INVESTIGATING THE EFFECTS OF PROMOAT BETA-GLUCAN ON IMPROVING AND MAINTAINING THE DESIRED STRUCTURE AND PHYSICAL PROPERTIES OF SPONGE CAKES OVER TWO WEEKS OF STORAGE.

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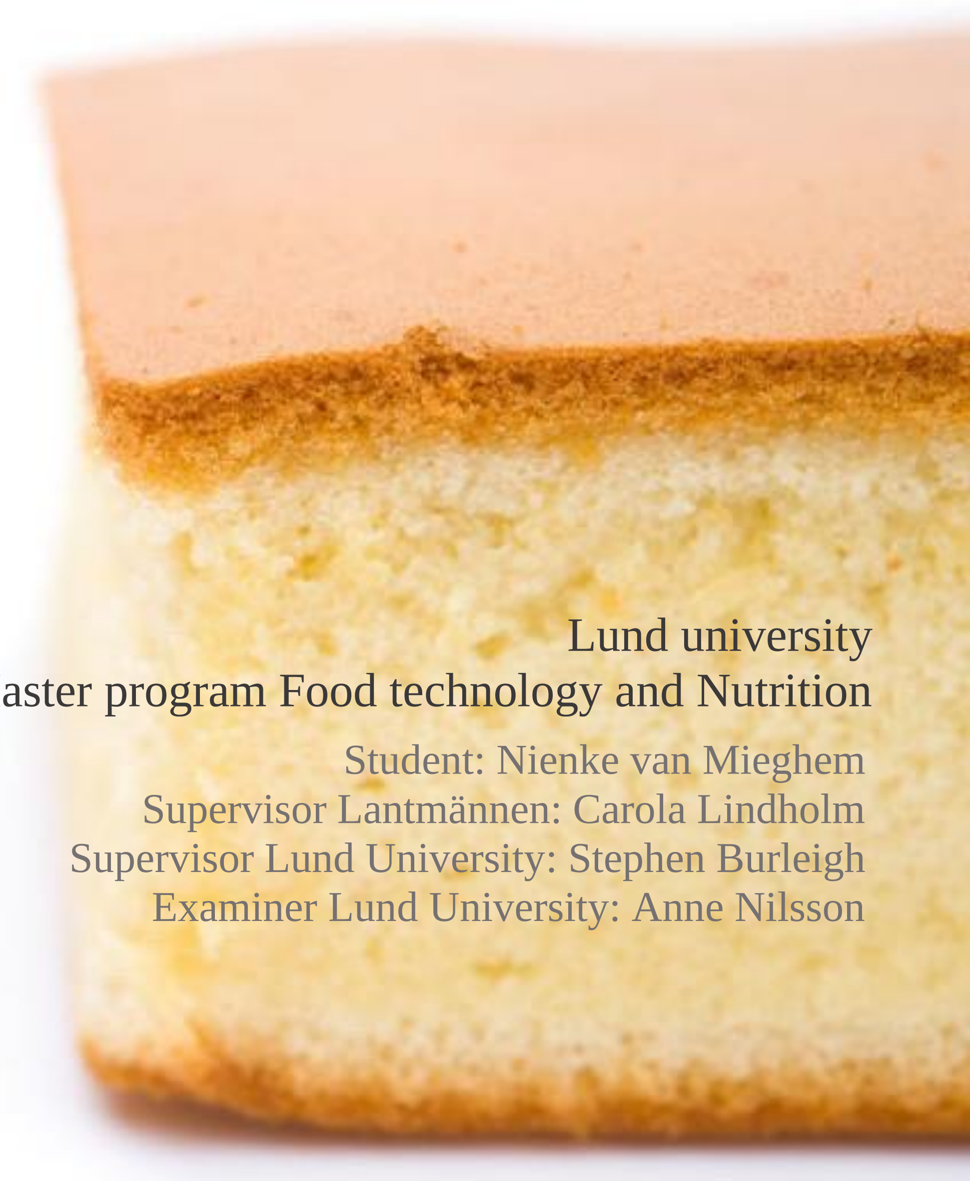
Lantmännen



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Abstract

This project investigated the impact of beta-glucan on sponge cake's desired texture and physical properties. Sponge cakes with different percentages of Lantmännens PromOat beta-glucan (0.0%, 1.0% and 2.0%) were produced and compared on the following parameters: volume, density, water activity, moisture content and texture profile analyses. These parameters were measured on different storage days (1, 3, 7, 10 and 14) to evaluate structural changes over time. Matlab's ANOVA and multivariate PCA analysis were used for data analysis.

The results revealed significant treatment, batch and storage influences on cake characteristics. The beta-glucan percentage increased hardness, gumminess, moisture content, water activity and density, and it decreased the cake's volume, cohesiveness and springiness. Storage time was found to influence hardness, chewiness and gumminess the most.

Contrary to previous research, increased beta-glucan levels led to a decrease in volume and springiness and an increase in density and cohesiveness, probably due to the formation of smaller air bubbles and hindered expansion. However, cakes with 2.0% PromOat beta-glucan unexpectedly increased in springiness.

Moisture content and water activity increased with increasing beta-glucan percentages due to its water-absorbing properties. Nevertheless, cakes fortified with PromOat beta-glucan showed a greater decrease in moisture content, probably related to the increasing hardness.

Hardness, chewiness and gumminess increased with increasing storage time, in which cakes with 2.0% PromOat beta-glucan had the highest values. These changes were likely related to retrogradation, ingredient composition and reduced moisture content. Furthermore, unlikely batch variations were observed, pointing out the importance of maintaining batch consistency in future research.

In conclusion, this research provided insights into the effects of different beta-glucan percentages on structural and physical factors over a two-week storage period. Complex interactions between beta-glucan, storage time, ingredients and cake characteristics were presented. Further research is needed to investigate these interactions and demonstrate the optimal use of beta-glucan levels in sponge cakes.

Popular Science Abstract

This project investigated the effect of a beta-glucans product, a good source of fibre, on sponge cake textures. The beta-glucan product was added to sponge cake in various percentages (0.0%, 1.0% and 2.0%) and compared and measured over different storage days (days 1, 3, 7, 10 and 15) to evaluate changes over time. Various cake characteristics were assessed to identify the influence of beta-glucan on these properties. The results were analyzed using two types of statistical tests and compared to results from previous studies. These results presented variations in Treatment, Batch and Days, implying a relation between beta-glucan levels, storage time and cake characteristics.

Considering Treatment, the results represented that beta-glucan levels had the most impact on and increased hardness, gumminess, moisture content, water activity and density the most, and it decreased the cake's volume, cohesiveness and springiness. Storage time was found to influence hardness, chewiness and gumminess the most.

The results showed that increasing beta-glucan levels positively and negatively affected the cake’s quality. Higher levels of beta-glucan led to less growth of and smaller air bubbles in the cake, which resulted in a decreased volume and increased density. In addition, cakes with higher beta-glucan percentages became less springy and more cohesive, resulting in a firmer structure. The moisture content and water activity increased as expected with increasing beta-glucan values, and cakes with higher levels demonstrated more significant moisture loss over time, likely related to the increased hardness of the cake. These cakes also experienced more mold growth over time.

Hardness, chewiness and gumminess increased with increasing storage time, conflicting with previous findings, resulting in a firmer cake structure. In addition, unlikely batch variations indicated possible errors in the production process.

In conclusion, this research has given insights into the effects of different beta-glucan percentages on cake characteristics. It highlights the need for further investigation in optimizing beta-glucan levels in sponge cakes and ingredient interactions.

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During my project, I have received valuable support from a group of people I would like to thank. First of all, a big thank you to Carola Lindholm and her department from Lantmännen Cerealia for being my supervisor with lots of involvement and guidance through my process, especially experiments. Also, thank you to my supervisor Stephen Burleigh at Lund University, for our weekly meetings, advice and insights through the process and valuable help in data analysis using MATLAB. In addition, I would like to thank the Food Department of Lund University for providing the pilot kitchen, laboratory rooms and equipment and for helping me with technical and practical guidance and support. Also, thank you to Anne Nilsson for taking the role of the examiner. Lastly, my final gratitude goes to family and friends for their mental support.

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Abbreviations

REF	Reference
BG1	1% Promoat Beta-glucan
BG2	2% Promoat Beta-glucan
PCA	Principal component analyses
BVM	Bread volume meter

1. Introduction

1.1 Project background

Lantmännen (https://www.lantmannen.com/) is an agriculture cooperative in Sweden, refining different kinds of cereals with focus on agriculture and foods. The company has multiple locations within Europe, and is the leader within the agriculture, machinery, bioenergy and food products in Nothern Europe. One of the cereals Lantmännen refines are oats, for which Lantmännen has a department solely focusing on oats and oat-based products: Lantmännen Oats. This department produces oat-based ingredients for the nutrition, health and cosmetics industries. One of the products they produce is PromOat, providing dietary fibre the form of oat bran. This natural oat beta-glucan source contributes to health and functional benefits.

Dietary fibre

With the increasing interest in plant-based foods (Graça et al., 2019), more companies and consumers are focusing on meeting these upcoming trends. Plant-based diets are often associated with a healthier lifestyle, especially products containing a higher fibre content, which is known to reduce the risk of heart disease and diabetes and contribute to satiety. The recommendations for dietary fibre are 25g/day, which is infeasible for most people. Therefore, methods to increase fibre intake are necessary. ("Beta-Glucan: Health Benefits and Product Applications", 2014).

Beta-glucan also contributes to positive functionalities within (bakery) products, meaning it can contribute to positive physical properties and is normally added as an ingredient for improving texture. These properties are due to beta-glucan's thickening, stabilizing, emulsifying and gelatinizing functions and properties.

Many different types of research about beta-glucan and its effects on bakery products have been performed. These previous studies have established that beta-glucan, added to bakery products in lower percentages (<3.0%) can have a more positive effect compared to higher percentages (>3.0%). However, higher additions of beta-glucan can lead to a rapid increase in density, staling and a rubbery texture.

Overall, lower amounts of beta-glucan in bakery products showed a better improvement in nutritional value without compromising the quality of the product. However, the optimal amount of beta-glucan can differ for each individual product and composition of ingredients. The range within this 3,0% and the effects in a product vary from study to study, with the optimal amount for beta-glucan ranging from 0.5% to 2.0-3.0%. (Karp et al., 2020), (Kalinga & Mishra, 2009a). (Han-Young, 2005). (Hajmohammadi et al., 2013).

Because of the positive health benefits and the functional benefits beta-glucan can have and give to products, Lantmännen is working on implementing the rich-in-fibre PromOat into different products. This application is mainly focused on dairy and bakery product areas and, within this project, is specified on sponge cakes. Further concepts of these health and functional benefits and relations between cake characteristics will be discussed more briefly in the literature study in Chapter 2.

1.2 Aim

The project aims to investigate the effect of PromOat beta-glucans on improving and maintaining a desired texture and physical properties of sponge cake over two weeks of storage. In this project, sponge cakes with different percentages of PromOat beta-glucan (0.0%, 1.0% and 2.0%) are produced and compared on textural and physical characteristics consisting of batter density, volume, water activity, moisture content and texture profile analyses. Including different measure points on different storage days (1, 3, 7, 10 and 14) to evaluate structural changes over time.

The project was divided into several phases to reach the final goal. The first phase consisted of a literature review and conducting test practices. The second phase consisted of baking sponge cake samples and performing the textural and physical tests on the scheduled test days. The results were analysed using Matlab's ANOVA test and multivariate analysis Principal component analysis (PCA) to compare the measured features. In this master's thesis report, the analyses' results were combined with a literature review.

2. Literature study

This chapter highlights information on sponge cakes, beta-glucan and their complex structure, characteristics and functionalities, and the methods and equipment used. The literature review was created by reading previously completed studies similar to the same products. These findings were then compared, and the most similar results were used to further develop the methodology for the project. Unclear concepts and topics were additionally developed.

2.1 Sponge cakes

Sponge cakes are a type of cake that is known for their fluffy and delicate texture and airy crumb. The cakes have a springy, light and soft structure with small air pockets, a high moisture content and a mild taste. The recipe for a sponge cake is simple, and the cake-baking process is less complicated than when baking bread. Unlike bread, to form a cake, no high-gluten flour is required to create a dough; a flour-based batter with egg protein is mostly sufficient. Starch gelatinization and egg protein denaturation are prominent in the cake structure. The denatured egg protein helps form an aerated structure, a structure with small air bubbles, giving rise to volume due to its foaming and emulsifying properties. The addition of emulsifiers can, therefore, also contribute to the desired structure of sponge cakes to help stabilize and absorb the air bubble in the batter and to contribute to keeping the structure soft and moist. (Moza & Gujral, 2017).

The mixing process is crucial for securing the desired structure of the end product. The mixing step is responsible for blending and folding the dry and wet ingredients until a smooth batter is obtained. It gives away the airy structure of the dough by incorporating air bubbles into the batter. The mixing time is also essential as over- or undermixing directly affects the texture of the batter. Overmixing can lead to higher batter viscosity as a gluten network is formed, resulting in a thicker batter with more resistance to flow (Lante et al., 2023). The cake will become dense and less airy, and a batter of high density is not suitable for the production of cake. Therefore, a short mixing time to incorporate the ingredients is sufficient. Another critical aspect of the baking process is the baking time and temperature. A lower temperature with a longer oven time is more beneficial to not over- or undercook the sponge cake and prevent a hard crust from forming.

Additionally, the humidity during baking should be kept low as an excess of moisture may result in a collapse of the cake. The moisture inside the cake needs to leave the cake and turn into steam to let the cake form an airy structure. When there is a moisture overload inside the oven, the moisture cannot be released, making the cake too dense and heavy, which causes the structure to break. Lastly, resting time after baking is essential to form the final structure. The cakes should be cooled down before further handling. The temperature during this resting period should not fluctuate to prevent the cake from drying out. (Moza & Gujral, 2017).

2.1.1 Bubble size

The formation of bubbles is essential to form the airy structure of the cake. Here, the air bubbles' number, size and distribution is important and dependent. Different factors influence these aspects—one reason being the batter's characteristics. The viscosity of the batter is dependent on bubble movement and distribution. Low viscosity can lead to the rise of bubble movement and lower bubble stability, causing loss of bubbles. Moreover, the batter's density can depend on the bubble size, where overall, a higher density leads to a smaller bubble size and a less varied size distribution. However, this can be influenced by adding specific ingredients to the batter. For example, previous studies found that fat and sugar can affect bubble expansion, and depending on the composition, it can either decrease or increase. In addition to this, emulsifiers can help with stabilizing bubbles and usually lead to an increase in the viscosity of the batter and bubble size. The batter's viscosity helps retain the gas bubbles and maintain the cake's volume. (Sahi & Alava, 2003), (Rodríguez-García et al., 2014b).

2.1.2 Shelf life and retrogradation

Sponge cakes' shelf life can vary from days to weeks to a month, depending on the baking process, preservative steps, storage conditions, type of sponge cake, ingredients and packaging. The main reasons for spoilage in sponge cakes are mold growth and the emergence of retrogradation, a process that occurs in most baked products containing starch when ageing. The water activity (aw) of sponge cakes lies between 0.8 and 0.9, and the pH is 6.0- 7.5. Sponge cakes can, therefore, still be susceptible to spoilage as the water activity should be lowered to 0.78 for preventing mold growth. Mold growth increases as the cake ages and is also influenced by the water content of the overall cake. (An Industrial Cake Manufacturing Guide (Eng), 2021).

Retrogradation

Starch is a complex carbohydrate made of all glucose molecules linked together, with amylose and amylopectin molecules as the main components. Starch has different pasting properties, such as gelatinization and retrogradation, affecting the final cakes' quality. Starch is present in the flour used for the batter of the cake. The starch granules start to adsorb water when the cakes are cooled down after baking, and the granules begin to swell. This results is a soft an moist structure, which is desirable for cakes. However, over time, the ability to hold the water inside the granules is lost, and the starch starts to recrystallize and form a more fixed structure. As a result, the gel-like structure comes to break down and doesn't exist anymore. This recrystallization is due to rearranging of amylopectin and amylose molecules into a crystalline structure. In this structure both amylose and amylopectin have a different part in this process. Amylose is a linear molecule, and amylopectin has a branched structure. During the recrystallization, amylose molecules adjust themselves in a way they can form tightly packed crystalline structures, decreasing the movement of water and other ingredients. In contrast, amylopectin forms less packed and more open crystalline structures during this phase allowing water and other ingredients to move. The ratio of amylose and amylopectin is therefore correlated to the rate of retrogradation. Considering retrogradation, the cake structure becomes more firm and dry and less accessible for consumption. Retrogradation appears more rapid at lower temperatures. Also, the quality of the starch and the composition of amylopectin and amylose quantities play a role, where a higher amylose percentage can result in lower resistance to retrogradation. (Choi & Baik, 2014b), (Oh et al., 2020).

2.2 Dietary fibre and beta-glucans

Dietary fibres are edible plant parts and non-digestible carbohydrates, defining that the fibre offers resistance to digestion and absorption in the small intestine. The small intestine cannot digest dietary fibres, so the fibres will continue to travel to the large intestine. Here they are partially or entirely fermented by microbiota in the large intestine (Ahmad et al., 2012).

Products high in fibre are beans and lentils, mushrooms, whole grain products such as barley and quinoa, oats and some fruits and vegetables. Dietary fibre exists in several forms; they can be either soluble or insoluble, as well as viscous or non-viscous. The dietary fibre present in the cell wall of grains (cereal crops), including oat bran, is beta-glucan and belongs to the category of viscous soluble fibres ("Beta-Glucan: Health Benefits and Product Applications", 2014). Beta-glucans are polysaccharides consisting of long linear or branched chains of glucose molecules linked together by β-1,3 and β-1,4 linkage in glycosidic bonds. The size of the chains and the linkage can differentiate. There are two main categories of beta-glucans, classified based on their type of linkage: mixed beta-glucans and beta-1,3 glucans. Mixed beta-glucans contain both β-1,3 and β-1,4 linkage, typical for cereal grains, whereas beta-1,3 glucans consist of the 1,3 linkage, which is more common for fungi species. (Ahmad et al., 2012b).

There are different techniques to extract and purify beta-glucan in a way the production produces consistent raw material: Chemical, enzymatic and physical methods. (Zhu et al., 2016).

2.2.1 Health benefits of beta-glucan

Beta-glucan consumption is associated with several health benefits, especially for the cardiovascular and immune systems. The group of viscous soluble fibres are known to reduce the total and LDL cholesterol levels. Furthermore, they also contribute to reducing risks of cardiovascular diseases, improving insulin sensitivity and blood glucose levels, assisting with weight loss and supporting the gastrointestinal tract. (Ahmad et al., 2012b), ("Beta-Glucan: Health Benefits and Product Applications", 2014).

2.2.2 PromOat® Beta Glucan

PromOat® Beta Glucan is a product of Lantmännen Oats. Typical PromOat® Beta-glucan is rich in beta-glucans with β-1,3 and β-1,4 linkage and contains 34% soluble beta-glucan fibre, carbohydrates, fat and hardly any protein. The nutritional values of PromOat® Beta Glucan obtained from the product specifications are presented in Table 1. ("Specification Sheet PromOat® Beta Glucan", 2022). PromOat® Beta Glucan is highly viscous due to its high molecular weight, and they have strong water-binding capacity and emulsifying properties. This beta-glucan source is derived from non-genetically modified (non-GM) Swedish oats, utilized by a chemical-free, aqueous and enzymatic method. The process steps are visualized in a flowchart in appendix 1. PromOat® Beta Glucan contributes to healthy cholesterol levels, increases dietary fibre intake and demonstrates positive health aspects to the abovementioned aspects ("Beta-Glucan: Health Benefits and Product Applications", 2014).

Table 1 Nutritional information of PromOat Beta-Glucan

Nutritional information	**PromOat® Beta Glucan**
Energy	3.3e02 kcal or 1.4e03 kj (per 100g)
Total fat	6.5 g
Saturated fat	1.2 g
Carbohydrate	43 g
Fibre	40. g
Sugars	2.0 g
Protein	3.5 g
Salt	1.7e-01 g
Beta-Glucan	34 g

2.2.3 Application in baking Products

Other than health benefits, Beta-glucans are nowadays applied to a wide range of products, including various baking products, to contribute to functional and nutritional benefits. The nutritional benefits rely on the dietary fibre content. Baking products often contain wheat flour or other ingredients with a lower to none-fibre content and have a lower nutritional value. By enriching a product with the addition of beta-glucan, the dietary fibre amount increases, bringing the product's nutritional value to a higher level. Another nutritional aspect of baking products is reducing the fat content by using beta-glucan as a fat replacer. (Ahmad et al., 2012). Fat has the highest calorific value (9.0 kcal/g) compared to the other macromolecules, carbohydrates, and proteins (4.0 kcal/g). Reducing the fat percentage in a product increases the nutritional value as the calorie content decreases.

In most products, beta-glucan is added as a functional ingredient for improving the texture of the products after baking, as it can perform thickening, stabilizing, emulsifying and gelatinizing functions and properties. Beta-glucan's thickening, gelatinizing and emulsifying properties are most beneficial for baking products. The majority of these qualities come from the water-binding properties and the complicated structure of the beta-glucans. When in contact with water, beta-glucan forms a three-dimensional gel-like network of long linear or branched chains entangled with one another. The ability to form gel-like networks increases the water absorption capacity in dough by trapping water into the network and preventing it from syneresis during baking. Because of this complex structure, the hindering of components in the dough increases and leads to a rise in viscosity and elasticity. In this way, the texture of the dough becomes softer, has higher moisture content and has higher resistance against deformation. The ability of beta-glucan to retain moisture in the structure also reduces the rate of retrogradation. The resistance against deformation is beneficial during fermentation, where the elasticity will help maintain the formed gas bubbles making the crumb structure airier after baking. Additionally, the cake volume increases as more gas bubbles stay intact. (Kalinga & Mishra, 2009), (Sharma et al., 2018). However, it is important to note that this ability can also cause the opposite effect if beta-glucan creates a synergestic effect with other ingredients, showing similar behaviour to hydrocolloids (Sánchez, 2018). Due to the gel-like network formed, air bubbles may be trapped inside the structure and no longer have the possibility to expand during baking, resulting in a more compact and firm structure. This process is also related to a lower cake volume or reduction of the volume. (Sánchez, 2018) (Żbikowska et al., 2020).

The gel-like construction also creates a texture similar to that of fats, leading to a fuller and creamier mouthfeel (Onacik-Gür et al., 2016).

2.3 Theory behind Analysis methods

2.3.1 Texture Analyzer TA-XT2

Texture is a multi-parameter attribute, meaning it considers more than one characteristic. Texture analyses using testing instruments, such as a texture analyzer, can only be identified and quantified by particular parameters that need to be translated into sensory perception. (Szczesniak, 2002).

The principle of the texture analyzer relies on the relation between the force applied by the arm of the equipment and the deformation of the product, where the sample can change and undergo a deformation. The change of the product is registered by a probe which transmits the information to the force transducer, which can translate the information into the different texture parameters: hardness, chewiness, fractures, springiness, firmness, toughness, cohesiveness, fibrousness, gumminess, stickiness, resilience, yield, extension and gel strength. (Liu et al., 2019). A method that describes the relationship between these texture characteristics and the physical measurements of the samples is Texture profile analysis (TPA). This process and the characteristics are used to represent a realistic imitation of biting into a product, presenting a force-time graph as presented in Figure 1. (Yusof et al., 2019).

2.3.1.1 Characteristics

Hardness, Springiness, Cohesiveness, gumminess, chewiness and resilience are the parameters measured and used to identify the texture of food products. These characteristics can be classified into primary and secondary parameters. Primary parameters are derived from the force-time graph, and secondary parameters are calculated based on the primary parameter values.

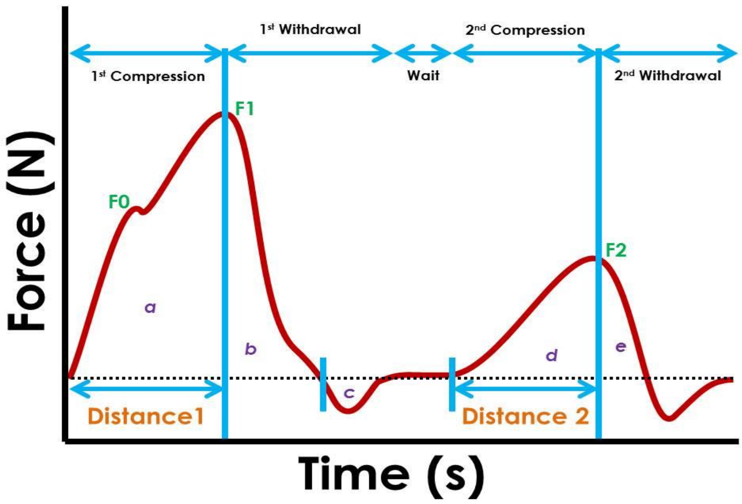

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Figure 1 TPA graph: Hardness (F1), resilience, springiness, cohesiveness (d/a), gumminess (hardness x cohesiveness), chewiness (gumminess x springiness). (Centre for Industrial Rheology, 2023)

Hardness
Hardness (H), a primary parameter, is explained by the force necessary to reach a given deformation. When describing the hardness of a product, the terms soft, firm and hard can be used. The hardness, in Newton, can be determined by the maximum force accessed through the first time of compressing. In the graph, hardness is represented as F1. (Szczesniak, 2002b).

Resilience
Resilience, a primary parameter, is described by a product's power to return to its original height after being deformed by a specific force and speed for the first time. The resilience is determined by dividing the area under the curve of the first compression after the peak force is reached by the area under the curve before the peak force is reached. (Singh et al., 2019). It is important to set enough time for sample recovery during the first and second compression measurements. A slower test speed can, in this case, be more beneficial than a higher test speed.

Springiness
The springiness (S), a primary parameter, is described as the rate a product turns back into its original shape after being deformed by a specific force for the second time. The springiness is inversely proportional to the hardness of a product. Springiness can be determined by measuring the height of the cake after the second time of compressing. When explaining the springiness of a product, the terms plastic and elastic can be used. (Szczesniak, 2002b).

Cohesiveness
Cohesiveness can be explained by the connection between the strength of the internal structure of a product and the ability to which a material can be distorted before breaking. Cohesiveness is often correlated to brittleness, chewiness and gumminess (Yusof et al., 2019). The cohesiveness (C = d/a) can be determined by taking the relation of the positive force area during the second time of compressing to the positive area during the first time of compressing.

Gumminess
Gumminess (G) is a secondary parameter, and is described as the energy necessary to break up a semi-solid product to a case suitable for swallowing. The gumminess is calculated by the hardness value times the cohesiveness value (H x C). A product with a gummy structure has a lower value for hardness and a higher value for cohesiveness. (Szczesniak, 2002b).

Chewiness

Chewiness, a secondary parameter, defines the energy necessary to masticate a solid product to a case suitable for swallowing. (Yusof et al., 2019). Chewiness can be calculated by the value of gumminess times the value of springiness (G x S) and is therefore also correlated to the product's hardness.

2.3.1.2 Characteristics in sponge cake

Favourable factors for sponge cake are cohesiveness, springiness and chewiness; the higher the value, the more these properties are characterised. These characteristics indicate a light and fluffy texture. Cohesiveness and springiness are connected to developing the three-dimensional protein network formed during baking. Therefore, a higher value for these characteristics is connected to a favourable internal bonding of the protein network. (Sharma et al., 2018). Resilience, gumminess, and hardness are the acceptability factors of sponge cake. A low value for hardness, gumminess, and a high value for resilience is considered acceptable regarding sponge cakes. A high resilience is like cohesiveness and springiness, often related to the light and fluffy structure of the sponge cake; a low resilience would give rise to a dense and heavy cake. Hardness is connected to the development of retrogradation and a dense cake. High gumminess can result in a sticky and heavy cake. Furthermore, chewiness is dependent on the firmness, where often a similar trend can be observed in the TPA analysis (Sánchez, 2018). The TPA results are often compared with the results of sensory experiments to give more meaning to acceptability, focusing on acceptability for consumers. (Sharma et al., 2018).
The texture characteristics of sponge cakes are also close related to the volume and density of cakes. Previous research has found that volume can have an inverse relationship with hardness. Meaning the smaller the volume the harder the cake. (Żbikowska et al., 2020). Furthermore, springiness was shown to be associated with the number of gas cells in the crumb, important for the determination of the density. When the springiness decreased, the number of gas cells decreased leading to a denser structure of the cake. (Rodríguez-García et al., 2014).

2.4.2 Volume meter

The Bread volume meter (BVM volume meter, Delta) is a testing equipment developed for the baking industry to analyze the main properties of ingredients, mixtures, dough and baked goods.

The volume meter can determine the volume (mL), Height (mm), Width (mm), Depth (mm), Specific volume (mL/g), Density (g/mL), Weight (g), Max Diameter (mm) and the Shape ratio (height/width) of the baked products, all within one measurement. The volume meter is connected to the program VOLCALC. It relies on the action of a laser that creates a crescent around the rotating object, resulting in a 3D structure of the object and the connected named properties.

2.4.3 Oven drying method

The moisture content can be determined using various oven-drying methods. The principles of the oven drying method rely on releasing and removing moisture from a material by heating it under specified conditions until a predetermined moisture content level is reached. The amount of moisture determined differs per type of oven and the set conditions within the oven. Furthermore, the temperature and drying time depend on the sample's size, shape and material. ("Food Analysis", 2010).

2.4.4 Water activity

Water activity (aw) measures the water molecules available for microbial growth and chemical reactions in food and is defined by the rate of vapour pressure of water in a food product and the vapour pressure of pure water, both measured at the same temperature and pressure. The water activity for pure water is 1.0, and the water activity for most foods is less than 1.0. ("Food Analysis", 2010).
Water activity instruments are designed to determine the water activity of a food product by reading and measuring the vapour pressure of the sample. The AQUALAB instrument uses a chilled-mirror sensor

with a dew point to fulfil this measurement. (*AQUALAB 4TE Accurate and reliable water activity meter*, 2023).

2.5 Statistical analyses

Matlab is a platform used to analyze statistical data, make algorithms and creating matrixes on a programming and numeric language. In this way statistical output of experiments can be combined into a script together with codes and formatted text. Furthermore, data can be visualized and explored with graphics. (Matlab - MathWorks, z.d.). Matlab was used for analyzation of the results on variance (ANOVA), Kruskal-Wallis test and on Principal Component (PCA).

ANOVA is an one-way analysis method commonly used to test significant differences between two or more groups. This analysis assumes that the data is normally distributed and variances are equal. The variation of both within and between groups is determined and calculated, using a F-test which indicates whether the variation between the group means is larger or smaller than the variation within the groups. An alternative to the ANOVA test is the Kruskal-Wallis test, which is a non-parametric test that can be used to compare three or more independent groups measured on different variables. The Krustal-Wallis test is used when there is a non-normal distribution of the variables, meaning normality and homogeneity of variances are not met. This test relies on ranking values of dependent variables in different groups. The ranking is then compared between the groups to indicate whether there is a significant difference between groups. (Analysis of variance (ANOVA) results - MATLAB - MathWorks Benelux, z.d.)

PCA is a multivariate analysis method used to compare relations between variables. For PCA, the data is transformed to allow variables with different magnitudes and units to be compared side-by-side in the same figure. (Principal component analysis of raw data - MATLAB pca - MathWorks Benelux, z.d.).

3. Material and Methods

The project was carried out at Lund University, Kemi Centrum. The materials and equipment used were mostly provided by Lantmännen Cerealia, Lantmännen Oats AB and Lund University.

3.1 Sponge Cake Preparation

Sponge cakes were prepared according to the recipe by Richardson (2003), with addition to the amount of beta-glucan added. The recipes are presented in Table 2. PromOat® Beta-Glucan (35% beta-glucan, 3.0% moisture) was provided by Lantmännen Oats AB, Kimstad. Wheat flour and wheat starch were supplied by Lantmännen Cerealia AB (Malmö, Sweden), Colco gel-like emulsifier by Bakels Sweden AB (Göteborg, Sweden), Fresh whole eggs, Sucrose and Baking powder by ICA supermarket, Sweden. The sponge cake batter was enriched with 0.0 (reference), 1.0 or 2.0% of beta-glucan.

Table 2 Ingredient list for all treatments used for the production of the spongecakes. PO= PromOat beta-glucan.

INGREDIENT %(W/W)	REFERENCE 0.0% PO	RECIPE 1.0% PO	RECIPE 2.0% PO
FRESH EGG	23	23	23
WATER	16	16	16
SUCROSE	27	27	27
EMULSIFIER	2.0	2.0	2.0
WHEAT FLOUR	27	26	25
WHEAT STARCH	4.0	4.0	4.0
BAKING POWDER	1.0	1.0	1.0
PROMOAT BETA GLUCAN	-	1.0	2.0

A large mixer (Varimixer, 1987R20) with a wire whip included was used to beat the eggs and mix the emulsifier for 1 minute on high speed (85%). Sugar was then added simultaneously within 30. seconds and mixed at the same high speed. Baking powder, wheat flour and wheat starch were manually mixed together in a small bowl and sifted to prevent the mixture of clumps. For the reference recipe, it continues by adding half of the mixed dry ingredients into the mixture with half of the water for 30. seconds on middle-high speed (55%) to fold the dry ingredients into the batter. A quart of the dry ingredients and the remaining water is then added and mixed again for 30. seconds on middle-high speed. For the recipes with beta-glucan, a quarter of the dry ingredients are manually mixed together with the beta-glucan and sifted into a small bowl. This mixture is then manually mixed with a part of the water to dissolve the beta-glucan before adding it to the mixture of eggs, emulsifier and sugar. The beta-glucan mixture is then added and mixed for 30. seconds at middle-high speed. Together with another quarter of the dry ingredients, the remaining water is added and mixed for another 30. seconds on middle-high speed. For all recipes, the last part of the dry ingredients is manually added and mixed using a rubber spatula. Part of the batter was measured for density.

Round baking rings (24 cm diameter, RVS) were filled with $50.*10^{\wedge 1}$ g (± 2.0g) cake mix and baked at 180ºC (± 1ºC) for 25 minutes in an electric oven. The cakes were left to cool at room temperature for ±1.5h. After cooling down, the cakes were packed in a plastic bag (Polyamide (PA) / Polyethylene (PE), 30.x40. cm), heat sealed and stored at 20.ºC (± 1ºC). The first cakes were measured on volume, texture, Water activity and moisture content after keeping them overnight. Other cakes were stored for 3, 7, 10 and 14 days before being measured on the same characteristics. A total of 4.0-5.0 cakes were evaluated per measurement day. In total, ± 20-30 cakes per recipe were made. Each recipe was made on a separate day. The cakes made on one day were divided over two-three batches of batter, where each batter delivered around 10 cakes. This led to 4-5 samples (replicates) that could be investigated on each measurement day.

3.2 Batter quality measurement

3.2.1 Density

The density of the batter was determined after mixing. A cup of known weight (±13g) was filled with batter. The batter was levelled off using a rubber spatula and weighed (W1). The same cup was then filled with distilled water and weighed again (W2). The volume of the water (V1) was now calculated by dividing the weight by the density (P1) of water (W2/P1), which represents 1.0 g/cm3. The batter density was now calculated by dividing the batter's weight by the water volume (W1/V1). Each batch of batter was tested in triplicate, and the average was calculated.

3.3 Cake quality measurement

3.3.1 Specific volume

The volume of the cakes was analyzed using a volume meter (BVM volume meter, Delta). In the program, Volcalc all settings are filled in to start the measurement. First, the machine is calibrated using a weight, followed by weighing the whole cake on a scale. Continuing by placing the cake on the pin with a flat support plate (100mm), connecting it to the machine, and starting the program. The laser creates a crescent around the rotating cake, determines the parameters and visualizes a 3D structure of the cake. The following settings were used during the experiments: Test duration: 60.s, finishing position: 90.°C, laser distance: medium, custom arm distance: 50. mm, Rotation speed: 470 /s, sampling rate: $60*10^{\wedge 1}$ s/sec, object height: 6.0 cm, object width: 24. cm and smoothing factor 5. The analyses for each cake were performed in duplicate.

3.3.2 Sampling

The moisture content, texture analyses and cake structure samples are prepared after measuring the specific volume. The cake is then cut in half. For both sides of the cake, a section of cake with a length of ±7.5 cm and a width of ±2.0 cm is cut out from the side of the cake, facing the side where the cake was sliced in two. This part is later used to observe the cake structure with a camera. For the moisture content and the texture analyses, blocks of 2.5 cm^3 without crust were taken from the same side, in the middle of the cake. This was done for both sides of the cake, giving six cubes of 2.5 cm^3 per cake. The blocks and the slices were stored in a bag together until the measurements were carried out. When cutting out the cake, all leftovers were collected in a separate bag and later used to determine the water activity.

3.3.3 Moisture Content

The moisture content was conducted following the drying oven method (AOAC, method no. 934,06).. Aluminium plates were dried for 1 hour at 100°C, stored in a desiccator to cool down for 30. minutes and weighed afterwards (Wa). After this, the cake samples were placed onto the aluminium plates and weighed (W1). Then the samples were heated at 105°C for four hours inside the drying oven (Salehi et al., 2016) and stored for 30. minutes in a desiccator to cool down. After cooling, the samples were weighed again (W2-Wa). The moisture loss (A) was calculated by the weight of the sample after the drying oven minus the weight before the drying oven (W2-W1). The moisture content was calculated by moisture loss (A) divided by the original weight (W1) of the sample and multiplied by 100% (A/W1*100%). The analyses per cake were performed in duplicate.

3.3.4 Texture analyses

The texture profile of the cakes was calculated using a texture analyzer (TAX-XT2I). The sample project TPA.PRJ was selected to analyze the texture profile of the cake. First, the weighed and height of the machine were calibrated. The cake samples were then placed on a flat stage and subjected to a double compression of 50.% of the original height using a 25.-mm aluminium probe. The following settings were used to establish the current compression: Pre-test speed: 2 mm/s, Test speed: 1 mm/s, Post-test speed: 5.0 mm/s, Strain 50.%, trigger force: 5.0 g, and returning height: 3.5 cm. The time between the compressions was set to 20. seconds. All results are connected to the TPA program and visualized in a table and graph. The analyses were performed in quadruplicate per cake.

3.3.5 Water Activity

Water activity was measured using the Aqualab water activity meter and following the equipment protocol. The water activity meter was calibrated before use. The samples were prepared in a plastic cup. Several pieces of cake from different places, with and without crusts, were taken to present a representative cake sample and placed into the plastic cup. The plastic cup was then placed in the water activity meter and analyzed. All analyses were performed in quintuplicate.

3.3.6 Cake structure

A digital camera (Nikon DX AF-S NIKKOR 18.-55.mm 1:3 5.0-5.6GII ED) was used to take pictures of a cake slice to observe the cake structure. The cake slice was set next to a ruler to indicate the dimensions. The settings of the camera were set on: Camera's sensitivity (Iso): 400, Maximum aperture (F): f/6.3 - f/7.1, Shutter speed (E): 1/125 s, Focal length (FL): 28. mm and Exposure value (EB): 0 EV.

3.4 Statistical analyses

3.4.1 Significance between variables

ANNOVA

Matlabs' ANOVAs were used to evaluate the statistical difference between beta-glucan treatments, batch, replications, days and resting time (independent variables) for each of the dependent variables (volume, density of the batter and cake, water activity, moisture content, hardness, cohesiveness, resilience, gumminess, chewiness, springiness). This test gives the p-values to indicate the significance and presents the data distribution (normal/non-normal). P-values lower or equal to 0.05 (95.% confidence) indicate a significant difference, and P-values higher than 0.05 show no significant difference.

Regression

Regressions were used for further comparisons between variables using Spreadsheet and Excel. This involves using a plot with a regression line to determine the strength of the relationship between two variables. An R2 value was used as an indication of this relationship. A value between 0.5 and 0.7 indicates a moderate connection, and a number higher than 0.7 shows a strong bond. (Correlation Coefficients, z.d.).

3.4.2 Posthoc tests

Variables of ANOVAs were indicated by colour coding. This colour coding shows whether there is a difference between the groups and which one exactly.

3.4.3 PCA

Matlabs' Principal Component Analysis (PCA) was used to observe relations between all the variables in one graph visually. The PCA figure explains which variables are closely connected and which are not by looking at how they are placed in the graph. Symbols closely located to one another are correlated, and symbols far away from each other are not. Symbols on opposites of one another and diagonal are negatifly related. Symbols farther from the centre have a larger influence in the experiment than symbols close or in the middle. The parameters were classified into independent (Treatment, Replicates, Days, Batch, Resting) and dependent (Hardness, Springiness, Cohesiveness, Gumminess, Chewiness, Resilience, Volume, Moisture content, Water Activity, Density batter, Density cake) groups.

Supervisor Stephen Burgleigh provided all statistical codes used for MATLAB.

4. Results

4.1 Significant Relationships of independent variables

Table 3 presents the values for the significant differences between the dependent variables compared to the independent factors and also shows the data distribution type (normally distributed or not). The results show that all of the dependent variables were significant for treatment, indicating that beta-glucan levels were of influence here. The independent variable batch also showed multiple variations with dependent variables, suggesting differences between batches in the same group, which is an undesirable and unlikely outcome. Lastly, Day was, to a limited extent, associated with dependent variables and Resting and Replica were more or less not associated with the variables analyzed. The relationship between these independent variables and the dependent variables will be explained in more detail in the rest of Chapter 4.

Table 3. Significant values for the dependent factors, P-values <0.05 or 0.05 = Significant difference and P-values >0.05 = No significant difference. The significant numbers are marked red. Normality: Y=yes, N=no.

Parameter	*Normality*	*P-value Treatment*	*P-value Replica*	*P-value Day*	*P-value Batch*	*P-value Resting*
Hardness	Y	0.1e-01	5.3e-01	<0.5e-01	7.7e-02	2.5e-01
Springiness	Y	0.2e-07	6.4e-01	1.8e-01	1.3e-02	0.3e-03
Cohesiveness	Y	0.3e-01	1.8e-01	1.3e-01	6.8e-01	6.2e-01
Gumminess	Y	7.9e-06	7.2e-01	<0.5e-01	5.3e-02	0.3
Chewiness	N	2.9e-07	7.1e-01	<0.5e-01	4.74e-02	0.1
Resilience	N	2.4e-03	4.5e-01	<0.5e-01	1.7e-01	0.4
Volume	Y	4.1e-10	7.9e-01	6.9e-01	3.1e-03	0.2
Moisture content	Y	1.9e-06	8.7e-01	<0.5e-01	2.9e-03	9.5e-01
Water activity	Y	5.6e-07	2.9e-01	5.9e-01	0.1e-03	0.3
Density Batter	Y	1.1e-17	6.6e-01	3.2e-01	<0.5e-01	9.6e-03
Density cake	Y	3.6e-09	9.4e-01	7.4e-01	1.2e-02	1.5e-01

An example of how the ANOVA test is conducted for one of the variables (hardness) with MATLAB can be seen in Figure 2. Here, the source represents the subjects to which the hardness is compared, Sum Sq. is the sum of squares and describes the measure of the deviation from the mean, d.f. The degrees of freedom indicate how many independent pieces there are. Mean sq. is the mean square, F is the F-value and Prob>F represents the probability that the null hypothesis is true. Values below or equal to 0.05 are considered significant.

Analysis of Variance

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob>F
Rep	8697.1	4	2174.28	0.81	0.5277
Day	262600.1	4	65650.01	24.38	0
Batch	24491	4	6122.75	2.27	0.0777
Resting	3621.4	1	3621.45	1.34	0.2529
Error	110414.9	41	2693.05		
Total	529586	54			

Figure 2. Example of the ANOVA test for hardness

4.2 Treatment

Treatment describes the difference in PromOat beta-glucan percentages (0.0%, 1.0% and 2.0%). The variables with significant differences in table 3 obtained from the ANOVA test were compared to the independent variable Treatment. The results indicate a significance between treatment and all dependent factors except.

4.2.1 Volume and Density of the cake

The cakes were evaluated on volume, and a significant difference was observed between treatments (table 3). The reference had the highest values for volume during all storage days, as seen in Figure 3, which shows the average of all values for volume per treatment and storage day. The lowest volume was evaluated for the cakes fortified with 2.0% PromOat beta-glucan (BG2). Figure 3 shows an R^2 value, the degree of correlation, of 0.4 for the reference, which indicates a low relationship between storage days and volume. For 1% PromOat beta-glucan (BG1) and BG2, the R2 value was lower, indicating little correlation between volume and storage days. When comparing volume against treatment, an R2-value of 5.4e-01 was obtained, indicating a moderate connection.
The overall decrease in the volume from the first measurement day (day 1) and the last measurement (day 14) was revealed to be around -16% for the reference, -5.8% for BG1 and -3.2% for BG2. These results demonstrate a more noticeable reduction for the reference and the lowest decrease over time for BG2. Nevertheless, the end volume for the reference was observed to stay the highest. The volume decreased with increasing beta-glucan percentage.

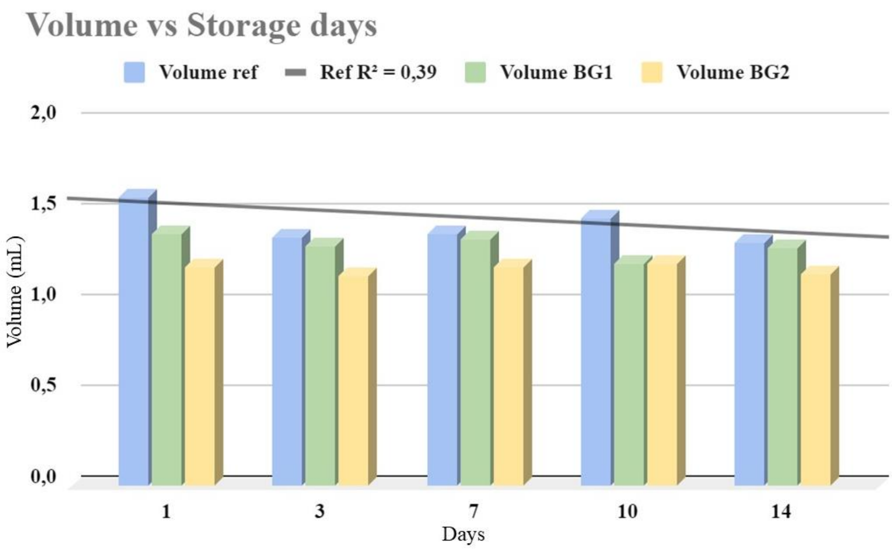

Figure 3. The volume of the cake for the three treatments over the storage time. R^2=0.4.

4.2.2 Density of the cake

The volume and the density were negatively related in this study. The density of the cake increased with increasing beta-glucan percentage, as seen in Figure 4. The density of the cake was also significant to treatment, indicating a significant difference between all treatments.

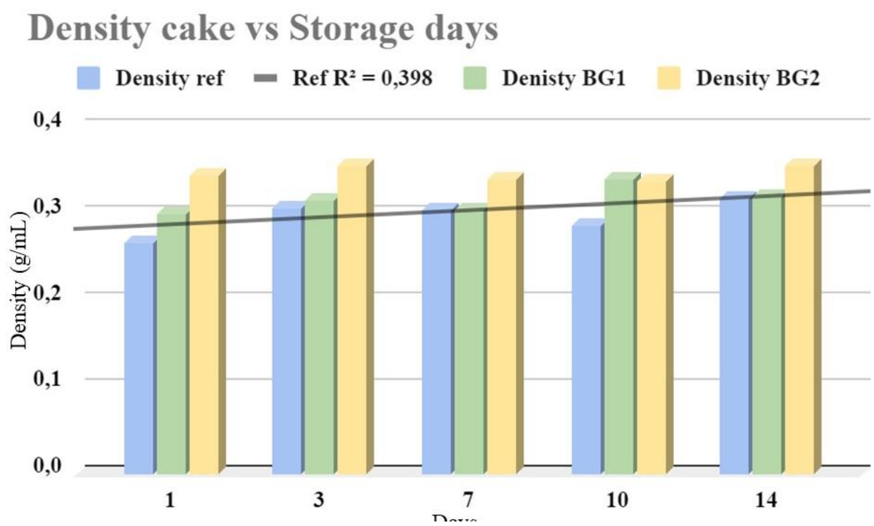

Figure 4. The density of the cake for the three treatments over the storage time. R^2=0.4

4.2.3 Density of the batter

The density of the batter was found to be significant for treatment and batch. The mean values of the batches of each treatment are presented in Figure 5. The data is not linear with increasing beta-glucan percentage. BG1 represents the cakes with the lowest density for the batter, whereas this was not the case for the density of the end product, as seen in Figure 4.

Figure 6 displays the figure of significant difference between the two variables batch and density of the batter obtained from the post hoc test. The colour blue and red indicate that there are significant differences between the values. The grey-coloured lines refer to no significance between the values. From these results, batch 2 differs significantly from all the other batches. All other batches showed no significant differences from one another.

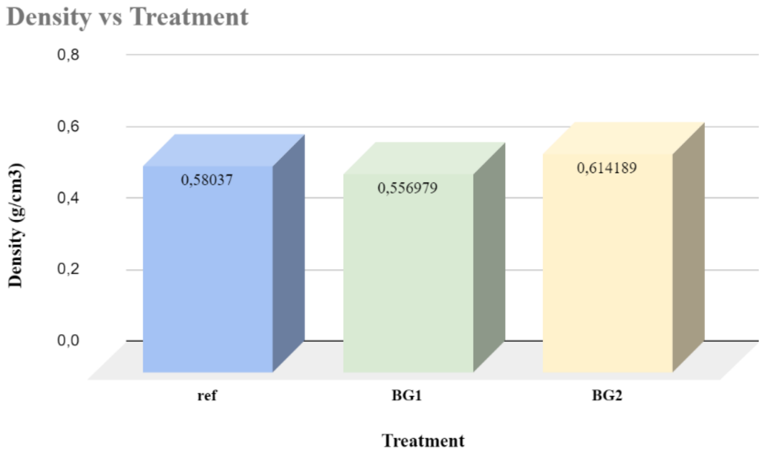

Figure 5. A comparison of the density of the batter for each treatment

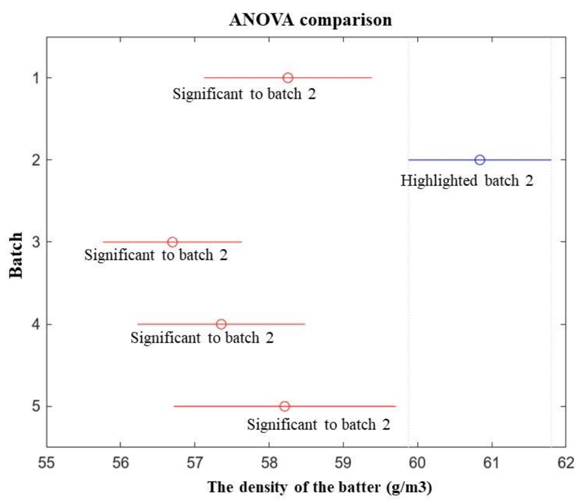

Figure 6. ANOVA test: significant differences between the density of the batter within correlation to batch.

4.2.4 Moisture Content and water activity

The moisture content for each treatment throughout the storage time is presented in Figure 7. The moisture content is significant to treatment and increased with increasing beta-glucan range. The R^2 value for the reference gave a value of 0.8, indicating an important relationship between the moisture content and storage days.

The moisture content was also significant to days. Figure 8 presents the significant differences between the moisture content of the treatments over storage time and highlights the significant differences between day one and days 7, 10 and 14. Day 3 is solely significant to day 14, and all other days are not significant to one other. Overall, the moisture content was the highest for the cakes with 2.0% PromOat beta-glucan. This is most noticeable on day 1, where the reference had a moisture content of about 34%, BG1 37% and BG2 39%. The moisture content for BG1 and BG2 had decreased the most over time with -12,4%. The moisture content on day 14 was about 31% for the reference, 33% for BG1 and 34% for BG2.

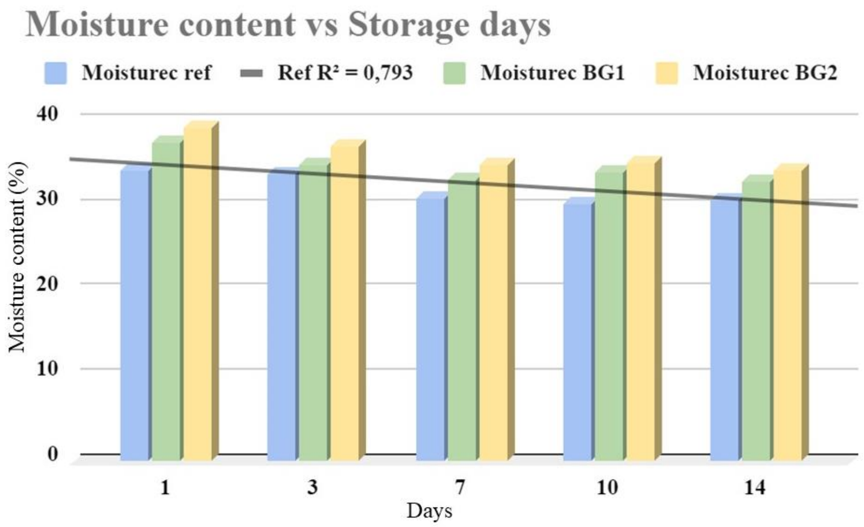

Figure 7 Moisture content of the different treatments over the storage time. R^2= 0.8

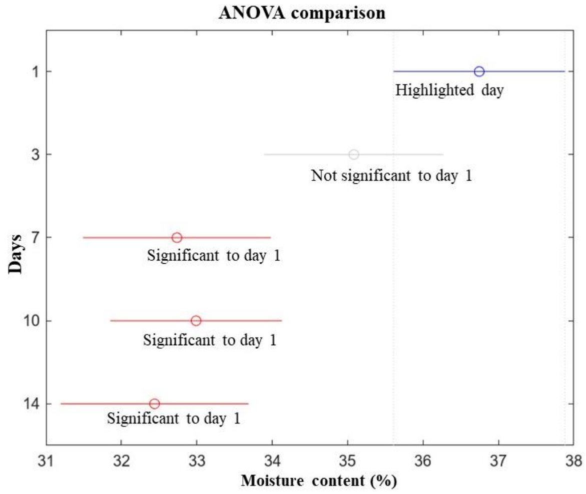

Figure 8. ANOVA test: significant differences between the Moisture content (x) over storage days (y)

Water activity was found to be significantly different to treatment. The water activity was found to be not linear to increasing beta-glucan amounts. Nevertheless, the water activity was higher for the cakes fortified with beta-glucan than for the reference without beta-glucan, as seen in Figure 9, showing similar behavior to the moisture content.

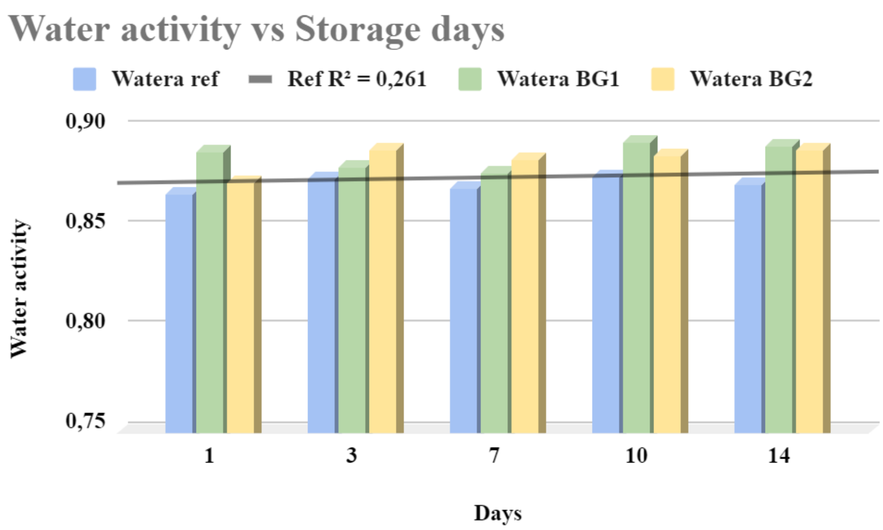

Figure 9. The water activity of the cake for the three treatments over the storage time. R^2=0.3

4.2.5 Springiness and Cohesiveness

The springiness and cohesiveness are both significant and positively correlated to treatment. The scatter plot in figure 10 presents the results for springiness throughout the storage period. The springiness of the cakes decreased after day 3 for all treatments. After day 7, the values for springiness started to increase for BG2 and the reference, although a decrease was shown again for the reference after day 10. BG1 started increasing again after day 10. BG2 showed the highest values for springiness. Given previous contradicting results for the volume and density of BG2, this was an unexpected result

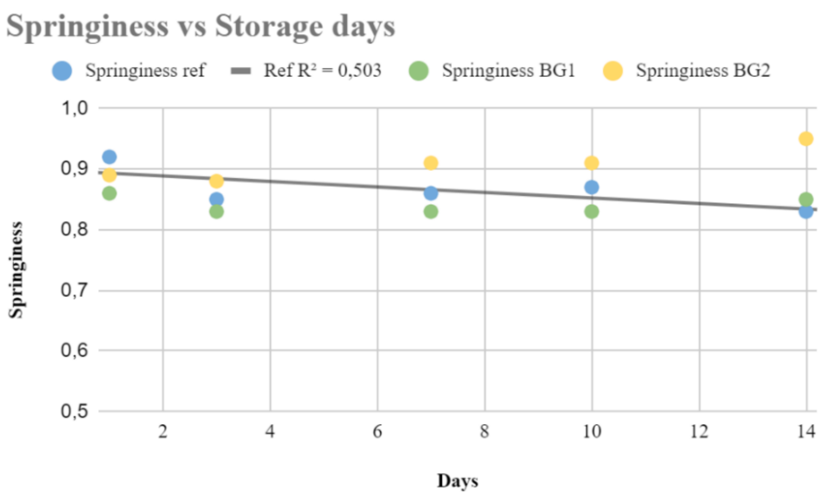

Figure 10. The springiness of cakes throughout the storage days for all treatments. R^2=0.5

In all treatments, the values of cohesiveness were decreased after three days. Nevertheless, after ten days of storage, the cohesiveness values increased for BG1 and after 14 days for the reference and BG2. The highest values were obtained for BG2. However, the reference showed the most significant decrease between day one and day 14.

4.3 Storage days

The influence of storage days on the cake characteristics was investigated using the significant values obtained from the ANOVA . From these results, it can be observed that hardness, gumminess, chewiness, resilience and moisture content are significant to days. For hardness, gumminess and chewiness, the association with storage days is positive, and for moisture content and resilience strongly negative.

4.3.1 Hardness

The hardness of all measurements is normally distributed and significantly correlated with storage days. Figure 11 illustrates the development of the hardness (N) of the cakes over time. The values for the hardness in the figure are averages of all samples per measurement day and are plotted against the storage days. The figure also depicts the linear line for the reference with the corresponding R^2 value of 0.9, implying there is a strong relationship between hardness and storage time.
Hardness was also significant to treatment and showed a negative relation. The overall increase in the hardness was 110% for the reference, 95% for BG and 79% for BG2. These results indicate that as time passed, the hardness for the reference and BG1 increased more rapidly than it did for BG2. The highest values were observed to be for BG2 for all storage days. However, the post hoc tests showed only a significant difference in hardness between BG1 and BG2, as seen in Figure 12.

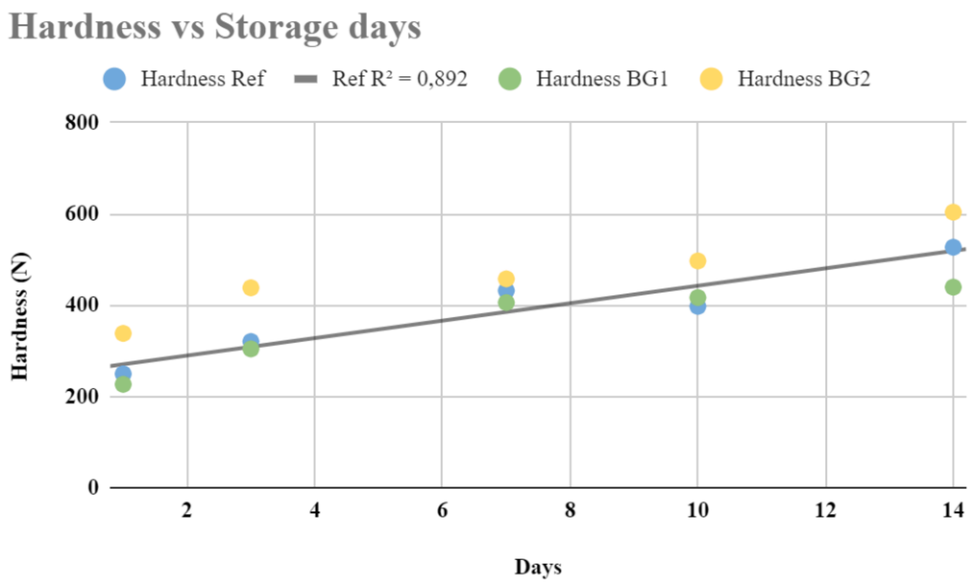

Figure 11. Scatter plot of the hardness throughout the storage time. R^2=0.9

Figure 13 presents the significant difference in hardness between the storage days. These results show significance for day one and all other days and for day three and all other days. Day 7 and day 14 solely have no difference from day 10. This indicates a large number of variations in hardness compared to storage time.

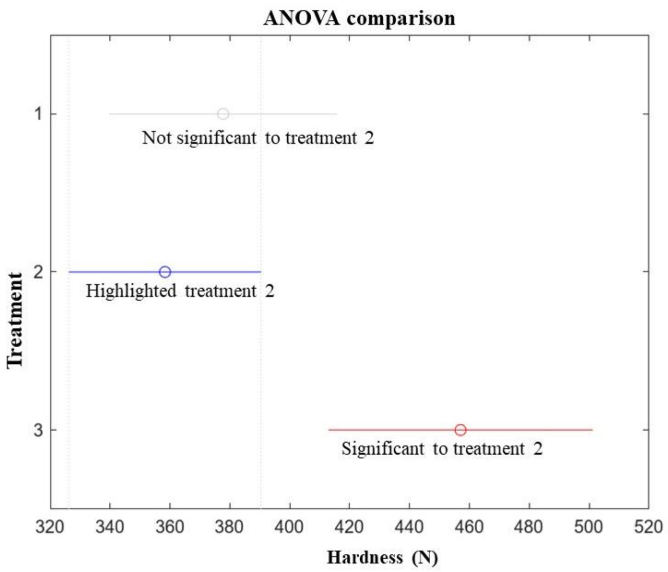

Figure 12. ANOVA test: significant differences between hardness and treatment

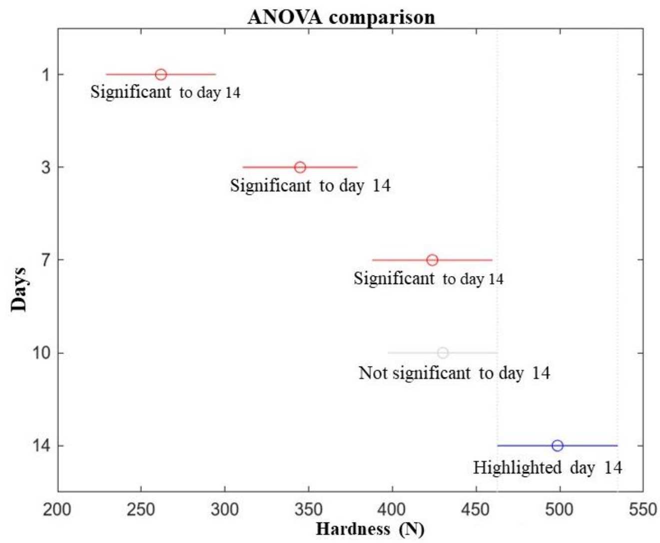

Figure 13. ANOVA test: significant differences between hardness and storage days

4.3.2 Gumminess and Chewiness

The results for gumminess and chewiness showed similarity to the results of hardness. Both parameters increased with increasing storage days and were observed to be the highest for BG2. BG1 led to an increase in the slowest and had the lowest gumminess and chewiness at the end of the experiment.

4.3.3 Resilience

The resilience is non-normal distributed; therefore, the statistical Kruskal Wallis test should usually be carried out. However, an ANOVA test was used to compare the results in this case. Figure 14 represents the development of the resilience of the cakes over time. The values in the figure for resilience are the average of all samples per measurement day. In addition, a linear line for the reference is shown with an R2-value of 0.9, demonstrating a strong correlation. The figure also presents the overall decrease in resilience over time. BG2 showed the highest resilience values, whereas the reference and BG1 showed similar lower results. The reduction in resilience was the highest for the cakes fortified with beta-glucan when compared to the reference.

The resilience shows a significant difference for days and treatment. The significance of resilience for the storage days was significant between days 1 and 3 and all other days, and there was no significant difference between days 7, 10 and 14, as seen in Figure 15.

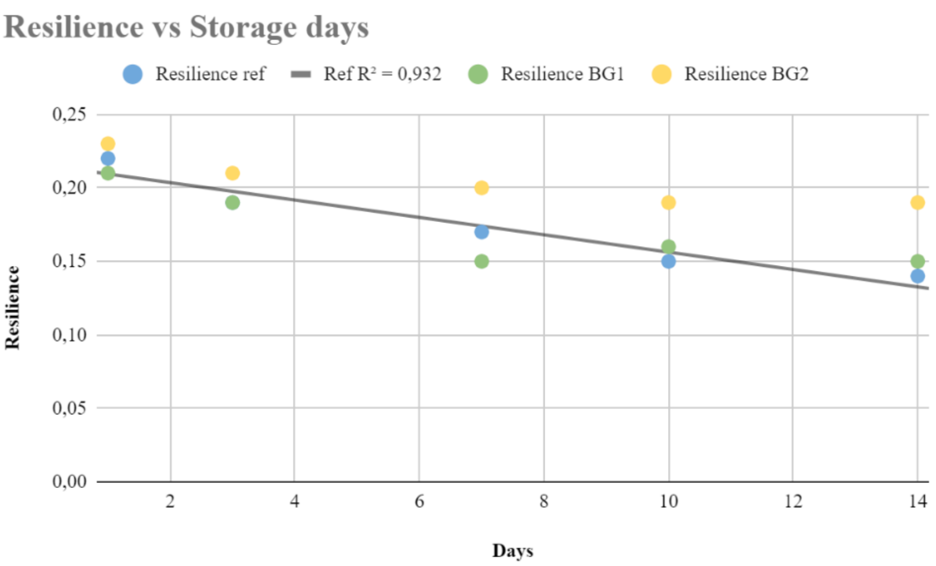

Figure 14 Scatter plot of resilience throughout the storage period for all treatment. R^2=0.9

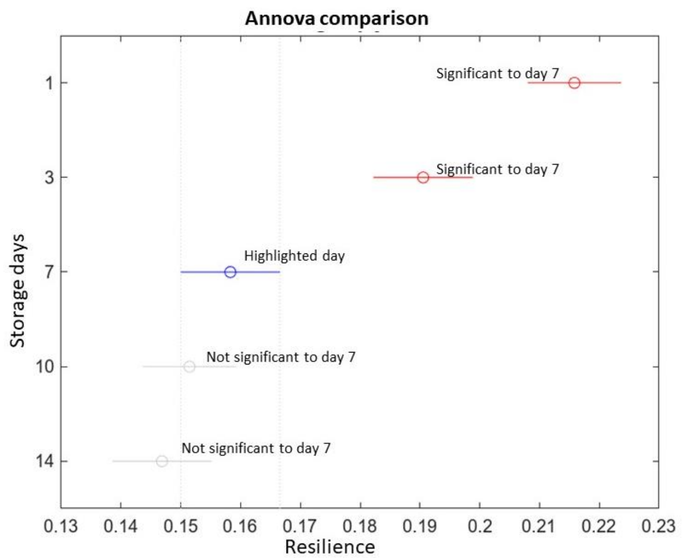

Figure 15. ANOVA test: significant differences between resilience (x) over storage days (y)

4.4 Bubble size

The size of the bubbles was not calculated but observed from photographs taken with a Nikon camera. Figure 16 depicts these images alongside each other and arranged by treatment and day. The exact bubble size cannot be observed from these images; however, general differences can still be noticed. The reference and BG1 show the most similarity between the three treatments, with several large and small bubbles. BG2, on the other hand, represents a finer structure, with many small gas bubbles and few to no large bubbles. Moreover, the reference structure slightly changes as the days pass, unlike BG1, where a clear decrease in larger gas bubbles can be observed between the first and last day. For BG2, a reduction in height can be observed slightly.

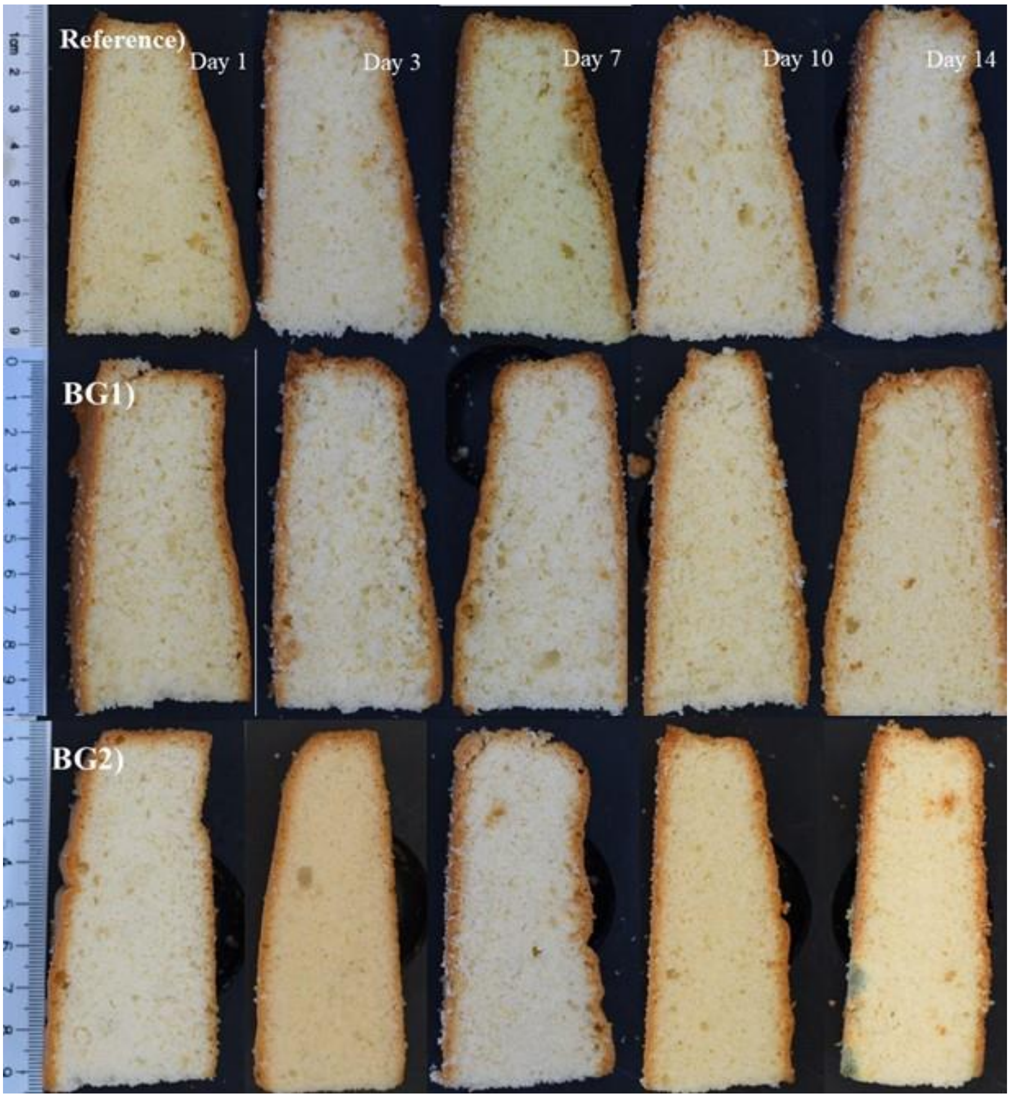

Figure 16. Cross-section of cakes by treatment and storage day for observation of the bubble size.

4.5 PCA

A multivariate PCA analysis was conducted on all measured variables to visualise all data in one figure. Figure 17 illustrates the corresponding PCA plot of the first two components. The scree plot in figure 18 shows that the first two components cover around 57% of the total.

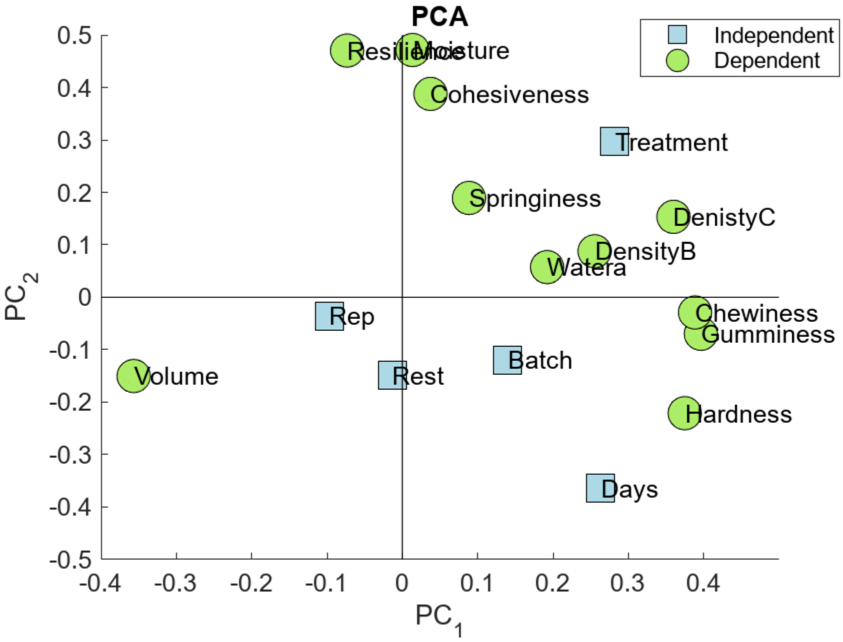

Figure 17. PCA model plot of comparing independent and dependent factors.

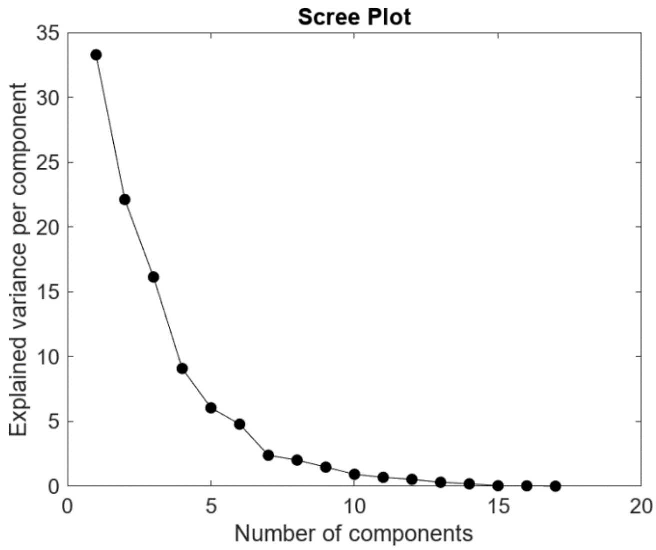

Figure 18. Scree plot based on PCA

The PCA plot confirms the relations of Treatment to the dependent variables. Treatment is placed in the middle of the positive end of PC1 and PC2, with many dependent factors around it, indicating a close relationship. This relation is positive, according to the placement of resilience, moisture content, springiness cohesiveness, water activity, and density of the batter and cake. This information is consistent with the post hoc and regression figures displayed in Chapter 4.2. Moisture content, water activity and cake density increased as the percentage of beta-glucan increased, showing a positive correlation. For resilience and cohesiveness, this relationship is more difficult to discern from the figures displayed in the previous chapters because the variables differ over the retention time. Nevertheless, all factors showed an increase in the order of increasing beta-glucan percentage at the end of the storage period.

Volume was negatively related to Treatment as it was oppositely located and on the diagonal to Treatment. For volume, this relationship was also visible in the regression figures in Chapter 4.2, where the volume decreased with increasing beta-glucan value indicating a negative correlation.

The relation of hardness, chewiness and gumminess with Treatment was observed to be not positive or negative. This relation is probably due to the position of days, which has much influence on these three factors and therefore tends to pull them to the negative end of PC2 in the PCA Plot. This, therefore, immediately affirms the relationship between days, hardness, chewiness, and gumminess, as reported in section 4.3.

The PCA plot also explains the same negative relationship between days and resilience: resilience decreased with increasing storage time. Moreover, the independent variable batch is also more off-centre, indicating that it influences the dependent variables.

4.6 Additional information

What was beyond the scope of the study but followed as an additional result was mold growth on the cakes. Mold growth for the reference was first observed on day 14, for BG1 on day ten and BG2 on day 7. However, days, when no measurements were taken, were not counted, so mold growth may have started earlier. Still, it is interesting to record the difference in the days evaluated.

5. Discussion

This experiment was conducted to investigate the effects of different PromOat beta-glucan percentages (0.0%, 1.0% and 2.0%) on improving and maintaining the desired structure and physical properties in sponge cakes over two weeks of storage. Different cake characteristics were measured and collected for a clearer understanding of whether beta-glucan truly had a positive effect on cake characteristics.
The results showed that the most significant variations could be seen for Treatment, Batch and Days. The difference in beta-glucan percentage was found to be positively related to an increase in hardness, gumminess, moisture content, water activity and density of the cake, and related to the decrease in volume, cohesiveness and springiness. Furthermore, the storage time was evaluated to have the largest influence on hardness, chewiness and gumminess. Table 4 presents a summary of the results per treatment and storage time. The first column represents the variations because of the increasing beta-glucan percentage compared to the reference. The second column describes the changes over time between day 1 and day 14. Values without a linear increase or decrease are presented as fluctuations. Lastly, the third column shows the significance with batch.

Table 4. Visualised summary of the results obtained from the experiment, Fluct=Fluctuation, ^= Increase, ∨ =Decrease. Significant: Y=Yes, N=No.

Parameters	***Increasing beta-glucan %***			***Increasing storage time***			***Batch***
	Ref	*BG1*	*BG2*	*Ref*	*BG1*	*BG2*	*Significant*
Hardness	-	v	^	^	^	^	Y
Springiness	Fluct.	^	^	v	v	^	Y
Cohesiveness	-	^	^	v	v	v	N
Gumminess	Fluct.	^	^	^	^	^	Y
Chewiness	-	v	v	^	^	^	Y
Resilience	-	^	^	v	v	v	N
Volume	-	v	v	v	v	v	Y
Moisture content	-	^	^	v	v	v	Y
Water activity	-	^	^	^	^	^	Y
Density batter	-	v	^	-	-	-	Y
Density cake	-	^	^	^	^	^	Y

5.1 Treatment

5.1.1 Volume and density of the cake and related factors

In this study, volume and density had the expected inverse relation with each other, but showed a contrasting outcome in relation to increasing beta-glucan levels compared to the literature. Namely, the volume decreased with increasing beta-glucan percentage. Focusing on the decrease in volume, there could be several reasons for this.

The first reason is the connection between the volume and the size of air bubbles. In most cases, beta-glucan addition has a positive effect on volume retention. However, in some cases, as described in the literature review, a hindrance to the expansion of air bubbles can lead to a decrease in volume and can be expected if beta-glucan is added as an ingredient in bakery products, which was the case in this experiment.

Smaller gas cells and almost no larger air pockets were observed for B2G, as seen in Figure 16. This treatment also corresponded to the lowest volume and highest density for the cake and batter obtained throughout the storage time. The reference and BG1 showed similar crumb structures with smaller and larger cells. However, the cake volume did differ between the reference and BG1, with BG1 giving a lower volume over the storage period. The density of the batter was highest for BG2, and the reference and BG1 showed similar lower densities corresponding to the bubble structures in Figure 16. It is, therefore, possible that fewer bubbles entered the batter during mixing because the beta-glucan hindered them from forming.
Overall, it must be noted that it is challenging to evaluate the cake structure by bubble size based on pictures; measurements could be attached to that in the future. However, generally, it is, in this case, considering the changes in volume and density, possible to say that the increase in beta-glucan values may have caused the difference in cake structures and bubble size and support the theory mentioned in the literature review.

Springiness and cohesiveness

As expected, the springiness showed a relation to the bubble size, volume and density of the cake. The springiness of the cakes decreased for the reference and BG1, which is the opposite of the desired result for baking products. The study of Żbikowska et al., 2020 showed the same effect, where the springiness decreased with increasing the beta-glucan percentage and storage time. This result showed a correlation to the lower volumes and higher densities of the cakes. However, the increase in springiness in cakes with 2.0% beta-glucan is remarkable and difficult to explain. After all, the volume and density of BG2 are directly opposite to the behaviour that occurs for springiness. It may be that interactions between beta-glucan and other ingredients have occurred that have led to alternations in the structure. However, this cannot be explained with the current study data and will therefore need to be investigated in a follow-up study.

Cohesiveness is related to forming the three-dimensional structure, as mentioned in the literature background. Higher cohesiveness is often associated with a firmer structure held together and less crumbly. This description corresponds to the structure of BG2, which also showed the highest values for cohesiveness

Hardness

Another attached reason for the decrease in volume is the relationship between volume and hardness. Considering the PCA model in figure 17, the results reflect this relationship. Hardness and volume are oppositely placed and thus show a negative relationship. This negative relationship also applies to the variations between treatments. The cake with the highest beta-glucan level showed the lowest volume and hardest texture compared to the reference. This cake was, as mentioned above, also the cake with the smallest air bubbles.

5.1.2 Moisture content and water activity

From previous research, moisture content had been expected to have a positive relationship with increasing beta-glucan content due to its water-absorbing properties. This was reflected in the results where moisture content increased as beta-glucan levels increased and was the highest for BG2. Thus when the percentage of beta-glucan increases within the cake, the formed gel-like structure can take up more water, resulting in higher moisture content. (Sharma et al., 2018). It is, however, remarkable that the moisture in the cakes with a higher beta-glucan content decreased more over time. It is difficult to determine where this originates from with the current results. Still, one possible reason could be the link to the observed hardness that increases with storage time. This will be addressed in greater detail in section 5.2.2 Hardness.

Water activity is a measure of available water for microbial and chemical purposes. In this case, the water holding capacity of beta-glucan causes a similar increase in water, and hence available water as for the moisture content. In addition, the increase in available water corresponds to mold growth on the cake. As mentioned in the literature review, available water is one factor in stimulating mold growth. Therefore, the results are thus in line with the expectations where the cakes with the highest moisture content (BG2) also showed to have the fastest growth of mold. For this reason, it may be engaging in the future to further investigate the relationship between shelf life and beta-glucan in sponge cakes.

5.2 Storage days

5.2.2 Hardness

It is desirable that hardness as a characteristic is not too pronounced in sponge cakes. The results indicate that hardness was increased for all treatments, with BG2 having the highest values. The BG1 cakes showed the lowest hardness after two weeks of storage. This points out that a higher beta-glucan percentage did not demonstrate a decrease in hardness in this case. These results contradict the findings of previous studies as described in the literature review. There could be several explanations for this.
The most apparent motive is ageing. Hardness is connected to retrogradation, an ageing-related property for products with starch as explained in the literature review. Considering the results, the percentage of beta-glucan in the BG2 cakes did not affect incrementing of the hardness compared to the reference. Nevertheless, BG1 decreased in hardness compared to the reference for most days. This suggests that, in this case, adding 1.0% PromOat beta-glucan had a more diminishing effect on retrogradation and a better impact on maintaining a softer texture than adding 2.0% PromOat beta-glucan.
In addition, during the retrogradation process, water is exuded from the structure. Therefore, the overall moisture content in the cake also became lower. Not only can retrogradation and ageing be related to the increase in hardness, this also explains the evaluated faster reduction of moisture content compared to the initial value on day 1.

However, what is interesting to note is that, in previous studies, equivalent percentages of beta-glucan resulted in a positive difference in hardness and moisture retention compared to the reference. In the study by Kalinga & Mishra (2009), an increase in hardness was observed when beta-glucan was added in excessive percentages (>7.5%) and a decrease in hardness with beta-glucan levels from 2.5 to 5.0%. Another study by Hajmohammadi et al. (2013) showed positive results for softening cakes by adding up to 2.0% beta-glucan. This shows different outcomes from the results of this study. One explanation may be that both studies used different compositions of ingredients. Hence, a different composition of the cake combined with beta-glucan may produce a different result and a variation in percentage. Elaborating on this, the interaction of ingredients with beta-glucan or one another might also contribute and cause a synergetic effect.
The quality and quantity of an ingredient may also affect the interaction. For example, as mentioned earlier, retrogradation can occur in products containing starch. However, the difference in quality and quantity of starch can affect the processing properties of the flour, resulting in a lower or higher percentage of starch and different composition of amylose and amylopectin. This results in various expressions in retrogradation. Also, beta-glucan can come in different species, where solubility varies, which may affect the desired result.
These aspects should be considered when a similar study with the same recipe and other percentages of beta-glucan will have to be conducted to rule out these possible influences.

5.2.3 Chewiness and Gumminess

The same findings for hardness apply to chewiness and gumminess. This was an expected outcome since these factors behave similarly to hardness. As mentioned in the literature review, gumminess is calculated using hardness values, and chewiness is dependent on hardness. Therefore, the same characteristics here can explain the changes over time: moisture content, density and beta-glucan percentage as described above.

5.2.4 Resilience

The resilience is determined by the ability of the sponge cake to return to its former shape after a specific force has been applied. When the resilience remains stable, it can translate to maintaining the cake's structure during storage. However, in this case, resilience mostly decreased for all treatments which was not in line with the expectations. This addresses that the cake's structure did not maintain and changed over time. Again, this can be explained by the increase in hardness and the decrease in moisture content, which negatively affected the cake's structure.

It is to be noted that the properties of the TPA analysis individually are only one aspect of the overall textural profile of the cake. Therefore, it is feasible that these factors reinforce each other's changes negatively or positively during the process. For example, in an ideal case, if resilience is high, hardness and gumminess are low. Since the reverse happened, it can be expected that the results are distributed logically. However, assigning a specific value to the factors for what is right and what is wrong is difficult. This preference may vary from product to product and from person to person. In the future, a sensory test could be conducted comparing the factors of the TPA analysis with individuals' acceptance and rating. For example, it would be possible to test whether a greater hardness in a product like cake is less accepted and where the acceptance boundaries lay. As a result, factors could be more easily linked to preferences.

5.3 Batch

Batches are often necessary since baking facilities do not always have the equipment to produce all batter in one go. A difference between batches is, therefore not favourable. In this study batch in some cases did influence results, perhaps due to the process and preparation of the cakes are prone to errors because, as described in Chapter 3, many operations are performed or tracked manually.
The difference in batch and the expression of variations in hardness, springiness, gumminess, chewiness, volume, moisture content, water activity and the density of the batter and cake could have arisen from different steps in the process. The first step in the process is weighing the ingredients and timing the mixing time. Mistakes made in this step are crucial for the composition of the cake and the formation of the basis for its structure, namely an airy batter. All treatments showed a significant difference between in density of the batter depending on batch, with BG1 having the lowest density. In addition, batch 2 was found to differentiate from all other batches. This indicates that batch 2 is responsible for most of the variances within parameters related to the batch. Since density is measured immediately after mixing, no other manual factors could have influenced this difference outside of weighing and mixing. Since the density of the batter does not increase or decrease linearly with increasing beta-glucan percentage, it is difficult to say whether this difference is solely due to possible human error, synergies of ingredients or the water-binding properties of beta-glucan, as mentioned previously.

6. Conclusion

In conclusion, this study aimed to investigate the effect of fortifying sponge cakes with different beta-glucan percentages (0.0%, 1.0% and 2.0%) and evaluate its effect on structural and physical properties over a two-week storage period. Using statistical analyses, the results showed significant differences in cake characteristics based on treatment, batch and retention time.

Regarding Treatment-based results, beta-glucan percentage demonstrated a positive relation to hardness, gumminess, water activity, moisture content and density of the cake. A negative relation was seen with volume, cohesiveness and springiness. An increasing beta-glucan percentage led to a decreased volume and increased density, which was in contrast with previous studies. Springiness and cohesiveness showed similar behavior, where higher beta-glucan levels resulted in a firmer structure. Treatment-related changes were likely related to the hindrance in bubble expansion.

In addition, storage time was of influence and had the most impact on hardness, gumminess and chewiness. Hardness increased for all treatments, conflicting with previous research. Moisture content and water activity increased with increasing beta-glucan levels, where a higher percentage presented a more significant decrease in moisture content over time and was likely related to the increasing hardness.

Lastly, batch variations suggested possible process errors, highlighting the need for improved consistency.

To summarise, this research has given insights into the effects of different beta-glucan percentages on structural and physical factors during a two-week storage period. The results have revealed several complex interactions between beta-glucan, storage time and possibly other ingredients and the investigated cake characteristics. However, further research is needed to investigate these interactions and the optimal use of beta-glucan levels in sponge cakes.

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Appendix

Appendix I: Flowchart beta-glucan process

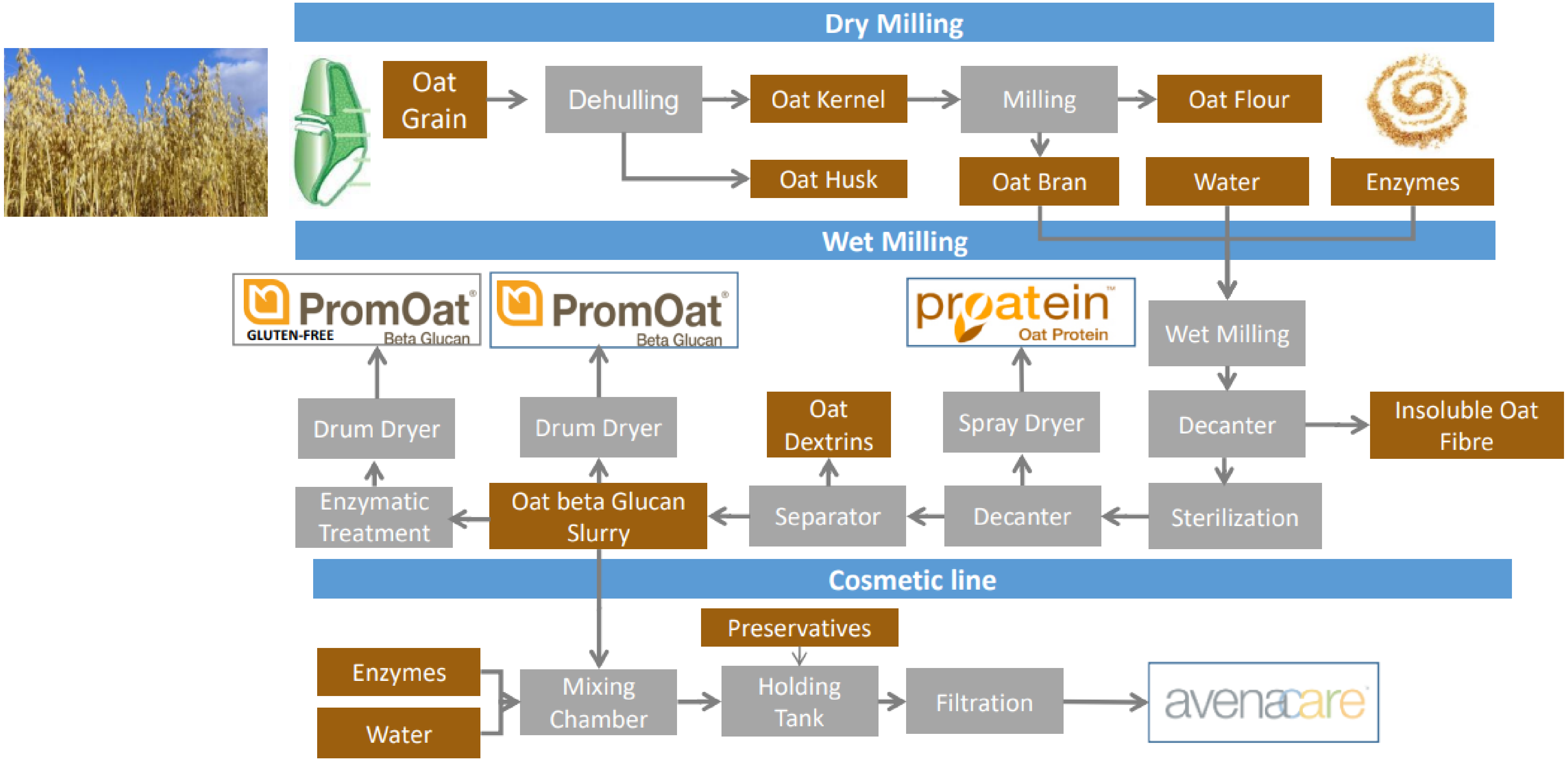

Figure 19 Flowchart of PromOat beta-glucan process, Lantmännen Oats.