the ingredients without changing the properties of the final product (Cauvain 2020).

### 2.1.1 Flour

Wheat is one of the most important crops in the world and wheat flour is the most common type used in bread production (Goesaert et al. 2005). Wheat contains two important proteins, gliadin, and glutenin, which create gluten. An optimal gluten network development is an important parameter that determines the quality and the viscoelastic behavior of the dough. It results in an aerated foam structure giving structure and texture to the crumb (Nwanekezi 2013). The gluten development determines the bread´s ability to entrap the carbon dioxide, which increases the volume of the bread (Cauvain 2020). Wheat flour consists of 70-75% starch (Goesaert et al. 2005) where amylose and amylopectin are the two glucose polymers that are present in the starch. The structure of amylose is mainly linear with α-1,4 glycosidic bonds, whereas amylopectin has a branched structure build-up with short linear chains with α-1,4 glycosidic bonds and side chains attached with α-1,6 glycosidic bonds (van der Maarel et al. 2002).

### 2.1.2 Yeast and sourdough

Baker's yeast also known as *Saccharomyces cerevisiae,* is the most common yeast used in breadmaking for fermentation. The yeast cells provide the bread with different end products that contribute to taste, texture, and volume, which is a critical part of the final quality of the bread. The different ingredients in bread may affect the yeast and give rise to different chemical reactions. Due to that, several factors may affect the fermentation rate of the yeast cells, but the availability of sugar in dough has raised the most interest. Changing the content of sugars and their availability for yeast cells may impact the fermentation and need to be taken into consideration when replacing and reducing sugar in yeast leavened bread (Struyf et al. 2017b).

A study by Corsetti et al. (1998) showed that sourdough may delay staling and firmness in bread and was suggested to be used to increase the shelf-life. Also, they implied that bread with sourdough had more flavor compared to bread baked with baker´s yeast. Sourdough increases the free amino acids concentrations that will contribute to flavor and aroma. Bread baked with only baker's yeast will have a different rate of amino acids and therefore have different flavor compounds compared to bread baked with sourdough (Struyf et al. 2017b).

## 2.2 The role of sugar in bread

This section introduces the function of sugar in bread, its interaction with the ingredients, and the reactions that occurs when sugar is added to bread.

The main function of sugar in bread is to provide sweetness and color. Sucrose is the most common sugar used in breadmaking (Timmermans et al. 2022). It is harvested from either sugar cane or sugar beet and results in a white crystalline shape (Clemens et al. 2016). Sucrose is a disaccharide with one glucose and one fructose unit providing a sweet taste (Cauvain 2017:2). In bread, sucrose itself does not provide any sweetness since it is rapidly hydrolyzed by the yeast invertase into glucose and fructose units. The reducing sugars, glucose, and fructose, are involved in the Maillard reaction, contributing to color and aroma. Depending on the different sugars that are available, it will influence with different levels of crust color and sweetness (Timmermans et al. 2022). Sucrose binds water easily, which results in an increase in the gelatinization temperatures (Sahin et al. 2019) and increases the protein denaturation's temperature when thermally heated (Tsatsaragkou et al. 2021). When temperatures rise in the dough, the wheat proteins denature and release water. The starch granules begin to swell due to the absorption of the released water, which leads to leakage of amylose and amylopectin that increase the viscosity. Sucrose delays the gelatinization phase by its ability to bind water molecules, leading to less water that is available to the starch granules. Therefore, an increase in the gelatinization temperatures occurs when sucrose is present in the dough. A delay in gelatinization has shown to give enough time for the expansion of air bubbles in the dough providing volume to the bread (Struck et al. 2014).

The amount of added sugar in bread varies greatly, from zero up to 20% of the flour weight. Due to the relative high-water content and the long process when making bread the sugars are easily dissolved, which shows that different shape of sugar crystals can be used in bread without affecting the bread. Sugars are existing natural in flour, with approximately 2-3% (Sumnu & Sahin 2008; Cauvain 2017). When sugar is available to the yeast, end products such as carbon dioxide and organic acids, that provides the bread with volume and aroma are produced. Bread containing around 2% sugar does not give any notable sweet taste. However, bread with more than 10% sugar yields a sweet taste (Müller et al. 2021). Too much sugar can give negative results on the bread, it has been shown that with more than 10% sucrose of the flour weight the gluten network may be weakened and delay the formation of the gluten network. This is part of the competition of the accessible water between sugar and gluten (Trinh et al. 2015; Sahin et al. 2019). The activity of the yeast may be inhibited with too high sugar levels as well (Cauvain 2017). The fermentation stage on yeast leavened bread has shown to have a great impact on the sugar levels of the finished end-product (Timmermans et al. 2022).

### 2.2.1 Perception of sweetness

Consumers have been shown to detect sucrose within 1 second and the taste lasts for 30 seconds. Any alteration in the taste of sweetness and its duration may be detected when using alternative sweeteners to sucrose in food products (Setser & Brannan 2003). Sucrose is used as the standard reference with a value of 1 or 100, when comparing the relative sweetness levels between different sugars (Cauvain 2017). The perception of sweetness varies between different sugars depending on their concentration and conformation (Wang et al. 2012). This is due to the various stereochemical arrangements of their attached groups or their hydroxyl groups. For example, glucose is a monosaccharide with a relative sweetness score between 50-70 compared to sucrose with a value of 100. The alterations in sweetness levels depend on the anomeric shape of glucose, where α-D-glucose is perceived as much sweeter than the β-D-glucose. Furthermore, the monosaccharide fructose is sweeter than both glucose and sucrose, estimated to have 115 to 180 relative sweetness score. Comparison of sweeteners can only be done by sensory evaluations (Clemens et al. 2016).

### 2.2.2 Fermentable sugars

During the fermentation stage, the yeast cells use fermentable sugars (e.g., glucose, fructose, sucrose, maltose) in the bread to produce carbon dioxide, ethanol, organic acids, and aroma compounds, which results in dough leavening and flavor (Struyf et al. 2017b). The naturally occurring enzyme in flour, amylase, acts on starches, converting it into the fermentable sugar maltose that further gets hydrolyzed into two glucose units (Cauvain 2017). α-Amylase acts on the linkages of the starch polymer resulting in oligosaccharides and dextrins, whereas the β-amylase acts on the non-reducing ends and release maltose from the starch chain. To increase the content of maltose, α-amylase can be added to the flour by adding malt. The levels of maltose are determined by the α -amylase activity and the damaged starch content. With proper amount of α-amylase it has seen to increase the volume of the bread (Struyf et al. 2017b). Maltose starts to be consumed by the yeast cells when the fermentable sugars, glucose, fructose, sucrose, and fructan are depleted (Sahlström et al. 2004). Glucose and fructose are the preferred sugar sources by the yeast cells, consumed first, and affect the bread volume the most (Gabriela & Daniela 2010). Glucose has a higher fermentation rate by the yeast cells compared to fructose, which is due to fructose and glucose having the same carrier and glucose having a better affinity to the carrier. Sucrose and fructan are hydrolyzed by the enzyme invertase into glucose and fructose (Sahlström et al. 2004; Struyf et al. 2017a). Too high levels of fermentable sugars in bread may give a too dark crust and a sharp flavor by the Maillard reaction (Martínez-Anaya 1996).

### 2.2.3 Maillard reaction

The Maillard reaction is one of the substantial non-enzymatic reactions in bread contributing with color, aroma, taste, and nutrition when being thermal processed (Wang et al. 2012). It is an important chemical reaction that results in the brown pigment melanoidin which is the main responsible product for the brown color, which is an important quality aspect. The structure of melanoidins is still unknown due to being a complex heterogenous polymer (Murata 2021). The Maillard reaction involves reducing sugars and amino acids that are present in the food. The reducing sugar and amino acid convert to aldimine, which further rearranges spontaneously to a ketoamin which is a stable product known as the Amadori product. Depending on the pH, the amadori products are degraded in various pathways. In alkaline conditions, it converts to reductones whereas under acidic conditions they become furfural or hydroxymethylfurfural. The products undergo several chemical reactions producing compounds that contribute to aroma and color in heated food. The final stage is when melanoidins in the Maillard reaction are produced (Wang et al. 2012). The food industry relies on the browning effect by the Maillard reaction, which results in different flavor compounds giving a variation in food products. However, the Maillard reaction can also be undesirable and affect the quality negatively (Ogutu et al. 2017). This study used inulin and glucoamylase that could potentially affect taste and color in the bread by alteration in the amount of reducing sugars being involved in Maillard reaction.

## 2.3 Clean label

A clean label trend has increased in the food industry. Consumers find natural ingredients more appealing when choosing food due to the increased awareness of healthier diets. The term clean label is not scientifically defined, but it means that there should be no additives or preservatives that are artificial or chemical. The ingredients list should contain familiar ingredients that are easy to understand as a consumer and not too processed. A clean label is highly desirable in bread but comes with challenges for the bakery industry. When reducing sugar in bread the sensory properties should not be affected, and the ingredient list should be indicated as a clean label (Vargas & Simsek 2021).

### 2.3.1 Labelling sugar

There are some definitions to consider when labelling sugar on food products. With no additional words added to sugars in the list of contents, it is defined to be the monosaccharides glucose, fructose, and galactose or the disaccharides sucrose, lactose, and maltose. These are the most common sugars in food (Tiefenbacher 2017a). According to the Swedish Food Agency, the total amount of sugars is included with the total amount of carbohydrates in the declaration of nutrition. The mono- and disaccharides in the food are listed as “varav sockerarter”, and compile both the naturally occurring sugars and the added sugars. Added sugar in products is the sugars that are added as an ingredient, and not naturally present in the raw ingredients. The most common added sugars found in food declarations are fructose, honey, invert sugar, high fructose syrup, sucrose, glucose (dextrose), and lactose (Livsmedelsverket 2022).

### 2.3.2 Enzymes in food

According to the Regulation of the European Parliament (Regulation 1332/2008/EC, 2012) food enzymes are defined as products that originate from either plants, animals, or microorganisms. The enzymes should have the capability to catalyze biochemical reactions, and the addition of enzymes in food products should only be for technological purposes. For example, usage of preparation, processing, treatment, packaging, transport, or storage, of food.

Enzymes exist naturally in flour, yeast, and lactic acid bacteria and can be added intentionally in the bread. Enzymes are being more used in the baking industry as natural improvers, prolonging shelf life and give consistent quality to the product (Barrera et al. 2016a). The usage of enzymes has increased due to the fact that consumers are demanding more natural ingredients in their food, and enzymes have replaced ingredients that are perceived as less natural ingredients (Tebben et al. 2018). In bread, enzymes may give beneficial effects such as improving the volume of the loaf, crumb, and texture and having anti-staling properties, generating flavor, and can improve the nutritional qualities (van der Maarel et al. 2002). Amylase is one common enzyme added to the dough, which hydrolyzes the starch into reducing sugars. The role of amylases in bread is necessary for the sugar availability to yeast cells. Hence, it enhances the color and flavor and the consistency of the dough (Martínez-Anaya 1996).

## 2.4 Alternatives to sugar in bread

Replacing sugar is a challenging task and can affect the quality notably due to its great interactions with the ingredients in breadmaking (Clemens et al. 2016). When doing a sugar reduction, the common way is to combine bulking agents and sweeteners to replace the lost functions that sugar provides (Sahin et al. 2019). A full reduction of sugar is more difficult and therefore partial reduction is more achievable. With a partial reduction the qualities of sucrose remains, sweet taste and texture, but the sugar content is still lowered (Struck et al. 2014). In this section alternatives to sugar replacers are presented; glucoamylase, inulin and aroma.

### 2.4.1 Glucoamylase

Glucoamylase, also known as amyloglucosidase (AMG) is an exo-amylase. It hydrolyses the terminal glucose unit from the non-reducing ends on the starch polymer chain, hydrolyzing the α-1,4 and α-1,6 linkages, which results in single glucose units (Douglas Crabb & Mitchinson 1997). Glucoamylase cleaves the α-1,4 glycosidic bond 30 times more efficient compared to the α-1,6 glycosidic bond (Belitz 2004). The most optimal condition for starch hydrolysis by glucoamylase is a pH around 4.5-5 and a temperature at 50-60 °C (Struyf et al. 2017c; Diler et al. 2021). Glucoamylase is used in food industries such as in the production of glucose syrups, juice production and brewing, and in baking processes (EFSA Panel on Food Contact Materials et al. 2018).

*Glucoamylase in bread*

Glucoamylase can potentially increase the sweetness levels in bread and alter the fermentation stage when it converts starch into glucose units (Struyf et al. 2017c). The glucoamylase activity has shown to be linear with glucose levels (Diler et al. 2015). Higher glucose levels make it available to act as an energy source for the yeast cells. This results in higher carbon dioxide production and a faster initial fermentation rate, but also affect the fermentation productivity due to more glucose available that can be utilized by the yeast cells (Struyf et al. 2017c). In addition, glucoamylase can improve the crust color of the bread and contribute to flavor due to the increase of the reducing sugar, glucose, that are participating in the Maillard reaction (Altamirano-Fortoul et al. 2014). However, excessive glucose production by glucoamylase may lead to a too dark crust, a tart flavor, and a sticky crumb (Martínez-Anaya 1996). Further, glucoamylases hydrolyze damaged starch granules easily and can therefore be used to decrease the levels of damaged starch and improve the bread quality (Barrera et al. 2016b). Too high levels of damaged starch granules in the flour may give undesirable effects on the bread. It increases the absorption of water and promotes the swelling of the granules (Barrera et al. 2016b). This leads to different rheological behaviors on the bread and the starch systems, such as less elasticity and extensibility, which can give a lower volume of the bread. Also, too high levels of damaged starch can lead to a sticky dough (Ghodke et al. 2009). However, a study by Barrera et al. (2016a) presented that a combination of both glucoamylase and α-amylase gave a positive impact on flour with a high degree of damaged starch granules and might give anti-staling properties.

*Previous studies*

A study by Struyf et al. (2017c) showed that the addition of glucoamylase in bread dough increased the levels of the sugars maltose and glucose and indicated that glucoamylase can potentially increase the sweetness in bread. Struyf et al. (2017) also indicate that after a longer resting time of the dough and the addition of glucoamylase the glucose levels were increasing whereas maltose levels decreased. They implied that glucoamylase cleaved maltose into glucose units giving higher levels of glucose, which results in a browner crust color due to the Maillard reaction. By adding glucoamylase to the dough, the study indicated that the fermentation rate increased, caused by higher glucose levels. However, the different concentrations of glucoamylase did not affect the total amount of the sugar levels significantly only the glucose content. The study by Diler et al. (2021) suggests that the amount of glucoamylase is a factor for the levels of glucose in bread and implies that the bread containing the highest concentration of glucoamylase generates more glucose. They also emphasize that dough with high hydration, and low oil content and having a longer resting time before baked may promote glucoamylase activity. A possible factor for higher glucoamylase activity could be a lower pH in the dough by adding sourdough.

### 2.4.2 Inulin

The attention to fiber rich foods has grown due to people's awareness of a healthier diet. But fibers change the characteristics of the bread. To keep the same quality but still be able to increase the fiber content, inulin has been shown to be a potential ingredient. Inulin is a soluble dietary fiber that has been given attention in the bread industry due to its versatile functions. It is applied to increase the dietary fiber content, used as a fat and sugar replacer, and is recognized as a clean label ingredient (Flamm et al. 2001; Peressini & Sensidoni 2009). Also, inulin may be a functional ingredient improving digestive health and is classified as a prebiotic ingredient (Gibson et al. 2004). It contributes with low energy, has a neutral flavor, and gives minimal aftertaste. It can also be used as a bulking agent and works well with a combination of high intensity sweeteners giving a good mouth feel and masking the aftertaste that may develop from sweeteners (Shoaib et al. 2016). Inulin can act as a sugar replacer due to it gives a sweetness level from 30% to 50% compared to sucrose and the sweetness level depends on the length of the chain of the inulin (Niness 1999). Due to its diverse properties inulin might be a promising ingredient as it provides with nutritional value as well as technological benefits to food (Franck 2002; Tungland & Meyer 2002).

Inulin is present as a storage carbohydrate in plants and vegetables. It is a polymer of D-fructose units by β-1,2-linkage with usually a terminal α-glucose residue linked to the reducing end of the fructan chain (Peressini & Sensidoni 2009). It is a non-digestible carbohydrate, meaning it is resistant to hydrolysis in the small intestine by human enzymes. Instead, it is fermented by bacteria in the large intestine, acting as a prebiotic (Shoaib et al. 2016). Inulin is included in a class of carbohydrates that are called FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) which means it is fermented in the colon at a high rate and produces end products like gas and makes water enter the colon. Due to this, people with IBS (irritable bowel syndrome), can be sensitive towards inulin, and therefore inulin is recommended at levels not more than 5g per portion (Tiefenbacher 2017b).

Inulin can be extracted from chicory root or Jerusalem artichoke (Flamm et al. 2001). The process for extracting inulin is done by slicing the root and extracting it gently with hot water. Thereafter it is hydrolyzed at different degrees depending on the length of the chain that is wanted (Beneo n.d). Depending on the extraction process, it can be produced in various degrees of polymerization (DP), from 2 to 60 (O'Donnell et al. 2012).

*Properties of inulin*

The change in texture may be noticeable when reducing the high amount of sugar in bread. For that reason, inulin might be a possible replacer that acts as a bulk ingredient and has physical properties that can be similar to sucrose. Depending on the DP it can affect the physicochemical properties and have different levels of sweetness. Therefore, DP is important to take into consideration when using inulin in breadmaking as a sugar substitute (Luo et al. 2017).

Inulin is divided into three different groups, first the short-chain inulin, oligofructose, having a DP of 10 or less than 10. Secondly, the medium chain can have DP between 2 and 60 and last, the long chain inulin with DP of 23 or more. Inulin is considered a carbohydrate with a relatively low DP (Meyer et al. 2011). The various length of the chains will give different levels of sweetness, the shorter the chain length the more perceived sweet taste (Tiefenbacher 2017b). Oligofructose has around 30% to 50% sweetness compared to sucrose (Niness 1999). The short chain inulin gives a sweeter taste and a better mouthfeel that will resemble more like sugar compared to longer chains (Meyer et al. 2011). Low DP generates a higher water solubility due to the molecules being more tightly bounded to water and reducing the water availability for other ingredients, such as starch granules and proteins. Inulin with higher DP could give less solubility, resulting in higher viscosity (Meyer et al. 2011). Also, higher DP may increase the elasticity and strength of the dough which can give a reduction on the volume (Peressini & Sensidoni 2009), which could contribute to alterations in the texture of the dough (Nieto-Nieto et al. 2015). However, a stronger dough can allow longer proofing time without getting volume losses (Wang et al. 2002). Hence, it is important to choose the inulin specifically for the preferred feature due to various inulin types give different characteristics (Ozturkoglu-Budak et al. 2019).

*Previous studies*

The study by Peressini and Sensidoni (2009) implied that addition of low DP inulin leads to fewer changes in the rheological properties of the dough compared to high DP inulin. In the same way, the study from Tsatsaragkou et al. (2021) indicated that doughs that had shorter chain inulin added was closer to the control compared to doughs that had longer chain inulin added, especially when measuring the consistency. In contrast to Wang et al. (2002) the dough development time have shown to be reduced when adding inulin, meaning the dough needs less time to reach its maximum fermentation time. Also, the study implied that bread with inulin gave reduced moisture content and an increase in crumb firmness and chewiness. Inulin has also been shown to affect the bread characteristics negatively in studies by Bojnanska et al. (2015) and Peressini and Sensidoni (2009), which implied that high concentrations of inulin affected the quality of the bread negatively. The results also indicated that more than 5% fb (flour basis) inulin would give a too sweet taste and significant changes in the bread sensory quality.

Inulin might increase the Maillard reaction which results in a browner crust to the bread. This could be caused by the degraded fructans from inulin that results in higher levels of reducing sugars fructose and glucose (Poinot et al. 2010). However, Rubel et al. (2015) indicate that there are some inconclusive results regarding the rheological properties in breadmaking when adding inulin. Tsatsaragkou et al. (2021) highlight the great importance to have knowledge about the effects on DP of inulin and mention that the literature on inulin is scarce.

*Natural Sweet enhancer- Aroma*

It has been shown that sweet enhancers can work as a synergy increasing the sweet taste by acting on several binding sites on the taste receptors (Munger 2017). The perception of sweetness may get affected by the texture and the physical properties of the food product due to the textural hindrance of the taste buds compared to aqueous formulations. Therefore, solid products may taste less sweet compared to aqueous products (Setser & Brannan 2003). Natural sweet enhancer, aroma, has been used in this trial together with the addition of glucoamylase to further enhance the sweet taste.

## 2.5 Analysis of bread - sensory and instrumental measurements

The quality of bread is an important aspect and can be measured in different ways. The instrumental quality is performed objectively (Rosell 2011), whereas sensory quality involves the perception of appearance, odor, taste, flavor, and oral texture of the food product. The perception of sensory quality varies greatly between individuals, by being influenced in different aspects (Heenan et al. 2008). In this section, instrumental and sensory quality measurements are presented.

### 2.5.1 Digital image of crumb structure

The cellular structure of the bread is important to understand because it has great influences on the properties of the bread and its sensory attributes. Digital image analysis (DIA) analyzes the inside of the bread of a cut surface and gives a microstructural image of the crumb analyzing different parameters, see Appendix III for all measured parameters (Rathnayake et al. 2018). A high number of cells indicates a high crumb fineness or cell density, which is related to a fine crumb structure. Also, a finer grain structure gives a brighter crumb color. Cell wall thickness is influenced by the starch content, where a low amount of starch granules may lead to thinner cell walls (Scanlon & Zghal 2001; Gonzales-Barron & Butler 2008). The variation of porosity in crumb structure is caused by the ingredients, the process (kneading, proofing, and baking), the activity of the yeast, and its temperature when fermented. Sugar affects the porosity by increasing the gelatinization and protein denaturation temperatures, which may increase air bubbles (Rathnayake et al. 2018).

### 2.5.2 Texture analyzer

The texture analyzer measures the hardness of the bread by an attached probe that is pressed down toward the center of the bread slice. The force needed to press the bread is analyzed from the peak force in the first compression and provides information on the hardness. The measurements are done to mimic the consumer´s perception when biting and the way to squeeze the package in the supermarket, attempting to decide the freshness of the bread (Young 2012; Rathnayake et al. 2018). The hardness of the bread is correlated with its freshness. Over time, retrogradation will take place in the bread and increase the crumb firmness rate (Jekle et al. 2018). An increase in firmness is due to time, temperature, and formulation. The mechanism of staling is the recrystallization of the starches, amylopectin and amylose, and the migration of water, resulting in less crispiness of the crust and a stiff and dry crumb (Rayas-Duarte & Mulvaney 2012). Therefore, textural measurements over time are conducted to see if the firmness rate in storage time may be correlated with the different compositions in the dough.

### 2.5.3 pH and Acid content

Acid content measured the total content of propionic acid, acetic acid, and lactic acid in the samples. The pH of the dough should be around pH 5.2-6.0 and is influenced by the activity of the yeast and other microorganisms producing carbon dioxide that converts to carbonic acid in reaction to water (Miller et al. 1994).

### 2.5.4 Sensory evaluation

Sensory quality involves the perception of the eaten food, involving its appearance both visually and overall, odor, taste, flavor, and texture (mouthfeel). There are multiple sensory evaluation techniques and usually they are combined when conducting a sensory analysis, to get more information about the product and to get less subjective results (Heenan et al. 2008). When using analytical methods for sensory evaluation the subjects need to have the ability to discriminate the differences in the products. Subjects that have practiced on the products frequently are recommended. Analytical test presents the difference in the products whereas a descriptive test yields the differences that have been perceived (Stone 2018). A descriptive test is conducted in this report.

## 2.6 Summary of background

To meet the consumer´s demand and public recommendations, reducing sugar in bread is desirable. However, sugar has an important role in bread making, and it interacts with water, starch, proteins, and yeast in the bread. Reducing sugar is a challenging task when it affects many of the attributes in bread (Sahin et al. 2019). The aim of this thesis is to reduce the sugar content in three different bread products by 20% and 30% and add different levels of inulin, glucoamylase and aroma to analyze if any of the sugar replacers could be a possible sugar substitute without altering the quality in the bread.

# 3. Methods and Materials

## 3.1 Literature review

The literature review was performed by searching scientific journals in databases such as PubMed, Google Scholar, Scopus and scientific books available in the SLU Library to find relevant information about sugar substitutes in baking products and the properties of different ingredients in bread. The following key words were used: bread, sugar substitute, sugar reduction, inulin, oligofructose, amyloglucosidase, glucoamylase, dough, wheat dough, clean label.

## 3.2 Baking experiment

### 3.2.1 Baking pre-trials

Baking trials were conducted to find the correct amount of aroma and to examine the effect of inulin and glucoamylase in bread. The baking trials are provided in Appendix I.

### 3.2.2 Experimental design baking test

Three bread products, PE, BE and GA were prepared according to the recipe by the company Pågen. The shaping of bread was made manually. For the bread product PE all tests were leavened and baked in loaf pans. For the bread BE and GA they were weighed, shaped manually, and placed on a sheet pan to leaven and baked.

The test breads were baked with a 20% sugar reduction or 30% sugar reduction, and a reference with no sugar reduction. The sugar reduced samples had an addition of inulin or glucoamylase and aroma. All test breads were baked with duplicates, including the reference. For bread samples with inulin added, inulin A and inulin B were added separately in the breads. In PE inulin was added with 9.1% and 6.3 % (30% and 20% sugar reduction, respectively). In

BE 7% and 4.7% inulin was added (30% and 20% sugar reduction, respectively) and in GA 8.2% and 5.8% (30% and 20% sugar reduction, respectively). All the above percentages were measured in percentage of flour basis (% fb) for each product. Inulin A contained higher DP compared to inulin B.

Glucoamylase and aroma (AA) were added based on the total flour basis. Glucoamylase (AMG) was added in two different concentrations and aroma was added with the same concentration in all samples (0.25% fb). Bread samples with 30% sugar reduction had two different amounts of AMG added, as follows: PE had 0.20% and 0.15%, GA had 0.15% and 0.10% and BE had 20% and 0.15% (table 1). Bread samples with 20% sugar reduction had also two different AMG concentrations, as follows: PE had 0.15% and 0.10% AMG, GA had 0.10% and 0.05% and BE had 0.15% fb and 0.10% (table 1). Explanations of the different samples is in detail in Appendix II.

*Table 1. Concentrations of glucoamylase (AMG) in bread products PE, BE, GA (% flour basis)*

| **Bread type** | **30% less sugar (% fb of AMG)** | **20% less sugar (% fb of AMG)** |
|---|---|---|
| *PE* | *0.2* | *0.15* |
| | *0.15* | *0.1* |
| *GA* | *0.15* | *0.1* |
| | *0.1* | *0.05* |
| *BE* | *0.2* | *0.15* |
| | *0.15* | *0.1* |

## 3.3 Instrumental measurements

Measurements of texture, volume, weight, water activity, water content, crumb structure, pH and acid content were performed. Also, a digital image analysis on the crumb surface was done. All the test breads had been frozen first and were measured 24 hours after being taken out from the freezer. PE and GA were sliced mechanically when the measurements were performed. BE had a shape of a bun and was sliced manually, using a slicing tool, giving slices with 25 mm in height.

### 3.3.1 Volume

Volume measurements were conducted by using TexVol instruments BVM- L450. Weight and volume were measured by a laser that measures the whole bread during rotation. Whole bread loaves were used. The specific volume was calculated by dividing volume by weight (ml/g). PE and GA had two replicates of each sample (n=2) and BE had replicates of three (n=3).

### 3.3.2 Texture analysis

TexVol instruments TVT-300XP with cylinder probe 36mm diameter, 40% compression, 1.0 mm/s initial speed, 1.7mm/s test speed and 5.0 mm/s retract speed were used when conducting textural analysis. The sample heights were 25mm and the starting distance from the cylinder probe was 5.0mm. The cylinder probe moves downwards to the bread in a single cycle and the measurements start when the trigger force is initiated. Hardness was interpreted by the peak force.

PE and GA, had for each test two slices (sliced mechanically) placed on top of each other. BE was sliced at the top manually with one bread at each measurement. All tests were made in three replicates. Texture analyses were measured day 1, day 3, day 7 and day 10. Day 1 started after 24 hours from taking it out from the freezer.

### 3.3.3 Digital image analysis, moisture content and water activity

The crumb structure was analyzed by C-cell caliber (Modell CC.200.05) which gives a digital image of the sliced sample and a data analysis of the crumb structure with several attributes (Appendix III).

For the water content and water activity analysis, one slice of each sample (n=3) was mixed into crumbles at day 1 and placed in a plastic cup closed with a lid. Moisture content was determined by using a moisture analyzer HE73 (Mettler Toledo), 2 grams of crumbles from each sample and heated at 130 °C. Water activity ($a_w$) was determined by using Lab Touch – aw (Novasina), analyzing the crumbs at 25°C.

### 3.3.4 pH and acid content measurements

pH and acid content were measured by Titrator Excellence T50 (Mettler Toledo) using sodium hydroxide. 2 g of crumbles from each sample (n=3), was filled with 50ml distilled water and analyzed by acid-base titration with 0.1 mol/L NaOH.

## 3.4 Sensory evaluation

An internal taste evaluation of all samples was performed to choose two of the products with two samples each that would be most similar and have the highest probability to resemble the reference sample. PE and GA were the products chosen after the internal evaluation. For PE the sample with 20% less sugar and an addition of inulin B, and the sample with 30% less sugar and an addition of inulin B were chosen. For GA the sample with 20% less sugar and an addition of inulin B, and the sample with 20% less sugar and addition of inulin A were chosen (table 2). A sensory evaluation was conducted by a hedonic test and a ranking test with 12 participants for the chosen products. The attributes for the hedonic test were following; visual appearance, odor, taste, texture (mouthfeel) and overall impression. They were evaluated on a 7-point hedonic scale (1-dislike very much, 2-dislike, 3-dislike slightly, 4-neither like nor dislike, 5-like slightly, 6-like, 7-like very much). The ranking test was performed by first, second and third place, where first place was the most preferable sample and third place the least preferable sample.

*Table 2. Samples chosen from the internal sensory evaluation*

| **Type of bread** | **Sample 1** | **Sample 2** | **Sample 3** |
|---|---|---|---|
| PE | Reference | 20% Inulin B | 30% Inulin B |
| GA | Reference | 20% Inulin B | 20% Inulin A |

Hedonic tests were chosen due to being best suitable for comparing two different samples with a control sample. The sensory evaluation was conducted to see if there are similarities between the samples and the reference. Ranking tests were applied to find out which sample was most preferred in taste.

# 4. Results

The parameters which were analyzed were slice brightness, slice area, number of cells, number of holes, area of cells, area of holes, average height, wall thickness, volume, weight, moisture content, water activity, acid content, pH and texture (day 1, day 3, day 7, day 10).

The parameters slice brightness, slice area, number of cells, number of holes, area of cells, area of holes, average height and wall thickness were first analyzed by Principal Component Analysis (PCA) by SIMCA® 17. All parameters were further analyzed by Minitab © 2020 where an analysis of variance (ANOVA) by general linear model with Tukey pairwise comparison method was used at 95% significance level to determine any significant differences between the samples.

*Principal Component Analysis*

PCA is a multivariate technique which analyses several dependent variables from a data table. The goal of using PCA is to find the most important information about the variables from the data and express it into two dimensions called principal components PC 1 and PC 2, giving a two-dimensional score plot. PC 1 has the maximum variance and PC 2 has the second largest variance in direction of the data. By using principal components, it is possible to extract and identify the most important information from the data. In this way PCA makes it easier to analyze variables and their observations. A loading plot identifies how the variables are included in each component and gives an easier overview to find systematic variations in the pattern (Abdi & Williams 2010).

PCA loading and score plots were interpreted from the digital image analysis data (Appendix III). The chosen variables form PCA plot in this study were slice brightness, slice area, number of cells, number of holes, area of cells, area of holes, average height, and wall thickness. These were parameters found to be distinctive in the PCA (figure 1) and they were thereafter analyzed by ANOVA with Tukey comparison test to identify significant differences.

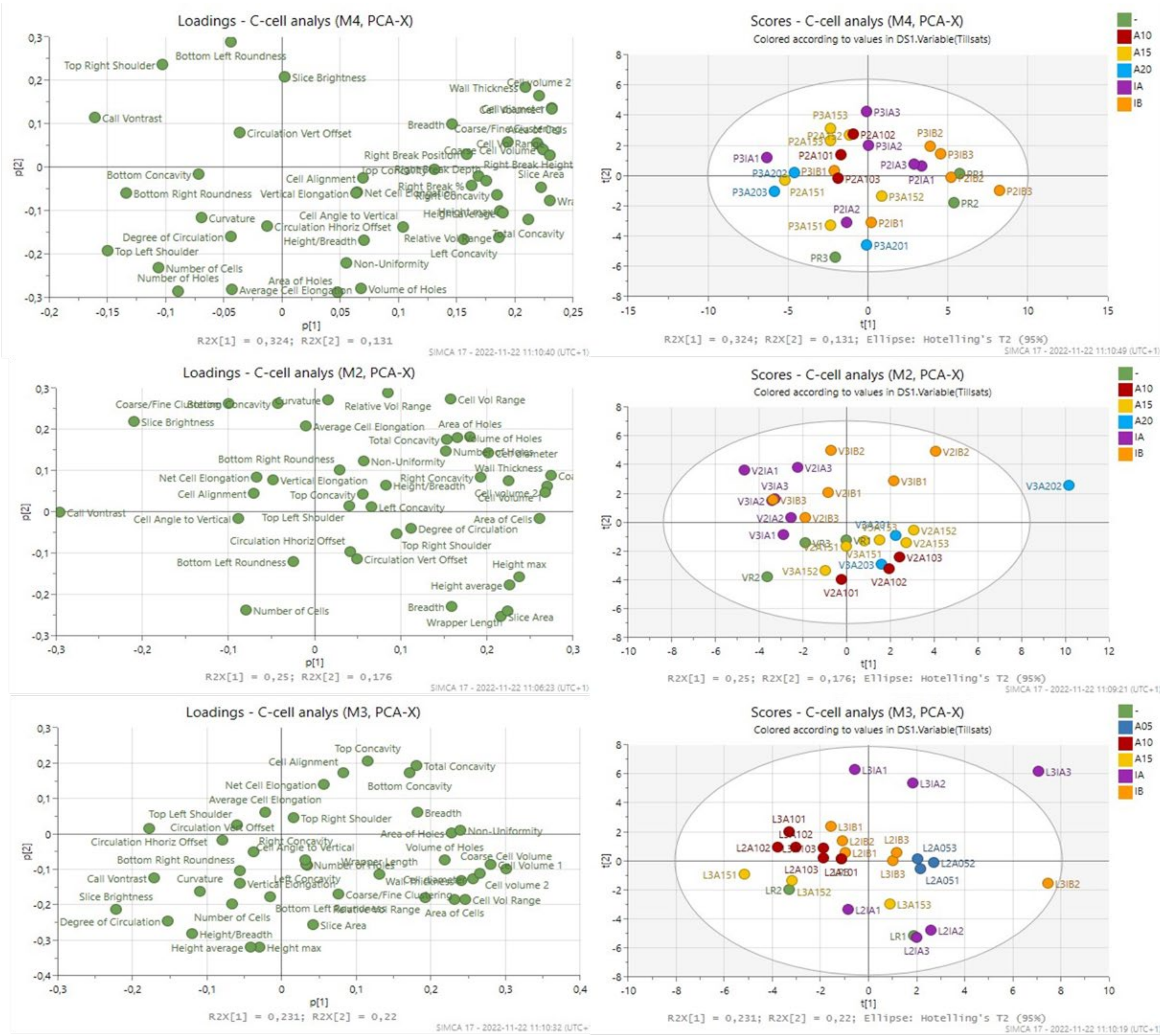


*Figure 1. PCA loading and score plot from bread products beginning from top; PE (a), BE (b) and GA(c) see appendix II for abbreviations.*

## 4.1 Bread PE

*Texture analyzer*

To see if the texture regarding firmness of the bread samples changed over time, texture analysis was conducted for day 1, day 3, day 7 and day 10 (figure 2).

Day 1 did not show any statistically significant difference between the samples. For day 3, the peak force for breads made with 30% inulin A and 30% AA 0.20 was significantly higher than for the reference bread. At day 7 and 10, the peak force for 30% inulin A was significantly higher than for the reference.

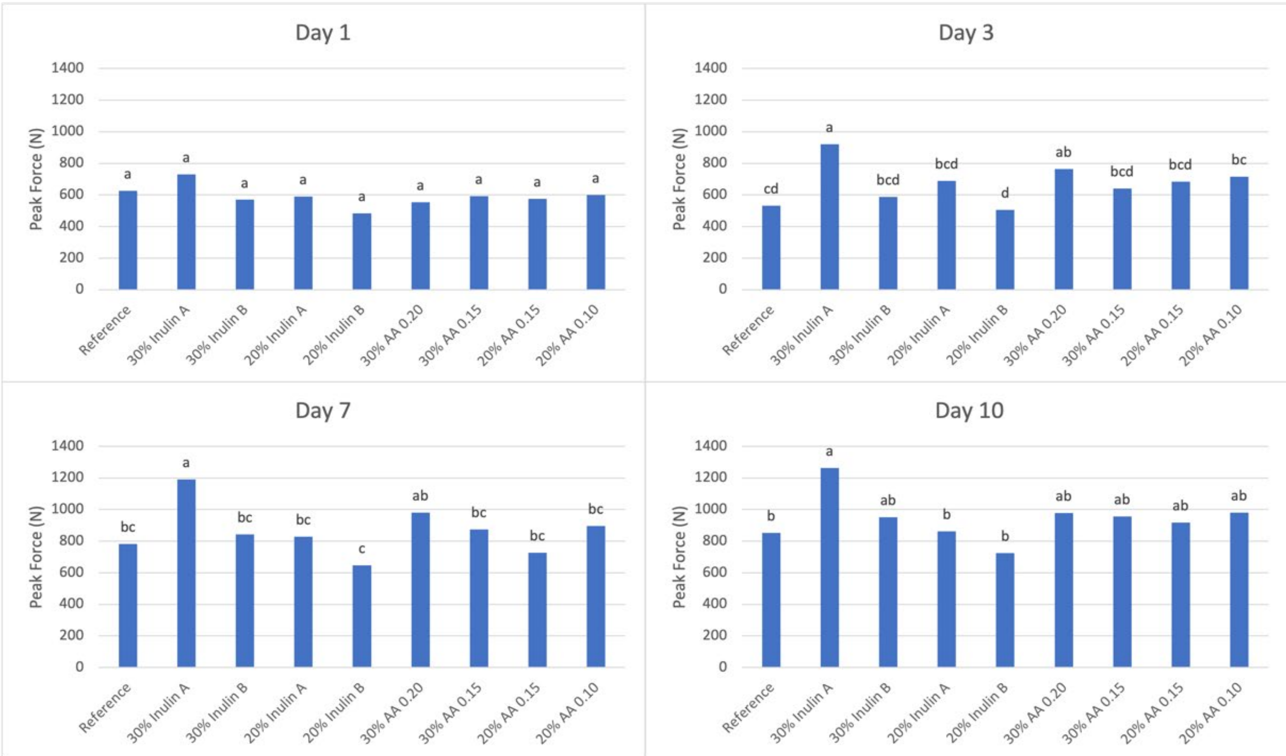


*Figure 2. Mean value for peak force (N) in reference bread and the bread product PE with 20 or 30% reduction of sugar, and added inulin A or inulin B, or added glucoamylase and aroma (AA) at different concentrations (0.20, 0.15 and 0.10 % fb of glucoamylase). Measuring firmness at day 1, day 3, day 7 and day 10, (n=3)[a] .*

a. Means with the same letter are not significantly different (P>0.05).

*Quality parameters*

Table 3 shows moisture content, slice area, slice brightness, average height, number of cells, area of holes, acid content and pH for all the samples in PE. Samples with 30% inulin A had the lowest moisture (27.2%), followed by samples with 30% inulin B (29.7%), although no significant differences were detected. For slice area, breads with 30% AA 0.20 (12304.3 $mm^2$) and 30% AA 0.15 (12711.3$mm^2$) had significantly lower slice area than reference

(13947.3mm$^2$). Regarding the slice brightness, breads with 30% AA 0.20 (97.2) had lower slice brightness compared to the reference bread (101.6), indicating having a darker crumb. For samples with 30% inulin B the slice brightness was highest (105.3), but no significant difference was revealed regarding slice brightness. For the measurements of average height, no significant difference was detected, although breads with 30% AA 0.20 had the lowest average height (109.6 mm).

The attributes Number of cells and Area of Holes showed no significant differences between any samples (table 3), although reference bread had the highest amount of number of cells (7129), followed by breads with 30% AA 0.20 (6966) and 20% inulin B (6828). This indicates a finer cell structure in the reference bread but lacked significant difference. Regarding the area of holes, breads with 20% AA 0.15 and 30% inulin A had lowest mean value, having less number of holes (1.2 and 1.6). This can be seen visually in Appendix VI for the digital images. However, no significant difference was revealed between the samples. Acid content was showing no significant difference between the samples and reference. The bread with 30% AA 0.20 had significantly higher acid content (6.02) compared to the bread with 30% inulin A (5.46).

*Table 3. Quality measurements for reference bread and bread PE with added inulin A or B, or glucoamylase and aroma (AA) (n=3) [a]*

| Sample | Moisture Content (%) | Slice Area (mm$^2$) | Slice Brightness | Average height (mm) | Number of Cells | Area of Holes | Acid Content | pH |
|---|---|---|---|---|---|---|---|---|
| Reference | 31.3$^{ab}$ | 13947.3$^{ab}$ | 101.6$^{ab}$ | 122.4$^{a}$ | 7129 $^{a}$ | 4.9 $^{a}$ | 5.81$^{ab}$ | 4.68 $^{a}$ |
| 30% Inulin A | 27.2$^{b}$ | 12790.3$^{bc}$ | 103.3$^{ab}$ | 113.8$^{ab}$ | 6607.7 $^{a}$ | 1.6 $^{a}$ | 5.46$^{b}$ | 4.78 $^{a}$ |
| 30% Inulin B | 29.7$^{ab}$ | 13375.0$^{abc}$ | 105.3$^{a}$ | 117.3$^{ab}$ | 6469.7 $^{a}$ | 3.0 $^{a}$ | 5.91$^{ab}$ | 4.70 $^{a}$ |
| 20% Inulin A | 31.1$^{ab}$ | 13409.3$^{abc}$ | 102.8$^{ab}$ | 117.9$^{ab}$ | 6676.3 $^{a}$ | 2.8 $^{a}$ | 5.85$^{ab}$ | 4.68 $^{a}$ |
| 20% Inulin B | 31.1$^{ab}$ | 13989.7$^{a}$ | 102.9$^{ab}$ | 120.3$^{a}$ | 6828 $^{a}$ | 3.7 $^{a}$ | 5.72$^{ab}$ | 4.69 $^{a}$ |
| 30% AA 0.20 | 32.8$^{a}$ | 12304.3$^{c}$ | 97.2$^{b}$ | 109.6$^{b}$ | 6966 $^{a}$ | 5.7 $^{a}$ | 6.02$^{a}$ | 4.61 $^{a}$ |
| 30% AA 0.15 | 33.1$^{a}$ | 12711.3$^{c}$ | 100.4$^{ab}$ | 114.0$^{ab}$ | 6202 $^{a}$ | 4.4 $^{a}$ | 5.87$^{ab}$ | 4.62 $^{a}$ |
| 20% AA 0.15 | 32.0$^{ab}$ | 12756.3$^{bc}$ | 105.0$^{a}$ | 114.8$^{ab}$ | 6573 $^{a}$ | 1.2 $^{a}$ | 5.76$^{ab}$ | 4.67 $^{a}$ |
| 20% AA 0.10 | 32.7$^{ab}$ | 13154.7$^{abc}$ | 101.1$^{ab}$ | 115.9$^{ab}$ | 6646.3 $^{a}$ | 3.4 $^{a}$ | 5.73$^{ab}$ | 4.70 $^{a}$ |

a. Means with the same letter in the same column are not significantly different (P>0.05)

## 4.2 Bread BE

*Texture analyzer*

Figure 3 shows the measurements for BE regarding hardness over time. Day 1, bread with 20% inulin B was significantly different from reference sample with a lower peak force, indicating that the bread had a softer texture compared to reference at day 1. At day 3 all the samples with inulin had a lower peak force compared to reference but was not significantly different. Day 7, breads with 30% inulin B, 20% inulin A and 20% inulin B were significantly different towards the reference with lower peak force. Day 10, the samples with inulin B (30% and 20%) showed a significant lower value in peak force compared to reference. The samples with addition of inulin resulted in a lower peak force for all measurements (except day 1 for 30% inulin A), but there was lack of significance regarding all the inulin samples, and therefore the results cannot be confirmed.

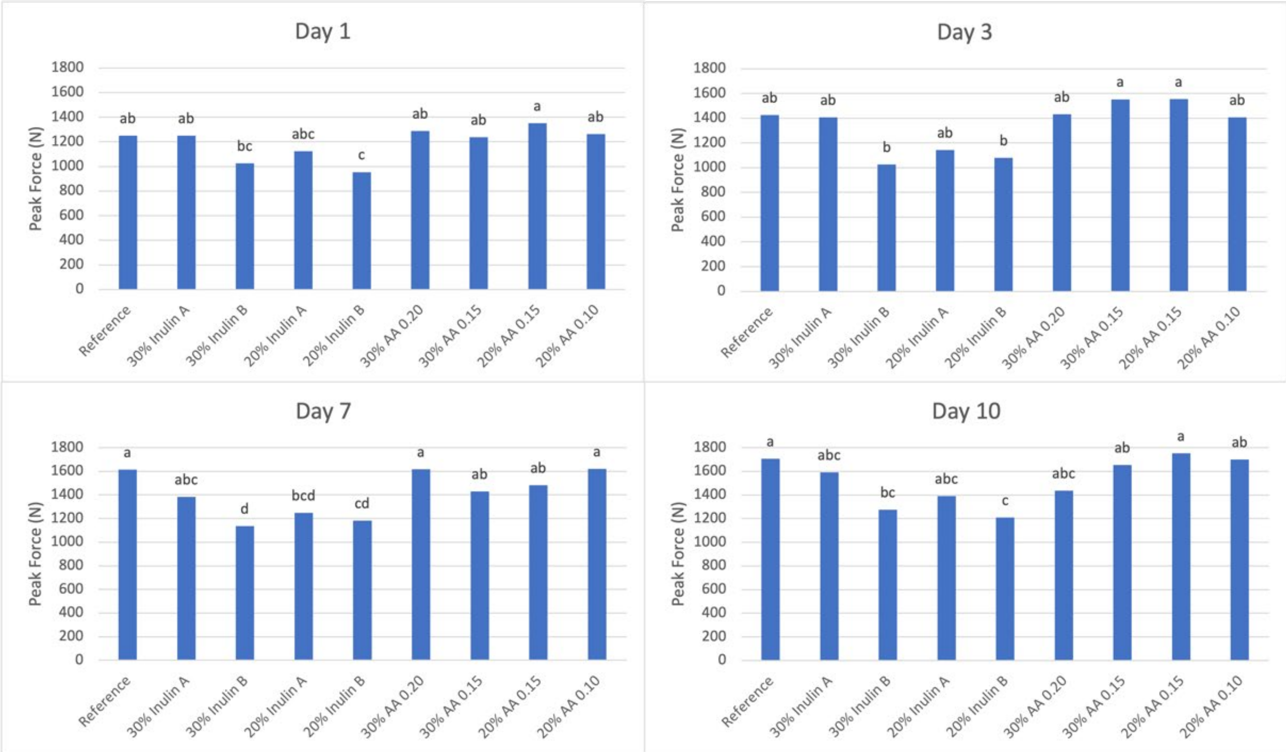


*Figure 3. Mean value for peak force (N) in reference bread and the bread product BE with 20 or 30% reduction of sugar, and added inulin A or inulin B, or added glucoamylase and aroma (AA) at different concentrations (0.20, 0.15 and 0.10 % fb of glucoamylase). Measuring firmness at day 1, day 3, day 7 and day 10, (n=3)* [a] *.*

a. Means with the same letter are not significantly different (P>0.05).

### *Quality parameters*

Values for moisture content, water activity, acid content and pH on BE are shown in table 4. 20% AA 0.15 had the highest moisture content (26.4%) and 30% inulin A hade the lowest moisture content (25.2%), although there was no significant difference. In water activity, all samples, except breads with inulin A, were significantly different towards the reference. Reference had the lowest water activity (0.876) followed by bread with 20% inulin A (0.879) and 30% inulin A (0.879). For acid content, reference had the lowest acid content (3.87) of all samples, whilst breads with 30% AA 0.20 and 30% AA 0.15 had significantly higher acid content. The samples containing inulin A and 20% inulin B were not significantly different from the reference. pH was significantly lower in all samples with AA compared to the reference bread.

*Table 4. Mean values for quality measurements on reference bread and BE breads with added inulin A or B or glucoamylase and aroma (AA) (n=3) [a]*

| Sample | Moisture Content (%) | Water activity (aw) | Acid content | pH |
|---|---|---|---|---|
| Reference | 25.9$^{ab}$ | 0.876$^{e}$ | 3.87$^{e}$ | 5.79$^{a}$ |
| 30% Inulin A | 25.2$^{b}$ | 0.879$^{de}$ | 3.99$^{cde}$ | 5.8$^{a}$ |
| 30% Inulin B | 25.9$^{ab}$ | 0.882$^{cd}$ | 4.05$^{bcd}$ | 5.71$^{a}$ |
| 20% Inulin A | 25.7$^{ab}$ | 0.879$^{de}$ | 3.94$^{de}$ | 5.76$^{ab}$ |
| 20% Inulin B | 26.1$^{a}$ | 0.881$^{cd}$ | 3.93$^{de}$ | 5.73$^{abc}$ |
| 30% AA 0.20 | 26.0$^{a}$ | 0.887$^{ab}$ | 4.32$^{a}$ | 5.58$^{d}$ |
| 30% AA 0.15 | 26.2$^{a}$ | 0.89$^{a}$ | 4,3$^{a}$ | 5.57$^{d}$ |
| 20% AA 0.15 | 26.4$^{a}$ | 0.883$^{bcd}$ | 4.1$^{bc}$ | 5.63$^{cd}$ |
| 20% AA 0.10 | 26.1$^{a}$ | 0.885$^{bc}$ | 4.16$^{b}$ | 5.66$^{bcd}$ |

a. Means with the same letter are not significantly different (P>0.05)

In table 5, volume, slice area, slice brightness, cell wall thickness, number of cells and area of cells are shown. The following samples gave a significant effect when measuring volume; breads with 30% AA 0.20 (131ml), 20% AA 0.10 (128ml) and 20% inulin B (126ml). The samples had higher volume compared to reference (119ml). For the slice area, the samples containing inulin A and 30% inulin B had significant lower slice area than the reference. The mean values for slice brightness showed that 20% inulin A had a significantly brighter crumb color (150.2) compared to reference (142.7). The samples containing AA had lower slice brightness but showed no significant difference. Results from cell wall thickness showed that the sample with 30% AA 0.20 had significantly higher mean value (0.450) than the reference (0.426). Higher value indicates thicker cell walls. Reference had highest number of cells (3032.7) and bread 30% inulin B had significantly lower amount (2547). All the samples, except breads with 20% AA 0.10 and 30% AA 0.15, were significantly different from reference. No significant difference was shown regarding the area of cells in the different BE samples.

*Table 5. Mean values from measuring the parameters, volume, slice area, slice brightness, cell wall thickness, number of cells and area of cells in reference bread and bread BE with added inulin A or B, or glucoamylase and aroma (AA) [a]*

| Sample | Volume (ml) | Slice Area ($mm^2$) | Slice Brightness | Cell-wall thickness (mm) | Number of Cells | Area of Cells |
|---|---|---|---|---|---|---|
| Reference | 119$^{de}$ | 3710$^{abc}$ | 142.7$^{bcde}$ | 0.426$^{e}$ | 3032.7$^{a}$ | 48,2$^{ab}$ |
| 30% Inulin A | 114.7$^{e}$ | 3405$^{d}$ | 144.9$^{abcd}$ | 0.429$^{de}$ | 2770.7$^{bc}$ | 48,17$^{ab}$ |
| 30% Inulin B | 124.7$^{bcd}$ | 3445$^{d}$ | 148.1$^{ab}$ | 0.440$^{abcd}$ | 2547.0$^{c}$ | 48,87$^{ab}$ |
| 20% Inulin A | 119.3$^{cde}$ | 3359$^{d}$ | 150.2$^{a}$ | 0.436$^{bcde}$ | 2643.7$^{bc}$ | 48,13$^{b}$ |
| 20% Inulin B | 126$^{abc}$ | 3525$^{cd}$ | 145.8$^{abc}$ | 0.443$^{abc}$ | 2635.7$^{bc}$ | 49,17$^{ab}$ |
| 30% AA 0.20 | 131$^{a}$ | 3853$^{ab}$ | 137$^{e}$ | 0.450$^{a}$ | 2603.3$^{bc}$ | 49,77$^{a}$ |
| 30% AA 0.15 | 123.5$^{abcd}$ | 3669$^{bc}$ | 139.1$^{de}$ | 0.435$^{cde}$ | 2802.3$^{ab}$ | 48,87$^{ab}$ |
| 20% AA 0.15 | 125.3$^{abcd}$ | 3762$^{ab}$ | 142.4$^{bcde}$ | 0.447$^{ab}$ | 2700.3$^{bc}$ | 49,33$^{ab}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 20% AA 0.10 | $128^{ab}$ | $3861^{a}$ | $140.4^{cde}$ | $0.442^{abc}$ | $2834.0^{ab}$ | $49,17^{ab}$ |

a. Means with the same letter are not significantly different (P>0.05)

## 4.3 Bread GA

*Texture analyzer*

Figure 4 shows the measurements for the texture analysis during day 1, day 3, day 7 and day 10. Regarding all days, breads with 30% inulin A was significantly different from all other samples, showing a larger peak force, indicating a harder texture. For day 7, breads with 30% inulin A had a lower peak force (3990 N) compared to day 3 (4809 N).

Results from day 1 show that all samples were significantly different from reference except the sample with 30% AA 0.10. Day 3, breads with 30% inulin A was the only sample that was significantly different, with higher degree of firmness than any other sample. Day 7, breads with 30% inulin A and both of the inulin B samples were significantly higher in peak force than the other samples. For day 10, breads with 30% inulin A showed a significantly higher peak force, whereas breads with 30% inulin B, 20% inulin A, 30% AA 0.15 and 20% AA 0.10 were significantly lower compared to reference (figure 4).

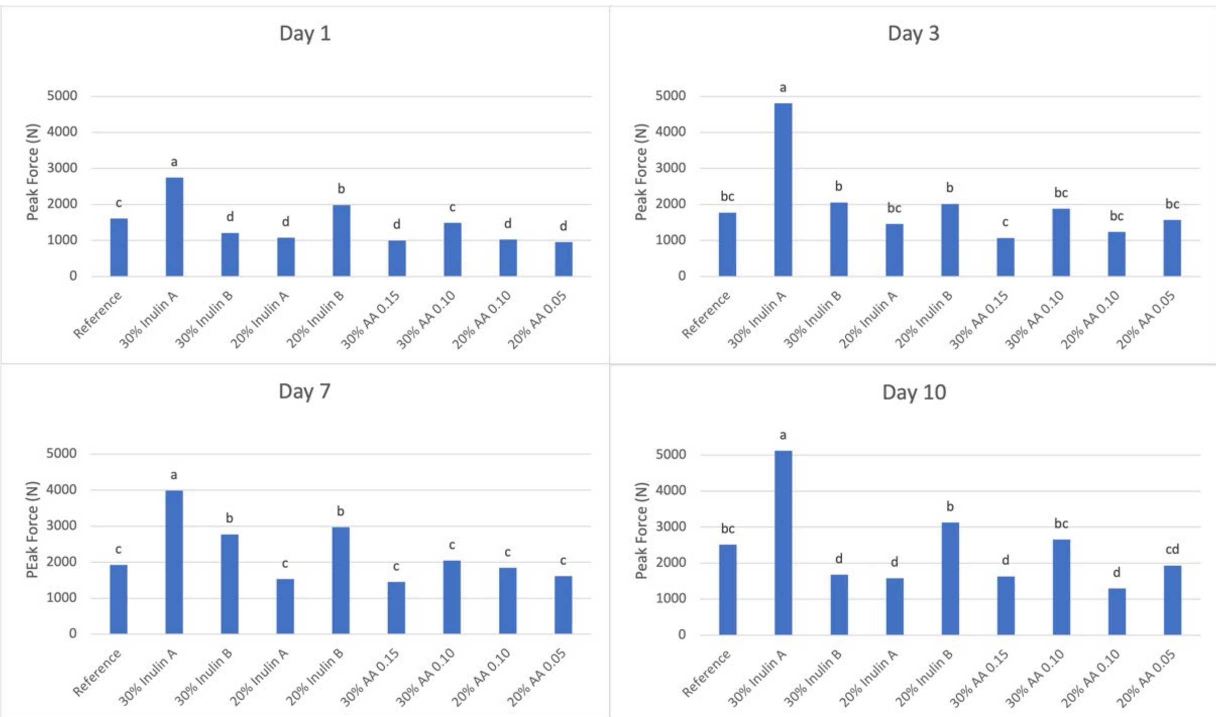


*Figure 4. Mean value for peak force (N) in reference bread and the bread product GA with 20 or 30% reduction of sugar, and added inulin A or inulin B, or added glucoamylase and aroma (AA) at different concentrations (0.20, 0.15 and 0.10 % fb of glucoamylase). Measuring firmness at day 1, day 3, day 7 and day 10, (n=3)* [a] .

a. Means followed by the same letter are not significantly different (P>0.05).

*Quality parameters*

In table 6, results from the measurements of volume, slice area, slice brightness, average height and cell wall thickness are shown. Volume did not show any significant difference between the samples and the reference. However, breads with 20% inulin A had significantly higher volume (1023.5ml) towards breads with 30% inulin A (733ml), which can be seen visually in Appendix VI. For average height, breads with 30% inulin A and 20% inulin B were significantly lower (61.7mm and 73.4mm) than the reference. For slice area, breads with 30% inulin A had significant lower slice area (7799.3mm$^2$), whereas 20% inulin A and 20% AA 0.05 had significant higher slice area (11710.3mm$^2$ and 11412.7mm$^2$) than the reference. Regarding the slice brightness, breads 30% inulin A and 30% inulin B were shown to have significant lowest slice brightness (54.2 and 57.1), which indicates that they had the darkest crumb color. Results from cell wall thickness showed no significant difference for any of the samples (table 6).

*Table 6. Measurements of the quality parameters volume, slice area, slice brightness, average height and cell wall thickness in reference bread and GA breads with added inulin A or B, or glucoamylase and aroma (AA) (n=3) [a]*

| Sample | Volume (ml) | Slice Area (mm$^2$) | Slice Brightness | Average height (mm) | Cell wall thick-ness (mm) |
|---|---|---|---|---|---|
| Reference | 879$^{ab}$ | 10046.3$^{bc}$ | 61.7$^{ab}$ | 84.2$^{ab}$ | 0.438$^{ab}$ |
| 30% Inulin A | 733$^{b}$ | 7799.3$^{d}$ | 54.2$^{d}$ | 61.7$^{d}$ | 0.439$^{ab}$ |
| 30% Inulin B | 879$^{ab}$ | 9983.7$^{c}$ | 57.1$^{cd}$ | 76.9$^{bc}$ | 0.434$^{ab}$ |
| 20% Inulin A | 1023,5$^{a}$ | 11710.3$^{a}$ | 60.2$^{abc}$ | 89.8$^{a}$ | 0.450$^{a}$ |
| 20% Inulin B | 800$^{ab}$ | 8887.7$^{cd}$ | 59.1$^{bc}$ | 73.4$^{c}$ | 0.438$^{ab}$ |
| 30% AA 0.15 | 917,5$^{ab}$ | 9647.3$^{c}$ | 63.5$^{a}$ | 82.9$^{abc}$ | 0.428$^{ab}$ |
| 30% AA 0.10 | 803,5$^{ab}$ | 9386.3$^{c}$ | 60.1$^{abc}$ | 75.1$^{bc}$ | 0.423$^{b}$ |
| 20% AA 0.10 | 878,5$^{ab}$ | 10280.3$^{bc}$ | 61.4$^{ab}$ | 79.4$^{bc}$ | 0.428$^{ab}$ |
| 20% AA 0.05 | 993$^{ab}$ | 11412.7$^{ab}$ | 58.7$^{bc}$ | 79.7$^{bc}$ | 0.438$^{ab}$ |

a. Means with the same letter are not significantly different (P>0.05)

Acid content and pH is shown in table 7. Results from acid content showed that all the samples containing AMG had significant higher acid content than all the other samples. Samples with 30% inulin A and 30% inulin B had significantly lower acid content than the other samples. pH was significantly lower for the samples with 20% AA 0.10 and 20% AA 0.05 (table 7).

*Table 7. Measurements of the quality parameters acid content and pH in reference bread and GA breads with added inulin A or B or glucoamylase and aroma (AA) (n=3) [a]*

| Sample | Acid content | pH |
|---|---|---|
| Reference | 5.98$^{b}$ | 5.05$^{ab}$ |
| 30% Inulin A | 5.70$^{c}$ | 5.11$^{a}$ |
| 30% Inulin B | 5.78$^{c}$ | 5.05$^{ab}$ |
| 20% Inulin A | 5.82$^{bc}$ | 4.99$^{bc}$ |
| 20% Inulin B | 5.82$^{bc}$ | 4.99$^{bc}$ |
| 30% AA 0.15 | 6.25$^{a}$ | 5.014$^{ab}$ |
| 30% AA 0.10 | 6.21$^{a}$ | 4.96$^{bc}$ |

| | | |
|---|---|---|
| 20% AA 0.10 | 6.27[a] | 4.95[c] |
| 20% AA 0.05 | 6.38[a] | 4.942[c] |

a. Means with the same letter are not significantly different (P>0.05)

## 4.4 Sensory analysis with PE and GA

A two-way ANOVA with Tukey HSD was analyzing the sensory attributes of the two chosen bread, PE and GA. The panelists consisted of 12 participants that did a hedonic test and a ranking test. The participants did not have any information about the bread samples. Test for PE included the reference bread, the bread with 30% inulin B and the bread with 20% inulin B. Test for GA included the reference bread, the bread with 20% inulin A and the bread with 20% inulin B. The samples had been earlier chosen by an internal evaluation.

*Hedonic test - PE*

Figure 5 shows the results from the sensory evaluation. From the hedonic test for PE, the bread with 30% inulin B were chosen to have the best appearance whereas the reference sample were chosen to have the best smell, taste, texture (mouthfeel) and overall impression, lack of significant differences was however presented (figure 5). In the ranking test the reference sample was chosen as the most preferred sample. Bread with 20% inulin B were chosen as the second most preferable and bread with 30% inulin B were chosen as the least preferable sample, showing no significant difference (table 8). The motivations for choosing in the ranking test were that reference sample had the best taste and texture whereas the bread with 30% inulin B and 20% inulin B were perceived as too sweet, dry, and less elastic. Although, no significant differences were detected (Appendix V).

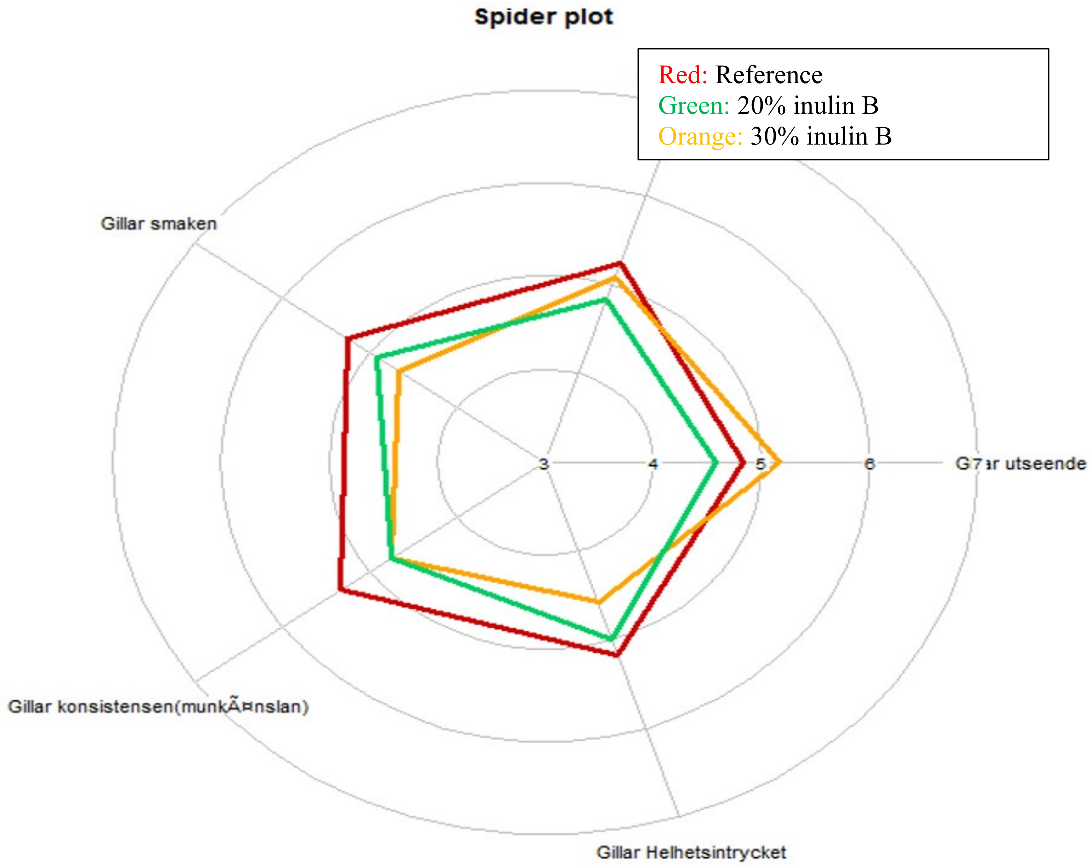


*Figure 5. Spider plot from the hedonic test of PE. Evaluation of (starting from the top) smell, appearance, overall impression, consistency (mouthfeel) and taste. Red as reference bread. Green as bread with 20% inulin B and orange as bread with 30% inulin B.*

Comments from the panelists experienced reference sample having an undesired appearance caused by too many holes in the bread. Both samples made with inulin B were observed having a dry crumb and a sweetness that was experienced as different and giving a delayed sweetness. Also, they were mentioned as being sweeter than the reference, giving a sugary taste, which was undesirable (Appendix V).

*Table 8. Results from the ranking test of reference and PE breads with 20% or 30% inulin. Lowest total sum means the most preferable sample*

| Ranking test of taste | Reference | 20% inulin A | 20% inulin B |
|---|---|---|---|
| Total sum | 20 ± 0.78 | 29 ± 0.67 | 23 ± 0.9 |

± indicate standard deviation

*Hedonic test – GA*

Figure 6 shows the Hedonic test for GA, that revealed statistically significantly best appearance for bread with 20% inulin A, while reference was found to have significantly best smell. With no significant difference, bread with 20% inulin A was chosen for best taste and best overall impression and reference sample were chosen to have the best texture (mouthfeel).

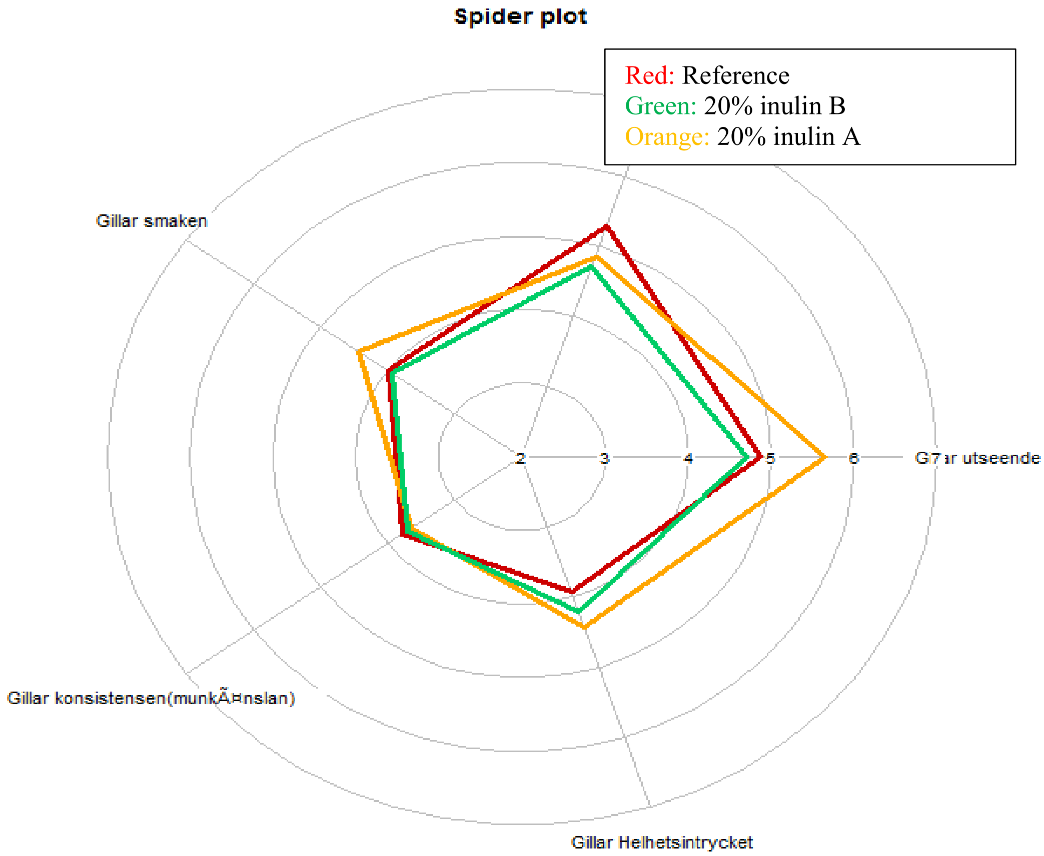


*Figure 6. Spider plot from the hedonic test of GA. Evaluation of (starting from the top) smell, appearance, overall impression, consistency (mouthfeel) and taste. Red as reference bread. Green as bread with 20% inulin B and orange as bread with 20% inulin A. Significant difference was shown with best smell and best appearance.*

In the ranking test analysis, breads with 20% inulin A and 20% inulin B were ranked as the most preferred samples, ended up with same score, and reference being the second most preferred sample. No significant difference was detected (table 9). By the comments some thought that breads with 20% inulin A had the best taste and others thought reference tasted better (Appendix V). However, in the comments all the samples were experienced being dry and were not likeable. This could have an impact on the results when panelists experiencing all the bread samples negative, including reference, and therefore might affect the sensory evaluation test.

*Table 9. Results from the ranking test of reference and GA breads with 20% or 30% inulin. Lowest total sum shows the most preferable sample.*

| Ranking test of taste | Reference | 20% inulin A | 20% inulin B |
|---|---|---|---|
| Total sum | 24 ± 0.75 | 21 ± 0.83 | 21 ± 0.94 |

± indicate standard deviation

# 5. Discussion

The aim of the study was to find sugar replacers that did not affect the quality on the bread products. Therefore, samples that did not show any significant difference towards the reference might be potential replacers for sugar. The addition of inulin or glucoamylase with aroma was shown to affect the bread quality differently depending on the type of bread.

*Texture analyzer- Firmness*

Regarding the textural measurements in firmness for PE and GA, samples with 30% inulin A were showing an increase, which signifies a higher firmness rate. This may be in accordance with earlier studies were they mention that inulin may increase the crumb firmness and chewiness (Wang et al. 2002). However, results from BE for day 1 and day 7 showed a decrease of firmness for the samples with inulin A and B. Inulin A contained higher DP and inulin B had lower DP. The study by Peressini & Sensidoni (2009) mentions that lower DP can give less changes on the consistency of the bread and be more similar to the control, compared to inulin with higher DP. However, in this study inconclusive results were given for the two different inulin types. The samples with AMG showed to affect the texture more in GA than in PE and BE. AMG addition was mentioned having anti-staling properties together with α-amylase (Barrera et al. 2016b) which can be seen in some degree in this study where some of the samples with AMG addition gave a softer texture, but not all samples containing AMG were significantly different from the reference bread.

*Quality measurements*

Regarding the acid content and pH, AMG gave the most effect in BE ang GA, whereas in PE there were no difference between samples. This could imply that AMG activity was higher in the products GA and BE. According to Diler et al. (2021), AMG activity can be increased by a lower pH and has an optimal pH range between 4.5-5. This is not in agreement with this study where product PE had the lowest pH with 4.68, whereas GA had pH 5.05 and BE had pH of 5.79. However, doughs with high hydration, low oil content and a long resting time might promote glucoamylase activity (Diler et al. 2021). All the products were dissimilar in ingredients and process, which could influence the glucoamylase activity differently.

Altamirano-Fortoul et al. (2014) indicate that addition of AMG may improve the crust color. This suggestion might be applied for the products PE and BE where addition of AMG resulted in a darker crust color, but the results were not significant. However, for GA this pattern was not followed, instead it was the samples containing 30% inulin A and B that gave darker crust color. The crust color is developed by the Maillard reaction, which involves reducing sugars and amino acids giving a browning effect. Depending on type of sugars that are present in the dough it may give different colors on the crust (Wang et al. 2012). The samples with improved color could be suggested having a higher amount of sugars and amino acids that were available for the Maillard reaction which resulted in a darker color in the bread. A possible cause of the great variation between all the quality parameters and the products could be that the products differentiated greatly in ingredients and in the baking process, which was performed manually and can affect the results.

*Sensory evaluation for bread PE and GA*

The sensory evaluation showed that no samples with AMG and aroma were chosen due to lack of sweet taste. This could imply that AMG did not provide with enough glucose to the dough and that the addition of natural aroma did not provide with enough sweetness. However, glucose is not as sweet as sucrose, having a lower sweetness level (Clemens et al. 2016). Also, this study had 0.20% fb of AMG as the highest dose, perhaps more AMG would be more efficient, but one need to take into consideration that other quality parameters might be affected. For the inulin B samples in bread PE, the participants tasted some difference from the reference bread, which as Setser & Brannan (2003) implied may be due to that sucrose is easy to recognize and other sugars are therefore easy to detect. Some of the participants commented that the taste was sweeter and perceived a different taste of sweetness. The results also support the implication of Peressini & Sensidoni (2009) that bread containing more than 5% fb inulin would give a too sweet and undesirable taste. Samples from PE with 20% inulin B (having 6.3% fb inulin), were more appreciated in taste compared to the samples with 30% inulin B (having 9.1% fb inulin). It can also indicate that replacing 30% sugar will give a more distinct taste due to it contains less sucrose. For product GA, samples with 20% inulin A and 20% inulin B were chosen further in the sensory evaluation due to that it had the best sweet taste. Samples with 20% inulin A was most liked in appearance for GA. It needs to be considered that all the bread in this study were made manually and not in an industrial manor. Therefore, the appearance could be influenced by how it was baked and not the sugar substitute itself. For both PE and GA, dryness and a different kind of sweetness was perceived in the samples with inulin. This might confirm that the sugar substitute inulin may not give the same taste and quality as sucrose. Furthermore, the hedonic test had only 12 participants which is too few to make any conclusions about the sensory evaluation.

# 6. Conclusion

The results in this trial showed that the reduction of sugar in bread affected the quality notably, especially in taste. It can be concluded that addition of either inulin or glucoamylase influenced the quality parameters firmness, color, volume, acid content and pH, but with great variation within the products. Glucoamylase did not give the desired sweet taste, whereas inulin could be a possible sugar replacer, and a future possibility could be to combine the different types of inulin, with different levels of DP that acts as both bulking agent and as sweetener. However, the different kind of sweet taste that comes with inulin compared to sucrose needs to be taken into account. Also, today´s consumer is aware of the ingredients which makes it even more important to find sugar substitute that are in the category “clean label” to retain the customers. More research is needed to find substitutes that act and taste like sucrose. Also, in this study there were too few samples to provide with any certainties.

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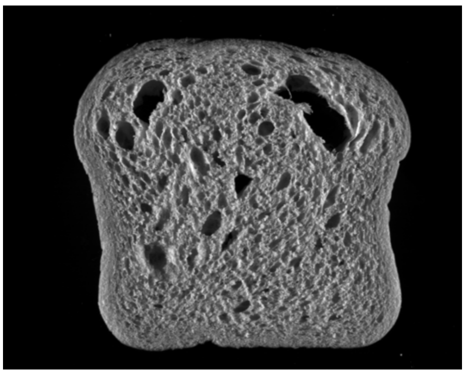
# Popular science summary

## Possible sugar substitutes in bread

*- Without affecting taste and quality*

Too much sugar in our diet gives negative effects on our health, and the World Health Organization are giving recommendations to reduce the sugar content of the total energy intake. One way to reach this recommendation could be to reduce the sugar content in bread that is a staple food consumed daily over the world. A reduction of sugar in bread could give more sustainable and healthier bread. However, it is not always that easy to just reduce the sugar. Sugar is involved in many reactions that occurs in the bread and without the sugar it won't give the same quality as consumers are used to. This thesis is a collaboration together with the Swedish bread company Pågen, with the aim to study the effect of two possible sugar substitutes, inulin and glucoamylase in three existing bread products.



This study used two different inulins, named "A" and "B", having different structures and different properties. Inulin is a sweet tasting dietary fiber from the chicory root. In this study it was presented as a powder and in liquid form. Glucoamylase is an enzyme that splits starch chains into glucose, which could give a sweeter taste due to higher amount of glucose in the bread. Also, natural aroma was added together with the glucoamylase to further enhance the sweet taste. These two substitutes, inulin and glucoamylase were added separately in the three bread products to replace sugar being reduced by 20% and 30%. The bread samples were analyzed for texture and taste to evaluate if they could act as possible sugar substitutes whilst not affecting quality or taste.

The study found that the addition of inulin and glucoamylase differentiated widely between the products and altered the quality and taste. In one of the products, there was a significant increase of firmness over time with the sample containing inulin A (with 30% less sugar), resulting in a harder bread. The bread containing glucoamylase was shown to affect pH and acid content in two of the products. For the perception of taste, consumers found clear differences when they tasted the products, and it was shown that addition of inulin gave a different kind of sweet taste. As a conclusion of this master thesis, addition of either inulin and glucoamylase altered the quality parameters; firmness, color, volume, acid content and pH, and the results differentiated within the products. This shows that it is difficult to find sugar substitutes that are similar to sucrose, giving the right taste and not affecting the quality. However, it was seen in this study that the inulin and glucoamylase gave different results within the products, which may confirm that depending on type of bread and its ingredients the additions of either inulin A, inulin B or glucoamylase will act differently. This shows that bread is a complex matrix with many different chemical reactions to be taken into consideration when changing the recipe. Further research is needed to find substitutes that taste and act as sucrose in bread to be accepted by consumers.

# Appendix I

Baking trials were conducted to test the baking machines and to adapt the concentrations of aroma, glucoamylase and inulin. Aroma was added with 0.25% fb (minimum recommended dosage), 0.33% fb and 0.40% fb (maximum recommended dosage). 0.33% fb and 0.40% fb yielded a slightly metallic after taste. Therefore, based on the results from trial test, 0.25% fb Aroma was chosen to be added in all bread products together with glucoamylase. Glucoamylase were added with 0.50% fb in the baking trials, yielding a much darker bread crust and a more caramelized taste, affecting the quality significantly. Addition of glucoamylase to each breads were customized considering the bread products total sugar content and previous scientific reports (Diler et al. 2015, 2021; Barrera et al. 2016b). The addition of glucoamylase in each bread are shown in table 1.

Inulin A and B did not give any noticeable changes of the visual appearance of the bread trials when replacing the sugar with 1:1 ratio and could therefore be used in this experiment.

# Appendix II

Explanation of the different samples and their concentrations of inulin, glucoamylase and aroma for GA.

| Bread sample | Explanation |
|---|---|
| Control | The reference bread |
| 30% Inulin A | 30% sugar reduction and addition of inulin A with ratio 1:1 |
| 30% Inulin B | 30% less sugar and addition of inulin B with ratio 1:1 |
| 20% Inulin A | 20% sugar reduction and addition of inulin A with ratio 1:1 |
| 20% Inulin B | 30% sugar reduction and addition of inulin B with ratio 1:1 |
| 30% AMG 0.15 | 30% sugar reduction and addition of 0.15% fb of Glucoamylase and 0.25% fb Aroma |
| 30% AMG 0.10 | 30% sugar reduction and addition of 0.10% fb of Glucoamylase and 0.25% fb Aroma |
| 20% AMG 0.10 | 20% sugar reduction and addition of 0.10% fb of Glucoamylase and 0.25% fb Aroma |
| 20% AMG 0.05 | 30% sugar reduction and addition of 0.05% fb of Glucoamylase and 0.25% fb Aroma |

Explanation of the different samples and their concentrations of glucoamylase and aroma for PE and BE

| Bread sample | Explanation |
|---|---|
| Control | The reference bread |
| 30% Inulin A | 30% sugar reduction and addition of inulin A with ratio 1:1 |
| 30% Inulin B | 30% less sugar and addition of inulin B with ratio 1:1 |
| 20% Inulin A | 20% sugar reduction and addition of inulin A with ratio 1:1 |
| 20% Inulin B | 30% sugar reduction and addition of inulin B with ratio 1:1 |
| 30% AMG 0.20 | 30% sugar reduction and addition of 0.20% fb of Glucoamylase and 0.25% fb Aroma |
| 30% AMG 0.15 | 30% sugar reduction and addition of 0.15% fb of Glucoamylase and 0.25% fb Aroma |
| 20% AMG 0.15 | 20% sugar reduction and addition of 0.15% fb of Glucoamylase and 0.25% fb Aroma |
| 20% AMG 0.10 | 20% sugar reduction and addition of 0.10% fb of Glucoamylase and 0.25% fb Aroma |

Explanation for the abbreviations in score plot (figure 1) for bread PE, BE and GA with three replicates

| **Abbreviations for bread PE** | **Explanation for bread PE** | **Replicate** | **Abbreviations for bread BE** | **Explanations for bread BE** | **Replicate** | **Abbreviations for bread GA** | **Explanation for bread GA** | **Replicate** |
|---|---|---|---|---|---|---|---|---|
| PR1 | Reference | 1 | VR1 | Reference | 1 | LR1 | Reference | 1 |
| PR2 | Reference | 2 | VR2 | Reference | 2 | LR2 | Reference | 2 |
| PR3 | Reference | 3 | VR3 | Reference | 3 | LR3 | Reference | 3 |
| P2A101 | 20% AMG 0.10 | 1 | V2A101 | 20% AMG 0.10 | 1 | L2A051 | 20% AMG 0.05 | 1 |
| P2A102 | 20% AMG 0.10 | 2 | V2A102 | 20% AMG 0.10 | 2 | L2A052 | 20% AMG 0.05 | 2 |
| P2A103 | 20% AMG 0.10 | 3 | V2A103 | 20% AMG 0.10 | 3 | L2A053 | 20% AMG 0.05 | 3 |
| P2A151 | 20% AMG 0.15 | 1 | V2A151 | 20% AMG 0.15 | 1 | L2A101 | 20% AMG 0.10 | 1 |
| P2A152 | 20% AMG 0.15 | 2 | V2A152 | 20% AMG 0.15 | 2 | L2A102 | 20% AMG 0.10 | 2 |
| P2A153 | 20% AMG 0.15 | 3 | V2A153 | 20% AMG 0.15 | 3 | L2A103 | 20% AMG 0.10 | 3 |
| P3A151 | 30% AMG 0.15 | 1 | V3A151 | 30% AMG 0.15 | 1 | L3A101 | 30% AMG 0.10 | 1 |
| P3A152 | 30% AMG 0.15 | 2 | V3A152 | 30% AMG 0.15 | 2 | L3A102 | 30% AMG 0.10 | 2 |
| P3A153 | 30% AMG 0.15 | 3 | V3A153 | 30% AMG 0.15 | 3 | L3A103 | 30% AMG 0.10 | 3 |
| P3A201 | 30% AMG 0.20 | 1 | V3A201 | 30% AMG 0.20 | 1 | L3A151 | 30% AMG 0.15 | 1 |
| P3A202 | 30% AMG 0.20 | 2 | V3A202 | 30% AMG 0.20 | 2 | L3A152 | 30% AMG 0.15 | 2 |
| P3A203 | 30% AMG 0.20 | 3 | V3A203 | 30% AMG 0.20 | 3 | L3A153 | 30% AMG 0.15 | 3 |
| P2IA1 | 20% Inulin A | 1 | V2IA1 | 20% Inulin A | 1 | L2IA1 | 20% Inulin A | 1 |
| P2IA2 | 20% Inulin A | 2 | V2IA2 | 20% Inulin A | 2 | L2IA2 | 20% Inulin A | 2 |
| P2IA3 | 20% Inulin A | 3 | V2IA3 | 20% Inulin A | 3 | L2IA3 | 20% Inulin A | 3 |
| P3IA1 | 30% Inulin A | 1 | V3IA1 | 30% Inulin A | 1 | L3IA1 | 30% Inulin A | 1 |
| P3IA2 | 30% Inulin A | 2 | V3IA2 | 30% Inulin A | 2 | L3IA2 | 30% Inulin A | 2 |
| P3IA3 | 30% Inulin A | 3 | V3IA3 | 30% Inulin A | 3 | L3IA3 | 30% Inulin A | 3 |
| P2IB1 | 20% Inulin B | 1 | V2IB1 | 20% Inulin B | 1 | L2IB1 | 20% Inulin B | 1 |
| P2IB2 | 20% Inulin B | 2 | V2IB2 | 20% Inulin B | 2 | L2IB2 | 20% Inulin B | 2 |
| P2IB3 | 20% Inulin B | 3 | V2IB3 | 20% Inulin B | 3 | L2IB3 | 20% Inulin B | 3 |
| P3IB1 | 30% Inulin B | 1 | V3IB1 | 30% Inulin B | 1 | L3IB1 | 30% Inulin B | 1 |
| P3IB2 | 30% Inulin B | 2 | V3IB2 | 30% Inulin B | 2 | L3IB2 | 30% Inulin B | 2 |
| P3IB3 | 30% Inulin B | 3 | V3IB3 | 30% Inulin B | 3 | L3IB3 | 30% Inulin B | 3 |

# Appendix III

List of the parameters given from the C-cell caliber (Modell CC.200.05) analyzing the crumb structure for the different bread samples. Slice Area, Height average, Slice brightness, number of cells, number of holes, area of cells, area of holes and wall thickness were parameters chosen to being further analyzed.

**Dimension**
Slice Area (mm2)
Height (max) (mm)
Height (avg) (mm)
Breadth (mm)
Height / Breadth
Wrapper Length (mm)

**Brightness**
Slice Brightness
Cell Contrast

**Shape**
Total Concavity %
Top Concavity %
Left Concavity %
Right Concavity %
Bottom Concavity %
Top Left Shoulder
Top Right Shoulder
Left Break %
Right Break %
Left Break Height (mm)
Right Break Height (mm)
Left Break Depth (mm)
Right Break Depth (mm)
Left Break Position (mm)
Right Break Position (mm)
Bottom Left Roundness
Bottom Right Roundness

**Cell size**
Number of Cells
Number of Holes
Area of Cells %
Area of Holes %
Cell diameter (mm)
Cell Volume
Coarse Cell Volume
Volume of Holes
Cell volume (map)
Cell Vol Range (map)
Relative Vol Range (map)
Coarse / Fine Clustering
Wall Thickness (mm)
Non-Uniformity

**Cell Elongation and Orientation**
Average Cell Elongation
Net Cell Elongation
Cell Angle to Vertical
Cell Alignment
Vertical Elongation
Degree of Circulation
Circulation Hhoriz Offset %
Circulation Vert Offset %
Curvature

# Appendix IV

Raw data from the hedonic and Ranking test by a two-way ANOVA with Tukey HSD. 12 participants were conducting the hedonic test and the ranking test with three samples from each bread product PE and GA.

Raw data from the hedonic test of bread PE

| **Summary Appearance** | | | |
|---|---|---|---|
| | **Reference** | **30% inulin B** | **20% inulin B** |
| Like Very Much | 25% | 16,67% | 0% |
| Like Moderately | 16,67% | 33,33% | 41,67% |
| Like Slightly | 8,33% | 16,67% | 8,33% |
| Neither Like nor Dislike | 25% | 16,67% | 25% |
| Dislike Slightly | 16,67% | 16,67% | 16,67% |
| Dislike Moderately | 8,33% | 0% | 8,33% |
| Dislike Very Much | 0% | 0% | 0% |
| N | 12,00 | 12,00 | 12,00 |
| Mean (arithmetic) | 4,83 | 5,17 | 4,58 |
| Standard Error | 0,51 | 0,41 | 0,42 |
| Top 2 Box (%) | 41,67% | 50% | 41,67% |
| Middle 3 Box (%) | 50% | 50% | 50,00% |
| Bottom 2 box (%) | 8,33% | 0% | 8,33% |
| **Summary Smell** | | | |
| | **Reference** | **30% inulin B** | **20% inulin B** |
| Like Very Much | 0% | 8,33% | 0% |
| Like Moderately | 50% | 33,33% | 25% |
| Like Slightly | 25% | 25% | 33,33% |
| Neither Like nor Dislike | 25% | 25% | 41,67% |
| Dislike Slightly | 0% | 8,33% | 0% |
| Dislike Moderately | 0% | 0% | 0% |
| Dislike Very Much | 0% | 0% | 0% |
| N | 12 | 12 | 12 |
| Mean (arithmetic) | 5,25 | 5,08 | 4,83 |
| Standard Error | 0,25 | 0,34 | 0,24 |
| Top 2 Box (%) | 50% | 41,67% | 25% |
| Middle 3 Box (%) | 50% | 58,33% | 75% |

| | | | |
|---|---|---|---|
| Bottom 2 box (%) | 0% | 0% | 0% |

**Summary Taste**

| | Reference | 30% inulin B | 20% inulin B |
|---|---|---|---|
| Like Very Much | 8% | 0% | 0 |
| Like Moderately | 41,67% | 8,33% | 0,1667 |
| Like Slightly | 25% | 66,67% | 0,5833 |
| Neither Like nor Dislike | 16,67% | 8,33% | 0,25 |
| Dislike Slightly | 8,33% | 16,67% | 0 |
| Dislike Moderately | 0% | 0% | 0 |
| Dislike Very Much | 0% | 0% | 0 |
| N | 12 | 12 | 12 |
| Mean (arithmetic) | 5,25 | 4,67 | 4.92 |
| Standard Error | 0,33 | 0,26 | 0.19 |
| Top 2 Box (%) | 50% | 8,33% | 16,67% |
| Middle 3 Box (%) | 50% | 91,67% | 83,33% |
| Bottom 2 box (%) | 0% | 0% | 0% |

**Summary Texture (mouthfeel)**

| | Reference | 30% inulin B | 20% inulin B |
|---|---|---|---|
| Like Very Much | 8,33% | 0% | 8,33% |
| Like Moderately | 50% | 33,33% | 16,67% |
| Like Slightly | 25% | 25% | 41,67% |
| Neither Like nor Dislike | 8,33% | 25% | 16,67% |
| Dislike Slightly | 0% | 16,67% | 8,33% |
| Dislike Moderately | 8,33% | 0% | 8,33% |
| Dislike Very Much | 0% | 0% | 0% |
| N | 12 | 12 | 12 |
| Mean (arithmetic) | 5,33 | 4,75 | 4,75 |
| Standard Error | 0,38 | 0,33 | 0,39 |
| Top 2 Box (%) | 58,33% | 33,33% | 25% |
| Middle 3 Box (%) | 33,33% | 66,67% | 66,67% |
| Bottom 2 box (%) | 8,33% | 0% | 8,33% |

**Summary Overall Impression**

| | Reference | 30% inulin B | 20% inulin B |
|---|---|---|---|
| Like Very Much | 8,33% | 0% | 0% |
| Like Moderately | 50% | 25% | 25% |
| Like Slightly | 25% | 33,33% | 50% |
| Neither Like nor Dislike | 16,67% | 16,67% | 25% |
| Dislike Slightly | 0% | 25% | 0% |
| Dislike Moderately | 8,33% | 0% | 0% |
| Dislike Very Much | 0% | 0% | 0% |
| N | 12 | 12 | 12 |

| | | | |
|---|---|---|---|
| Mean (arithmetic) | 5,17 | 4,58 | 5 |
| Standard Error | 0,39 | 0,34 | 0,21 |
| Top 2 Box (%) | 50% | 25% | 25% |
| Middle 3 Box (%) | 41,67% | 75% | 75% |
| Bottom 2 box (%) | 8,33% | 0% | 0% |

**Results Two Way ANOVA with Tukey HSD**

| Mean value | **Reference** | **30% inulin B** | **20% inulin B** |
|---|---|---|---|
| Like the Appearance | 4,83 | 5,17 | 4,58 |
| Like the Smell | 5,25 | 5,08 | 4,83 |
| Like the Taste | 5,25 | 4,67 | 4,92 |
| Like the Texture (mouthfeel) | 5,33 | 4,75 | 4,75 |
| Like the Overall Impression | 5,17 | 4,58 | 5 |

Level of significance (Tukey): A'<99.9% ; A<99% ; a<95% ; a'<90%.

The ANOVA performed is a two-way ANOVA.

## Raw data from the hedonic test of bread GA

**Summary Appearance**

| | **Reference** | **20% inulin A** | **20% inulin B** |
|---|---|---|---|
| Like Very Much | 7,14% | 21,43% | 7,14% |
| Like Moderately | 42,86% | 28,57% | 21,43% |
| Like Slightly | 7,14% | 42,86% | 28,57% |
| Neither Like nor Dislike | 28,57% | 7,14% | 21,43% |
| Dislike Slightly | 7,14% | 0% | 21,43% |
| Dislike Moderately | 0% | 0% | 0% |
| Dislike Very Much | 7,14% | 0% | 0% |
| N | 14 | 14 | 14 |
| Mean (arithmetic) | 4,86 | 5,64 | 4,71 |
| Standard Error | 0,43 | 25 | 0,34 |
| Top 2 Box (%) | 50% | 50% | 28,57% |
| Middle 3 Box (%) | 42,86% | 50% | 71,43% |
| Bottom 2 box (%) | 7,14% | 0% | 0% |

**Summary Smell**

| | **Reference** | **20% inulin A** | **20% inulin B** |
|---|---|---|---|
| Like Very Much | 7,14% | 0% | 0% |
| Like Moderately | 50% | 42,86% | 28,57% |
| Like Slightly | 7,14% | 14,29% | 28,57% |
| Neither Like nor Dislike | 35,71% | 35,71% | 28,57% |
| Dislike Slightly | 0% | 0% | 14,29% |
| Dislike Moderately | 0% | 7,14% | 0% |
| Dislike Very Much | 0% | 0% | 0% |
| N | 14 | 14 | 14 |

| | | | |
|---|---|---|---|
| Mean (arithmetic) | 5,29 | 4,86 | 4,71 |
| Standard Error | 0,29 | 0,33 | 0,29 |
| Top 2 Box (%) | 57,14% | 42,86% | 28,57% |
| Middle 3 Box (%) | 42,86% | 50% | 71,43% |
| Bottom 2 box (%) | 0% | 7,14% | 0% |

**Summary Taste**

| | Reference | 20% inulin A | 20% inulin B |
|---|---|---|---|
| Like Very Much | 0% | 0% | 0% |
| Like Moderately | 21,43% | 21,43% | 7,14% |
| Like Slightly | 7,14% | 35,71% | 35,71% |
| Neither Like nor Dislike | 28,57% | 14,29% | 14,20% |
| Dislike Slightly | 35,71% | 21,43% | 28,57% |
| Dislike Moderately | 7,14% | 7,14% | 14,29% |
| Dislike Very Much | 0% | 0% | 0% |
| N | 14 | 14 | 14 |
| Mean (arithmetic) | 4 | 4,43 | 3,93 |
| Standard Error | 0,35 | 0,34 | 0,34 |
| Top 2 Box (%) | 21,43% | 21,43% | 7,14% |
| Middle 3 Box (%) | 71,43% | 71,43% | 78,57% |
| Bottom 2 box (%) | 7,14% | 7,14% | 14,29% |

**Summary Texture (mouthfeel)**

| | Reference | 20% inulin A | 20% inulin B |
|---|---|---|---|
| Like Very Much | 0% | 0% | 0% |
| Like Moderately | 7,14% | 7,14% | 14,29% |
| Like Slightly | 21,43% | 21,43% | 21,43% |
| Neither Like nor Dislike | 28,57% | 14,29% | 7,14% |
| Dislike Slightly | 28,57% | 42,86% | 35,71%% |
| Dislike Moderately | 14,29% | 14,29% | 21,43% |
| Dislike Very Much | 0% | 0% | 0% |
| N | 14 | 14 | 14 |
| Mean (arithmetic) | 3,79 | 3,64 | 3,71 |
| Standard Error | 0,32 | 0,32 | 0,38 |
| Top 2 Box (%) | 7,14% | 7,14% | 14,29% |
| Middle 3 Box (%) | 78,57% | 78,57% | 64,29% |
| Bottom 2 box (%) | 14,29% | 14,29% | 21,43% |

**Summary Overall Impression**

| | Reference | 20% inulin A | 20% inulin B |
|---|---|---|---|
| Like Very Much | 0% | 0% | 0% |
| Like Moderately | 14,29% | 14,29% | 21,43% |
| Like Slightly | 0% | 35,71% | 21,43% |
| Neither Like nor Dislike | 57,14% | 35,71% | 28,57% |

| | | | |
|---|---|---|---|
| Dislike Slightly | 21,43% | 7,14% | 14,29% |
| Dislike Moderately | 7,14% | 7,14% | 14,29% |
| Dislike Very Much | 0% | 0% | 0% |
| N | 14 | 14 | 14 |
| Mean (arithmetic) | 3,93 | 4,43 | 4,21 |
| Standard Error | 0,29 | 0,29 | 0,37 |
| Top 2 Box (%) | 14,29% | 14,29% | 21,43% |
| Middle 3 Box (%) | 78,57% | 78,57% | 64,29% |
| Bottom 2 box (%) | 7,14% | 7,14% | 14,29% |

**Results Two Way ANOVA with Tukey HSD**

| Mean value | **Reference** | **20% inulin A** | **20% inulin B** |
|---|---|---|---|
| Like the Appearance | 4,86 | 5,64[a] | 4,71 |
| Like the Smell | 5,29[a'] | 4,86 | 4,71 |
| Like the Taste | 4 | 4,43 | 3,93 |
| Like the Texture (mouthfeel) | 3,79 | 3,64 | 3,71 |
| Like the Overall Impression | 3,93 | 4,43 | 4,21 |

* Level of significance (Tukey): A'<99.9% ; A<99% ; a<95% ; a'<90%.

The ANOVA performed is a two-way ANOVA.

# Appendix V

Comments from the hedonic test from bread PE and GA.

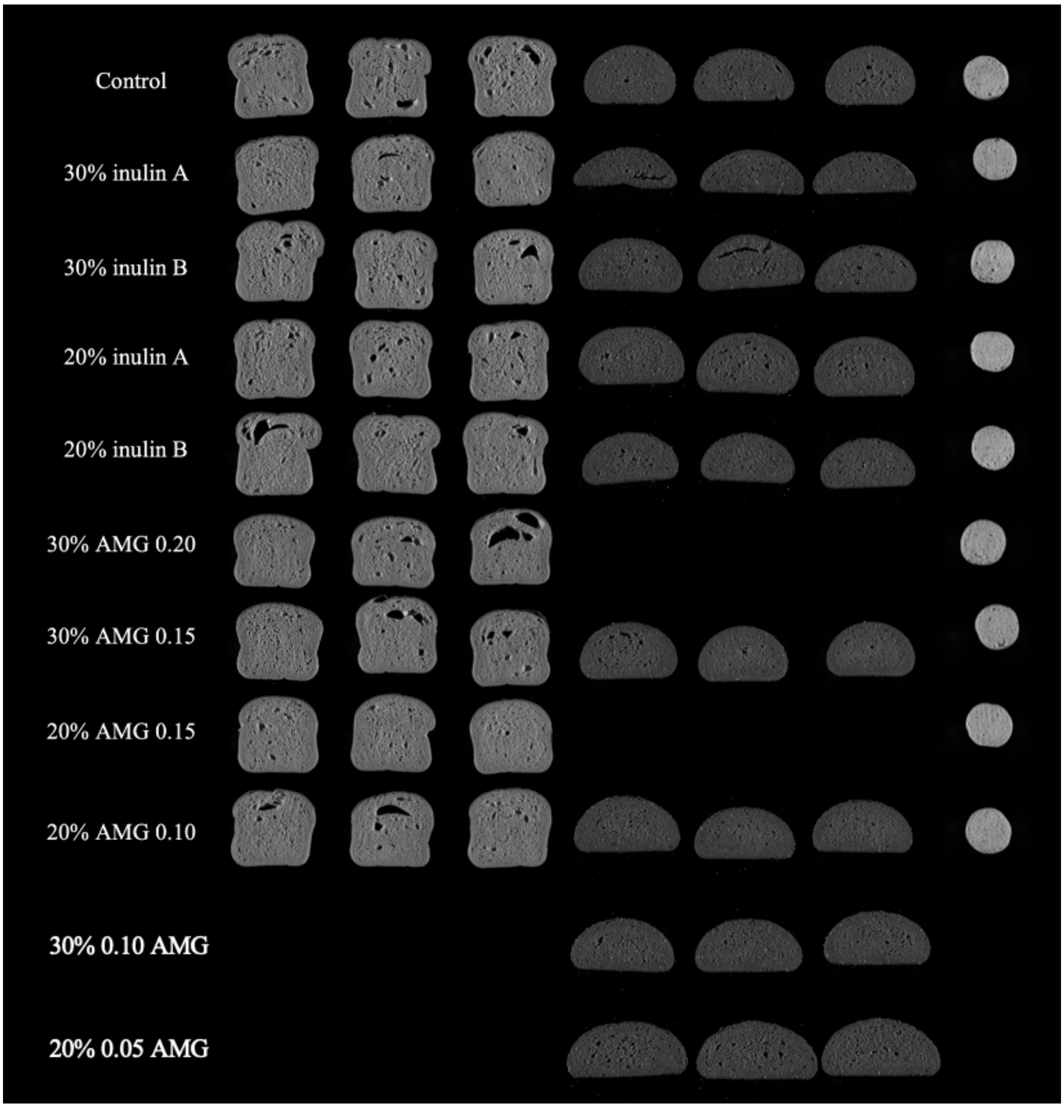
| Comments from the panelist for bread PE<br>Reference | 30% inulin B | 20% inulin B |
|---|---|---|
| *"Like the appearance but not the texture or the flavor"*<br>*"Balanced sweetness"*<br>*"Its ok, good"*<br>*"A little bit too much holes in the bread"*<br>*"Too big holes, very loose crumb"*<br>*"Big holes"* | *"Dry crust, dull appearance"*<br>*"Neither too sweet or too un-sweet"*<br>*"Boring and tasteless, a bit stale bread"*<br>*"Less taste compared to the others"*<br>*"Too many holes"*<br>*"Dry and not elastic, weird taste of sweet"* | *"Weird shape, otherwise ok"*<br>*"It was the least sweet of them all"*<br>*"A bit stale and boring, tasteless"*<br>*"Better chewing resistance"*<br>*"Missing taste of sweetness, sweetness appears afterwards"*<br>*"Little bit dry and not as elastic"*<br>*"Holes in the crumb"* |
| **Comments from the panelist about GA**<br>**Reference** | **20% inulin A** | **20% inulin B** |
| *"Very dry"*<br>*"Nice shape, a little bit dry, tasteless"*<br>*"All samples feels dry, like something is missing"*<br>*"Dry and boring"*<br>*"Dry, no sweetness, dry crumb"*<br>*"No volume, compact"*<br>*"Not so sweet, and not so tasteful"* | *"A bit dry"*<br>*"Taste good"*<br>*"Dry"*<br>*"Strong smell of sourdough"*<br>*"Similar to 458[1] sample"*<br>*"Not as sweet, bit dry"*<br><br>[1]. *458 was the reference sample.* | *"Also dry as the other ones"*<br>*"Dry, giving crumbs and tasteless"*<br>*"Not sweet enough"*<br>*"Dry"*<br>*"Not as dry as 371[2] sample"*<br>*"A bit sweet"*<br><br>[2]. *371 was the 20% inulin A sample* |

Comments from the ranking test from bread PE and GA.

| Reason for choice in ranking test GA | Reason for choice in ranking test PE |
|---|---|
| *"No one specific was the most preferrable, had to choose one"*<br>*"895* taste more"*<br>*"458* taste good"*<br>*"895* had the best taste and mouthfeel"*<br>*"458* and 895* was drier and less tasteful than 371"*<br>*"458* had rich taste"*<br><br>458*= reference<br>895*= 20% inulin A<br>371*= 20% inulin B | *"458* had the best taste and good texture"*<br>*"458* had a balance sweetness, 371* was too sweet and neutral in taste"*<br>*"458* best taste and texture"*<br>*"Not so big difference between the samples"*<br>*"458* had the best taste, 895* was dry and not so elastic, a too sweet taste and sugary taste,, 371* perceived as dry and not elastic, tasted sweeter than 458*"*<br><br>458*= reference<br>895*= 30% inulin B<br>371*= 20% inulin B |

# Appendix VI

Visual slices from the digital image analysis. From top to bottom: Control, 30% inulin A, 30% inulin B, 20% inulin A, 20% inulin B, 30% AMG 0.20, 30% AMG 0.15, 20% AMG 0.15, 20% AMG 0.10. From left: PE, GA and BE. GA did not have 30% AMG 0.20 and 20% AMG 0.15, instead it had 30% 0.10 AMG and 20% 0.05 AMG.



## Publishing and archiving

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