Master’s thesis by Martin Jönsson

How different fractions of soluble ß-glucans from oats affect staling of high oat flour containing breads

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Abstract

Introduction. Bread is one of the most consumed foods in the world today. It is also the cause for major food waste streams. Most breads have turned into high glycaemic index products with low nutritional value. This master's thesis investigated whether the soluble oat fiber ß-glucan (BG) would have positive effects on the abovementioned. There are health claims associated with different doses of BG for positive effects on blood glucose control and ways of preventing cardiovascular disease, but these were not investigated. The idea was that by including extra BG in already high oat flour containing wheat breads, would retard the staling aspects of bread in terms of moisture content, specific volume, springiness, firmness and starch retrogradation. **Methods.** Breads with a 50-50 blend of oat and wheat flour were baked including two different BG powders with BG concentrations of 32 % (BG32) or 75 % (BG75). The BG was added either as a powder or pre-solubilized in the water prior to mixing with the rest of the ingredients. BG concentrations of 1, 3 and 5 % based on total flour, with corrected water addition for comparable dough stiffness, were analysed. The breads were stored in room temperature for 0, 1 and 3 days before being analysed. All qualities were performed with 6 replicates except for specific volume which was only performed in duplicate. **Results and discussion.** Multivariate analysis showed positive correlations between the binary addition type of BG and the quality aspects of specific volume and springiness. There was also a positive correlation between a higher moisture content and higher concentration of BG. The latter was however negatively correlated with specific volume and springiness. The amount of storage days were negatively correlated with peak force, meaning that the longer the storage, the softer the breads were compared to their reference bread. The best overall effect was seen for the intermediate BG32 concentration (1.2 % of total weight) added as a powder.

Populärvetenskaplig sammanfattning

Bröd är ett av världens mest konsumerade livsmedel. Med en färskvara som bröd och i så stora mängder uppstår alltid mycket matsvinn. Bara i Sverige är det estimerat att det slängs bröd för 2,4 miljarder kronor varje år. Detta oftast till följd av att brödet inte håller sin kvalitet tillräckligt länge. Dessutom har brödet som många äter blivit mindre nyttigt till följd av dess låga fiberhalter då mer processade mjöler används. Syftet med det här arbetet var att undersöka hur det vattenlösliga havrefiberfraktionen av beta glukan (BG) påverkar åldringsprocessen av bröd samtidigt som tillsatsen ökar fibermängden i brödet. Bröden som bakades innehöll standardingredienserna vatten, vetemjöl, salt, socker och jäst. Ytterligare ingredienser som användes var havremjöl (i lika stor mängd som vetemjölet) och BG pulver.

Bröden bakades på ett reproducerbart sätt för att öka jämförbarheten. Under förstudien upptäcktes det att när mer BG pulver sattes till behövdes mer vatten för att få en motsvarande deghårdhet. Olika koncentrationer av BG och olika tillsättningssätt (antingen som pulver eller som förupplöst i vattnet) användes i bröden innan de analyserades efter noll, en och tre dagars lagring i rumstemperatur i förslutna plastpåsar. Bröden analyserades gällande deras densitet, fuktighet, hårdhet, elasticitet och en termisk analys som heter differentiell skanning kalorimetri som huvudsakligen mäter hur stärkelsen i brödet förändras vid bakning och under lagring.

Lagringsstudien visade att det fanns positiva korrelationer mellan tillsättning av pulver, högre elasticitet och lägre densitet. Det fanns också positiva korrelationer mellan fuktighet och ökad mängd BG i brödet. De senare var dock negativt korrelerade med högre elasticitet, lägre densitet. Bröden var generellt bättre än deras referenser utan tillsatsen av BG, främst vid en längre lagringstid.

Rekommendationerna från arbetet är att för att förlänga hållbarheten på brödet skall en koncentration på ca 1 % BG tillsatt som pulver finnas i bröden. Detta ger en optimerad effekt på fuktighet, elasticitet och densitet samtidigt som en högre koncentration inte behövs påkostas.

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1. Introduction

1.1. Relevance and scientific background

This master's thesis was in collaboration with Lantmännen. The organisation is in a constant search for improving their products and raw materials delivered to consumers. A previously researched topic of theirs has been using oats and its components in innovative ways. That was also what this project was about.

One of the interesting components in oats is one of its soluble fibers, namely the ß - glucan (BG) (Malmberg, 2020). Oats naturally contains about 4 % BG (SweOat, 2020). What Lantmännen has done is further concentrating into high fiber powders. One with 32 % BG (BG32) and one with 75 % BG (BG75). Lantmännen wanted to investigate how the incorporation of these products affect bread and their shelf-life and if there were any differences.

Retarding/prolonging the staling of bread is very important since bread contributes to very large food waste streams. Only in Sweden the food waste associated with bread is as much as 80 400 tonnes a year (Brancoli et.al., 2019). This equals 350 loafs of bread per minute and costs about 2.4 billion SEK per year if 30 SEK per kilo of bread is assumed.

There is a need for higher fiber diets since overeating is a worldwide problem (Ludwig et.al., 1999). By consuming BG, health claims regarding reducing the risk for coronary heart diseases and cholesterol control (EFSA, 2010) and post-prandial glucose regulation (EFSA, 2011) are possible to accomplish. Additionally, oat fiber (amongst them BG) acts as a resistant starch, improving the gut microflora by acting as a prebiotic (Mälkki & Virtanen, 2001).

1.2. Aim

The overall aim of this master thesis was to understand how breads containing high amounts of oat flour were affected regarding to the staling effects upon the addition of different concentrations of soluble BG, the way of adding the BG, and if changing the powder to a higher purified one (BG75) would knowledge wise be directly transferable or not.

The project started with a pre-study in order to investigate whether including high hydrostatically pressurized oat protein would improve 100% oat bread or not, which can be seen in Appendix 8.1.. The pre-study was otherwise intended for learning the baking procedure, analysis equipment and basic optimizations of the processes.

2. Background and Theory

2.1. Bread

2.1.1. Bread structure and staling

Breads are generally made from doughs containing high amounts of starch. Starch exists as granules and are composed of two main components, i.e. amylose and amylopectin. Amylose is an almost linear polymer consisting of glucose molecules linked together with (1→4) α-linkages. Amylopectin has the same backbone as amylose but also extensive branching with (1→6) α-linkages (Edwards, 2007). During baking of bread, the starch gelatinizes by granular swelling which increases viscosity because of the absorption of water. Ultimately, the granules will burst, leaching amylose and the gelatinization is from hereinafter irreversible (Benkeblia, 2014).

After baking the bread, even at the cooling, interesting phenomena are happening. Initially the amylose is starting to recrystallize (called retrogradation) which sets the crumb structure. Secondly, water migration from the crumb to the crust occurs, making the crust soft. Simultaneously, and this is the most prolonged step, amylopectin retrogradation takes place. This is one of the main factors to what is causing staling of bread (Corke et.al., 2008). For an illustrative view, see ***Figure 1***.

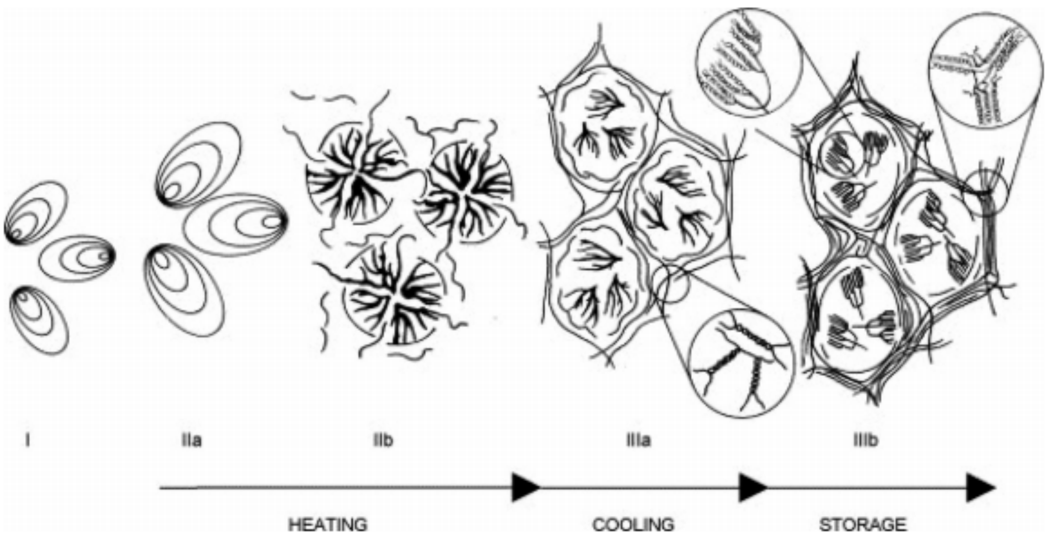

Figure 1. *Schematic view of starch gelation and retrogradation. (I) The starch granule in its original form. (IIa) During heating the starch granules start swelling while absorbing water. (IIb) Bursting of the starch granules and the amylose start leaching. (IIIa) During cooling the amylose retrogrades forming a starch gel. (IIIb) During the storage also the amylopectin starts retrograding. (Wang et. al., 2015)*

For breads structure development, a protein network is needed for the stability of the colloidally complex system (Benkeblia, 2014). Most commonly used is wheat flour containing 11-13 % of protein (King Arthur Flour Company, 2020). The primary on is gluten which is composed from gliadins and glutenins (Benkeblia, 2014). An image of the gluten network can be seen in ***Figure 2***. The long black strands are the glutenins and the white circles are the gliadins. Glutenins are providing the elasticity while the gliadins provide viscosity and extensibility (Edwards, 2007).

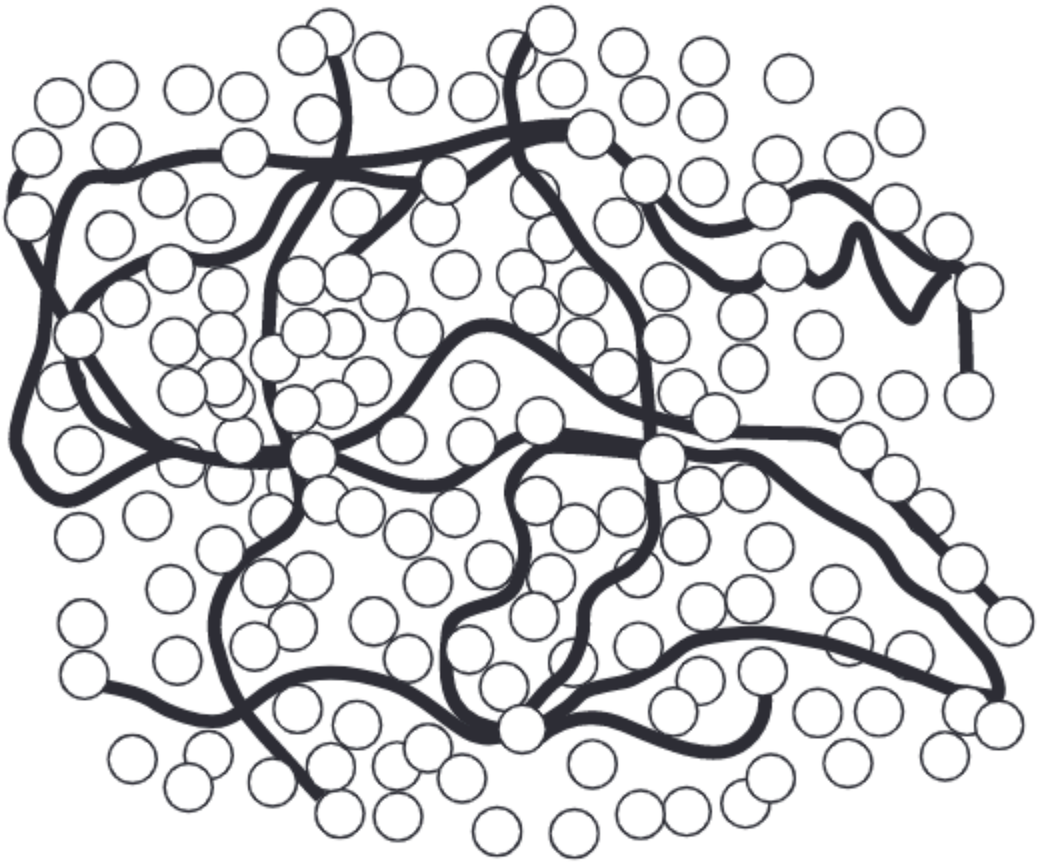

Figure 2. *A structural illustration on the gluten network* (Belton, 1999). The white circles are the gliadins and the black strands are the glutenins.

2.1.2. Bread components and their effects

Wheat flour

There are two main types of categorized wheat flour, namely hard and soft. The hard one has a higher protein content and is therefore more used in bread making while the soft one usually is for cake and cookie applications (Edward, 2007). Another quality aspect is the falling number of the flour. The higher the number the better. The lower the α-amylase activity, the higher the falling number. For flours to have low α-amylase activities, they should not have started to sprout before harvest, which is more likely to occur if there has been a more humid period (Pennington, 2017). α-amylase hydrolyzes starch to glucose monomers (Berg et.al., 2015).

The bread making possibilities of wheat flour are good because of its high gluten content (Benkeblia, 2014) and ability to gelatinize during baking (Lineback & Wongsrikasem, 1980).

Oat flour

The fat content in oat flour is around 8 % (Risenta, 2020) which would make it prone to go rancid if the high amounts of lipase were not inactivated by heat treatment (Moisio et. al., 2015). During the heat treatment some starch will also gelatinize, making the final oat flour partly pregelatinized, preferably between 25 and 50 % (Burri, 2000). A pregelatinized flour will absorb water even at lower temperatures (Kulp, 2000). This will generate stiffer doughs at the same hydration level compared to non-pregelatinized flours.

Hüttner et. al. (2010) investigated different sources of oat flour and baked bread with all of them. They noticed differences regarding damaged starch, BG content and particle size. They concluded that these factors affected the bread quality in terms of specific volume and texture. Since oat flours seem to differ this much between sources it is therefore difficult to predict in how breads made with 100 % oat would result.

Salt

Salt is an essential ingredient in bread making since it stabilizes the fermentation rate, strengthens the dough and enhances the flavour. Increasing amounts of salt has also shown to increase the specific volume up to a certain concentration, depending on the salt (Miller & Hoseney, 2008). McCann and Day (2013) showed however that this effect was more pronounced in flours with lower protein content.

Sugar, yeast and other ingredients

Sugar impacts bread on the taste, speed of fermentation, appearance, colour, structure and texture. The main function of the yeast in bread making is to produce carbon dioxide to give the bread rise, but it will also produce other volatile organic compound contributing to taste and smell. Apart from these essential ingredients, there are some who use emulsifiers, enzymes, and hydrocolloids to further increase the quality of bread (Corke et. al., 2008).

2.2. ß - glucans

2.2.1. Structure and origin

There are different kinds of BG depending on their origin. The main structural difference is in how the glucose monomers are linked together. The fungi and cereal BG has a mixture of (1→3) and (1→4) ß-linkages while microbial BG has (1→2), (1→3), (1→4) and/or (1→6) ß-linkages. The cereal BG is present in barley, oats, and wheat. The (1→3) and (1→4) ß-linkages are not randomly distributed across the polymer. The polymer consists mainly of glucose polymers with (1→4) ß-linkages in length of 3 or 4 and occasionally ≥5 monomers. The shorter polymer blocks are linked together by the (1→3) ß-linkages. These blocks of cellulose-like structures are however connected randomly since the length of the blocks between (1→3) ß-linkages are randomly long and the order between them is not repetitive, see Figure 3. It is this randomicity that makes up for the interesting soluble characteristic of cereal BG. If the polymers would only be composed of a single kind of equally long the cellulose structures would end up tightly connected to each other (as a result of many possible interpolymeric interactions) and the solubility would be absent. For a good demonstration of the solubility reasoning above, see Figure 4. If the polymers were to be so strictly bound to each other, they would form insoluble aggregates (Kale, Hamaker & Bordenave, 2013).

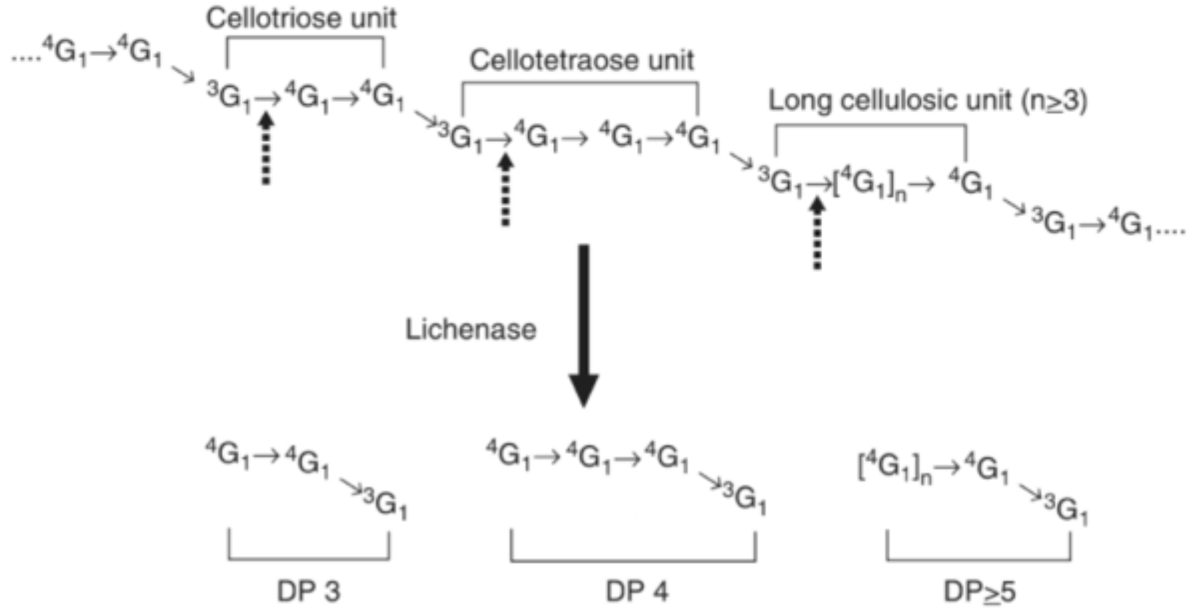

Figure 3. *The basic structure of oat BG. Cellotriose is composed of 3 glucose monomers and cellotetraose of 4. Linkages are indicated by the super- and subscripts at each G.* (Kale, Hamaker & Bordenave, 2013)

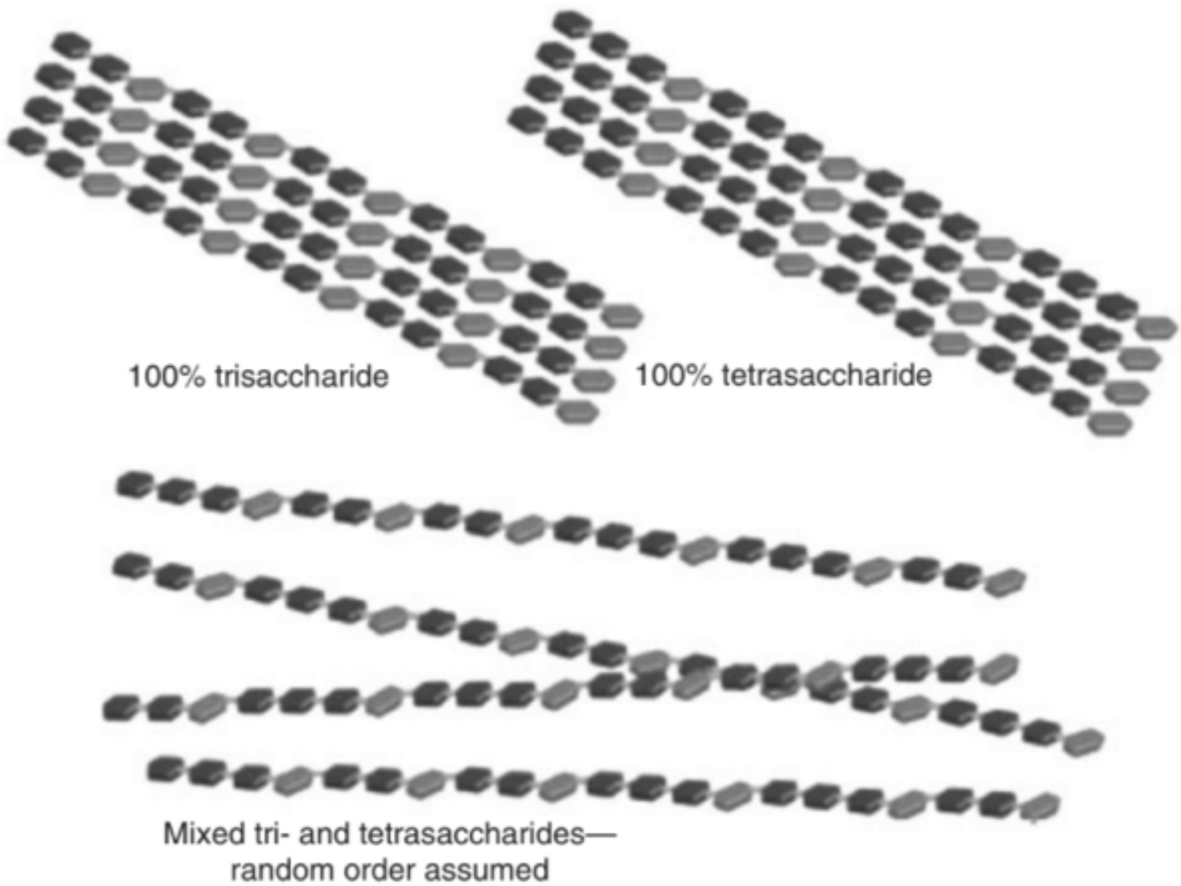

Figure 4. *What effect the structure has on the solubility.* (Kale, Hamaker & Bordenave, 2013)

In oat BG, the composition of the 3, 4 and ≥5 long sub-polymer are 53.4-66.1 %, 29.1-41.4 % and 3.6-9.7 %, respectively. This variability can depend on e.g. climate (Kale, Hamaker & Bordenave, 2013).

2.2.2. Properties and functionality

For every polymer there is a critical concentration. It is determined by plotting the logarithm of the viscosity on the y-axis and the logarithm of the concentration on the x-axis and then finding the concentration where the slope drastically increases. This point is the critical concentration. A common explanation for the critical concentration is when the polymers are not simply dispersed on their own but starting to interact and clump together forming a network and thus increasing the viscosity (Teraoka, 2002). At concentrations below the critical concentration the dispersion has Newtonian behaviour and above the critical concentration the dispersion shows shear thinning behaviour. For high molecular weight BG the critical concentration is around 0.2 % (Kale, Hamaker & Bordenave, 2013).

The gelling properties of oat BG depends on the ratio between the number of chains containing 3 and 4 subunits. The higher the ratio (more 3 subunits) the faster gel formation and firmer gel is produced. This is because of the higher chance to have junction zones in the network (Kale, Hamaker & Bordenave, 2013).

2.2.3. ß-glucans in bread

One principle to how BG would retard the staling of bread is by increasing the viscosity of the liquid phase of the bread, thus limiting the mobility of the amylopectin and therefore also its risk of recrystallizing (Rieder et.al., 2012). In literature this is also stated as a competition for water (Wang et.al., 2015).

There are a lot of studies investigating the effects from BG in breads. The reason behind this could be that many different mechanisms are happening simultaneously (Corke et.al., 2008). When it comes to how the specific volume of breads is affected by the addition of BG, Hager et.al (2011) concluded that BG actually decreased the specific volume for wheat breads. For gluten free breads however, they found no significant differences. Mohebbi et. al. (2018) have proposed three possible explanations for why the addition of BG would decrease the specific volume. The first one is a dilution of gluten and thus decreasing the network possibilities. The second one is a disruption, by interactions, of the gluten network which has the same effect as the dilution. The third is a competition for water and thus also less water vapour production during baking providing lift. Hager et.al (2011) also found that wheat breads had a higher moisture content compared to reference, also reported by Mohebbi et. al. (2018), in gluten-free and wheat breads.

2.2.4. Health effects and claims

European food safety authorities (EFSA) have made two claims regarding BG. The first one is that the consumption of 3 g of BG per day will lower/reduce blood cholesterol which in part may reduce the risk of coronary heart disease (EFSA, 2010a). EFSA:s other claim is regarding the effect that BG has on controlling blood sugar levels post meal. The claim requires 4 g of BG per 30 g available carbohydrates (EFSA, 2011). The mechanism BG has, according to Goff et.al. (2018), is primarily by increasing the viscosity and thus lowering the speed of absorption.

A standard slice of bread weighs about 40 grams. Assuming a normal person eats 2 slices of bread per day. To reach the cholesterol health claim the concentration would have to be approximately 4 % (3 g BG/80 g bread). Since the concentration of BG in dried rolled oats is around 5 % (Mason, 2002) it would be very difficult to even reach the cholesterol claim without the addition of extra BG.

Another very interesting investigation about BG was performed by Choromanska et.al. (2015) who investigated different soluble oat BG:s as anticancer treatment.

2.3. Theory about analysis methods

2.3.1. Differential Scanning Calorimetry (DSC)

DSC provide thermal properties by changing temperature of samples so that reactions, endothermic as well as exothermic (usually phase transitions but in this case melting of starch retrogradation crystals and amylose-lipid complexes), are occurring and the enthalpies, ΔH (J/g) can be calculated. For breads, mainly amylopectin retrogradation (Peak around 55 °C), since it is well known to correlate with staling (Gudmundsson, 1994), and amylose-lipid complexes (peak around 115 °C) (Jovanovich & Añón, 1999) are of highest interest. The amylose-lipid complexes have been shown to retard bread staling when they have been replacing shortenings (Lee et.al., 2020). DSC is measuring different heat flux for two samples while maintaining the same temperature profile. One sample contains the interesting sample and the other one acts as a reference. The difference in heat flux correlates with reactions occurring during the heating. The peak generated as the difference between the heat flux might be protracted since the heating to the material not always is immediate. The protracted peak could also be a result of the reactions occurring during a temperature interval (Laye, 2002). For a typical DSC curve showing oat flour retrogradation, see ***Figure 5***.

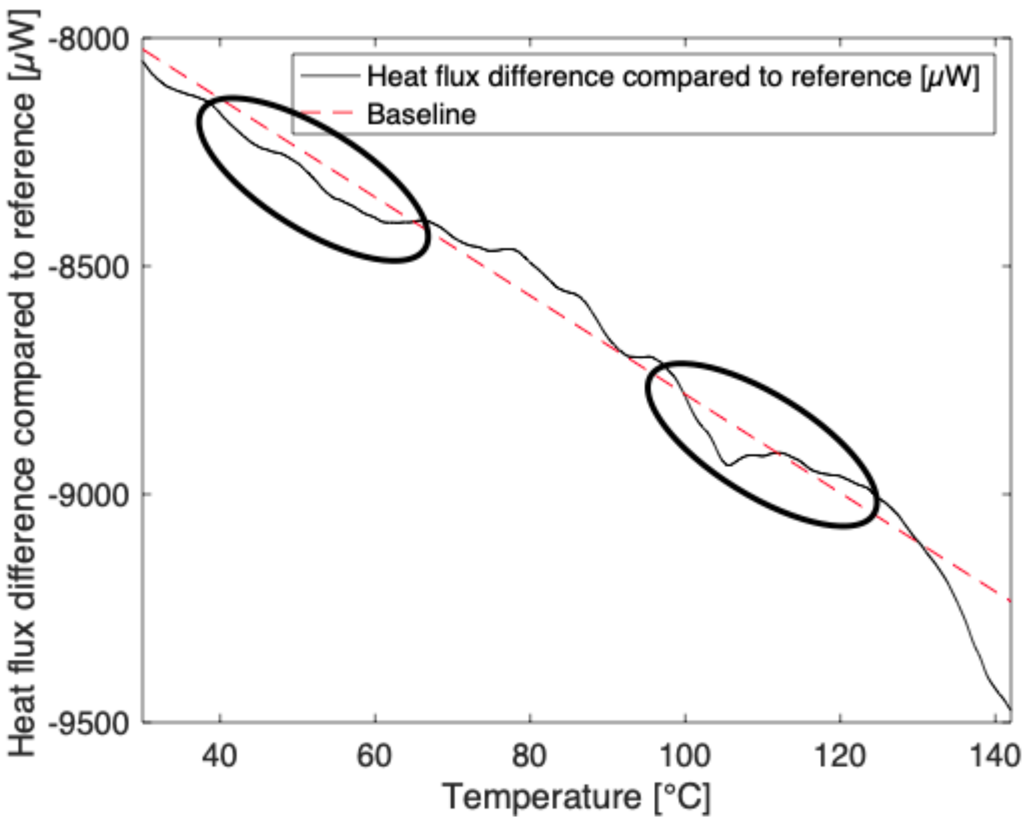

Figure 5. *A typical DSC curve for oat flour retrogradation. The amylopectin retrogradation is distinguishable as a peak around 50 °C and the amylose-lipid complex around 100 °C.*

2.3.2. Rapid Visco Analyser (RVA)

The RVA is a temperature-controlled viscometer. Samples are corrected to a reference water content to increase the comparability between different samples (e.g. how the pasting differs with flours with different water content). It is possible to use a temperature profile of choice and measure absolute viscosity in the unit centiPoise (cP). There are two types of RVAs, either open to the atmosphere or pressurized RVAs in which the temperature can reach 140 °C (Perten Instruments, 2020a). For different food applications there has been developed a number of standard methods approved by the American Association of Cereal Chemists (AACC). These methods have pre-determined temperature and stirring profiles. An RVA can be used to imitate temperature and shear conditions from processing equipment and/or to understand what reactions that are happening. For a typical graph for pasting properties of flour, see ***Figure 6***.

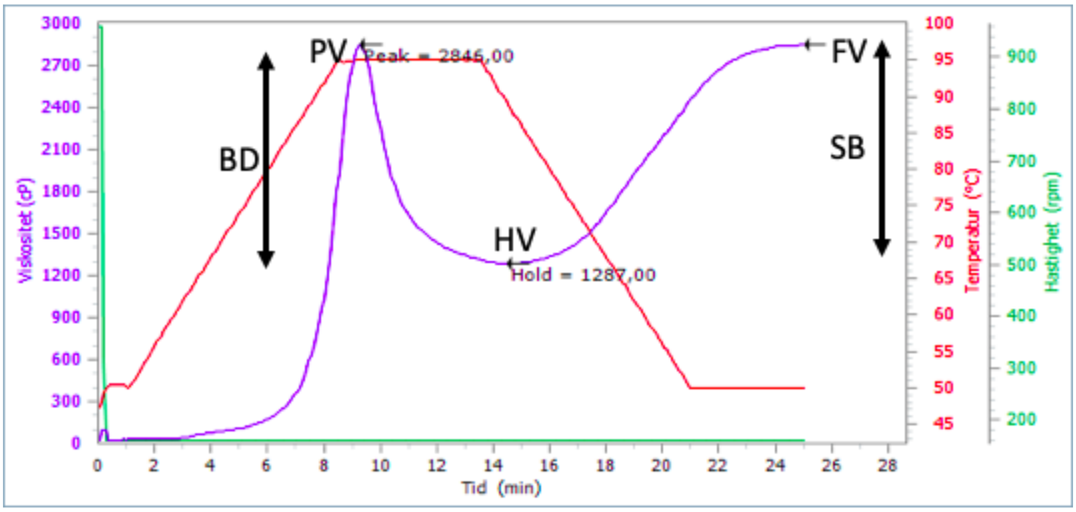

Figure 6*. An example graph for pasting properties of a flour. The blue curve is the viscosity (cP) and the red it the temperature (°C).*

During heating, see ***Figure 6***, the flour starch undergoes gelatinization reaching its peak viscosity (PV). When the temperature is constant, the gel undergoes breakdown (BD) due to the rotational stirring and the viscosity decreases until the hold value (HV). During the cooling phase of the graph, the viscosity increases, called set back value (SB), due to the phenomenon when viscosity increase with temperature decrease, and also due to starch retrogradation (mainly amylose, if present), finishing off at its final viscosity (FV).

2.3.3. DoughLAB

DoughLAB is a dough mixer with the ability to measure water absorption (measured in g water/g sample), dough development (measured in torque in farinograph units (FU)) and mixing tolerance, see ***Figure 7*** (Perten Instruments, 2020b). DoughLABs are used in many different applications, e.g. gluten analysis, carbohydrate functionality and processing characteristics (Perten Instruments, 2020f). By controlled heating, water addition and stirring, the machinery and software measures and presents data on how much water that it is possible to add, and the force required to maintain the profile. The software can also predict what changes to make in order to reach a target resistance of a dough, assuming the previous run did not meet the requirements (Perten Instruments, 2020c). A DoughLAB is similar to a farinograph apart from the controlled temperature, automated water addition and the iterative software (Bason et.al., 2005).

***Figure** 7. A doughLAB from PerkinElmer (Perten Instruments, 2020b).*

2.3.4. Texture analysis

Texture analysers are used extensively in the food industry because of their high versatility. They measure distance (in mm) and weight (in g) and can from that information determine specific parameters for what was measured. Examples are stretchability (e.g for tortilla breads), fracturability (e.g. for crackers), firmness (e.g. for bread and bakery goods) and adhesiveness (for pasting properties) (Perten Instruments, 2020g). During a texture analysis of bread, a probe compresses a representative slice of bread (thickness in millimeters) according to the profile input in the software. The force needed varies depending on how hard the bread is. A softer crumb will require less force than a hard crumb for the same compression method. For a typical output from a texture analysis with one compression and a holding time, see ***Figure 8***. Texture analysers can also be used to imitate chewing (Perten Instruments, 2020d).

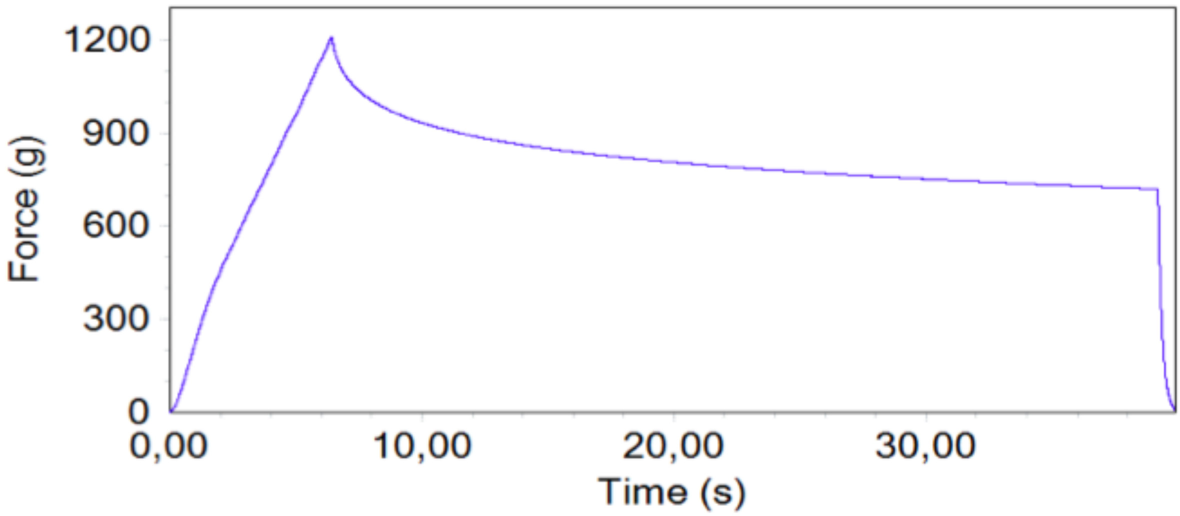

***Figure 8.** A typical measurement of a bread slice with a holding time of 30 seconds.*

Peak force

When compressing the bread slice with the probe, the crumb will become more compressed and thus also more dense. This makes the force for breads usually needed to accomplish the profile compression value higher the further down the bread slice the probe gets but must not always be the case. The peak force peak is seen in ***Figure 8***. When compressing a sample, the slope until the peak force is not always as straight. This could be a result of the structure simultaneously slightly breaking during the compression or differences between surface and interior parts of the examined sample.

Springiness

Springiness is a relative measure on how willing the compressed structure is to return to its original shape. This is shown in ***Figure 8*** as the plateau value after the hold at the profile compression divided by the peak force, see ***Equation 1***. Without a holding time, determining springiness in a single run of compression would not be possible.

$$Springiness = \frac{Plateau\, force}{Peak\, force} * 100 \qquad \textbf{\textit{Equation 1}}$$

2.3.5. Volume analysis

Volume analysis devices, see ***Figure 9***, producing a 3D-imaging use laser topography (Perten Instruments, 2020e). The machine measures the distance between the sensor and the surface of the bread and with the angle between the sent light beam and the reflected beam, the volume is calculated. It is as a result of the rotating laser arm and spinning bread that it is possible to make a 3D image. The volume analysis returns all the three-dimensional characteristics (length, width, and height in mm), total volume (in mL), surface area (in cm^2), specific volume (in ml/g) and mass (in grams).

Figure 9*. A Perten BVM volume analysis machine (Anderson, Purhagen & Bason, 2014).*

3. Materials and Methods

3.1. Materials

The main materials used in the project were oat flour, wheat flour, PromOat (BG32, consisting of 32 % BG) and purified BG (BG75, consisting of 75 % BG). All of them were provided by Cerealia AB, Malmö, Sweden. Apart from these four materials, salt, sugar, and yeast were bought in the local supermarket. Water was used from the tap.

3.2. Experimental plan of storage study

The different recipes and their respective storage days needed to be investigated. Due to the pandemic with covid-19, the number of baking trials and storage days had to be reduced and wisely chosen. The idea was to get as much differences as possible of what seemed the most interesting beforehand. For an overview of the experimental set-up, see ***Table 1***.

The different recipes' names were based on whether the BG was added as powder (PX) or as pre-solubilized (hydrated) (HX), where X is the concentration of either 1, 3 or 5 % based on the total flour. R(1-4) corresponds to replicate baking trails of the reference. P75 and H75 were with BG75 while the rest used BG32 as BG addition.

Table 1. *The storage study plan regarding what analyses on which days and the quantities of bread per recipe.*

<table>
<tr><th>Week</th><th>Recipe</th><th>Baking day</th><th>Storage day 1 & 3</th></tr>
<tr><td rowspan="3">1</td><td>R1 (3 breads)</td><td rowspan="12">Volume (duplicates for samples and one measurement for references)

Dry matter (six replicated for samples and triplicates for references)

Texture (six replicated for samples and triplicates for references)</td><td rowspan="12">Volume (duplicates for samples and one measurement for references)

Dry matter (six replicated for samples and triplicates for references)

Texture (six replicated for samples and triplicates for references)

DSC (six replicated for samples and triplicates for references)</td></tr>
<tr><td>P1 (6 breads)</td></tr>
<tr><td>P5 (6 breads)</td></tr>
<tr><td rowspan="3">2</td><td>R2 (3 breads)</td></tr>
<tr><td>H1 (6 breads)</td></tr>
<tr><td>H5 (6 breads)</td></tr>
<tr><td rowspan="3">3</td><td>R3 (3 breads)</td></tr>
<tr><td>H3 (6 breads)</td></tr>
<tr><td>P3 (6 breads)</td></tr>
<tr><td rowspan="3">4</td><td>R4 (3 breads)</td></tr>
<tr><td>P75.3 (6 breads)</td></tr>
<tr><td>H75.3 (6 breads)</td></tr>
</table>

Since investigating all conditions as described in ***Table 1***, a full factorial experimental design has been applied.

Each bread contributed with a triplicate of peak force, springiness, and moisture content, one measurement on specific volume and three breadcrumbs for DSC. This makes for 6 replicates per recipe and storage day for peak force, springiness, DSC and moisture and duplicates for specific volume. While for the references, all the response variables were halved.

3.3. Methods

3.3.1. Differential Scanning Calorimetry (DSC)

Gelatinization

The flours were measured to 2 mg in coated aluminum pans with added three times flour weight of water before closed and let sit to hydrate for 15 min. The method used in the DSC (Seiko Instruments Inc. (Shizuo, Japan) calibrated with Indium (melting point 156.6 °C) Exstar DSC 6200) started at 20 °C held for 1 min followed by a 10 °C/min increase until 160 °C was reached. The program temperature, i.e. the end target temperature 160 °C was actually only 150 °C for the ampule. Gelatinization properties in terms of T_O (°C), T_P (°C), T_C (°C) and ΔH (J/g dry weight) were analysed. The gelatinization of a starch indicates how it will behave in the bread when the dough bakes.

Retrogradation
The previously gelatinized samples were kept stored in room temperature. The temperature profile was the same as for the gelatinization. The specific retrogradation enthalpy, i.e. the enthalpy per dried sample, was calculated in the integrated software SII EXSTAR6000 Muse. Retrogradation properties in terms of T_O (°C), T_P (°C), T_C (°C) and ΔH (J/g dry weight) were analysed for both amylopectin retrogradation and amylose-lipid complexes. The samples were performed in triplicates and two different storages days of 5 and 7 days were measured.

Thermal analysis of bread
The procedure was the same with the crumbs as for the gelatinization apart from that no water was added, the sample size was aimed at 8 mg crumb, and the first minute held at 20 °C was removed, i.e. the temperature increase started directly. The small bread parts analysed were taken from the center of each slice of bread. Per recipe and day of storage there were three samples each.

After the measurements, the pans were punctured and dried overnight in an oven at 105 °C until constant mass (at least 12 h) to get to total dry weight of the sample initially added.

The integrated software SII EXSTAR6000 Muse was used to calculate the melting enthalpies of the peaks, according to section 2.3.1., in the graphs. It was performed by integrating the curve between T_O and T_C. The retrogradation enthalpy was calculated from the required energy divided by the dry weight of the sample and expressed in J/g.

3.3.2. Rapid Visco Analyser (RVA)
Two different methods were run with the RVA 4500 (Perten Instruments, Australia). One method started at 50 °C, followed by 160 rpm and a temperature increase from 50 °C to 95 °C until total time of 8.5 min at a speed of 5.3 °C/min and held at 95 °C for 5 min before cooling to 50 °C during 7.5 min at a rate of 6 °C/min and held there for 4 min. The rotational speed was the initial 10 seconds set to 960 rpm and then kept at 160 rpm for the remaining run. The first method was used for the wheat, oat and a 50-50 combination of the two flours (3.5 g per sample corrected to 14 % reference moisture content) and the BG powders (0.5 g BG32 and the same amount of BG from BG75). The samples were run with 25 g water and pre-mixed by hand to avoid clumps. The lower sample weights of the BG powders were due to their highly viscous dispersions.

The second method had the same rotational speed profile but run at a constant temperature of 25 °C. This method was only used for 0.5 BG32. All samples were run in duplicate.

3.3.3. DoughLAB
The dough ingredients were weighed to a total weight of 300 g and added in the dough bowl paired with a DoughLAB (DoughLAB 2500, Perten Instruments, Sweden). The program was run by an initial rotational speed at 63 rpm for 1 minute to mix the dry ingredients. Water was added in a steady stream until the desired water amount was reached while the rotation speed also was changed to 120 rpm and kept there until a total mixing time of 10 minutes. The peak resistance, measured in farinograph units (FU), and the time to reach the peak, measured in minutes, were noted. The software provided a suggestion on how much water to add to obtain the optimal dough consistency of 750 FU based on the run that just was performed. This meant that a clever way of iterations was performed with the DoughLAB.

3.3.3. Bread making

There were two dough preparations per BG, one with pre-hydrated BG and one with BG added as powder. The BG percentage was based on pure BG per total flour amount. For BG32, the one with BG added as powder the methods was the following: All ingredients were measured by weight and mixed in a table-top dough mixer (Electrolux EKM9000, France) on setting 3 for 2.5 minutes. For the doughs with BG added as powder, a dough hook was used. For the doughs when BG was pre-solubilized in the water, a paddle was used. The paddle attachment was used with the pre-solubilized samples since they did not mix well with the dough hook.

For BG75, when added as powder, the method was the same as for BG32. When pre-solubilizing BG75, the powder and all the water according to its recipe were heated until 80 °C and then let to cool before the thickened mixture was used the same way as pre-solubilized BG32 was.

The doughs were let to rest for 10 minutes before shaped into 400 g dough balls and put in molds and into the proofer set to 38 °C and 85 % relative humidity (RH) for a total of 30 min. BG75 added as powder was too runny and therefore not shaped, but rather poured and pressed into the molds. After proofed, the shaped and risen doughs were baked with steam added at 240 °C and then lowered to 225 °C until a total baking time of 40 minutes and an internal temperature of 98-99 °C (Therdthai, Zhou & Adamczak, 2002). When baked, the breads were let to cool in room temperature for 2 hours. The cooled breads were packed into sealed plastic bags and put in groups correlating with day of analysis and stored dark in room temperature (20-22 °C). For an overview of the baking procedure, see ***Figure 10***.

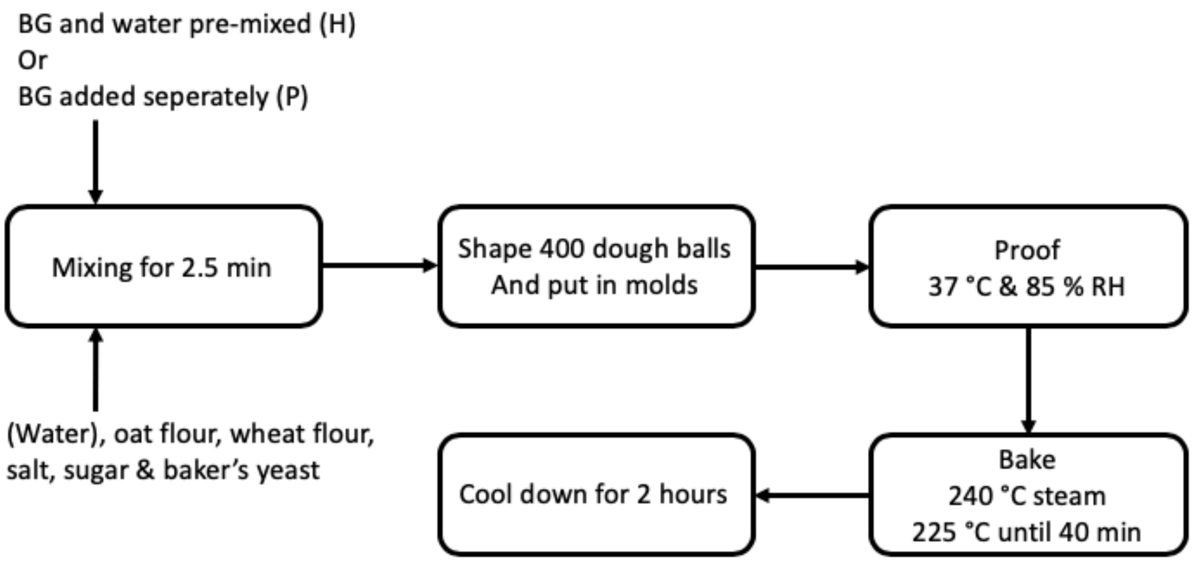

Figure 10. *A flowchart showing the baking procedure*

3.3.4. Texture analysis

The breads were cut into three 2.5 cm thick slices from the middle of the bread. A 25 mm in diameter cylindrical aluminum probe with rounded edges was used. Since increasing the shelf-life of bread was the important part of this master's thesis, and not assess how it would be judged by a sensory trial the method chosen to measure bread quality was a hold-until-time method. A texture analyser (TexVol TVT-300XP, Perten Instruments, Sweden) and a hold until time-method had a starting position 5 mm above the bread slice, approaching the bread with an initial speed of 1 mm/s, compressing the bread to 40 % of the bread slice's height at a test speed of 1.7 mm/s, holding for 32 seconds before releasing with a retract speed of 10 mm/s. The aluminum probe was placed over the center of each slice to avoid the harder crust. If larger holes were present in the center of the crumb, the cylinder was moved to slightly off-center but still making sure the impact from the crust would be as small as possible. The properties measured during the texture analysis were peak force (measured in g) and springiness (measured as a relative force between the plateau value and peak force, thus being unitless but typically a value between 0 and 100). Per recipe and day of storage, five breads and a total of 30 slices were analysed.

3.3.5. Specific volume analysis

The breads were weighed using a 0.001 g resolution scale and then measured in a volume meter (TexVol BVM - L370, Perten Instruments, Sweden). The method was set to run for 1 minute with corresponding plates to hold the bread in place, the 30 mm wide metal disk with two metal nails to attach the bread to. 3D images of the breads were illustrated in the software VolCalc and turned to ensure no spikes were affecting the result. The specific volume was calculated by doing the fraction between the volume (in mL) and the weight (in grams) according to ***Equation 2***.

$$Specific\ volume\ \left(\frac{ml}{g}\right) = \frac{volume\ (ml)}{Weight\ (g)}$$ ***Equation 2***

3.3.6. Moisture content analysis

Flours and other powders

2 grams of product were dried in aluminum pans at 135 °C for 2 hours. The samples were then let to cool in a desiccator to avoid reabsorption of water from the air humidity before weighing and calculations according to ***Equation 3*** could be made to obtain the moisture content with the unit of g water per g wet powder.

$$Moisture\ content = \frac{m_{before\ drying} - m_{after\ drying}}{m_{before\ drying}}$$ ***Equation 3***

Bread

The bread slices from the texture measurements were wrapped in aluminum foil to prevent loss of mass with bread crumbs falling off. The wrapping was slightly open to let water evaporate. The wrapped breads were dried in an oven at 135 °C for 2 hours until constant mass was obtained. The breads were then let to cool in a desiccator to avoid reabsorption of water from the air humidity. The breads, and their corresponding wrapping were weighed and the moisture content (wet basis) were calculated according to ***Equation 3***.

3.3.7. Visual evaluation

Photographs of each center-cut cross-section of the breads were performed. From the photos the shape of the bread, crumb size distribution and colour were signs to investigate and compare between the different recipes and storage days.

3.3.8. Statistical analyses

Grubb's outlier tests were conducted on all the storage days and different recipes for all responses/data. The detected outliers were replaced with the mean value from the other measurements. It was handled this way since the following multiple comparison tests required same sized data matrices. The data analysis was treated with an analysis of variance (ANOVA) in MATLAB. With subsequent Tukey's multiple comparison tests significant differences could be detected and demonstrated in figures. All tests were run with an uncertainty of 5 % ($p<0.05$). 95 % confidence intervals are presented based on the 1.96 times the standard error.

Multivariate analysis (MVA)

During a multivariate analysis there are two common ways of presenting the data to be able to make conclusions. The first one is a principal component analysis (PCA) and the second one is a more specific analysis made for process variables and responses, called PLS (partial least square). Both are based upon principal components (PC:s) which are thought to explain the remaining variance (only PCA) in the data after each addition of PC:s and also optimize the PC:s to the responses in PLS. The processing variables were concentration, way of addition and storage time while the responses were springiness, peak force, moisture content and specific volume. In PCA, the process variables and responses were all made into one data set while in the PLS they were kept separate. PLS is also commonly used to predict outcomes from new processing variables after it has been trained with previous data also containing responses (Brereton, 2003). The way of addition was treated as a binary variable where powder addition was 1 and pre-solubilized was 0.

4. Results and Discussions

4.1. Pre-baking trials

Investigating how the raw ingredients separately behave during baking and storage was a strategy to be able to better understand the different processes and the study's results. The pre-baking trials are separated into BG dry matter, pasting and thermal properties.

4.1.1. Pasting properties

Flours

Knowledge about how the different raw materials' properties is important to better understand their potential effect in the bread. E.g. flours with lower set-back viscosities generally make bread maintain freshness for longer (Wani et.al., 2016). Duplicates of oat flour, wheat flour and a 50-50 combination of both were run and is seen in ***Figure 11***. The calculations regarding the BD and SB values are seen in ***Table 2***.

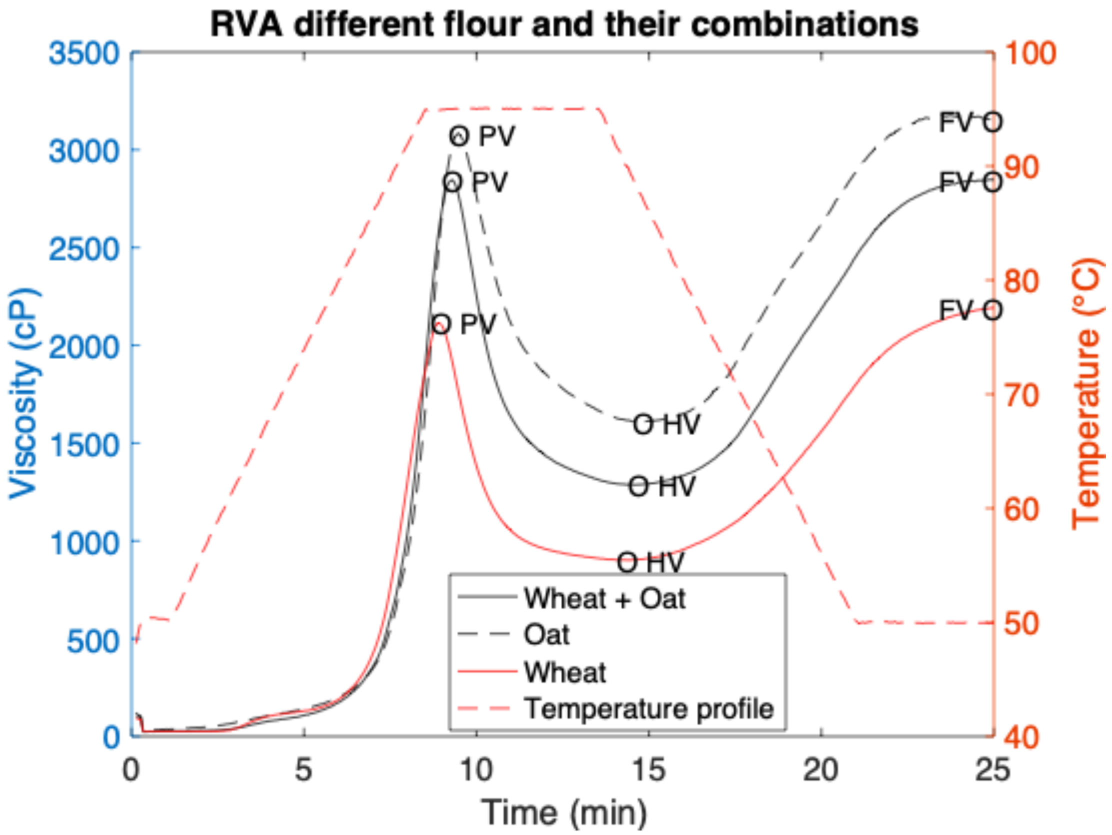

Figure 11. *How the viscosity changed during the temperature profile including both heating and cooling steps.*

Table 2. *The calculated data from the RVA measurements. The relative calculations are shown in parentheses. BD % is based on PV and SB % is based on FV.*

	PV	BD	HV	SB	FV
Oat	3084	1475 (48 %)	1609	1547 (49 %)	3156
Wheat	2115	1213 (57 %)	902	1289 (59 %)	2191
Combined	2846	1558 (55 %)	1288	1559 (54 %)	2847

The oat flour had the highest viscosity for all aspects of the RVA measurements. The peak viscosity was also reached the latest. This could indicate larger starch granules, making the diffusion time longer during swelling. The relative set-back effect from oat flour was also the lowest, indicating good freshness-maintaining properties. Therefore, something that would be well suited to be incorporated into the dough.

The combination of the two flours were basically averages from the two individual flours. Therefore, the more oat flour that becomes incorporated, the better. Also supported by Salehi and Shahedi (2007) where they included different amount of oat flour, to an otherwise wheat consisting dough, with improved bread qualities. During bread baking there are also e.g. protein networking that affects the dough and final product, something not noticed in the pasting trials.

Despite the higher starch content in wheat flour (68 % (Kungsörnen, 2020)) compared to 58 % in oat flour (Risenta, 2020) the overall viscosity was higher for oat flour. This was probably due to the higher fiber content in the oat flour.

BG

To even further understand the different components in the bread baking, and their effects, also simulating how the different BG powders behaved during a baking and cooling temperature profile was of interest, see ***Figure 12***.

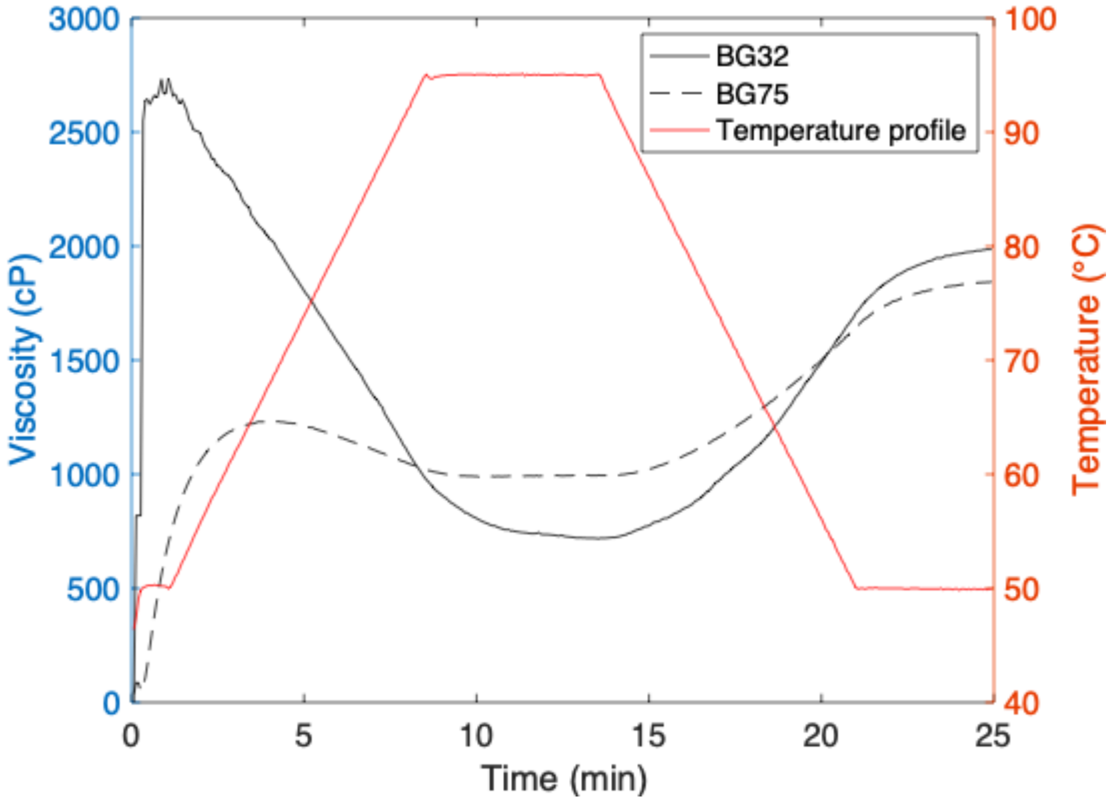

Figure 12. *RVA measurement of BG32 and BG75.*

The rapid increase in viscosity, seen in ***Figure 12*** for BG32, even without the increased temperature indicated a high solubility of BG. The gel breakdown was because of the shear stresses from the rotational mixer. The viscosity increased during the temperature decrease also indicates some impurities of e.g. amylose which retrogrades.

The increase in viscosity during the increased temperature, see ***Figure 12*** for BG75, indicated a solubilization of BG during heating. The gel breakdown was as before a result of the shear stresses but smaller compared to BG32. A lower SB contribute with maintaining freshness for flours (Wani et.al., 2016). BG32 had a HV of 719 cP and a final viscosity of 1986 cP, meaning a SB % of 63 %. B75 had a SB % of 46 %. Therefore, BG75 would likely behave better since prolonging the freshness was the goal.

4.1.2. Thermal properties

To simulate, and thus understand, the retrogradation of pure oat flour, DSC was a great method to utilize. Firstly, a gelatinization run was performed, see ***Figure 13***.

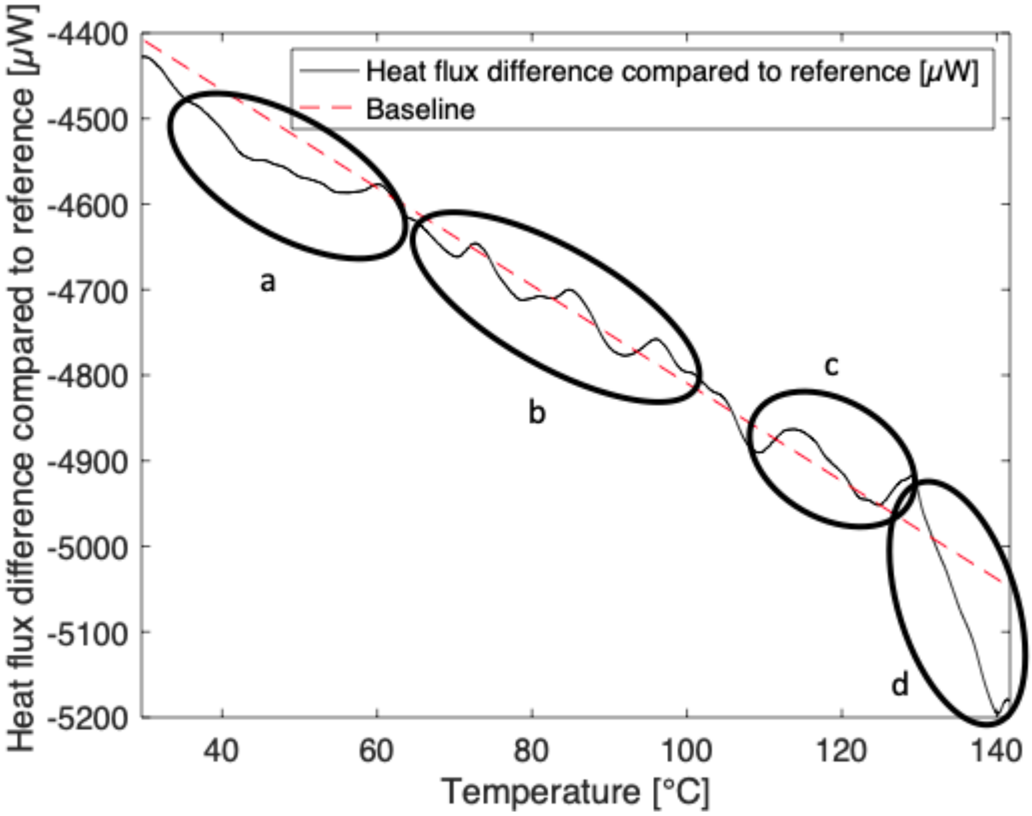

Figure 13. *The DSC curve for oat flour gelatinization.*

The first peak (a) in ***Figure 30*** around 50 °C was the gelatinization. The different peaks between 70 and 90 °C (b) were protein denaturations and the last peaks between 100 and 120 °C (c) were different complexes, amongst them amylose-lipid complexes. After 130 °C (d) there was a leakage from the pan, this caused the slope to increase.

The small peak shown around 50 °C for the oat flour in ***Figure 13*** indicated an already pre-gelatinized starch. The complexes peaks between 100 and 120 °C showed that the oat flour has the lipid content that was expected and amylose content in accordance with the increasing viscosity during the amylose retrogradation in the RVA measurements.

Secondly, to understand the potential staling effects oat flour could have, a storage time of five and seven days for the already gelatinized samples was performed. Two typical graphs without any leakage are shown in ***Figure 14*** and ***Figure 15***.

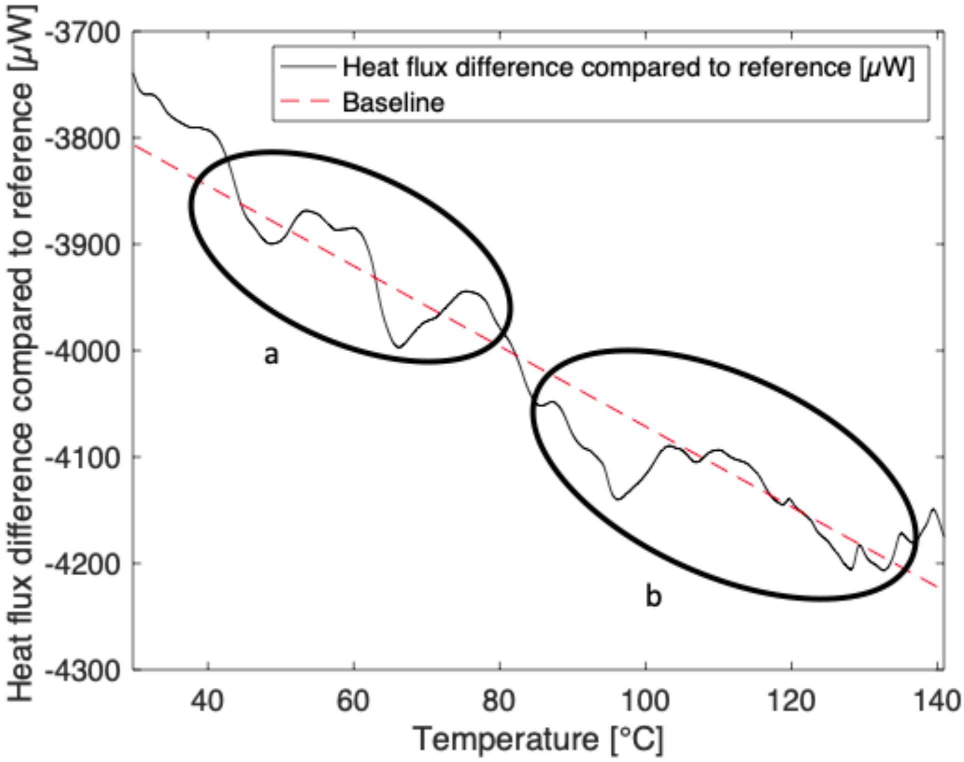

Figure 14. *The different peaks shown for the five-day retrogradation of gelatinized oat flour.*

Peaks shown between 40 and 70 °C in ***Figure 14*** (a) were thought to be starch retrogradation and the other peaks between 80 and 130 °C (b) were differently bound complexes.

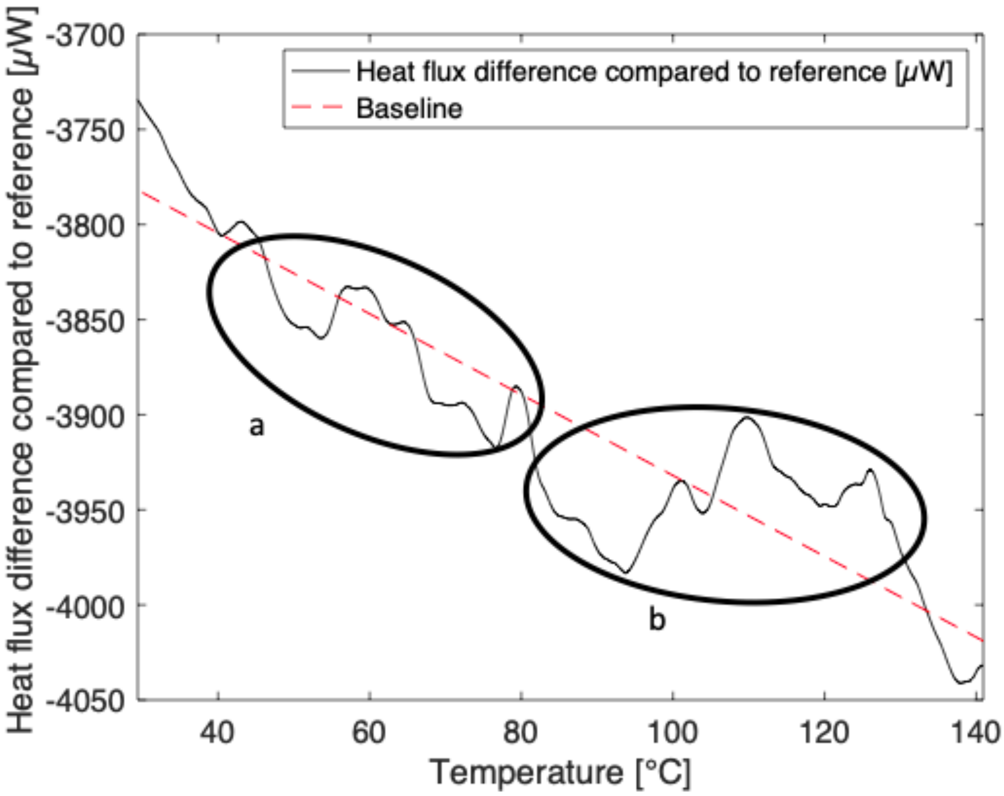

Figure 15. *The different peaks shown for the seven-day retrogradation of gelatinized oat flour.*

Peaks shown between 40 and 80 °C (a) were thought to be starch retrogradation and the other peaks between 80 and 130 °C (b) were differently bound complexes.

The melting enthalpies of the retrograded amylopectin retrograded were lower for day 7, see ***Figure 15***, than for day 5.

4.1.3. ß-glucan dry matter

The dry matter for BG32 was determined to 4.3 ± 1.1 (95% confidence interval, based on standard errors). This was later used when developing the recipe since 68% of BG32 was not BG, it had to be considered to rest from the oat flour, e.g. starch and fat. The assumption that is had the same pasting and retrogradation properties as the oat flour was done and used when calculating. This meant that when more BG was added, small amounts (corresponding to the extra 68% of the BG32-powder) of oat flour was replaced.

4.2. Recipe development

4.2.1. Base recipe

A base recipe was developed during a pre-study and was according to ***Table 3*** for one 400 g dough ball.

Table 3*. The base recipe for high oat flour containing breads without BG for one bread.*

Ingredient	**Oat flour**	**Wheat flour**	**Baker's yeast**	**Salt**	**Sugar**	**Water**	**Total**
Amount (g)	107	107	10	6	5	172	407

Scaling up of the recipe and the addition of the BG concentrations were based on baker's percentage (b%), e.g. BG divided by the total amount of flour. Salt and sugar were also calculated based on total flour. Yeast was used based on total weight of the dough. The 1, 3 and 5 % in b% were 0.49, 1.3 and 2.1 % respectively when pure BG was divided by the total dough. They became relatively smaller to their b% because of an increased addition of water to account for the higher water absorption BG32 had.

The full-scale recipes are shown in ***Table 4***. The total weight exceeded 6 breads with 400 g each since there always was some rests in the mixing bowl left.

Table 4. *The recipes for the different recipes when baking for storage study was performed.*

Ingredient	**R (3 breads) (g)**	**BG32, 1 % (6 breads) (g)**	**BG32, 3 % (6 breads) (g)**	**BG32, 5 % (6 breads) (g)**	**BG75, 3% (6 breads) (g)**
Oat flour	320	593	515	455	607
Wheat flour	320	620	590	570	620
Salt	19	37	33	31	37
Sugar	15	29	26	26	29
Baker's yeast	31	57	57	57	57
BG	0	38	103	160	49
water	517	1095	1128	1167	1193
Total	1222	2469	2452	2466	2592

The recipe for BG75 3 % was based on BG32 3 % but since the rest in BG75 was less, also less oat flour had to be replaced.

4.2.2. Water addition

During the pre-study it was found that when the BG32 was increased, the dough rheology changed during baking if no extra water was added. Therefore, a water addition optimization had to be done so that a good comparability and reproducibility was accomplished. It was dealt with by correcting the water to the point where the same dough torque was achieved, for a typical graph see ***Figure 16***. Through the iterative runs with the DoughLAB with 300 g flours, salt and sugar, the extra water needed for the 1, 3 and 5 % BG32 were 8.6, 16.4 and 23.6 % respectively compared to the water amount needed in the dough without BG to reach the dough torque of 750 FU. The same water content was used for both the pre-solubilized and powdered additions. The peak consistency was reached around 2-2.5 minutes for the different doughs, which therefore determined the mixing the in the bakery when preparing the doughs.

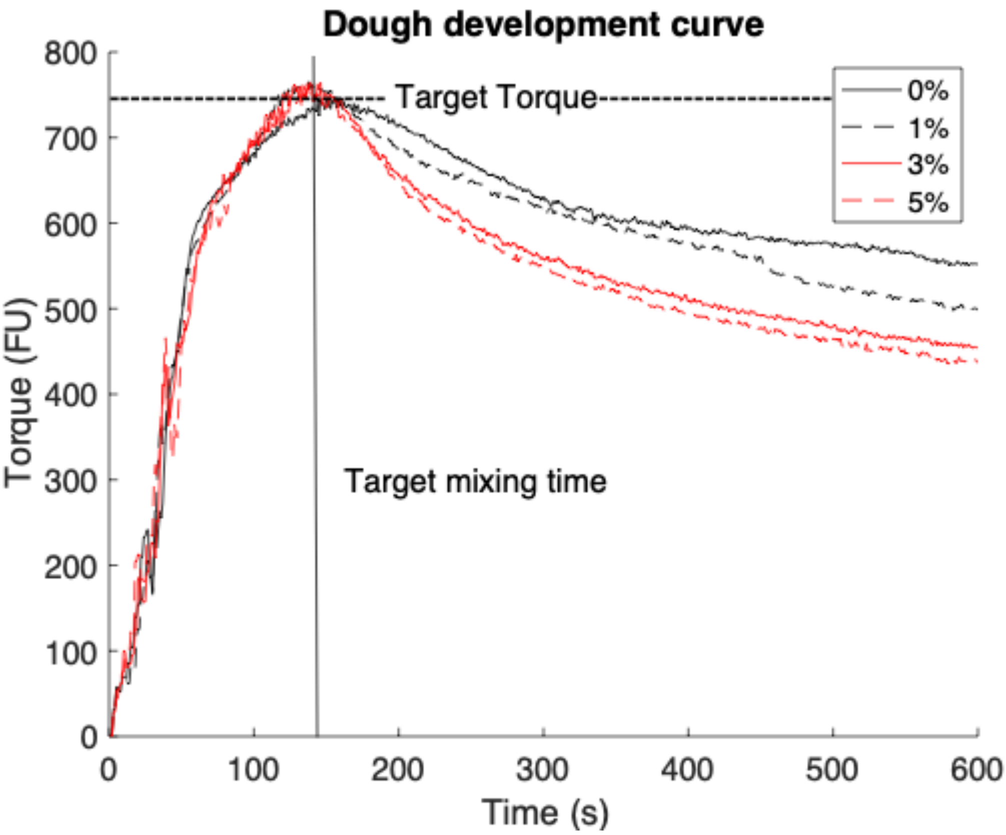

Figure 16. *Dough development curve with all concentrations of BG from BG32. Target mixing time at 150 seconds (2.5 min) and target torque at 750 FU.*

After mixing when baking, the dough was kept resting for 10 minutes. This was because during the constant 25 °C RVA measurement with BG32, it took approximately 10 minutes for the viscosity to reach its plateau, see ***Figure 17.***

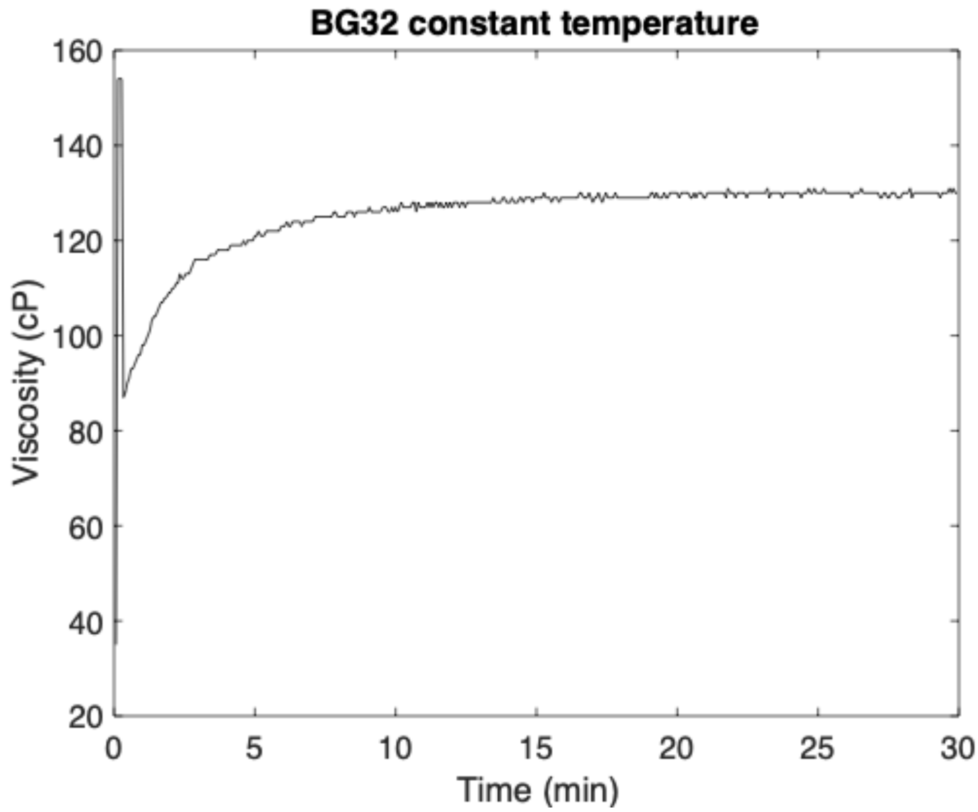

Figure 17. *The viscosity behaviour during stirring at constant temperature for BG32 in RVA.*

4.3. Storage study

4.3.1. BG75

The aim for BG75 was to investigate if it was directly transferable from BG32. It was concluded that the 3 b% recipes were the overall high performers from the BG32 storage study. When recalculating the recipes for BG75, assuming the same water absorption for BG75 as for BG32, the doughs and breads came out dramatically different. The crumb and crust showed big differences in texture and crispness, respectively, compared to reference and breads made from BG32. For comparison, see ***Figure 18*** below. It was therefore concluded that the comparison between the two BG powder origins were not valid.

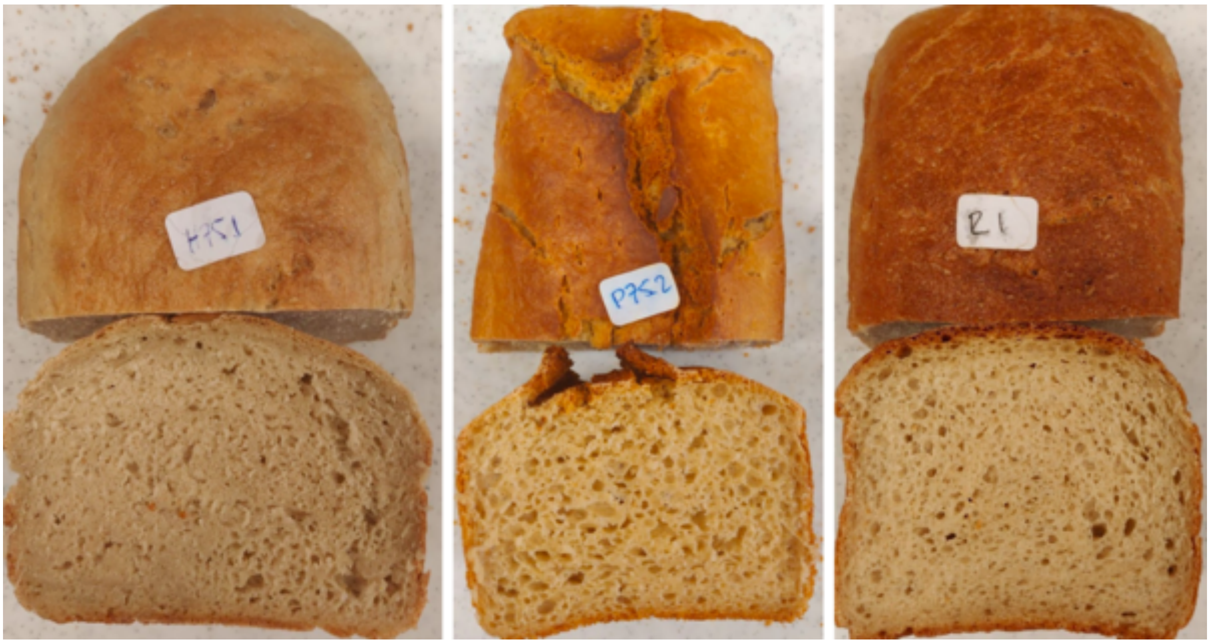

Figure 18. *To the left, BG75 3 % with BG added as pre-solubilized, in the middle BG75 3% added as powder, and to the right a reference bread.*

4.3.2. Data handling

There were in total six outliers, two per moisture content, peak force and springiness.

For a valid comparison between the different baking days, the idea was to subtract the references from their corresponding recipes. This was only required if the references were different. There were significant differences within one storage day for moisture content (day 1) and springiness (day 3) but not one for peak force, see ***Figure 19***, ***Figure 20,*** and ***Figure 21***. For comparison reasons, the same data handling was performed on all responses. It was not possible to investigate whether the references were significantly different from each other for specific volume due to a lack of replicates. The DSC measurements had too many missing values for any data analysis but will be commented on later.

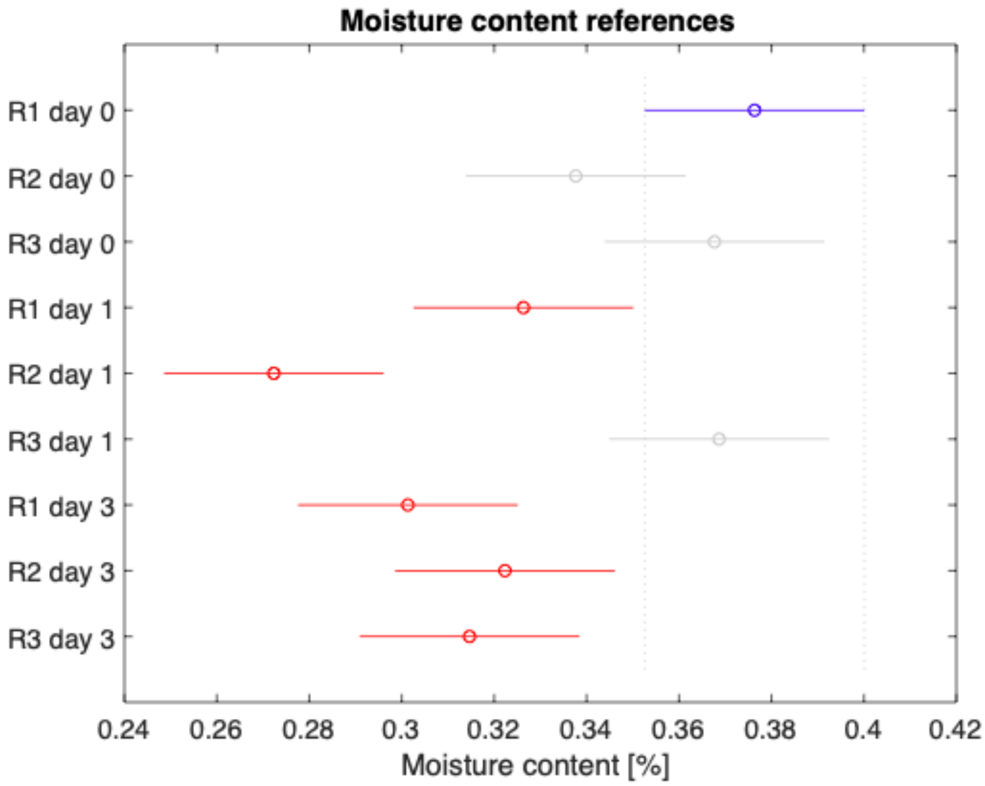

Figure 19. *How the references compare to each other regarding moisture content over the storage days.*

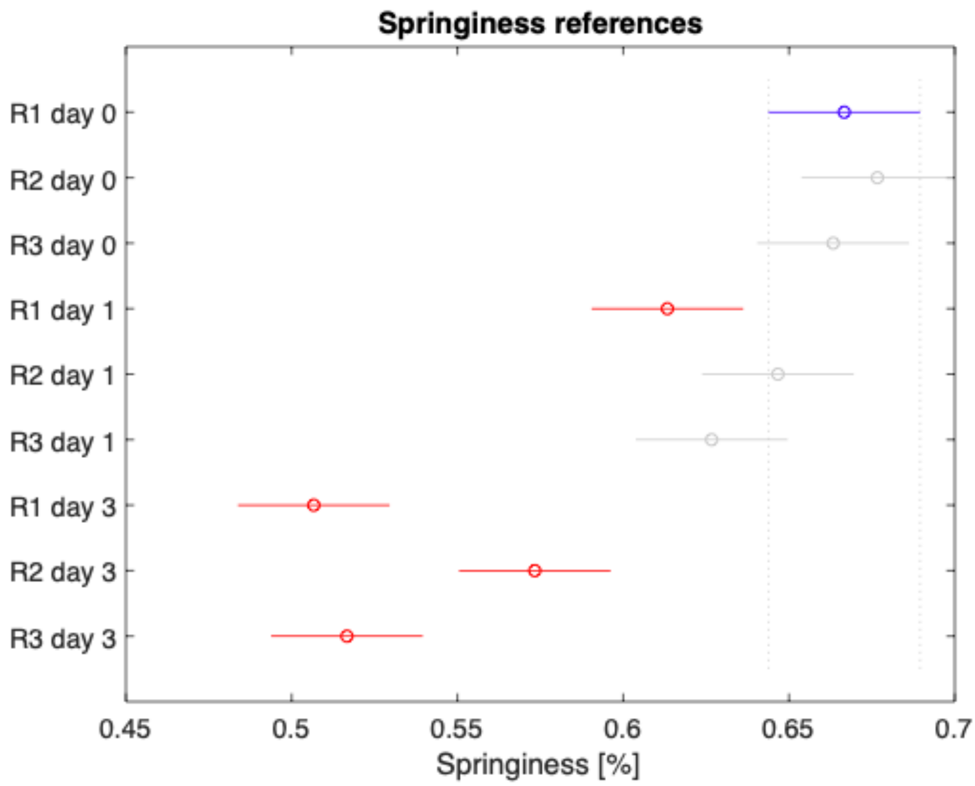

Figure 20. *How the references compare to each other regarding springiness over the storage days.*

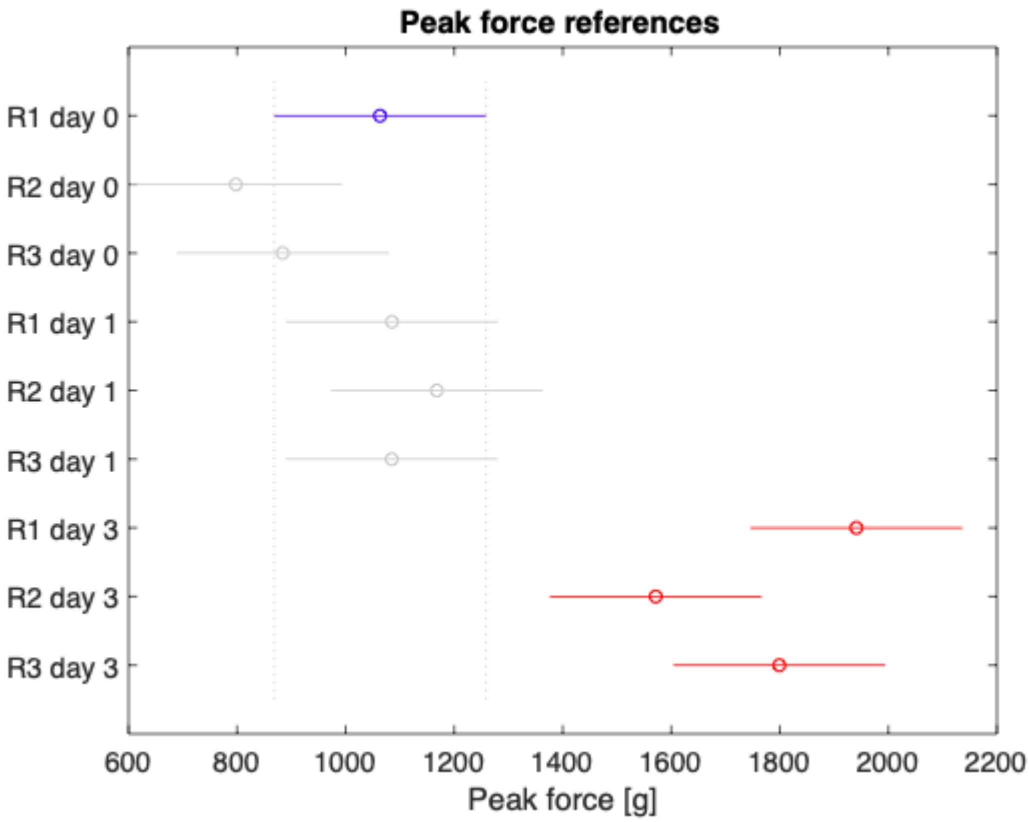

Figure 21. *How the references compare to each other regarding peak force over the storage days.*

The moisture content measurements told, when compared to reference, whether BG32 holds water better. A higher number is therefore better. It was the same for the specific volume and springiness measurements. For the peak force, however, when subtracting the stiffer references, the better, i.e. lower peak force, was the most negative value. E.g. if the reference was 2000 g in peak force and bread A was 1500 g and bread B was 780 g, bread B is better. The corrected values would be -500 g and -1220 g, respectively.

The statements made below, apart from the DSC measurements, were made based on an integrated software in MATLAB when performing the multiple comparison test on all the recipes and storage days where significant differences easily are shown with different colours, but changes by interest. The images show 95% confidence intervals based on all the observations for each response, i.e. all days and recipes contribute to the variability of the measurement means.

4.3.3. Multivariate analysis

The PCA showed some interesting correlations, see ***Figure 22***:

- Storage days and peak force were negatively correlated. Since a larger negative value was wanted for peak force data, an increase in storage days seemed to correlate well with a softer crumb. Since concentration and way of addition was neither positively nor negatively correlated, it was thought to be concentration and addition type independent. According to this PCA, the longer the storage, the softer crumb compared to corresponding reference the breads containing BG showed.
- Concentration and moisture content were positively correlated. The higher the concentration, the higher moisture content in the bread. This could be due to the higher water level in the initial dough or that BG bound the water more strongly.

- Addition, springiness, and specific volume were positively correlated. When BG32 was added as a powder, the springiness and specific volume was also higher. But, the addition was negatively correlated with moisture content, indicating a higher moisture content when BG was added as pre-solubilized.

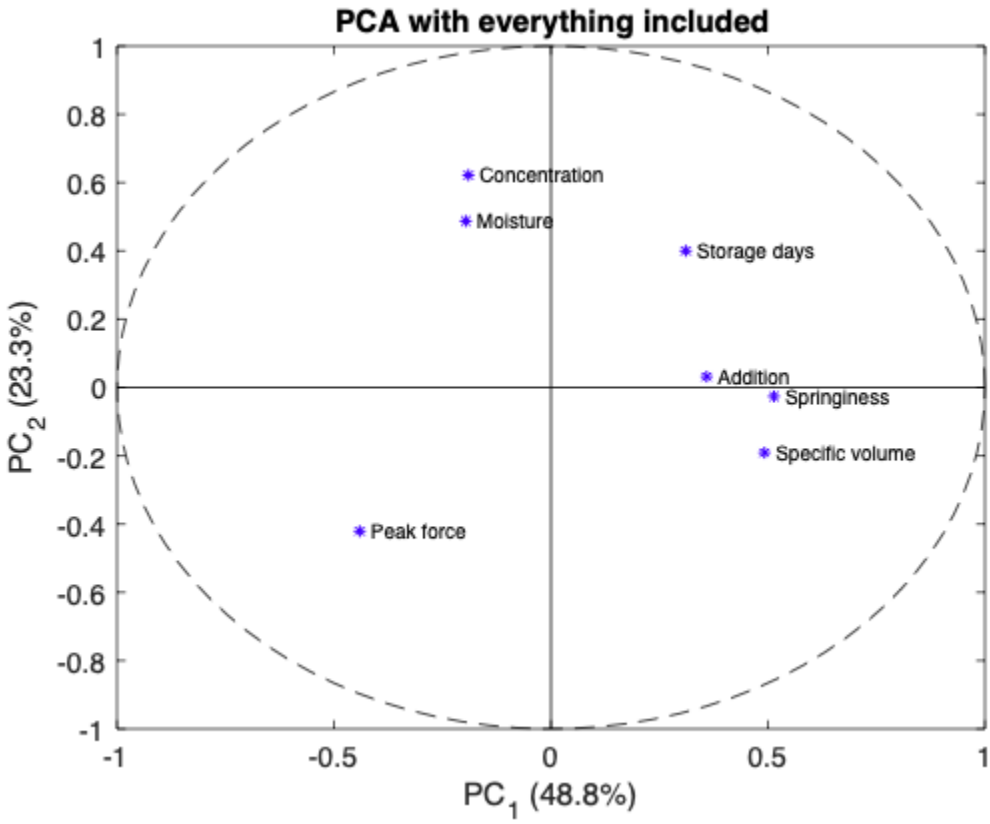

Figure 22. *The PCA of process variables and the responses. The explanation from the two PC:s were 72.1 % of the total variance.*

The PLS showed a similar result in its W*Q plot, see ***Figure 23*** where the process variables and responses were illustrated. The only differences were that concentration and moisture were not as correlated and that the orientation of PC_1 was in the opposite direction, since ***Figure 23*** was mirrored in the x=0-axis compared to ***Figure 22***.

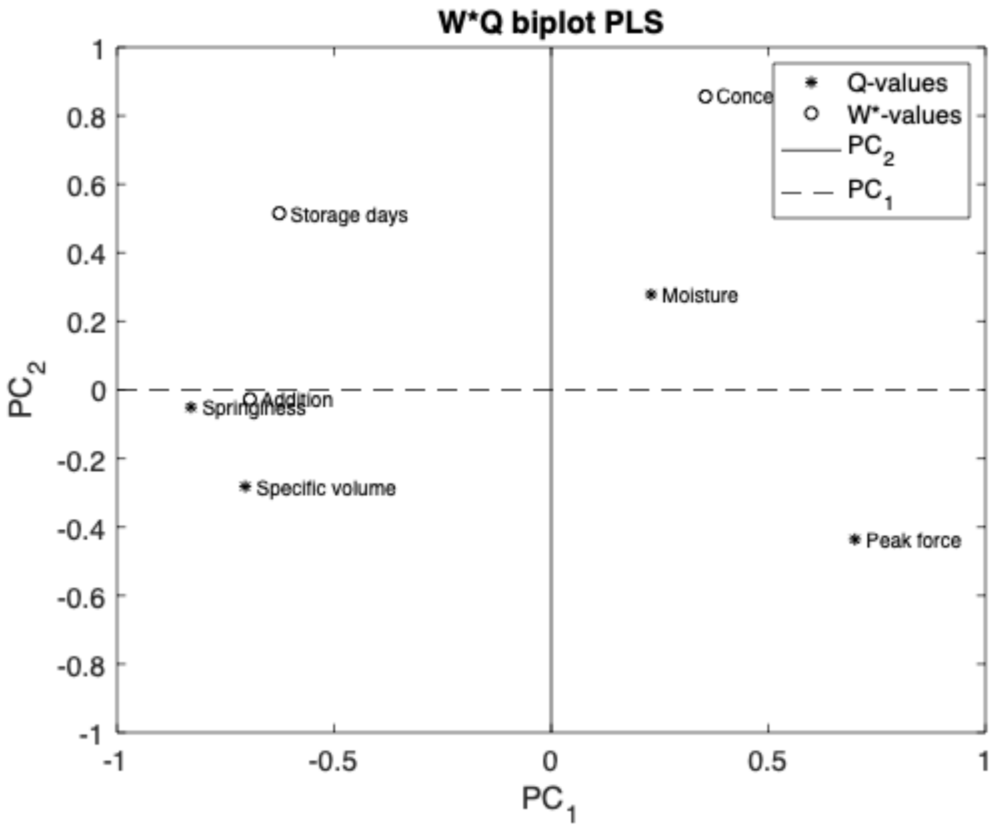

Figure 23*. The correlations between the processing variables and responses shown after an optimization was done when describing the responses from the variables.*

How the processing variables and all the observations were correlated is seen in ***Figure 24***. There is a visible trend towards the different processing variables in the different recipes and storage days. If a vector was drawn from the origin to the process variable the same direction was seen in the observations, i.e. when looking at the different observations and their storage days, the vector between storage days 0, 1 and 3 is pointing in the same direction. The same is true for concentration and addition type.

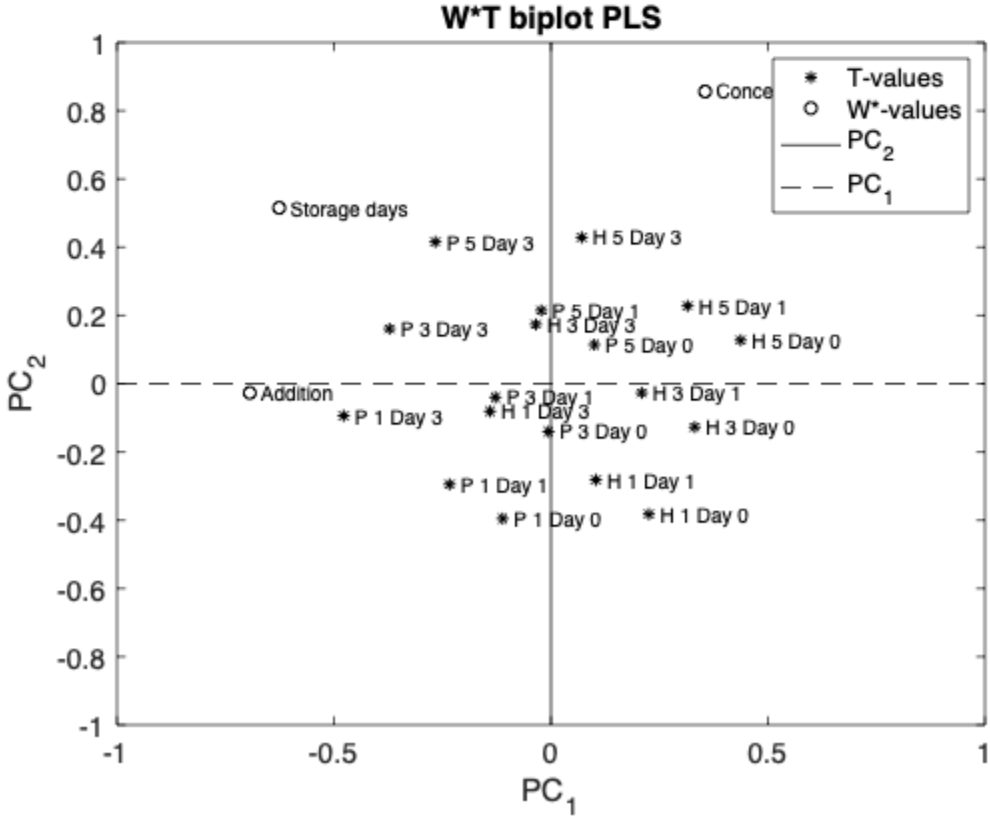

Figure 24. *A plot investigating the correlations between the observations and processing variables. Trends in the same direction from origo to the processing variables were seen in the observation scatter.*

4.3.4. Thermal properties

A higher retrogradation enthalpy would indicate a more prominent staling, which was not wanted. However, if the other responses indicate no staling, the retrogradation was wanted since it would act as an indigestible starch and therefore as a prebiotic improving gut microflora as discussed earlier in this master's thesis.

Zhang et. al. (1998) found that the addition of oat flour in wheat breads retards the retrogradation. They suspected the increased water content in the dough to be the main factor retarding the staling. By including more of the highly water absorbent BG in the dough, it means that the effect could be even greater.

4.3.5. Moisture content

The higher the moisture content the better since it would make the bread appear less dry. Day 0, 1 and 3 had total mean moisture contents (based on all recipes per storage day) of 0.373 ± 0.011 g/g, 0.357 ± 0.013 g/g and 0.331 ± 0.013 g/g, respectively. The following reasoning and conclusions are based on ***Figure 25***. The samples not significantly higher than the corresponding reference were H1-5, P1-3 storage day 0 and P1-3 storage day 3. That the first storage day did not show higher moisture content than corresponding reference for all but P5 was not surprising, since the effect of BG was expected to not have the possibility to retain the water due to too little time had passed. Storage day 1 showed significant higher moisture content values for P5 than P1. The same was true for the same recipes, storage day 3, but then not significantly better than reference, except for P5.

For moisture content, it seems as if the higher the concentration of BG from BG32, the better moisture retention. It also seemed as if the concentration gradient correlation (the higher the concentration, the higher moisture content) was clearer when BG from BG32 was added as a powder instead of pre-solubilized but the latter showed indications towards a higher overall moisture content. This could be a result of the lack of competition for water when the dough was mixed, since the pre-solubilized already had had full access to all the water.

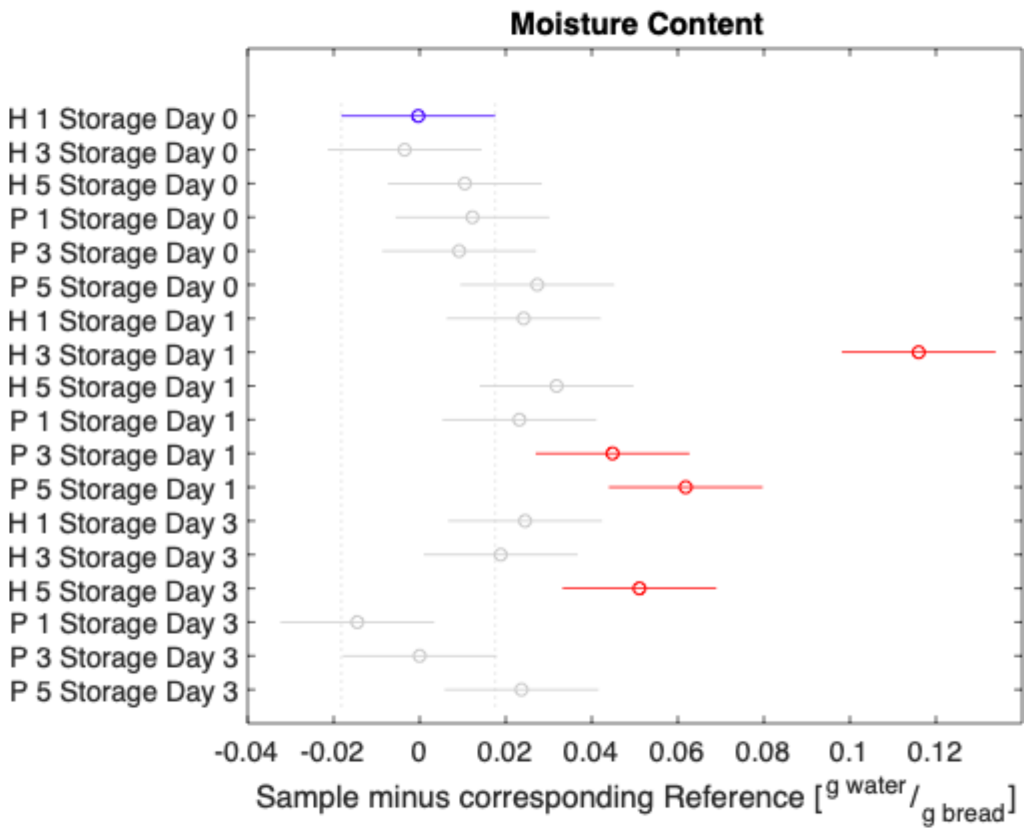

Figure 25*. How the different recipes compared to their corresponding reference regarding moisture content.. The higher value, the better.*

Water loss

During baking there were weight loss, most likely due to water evaporation, from the dough to final loaf between 53 and 65 g (total dough of 400 g). When the breads' crusts were investigated, there were noticed some colour differences. This was due to uneven temperatures in the oven. The immediate question that arose was whether this affected the water loss and thus also the moisture content. Either the darker crust provides a less permeable barrier for water to escape during baking, leading to a higher moisture content, or the higher temperature reaches the inside of the bread faster, starting to dry out the breads while the breads baked in the cooler part of the oven still were baking. The latter leading to a lower moisture content. Another question was if the dough water content affected the weight loss. If the water content in the dough was higher, there was also more water arguably more prone to evaporate. The probability for correlation between the dough water content and water loss was 61 % ($p=0.39$ with H_0: No correlation between the two data sets), i.e. not high enough to say that there was a correlation.

4.3.6. Specific volume

The following reasoning and conclusions are based on ***Figure 26***. Day 0, 1 and 3 had total mean specific volumes (based on all breads per storage day) of 2.04 ± 0.07 ml/g, 2.03 ± 0.05 ml/g and 2.08 ± 0.08 ml/g, respectively. The overall impression from specific volume is that there are not that many significantly different from each other. The only ones that was significantly better than their corresponding reference were P1 day 0, P1 day 3 and P5 day 3. There seem to be a trend towards a lower specific volume when the concentration of BG increases. This was due to the higher moisture content in the breads which made them heavier, thus decreasing the specific volume, which can be seen in ***Figure 22*** as a negative correlation between moisture content, concentration and specific volume.

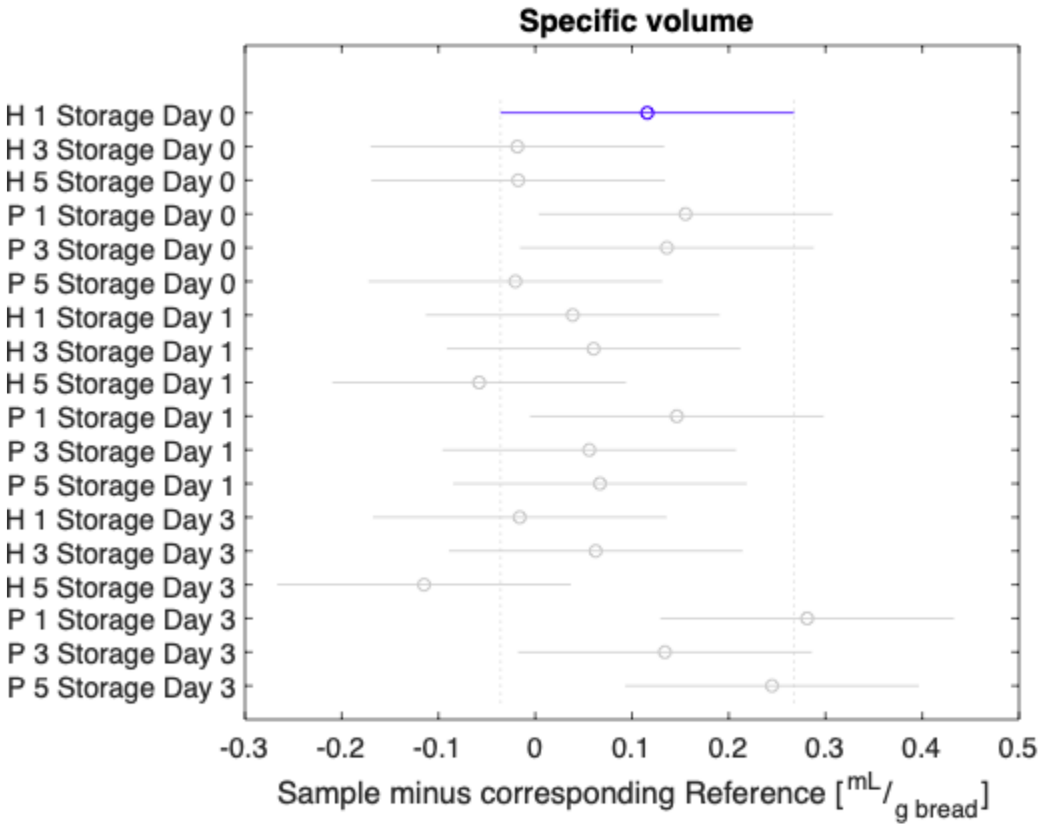

Figure 26*. How the different recipes compared to their corresponding reference regarding specific volume. The higher value, the better.*

4.3.7. Texture

Springiness

The following reasoning and conclusions are based on ***Figure 27***. Day 0, 1 and 3 had total mean specific volumes (based on all breads per storage day) of 0.66 ± 0.01, 0.64 ± 0.01 and 0.57 ± 0.01, respectively. The ones that were significantly better/higher than their corresponding reference were P1 day 1, H3 day 3 and P1-5 day 3. These five were not significantly different from each other. Breads with BG added as powder during the third day of storage were significantly more springy than H1 and H5 the same day. A trend towards a springiness decrease with increasing concentration was observed. It also seemed like that the powder addition of BG from BG32 generated an overall higher springiness compared to the pre-solubilized ones which were less springy.

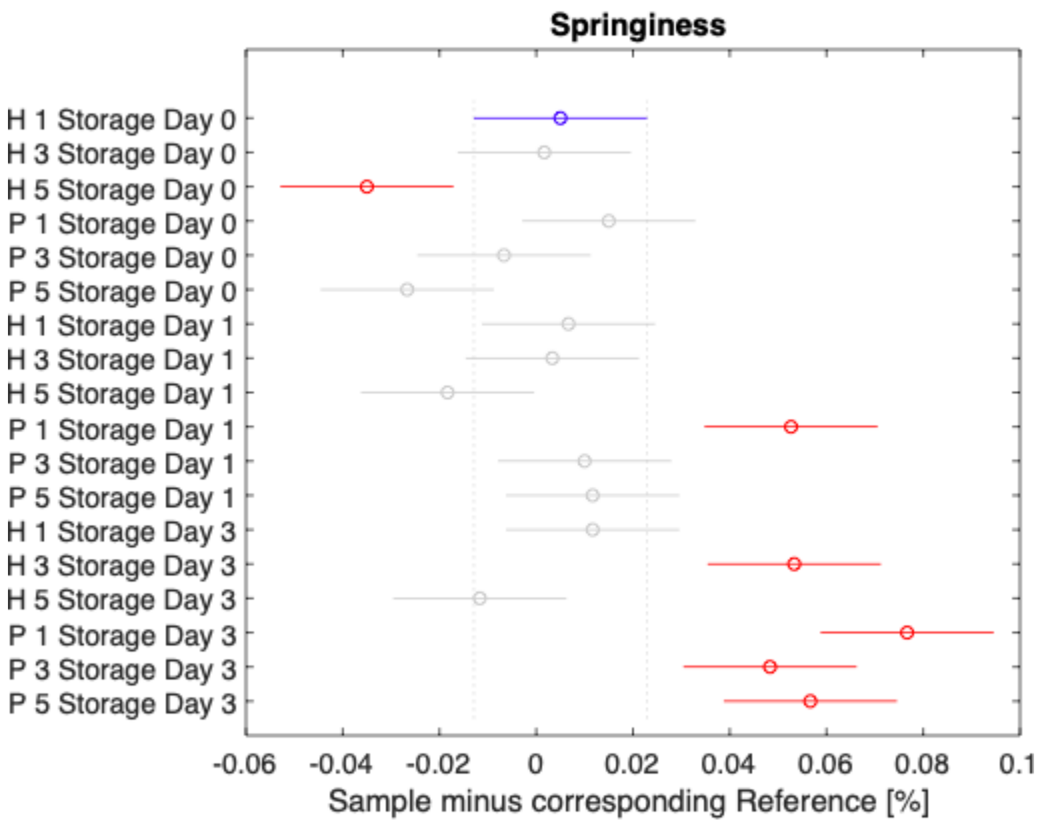

Figure 27*. How the different recipes compared to their corresponding reference regarding springiness . The higher value, the better.*

Softness

The following reasoning and conclusions are based on ***Figure 28***. Day 0, 1 and 3 had total mean peak forces (based on all breads per storage day) of 592 ± 45 g, 814 ± 56 g and 1109 ± 64 g, respectively. All samples except for H5 day 0 were significantly softer than their corresponding reference. The longer the storage time, the bigger differences were observed between the different concentrations and ways of addition. The trends noticed were a softer crumb compared to their corresponding reference with an increase in concentration and the samples when BG from BG32 was added as powder were also softer. E.g. was P3 day 3 significantly softer than both H1 and H5 the same day.

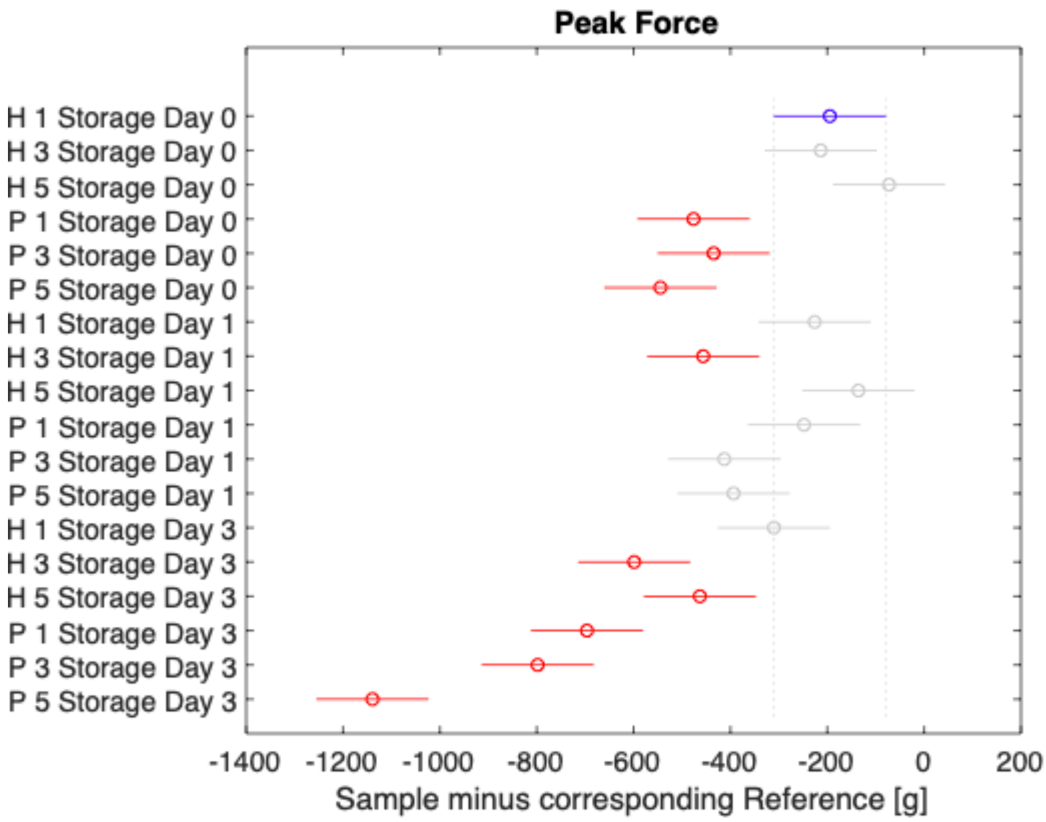

Figure 28. *How the different recipes compared to their corresponding reference regarding peak force. The lower value, the softer.*

4.4. Visual evaluation

According to ***Figure 29***, ***Figure 30***, and ***Figure 31*** no major differences were observed. Slight differences regarding crumb hole size could be noticed, but not statistically confirmed. The crust colour was as already mentioned different for different breads. Probably not due to differences in recipes but instead because of uneven heating in the oven. For images of all recipes and storage days, see ***Appendix 8.2. Visual evaluation***.

Figure 29. *Crust and crumb shot of reference bread.*

Figure 30. *Crust and crumb shot of powered BG32 5 b%.*

Figure 31. *Crust and crumb shot of pre-solubilized BG32 5 b%.*

4.5. Sources of error

During the lab work there were factors influencing the measurements and results. One part of them were uncertainty in measurements and the other part were the sources of error. The following section will focus on the latter.

During baking, the uneven temperature within the oven gave rise to colour differences between the breads, see ***Figure 32***. It shows how the colour looked on five different breads baked at the same time, the rightmost being the furthest out, closest to the oven door. The different hot parts in the oven would likely affect water loss during baking and therefore moisture content and thus also the specific volume. During the moisture content measurements, when the breads were taken out from the oven and put in a desiccator, there was condensation. This could have affected the moisture content to be less than what it really was for those breads that possibly contained some condensation. When performing the texture analyses, the probe was placed in the center of the crumb. If there was an air bubble at that position the probe was moved a bit off-center. Even though this was considered, the unevenness in the crumb was a factor not able to impact apart from the handling of the dough. The same heterogeneity was impactful when measuring staling with DSC. Taking a representative part of the bread crumb was not a simple or clear task. It was especially difficult since the sample size was around 7 mg. This was a known source of error due to the high variability in DSC measurements.

Figure 32. *The effect the uneven heating from the oven had on bread colour.*

5. Conclusions

To conclude, it was observed that the addition style from powder showed high correlations with the quality aspects high specific volume and springiness. However, pre-solubilized BG32 showed indications towards retaining higher moisture content in the bread.

Additionally, the concentration contributing with the highest bread quality had to be considered the intermediate 1.2 % BG when baking with BG32. This was concluded since the MVAs showed that springiness and specific volume was negatively correlated with an increasing concentration. The latter was nevertheless positively correlated with higher moisture content. Something being a property highly associated with less overall staling.

Unfortunately, the differences between BG75 pre-solubilized and powders samples were too big. They differed also compared to BG32 breads. To enable comparisons, a dough water optimization would be necessary.

For the breads made with BG32 as BG source, the specific volume was generally unaffected, the moisture content higher, the higher concentration and higher for pre-solubilized than for powdered addition. Regarding the peak force (softness), all but one breads were softer than their corresponding reference even from day 0. The springiness was better for breads made with powdered addition of BG32 and better compared to reference the longer the storage time.

The recommendations from the conclusions would be to use intermediate BG from BG32 concentrations (1.2 % of total weight) added as a powder.

5.1. Future aspects

To eliminate the potential sources of error there are a few possible corrections:

- Turn the breads in the oven halfway through the baking time to certain an even cooking and crust formation.
- Potentially dry breadcrumbs before performing the DSC for it to be easier when adding into the pans, thus avoiding leakage.
- Avoid any condensation in the desiccator when cooling the dried bread for moisture content measurements.
- Re-calculate the sugar, BG and salt concentrations based on total dough weight and not base it on total flour weight.
- Have a better knowledge about how the oven was run, regarding the program temperature profile and steam injection amounts.

If more time were available, it would also be highly interesting to optimize the water addition for BG75 so that comparable results could be obtained. Furthermore, it would be interesting to study any potential complex formations between flour components and BG. To get a clearer view on the DSC measurements, a sampling size sensitivity analysis could be suitable.

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7. References

Anderson, S., Purhagen, J. K., & Bason, M. L. (2014). AACCI approved methods technical committee report: Collaborative study on bread volume determination by laser topography using a bread volume meter. *Cereal Foods World*, *59*(6), 294-296.

Bason, M. L., Dang, J. M. C., & Charrié, C. (2005). COMPARISON OF THE DOUGHLAB AND FARINOGRAPH FOR TESTING FLOUR QUALITY. In *Using cereal science and technology for the benefit of consumers* (pp. 276-282). Woodhead Publishing.

Berg, J. M., Tymoczko, J. L., Gatto, G., & Stryer, L. (2015). Biochemistry (eight edition).

Belton, P. S. (1999). Mini review: on the elasticity of wheat gluten. *Journal of Cereal Science*, *29*(2), 103-107.

Benkeblia, N. (Ed.). (2014). *Polysaccharides: Natural fibers in food and nutrition*. CRC Press.

Brancoli, P., Lundin, M., Bolton, K., & Eriksson, M. (2019). Bread loss rates at the supplier-retailer interface–Analysis of risk factors to support waste prevention measures. *Resources, Conservation and Recycling*, *147*, 128-136.

Brereton, R. G. (2003). Chemometrics: data analysis for the laboratory and chemical plant. John Wiley & Sons.

Burri, J. (2000). *U.S. Patent No. 6,136,365*. Washington, DC: U.S. Patent and Trademark Office.

Choromanska, A., Kulbacka, J., Rembialkowska, N., Pilat, J., Oledzki, R., Harasym, J., & Saczko, J. (2015). Anticancer properties of low molecular weight oat beta-glucan–An in vitro study. *International journal of biological macromolecules*, *80*, 23-28.

Corke, H., De Leyn, I., Nip, W. K., & Cross, N. A. (2008). *Bakery products: science and technology*. John Wiley & Sons.

Edwards, W. P. (2007). The science of bakery products. Royal Society of chemistry.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2010). Scientific Opinion on the substantiation of a health claim related to oat beta glucan and lowering blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006. *EFSA Journal*, *8*(12), 1885.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2011). Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and “digestive function”(ID 850) pursuant to Article 13 (1) of Regulation (EC) No 1924/2006. *EFSA journal*, *9*(6), 2207.

Goff, H. D., Repin, N., Fabek, H., El Khoury, D., & Gidley, M. J. (2018). Dietary fibre for glycaemia control: Towards a mechanistic understanding. *Bioactive carbohydrates and dietary fibre*, *14*, 39-53.

Gudmundsson, M. (1994). Retrogradation of starch and the role of its components. *Thermochimica Acta*, *246*(2), 329-341.

Hager, A. S., Ryan, L. A., Schwab, C., Gänzle, M. G., O'Doherty, J. V., & Arendt, E. K. (2011). Influence of the soluble fibres inulin and oat β-glucan on quality of dough and bread. *European Food Research and Technology*, *232*(3), 405-413.

Hüttner, E. K., Dal Bello, F., & Arendt, E. K. (2010). Rheological properties and bread making performance of commercial wholegrain oat flours. *Journal of cereal science*, *52*(1), 65-71.

Jovanovich, G., & Añón, M. C. (1999). Amylose-lipid complex, physicochemical properties and the effects of different variables. *LWT-Food Science and Technology*, *32*(2), 95-101.

Kale, M., Hamaker, B., & Bordenave, N. (2013). Oat β-Glucans: Physicochemistry and Nutritional Properties. *Oats Nutrition and Technology*, 123-169.

King Arthur Flour Company. (2020). *King Arthur Unbleached Bread Flour - 5 lb.*. King Arthur Flour. Retrieved 12 May 2020, from https://shop.kingarthurflour.com/items/king-arthur-unbleached-bread-flour-5-lb.

Kulp, K. (Ed.). (2000). *Handbook of Cereal Science and Technology, revised and expanded*. CRC Press.

Kungsörnen. (2020). *Svenskodlat Vetemjöl Special från Kungsörnen*. Kungsornen.se. Retrieved 9 June 2020, from https://www.kungsornen.se/produkter/vetemjol-special-2000g/.

Laye, P. G. (2002). Differential thermal analysis and differential scanning calorimetry. *Principles of thermal analysis and calorimetry*, *52*.

Lee, H. S., Kim, K. H., Park, S. H., Hur, S. W., & Auh, J. H. (2020). Amylose-Lipid Complex as a Fat Replacement in the Preparation of Low-Fat White Pan Bread. *Foods*, *9*(2), 194.

Lineback, D. R., & Wongsrikasem, E. (1980). Gelatinization of starch in baked products. *Journal of Food Science*, *45*(1), 71-74.

Ludwig, D. S., Majzoub, J. A., Al-Zahrani, A., Dallal, G. E., Blanco, I., & Roberts, S. B. (1999). High glycemic index foods, overeating, and obesity. *Pediatrics*, *103*(3), e26-e26.

Malmberg, C. (2020). Introduction to Master thesis with Lantmännen [Skype-meeting].

Mason, R. (2002). *What is Beta Glucan?*. Safe Goods/New Century Publishing 2000

Master's thesis with Lantmännen. (2018). *Addition of high hydrostatic pressure treated oat fractions, in relation to improvement of the quality of oat bread.*

McCann, T. H., & Day, L. (2013). Effect of sodium chloride on gluten network formation, dough microstructure and rheology in relation to breadmaking. *Journal of cereal science*, *57*(3), 444-452.

Miller, R. A., & Hoseney, R. C. (2008). Role of salt in baking. *Cereal Foods World*, *53*(1), 4-6.

Mohebbi, Z., Homayouni, A., Azizi, M. H., & Hosseini, S. J. (2018). Effects of beta-glucan and resistant starch on wheat dough and prebiotic bread properties. *Journal of food science and technology*, *55*(1), 101-110.

Moisio, T., Forssell, P., Partanen, R., Damerau, A., & Hill, S. E. (2015). Reorganisation of starch, proteins and lipids in extrusion of oats. *Journal of Cereal Science*, *64*, 48-55.

Mälkki, Y., & Virtanen, E. (2001). Gastrointestinal effects of oat bran and oat gum: a review. *LWT-Food Science and Technology*, *34*(6), 337-347.

Pennington, D. (2017). *What causes low, falling numbers in wheat?*. Michigan state University, MSU extension. Retrieved 23 May 2020, from https://www.canr.msu.edu/news/what_causes_low_falling_numbers_in_wheat.

Perten Instruments. (2020a). Retrieved 8 May 2020, from https://www.perten.com/Products/Rapid-Visco-Analyser-RVA/.

Perten Instruments. (2020b). Retrieved 8 May 2020, from https://www.perten.com/Products/doughLAB/.

Perten Instruments. (2020c). Retrieved 8 May 2020, from https://www.perten.com/Products/doughLAB/Features-and-benefits/.

Perten Instruments. (2020d). Retrieved 8 May 2020, from https://www.perten.com/Products/texture-analyzer/.

Perten Instruments. (2020e). Retrieved 9 May 2020, from https://www.perten.com/Industries/Baking/BVM-Volume-Meter/.

Perten Instruments. (2020f). Retrieved 29 May 2020, from https://www.perten.com/Products/doughLAB/Applications/.

Perten Instruments. (2020g). Retrieved 29 May 2020, from https://www.perten.com/Products/texture-analyzer/texture-analysis-applications/.

Rieder, A., Holtekjølen, A. K., Sahlstrøm, S., & Moldestad, A. (2012). Effect of barley and oat flour types and sourdoughs on dough rheology and bread quality of composite wheat bread. *Journal of Cereal Science*, *55*(1), 44-52.

Risenta. (2020). *Havremjöl*. Risenta.se. Retrieved 23 May 2020, from https://www.risenta.se/produkter/mjol-flingor-socker/havremjol.

SALEHI, F. M., & Shahedi, M. (2007). Effects of oat flour on dough rheology, texture and organoleptic properties of taftoon bread.

SweOat. (2020). *Oat beta glucans*. Oat Fiber from Sweden. Retrieved 20 May 2020, from https://www.sweoat.com/oat-beta-glucans

Teraoka, I. (2002). *Polymer solutions*. John Wiley & Sons, Inc.

Therdthai, N., Zhou, W., & Adamczak, T. (2002). Optimisation of the temperature profile in bread baking. *Journal of Food Engineering*, 55(1), 41-48.

Wang, S., Li, C., Copeland, L., Niu, Q., & Wang, S. (2015). Starch retrogradation: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, *14*(5), 568-585.

Wani, I. A., Sogi, D. S., Sharma, P., & Gill, B. S. (2016). Physicochemical and pasting properties of unleavened wheat flat bread (Chapatti) as affected by addition of pulse flour. *Cogent Food & Agriculture*, *2*(1), 1124486.

Zhang, D., Moore, W. R., & Doehlert, D. C. (1998). Effects of oat grain hydrothermal treatments on wheat-oat flour dough properties and breadbaking quality. *Cereal Chemistry*, *75*(5), 602-605.

8. Appendices

8.1. HHP Protein in bread baking

Significance

The initial thought of this master thesis was to make 100 % oat flour breads. Since another previous master's thesis work back in 2018 (Master's thesis with Lantmännen, 2018) was successful with a high hydrostatically pressurized (HHP) oat protein fraction which was included in the dough (or rather a batter), it was something to investigate here as well. A developed recipe from the aforementioned study was provided. Their breads had a gluten-like crumb and high specific volume. Something that is not easily achieved without the addition of the HHP oat protein fraction.

Oat proteins

The oat protein fraction was supplied by Lantmännen with a composition according to ***Table 5***.

Table 5. *Composition of oat protein fraction.*

	Water content	Protein content	Beta glucan	Fat	Starch	Ash	Sum
Amount (%)	6.6	30.5	1.3	5.3	48.8	5.1	91

Bread making with and without HHP

The same recipe as the previous master's thesis work was used. The recipe without HHP was recalculated by dividing the water and protein powder fractions to the total water and oat flour parts respectively.

To compare how the HHP affected the bread quality, breads with (see ***Figure 34***) and without HHP (see ***Figure 33***) were baked.

Figure 33. *A crumb shot of 100 % oat bread without HHP*

Figure 34. *A crumb shot of 100 % oat bread with HHP*

Discussion and conclusions

The results were not replicable from the previous study. The reason behind it was probably due to a storage time dependency after treatment and/or oat flour variability and its baking properties.

8.2. Visual evaluation

During the visual evaluation a combined crust and crumb photo was taken. In ***Table 6.*** each recipe and storage day is shown.

Table 6. *A crust and crumb shot of all the recipes and storage days.*

Recipe	Storage day 0	Storage day 1	Storage day 3
R1			
R2			

R3			
R4			

<table>
<tr><td>H1</td><td>
</td><td>
</td><td>
</td></tr>
<tr><td>H3</td><td>
</td><td>
</td><td>
</td></tr>
</table>

H5			
P1			

P3			
P5			

P75.3			
H75.3