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– Effect of extraction method and particle size

Extraktion av β-glukan från havrekli – Effekt av extraktionsmetod och partikelstorlek

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Swedish University of Agricultural Sciences, SLU
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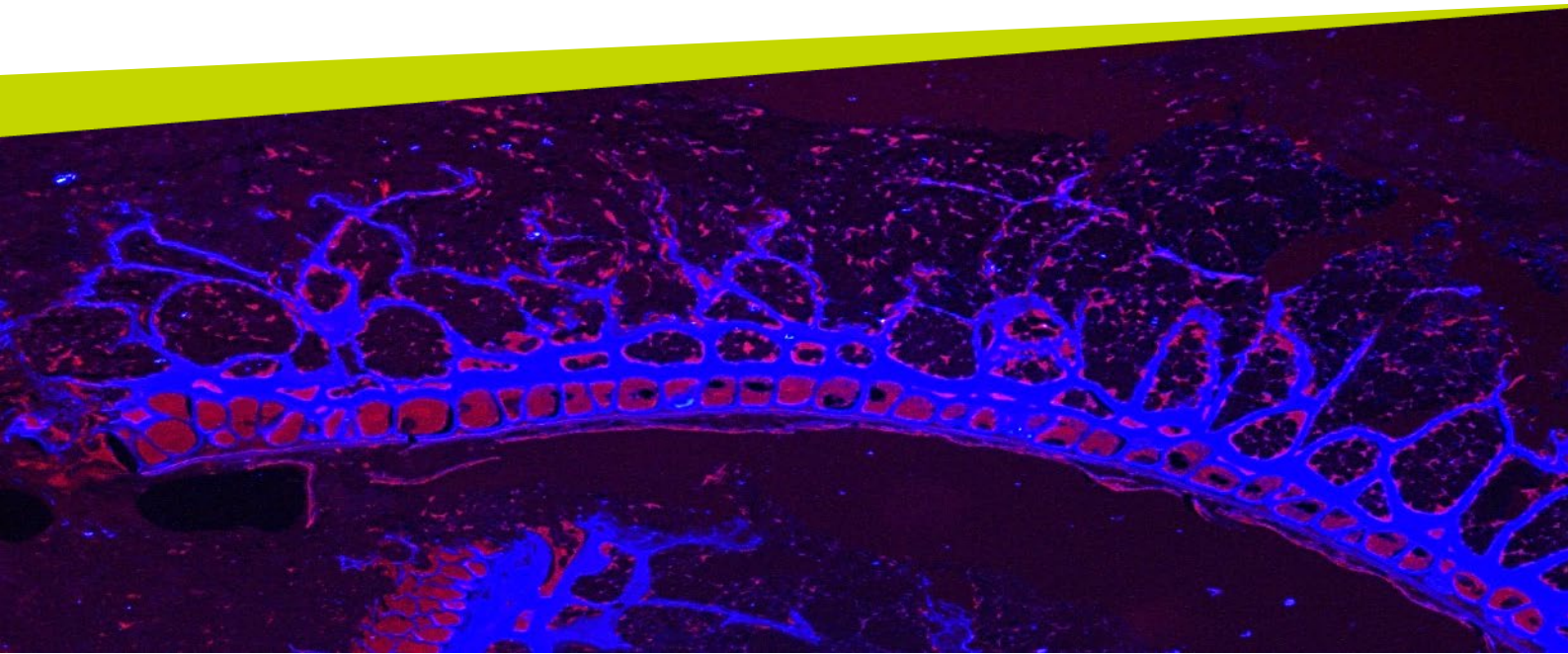

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Abstract

An increasing number of people are suffering from lifestyle related diseases due to poor dietary habits. The recommendation of a fibre intake of 25-35g per day is not achieved by many. There is a need for an optimization of already existing products in order to provide greater variety and making it easier for people to maintain healthy dietary habits. Oat bran is a by-product in the production of oat products although it is full of nutrients. It is rich in dietary fibre and β-glucan constitutes approximately 5-20% of the bran. A daily intake of β-glucan is known to reduce the risk of cardiovascular diseases and to lower the blood glucose levels and the LDL-cholesterol.

The purpose of this study was to investigate how different particle sizes of oat bran and different extraction methods affect the yield and molecular weight of β-glucan. The extraction methods used were wet extraction at 95°C and subcritical water extraction at 95°C, 150°C and 170°C. To evaluate this, particle size of milled oat bran, β-glucan concentration and viscosity, as well as the molecular weight as an estimate of those, were determined.

For the wet extraction both time of extraction and particle size had significant effects on the viscosity, and the statistical analysis showed an interaction between the two factors. The viscosity was increasing with decreasing particle size, and for all samples with a longer extraction time. The same conclusion was drawn for the concentration of β-glucan; a higher concentration was obtained with decreasing particle size and longer extraction time. Individually, time and particle size had significant effects on the concentration. For the molecular weight, only time had a significant effect and for all particle sizes a medium-molecular weight between 550-750 kg/mol was obtained.

With subcritical water extraction there was a significant effect of temperature as well as an interaction effect between temperature and particle size on viscosity. A lower temperature used gave a higher viscosity. The yield of β-glucan was almost two-fold higher with higher temperatures at 150-170°C compared to a lower temperature at 95°C. Both temperature and particle size had significant effects on the yield. With subcritical water extraction a high yield can be obtained with high temperatures, but the molecular weight decreases dramatically to a low-molecular weight around 70-200 kg/mol.

The subcritical water extraction has some advantages in time, cost-efficiency and β-glucan yield compared to the wet extraction. However, further research is needed to investigate how a high yield of β-glucan can be obtained, but still with a high or medium-molecular weight. The combination of time and temperature needs to be optimized in order to get high molecular weight.

Keywords: Beta glucan, oat bran, subcritical water extraction, viscosity, particle size

Sammanfattning

Allt fler människor lider av livsstilsrelaterade sjukdomar på grund av dåliga kostvanor. Rekommendationen av ett fiberintag på 25-35 g per dag uppnås av få. Det finns behov av en optimering av redan befintliga produkter för att ge större variation samt underlätta för människor att upprätthålla hälsosamma kostvanor. Havrekli är en biprodukt i produktionen av havreprodukter, även om den är full av näringsämnen. Den är rik på kostfiber och β-glukan utgör cirka 5-20% av hela kliet. Ett dagligt intag av β-glukan är känt för att minska risken för hjärt- och kärlsjukdomar samt att sänka blodsockernivån och LDL-kolesterolet.

Syftet med den här studien var att undersöka hur olika partikelstorlekar av havrekli och olika extraktionsmetoder påverkar utbytet och molekylvikten av β-glukan. Extraktionsmetoderna som användes var våtextraktion vid 95°C och subkritisk vattenextraktion vid 95°C, 150°C och 170°C. För att utvärdera detta beräknades partikelstorleken av malt havrekli, koncentrationen och viskositeten av β-glukan, samt molekylvikten som en uppskattning av de sistnämna.

För våtextraktionen hade både extraktionstid och partikelstorlek en signifikant effekt på viskositeten, och den statistiska analysen visade att det fanns en interaktion mellan de två faktorerna. Viskositeten ökade med minskad partikelstorlek, och för alla prover, även med en längre extraktionstid. Samma slutsats drogs för koncentrationen; en högre koncentration erhölls med minskande partikelstorlek och längre extraktionstid. Individuellt hade både tid och partikelstorlek signifikanta effekter på koncentrationen. För molekylvikten hade endast tiden en signifikant effekt, och för alla partikelstorlekar erhölls en medium-molekylvikt mellan 550–750 kg/mol.

Med subkritisk vattenextraktion fanns en signifikant effekt av temperatur, såväl som en interaktionseffekt mellan temperatur och partikelstorlek, på viskositeten. En lägre temperatur gav en högre viskositet. Utbytet av β-glukan var nästan dubbelt så stor vid de högre temperaturerna 150°C och 170°C än vid lägre temperaturer vid 95°C. Både temperatur och partikelstorlek hade signifikanta effekter på utbytet. Med subkritisk vattenextraktion kan ett högt utbyte av β-glukan erhållas vid höga temperaturer, men molekylvikten minskar drastiskt till en låg-molekylvikt runt 70–200 kg/mol.

Den subkritiska vattenextraktionen har vissa fördelar med avseende på tid, konstadseffekt och utbyte av β-glukan i jämförelse mot en vanlig våtextraktion. Ytterligare forskning behöver undersöka hur ett högt utbyte av β-glukan kan erhållas, men fortfarande med en hög- eller medium-molekylvikt. Kombinationen av tid och temperatur behöver optimeras för att få en hög molekylvikt.

Keywords: Betaglukan, havrekli, subkritisk vattenextraktion, viskositet, partikelstorlek

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Abbreviations

BG	Beta-glucan
DF	Dietary fibre
LDL	Low-density lipoprotein
RISE	Research Institutes of Sweden
SLU	Swedish University of Agricultural Sciences
SWE	Subcritical water extraction
WB	Water bath

1. Introduction

Oat is along with wheat, barley and rye the most cultivated crops in Sweden (Jordbruksverket, 2020). During 2019 the total weight of oat harvested in Sweden was 674 000 tons according to the statistics of Jordbruksverket making oat the third most harvested cereal crop (Jordbruksverket, 2019). Oats, *Atena Sativa*, is the youngest type of cereal and it has been cultivated in the Nordic region since the 18th century peaking in the end of the 19th century (Lantmännen, 2019). Oat is specially known for its high amount of dietary fibre (DF), and especially the high content of soluble dietary fibre (Maheshwari *et al.*, 2017). Dietary fibre are carbohydrates that do not break down in the small intestines (Hu *et al.*, 2015). In the colon some fibre can be fermented and broken down by microorganisms to short chain fatty acids while other dietary fibres bind water and contribute to a swelling of the content which gives a feeling of saturation.

Oat along with barley are the cereals with highest amount of the dietary fibre beta-glucan (BG) (Benito-Román *et al.*, 2011). BG is a soluble fibre that consists of non-starchy polysaccharides which can be found in the cell-walls of the aleurone layer, the sub-aleurone layer and in the starchy endosperm tissue (Hu *et al.*, 2015). The BG form a viscous solution when liquid is added, and it contributes to a positive impact on reducing the harmful LDL cholesterol in the blood as the BG binds the bile salt and bring them out with the stools (Tosh *et al.*, 2010). BG also flattens the levels of blood glucose that prevents diabetes type II and reduces the risk of coronary heart disease. Both the viscosity and the effect on lowering LDL cholesterol is depending on the molecular weight and the concentration of BG (Wolever *et al.*, 2010). A recommended intake of BG in high-molecular weight or medium-molecular weight can reduce the cholesterol by 5% while the same intake of BG in low-molecular weight has 50% less efficiency. A high-molecular weight for BG is usually above 2 000 kg/ mol, a medium-molecular weight around 500-800 kg/ mol and a low-molecular weight around 200 kg/ mol or lower (Wolever *et al.*, 2010).

The European Food Safety Authority (EFSA) has stated that we need to have an intake of minimum 4 grams BG per 30 gram of available carbohydrates each day to attain maximum health benefits (EFSA, 2010). As many people have an intake of less than the recommended dose there are companies providing pure BG that can be added to drinks or foods.

The oat bran and the outer layer of the endosperm contains around 70% of the total BG content in the oat grain and it is tightly bound with starch, protein and pentosan in the endosperm cell wall (Yoo *et al.*, 2020). There are many technologies in extracting BG from the oat grain and some are more efficient than others. The processes can be divided into two types: dry and wet processes (Vasanthan & Temelli, 2008). For the dry process it includes particle size reduction often by milling and then separation by density and size to get a flour enriched in BG. For the wet process, the separation and concentration of BG is often performed with aqueous or semi-alcoholic procedures and this can make the BG concentration of the fibre concentrate reach up to 95% (w/w). There are many factors affecting how much BG that can be extracted from grains. Both particle size, the temperature of extraction, the time of extraction, the strength of gel building and the solvent: flour ratio matters (Benito-Román *et al.*, 2011).

1.1. Purpose and objectives

There are many studies on how to extract as much oat BG as possible. There are fewer studies on how the molecular weight changes, and consequently the viscosity induced by the BG, depending on which extraction method or which particle size used.

The aim of this study was to investigate the effect of different particle sizes and extraction methods, both water extraction and subcritical water extraction, on yield and molecular weight of BG extracted from oat bran. To evaluate this, particle size of bran and BG concentration and viscosity, as an estimate of molecular weight, of the extracts was determined.

2. Background

2.1. Oat

Oat is one of the four most cultivated grains in Sweden (Jordbruksverket, 2020). The oat grain is surrounded by an unpalatable hull that comprises 25-36% of the total dry weight of the grain (Chu, 2014). The hull is mostly built up of cellulose and hemicellulose. When the hull is removed there are three mayor layers: the bran is the outer layer of the intact grain then the starchy endosperm in the middle and the germ in the center. The starchy endosperm represents about 70% of the dry weight of the grain and that is why the oat grain largely consists of carbohydrates.

2.1.1. Oat bran

The bran is the outer layer of the groats and it is rich in minerals, vitamins, phytates, phenolics and cell wall polysaccharides like cellulose, beta-glucan (BG) and arabinoxylan (Grundy *et al.*, 2018). From outside in the bran is covered of a pericarp and a seed coat. Below those components is the aleurone layer and the sub-aleurone layer with the endosperm innermost (see Figure 1). The oat bran is a by-product during grain processing, and it is produced by grinding and sieving to remove most of the starch. The composition of the bran can vary between species but it usually contains: 15-18% protein, 10-50% starch and sugars, 5-10% fat and 10-40% dietary fibres (DF) (Duţă *et al.*, 2018).

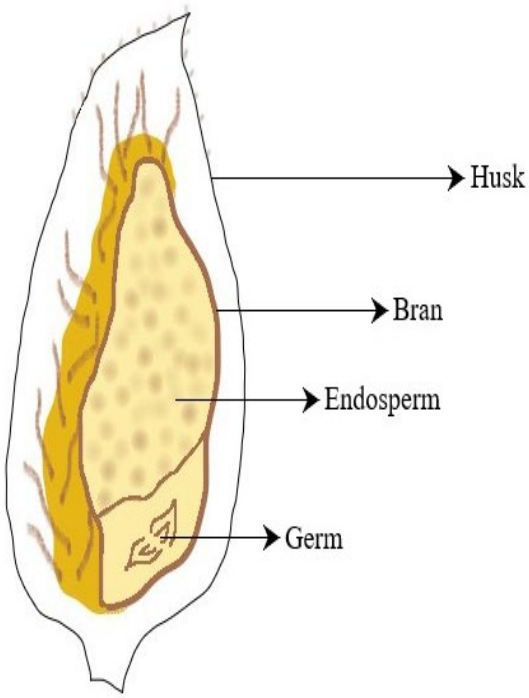

Figure 1. Cross section of an oat grain.

2.1.2. Oat dietary fibre

The oat DF can be divided into insoluble DF and soluble DF (Cui *et al.*, 2013). In the intact oat grain the insoluble dietary fibres constitutes 42% and the soluble dietary fibres constitutes 58% (Chu, 2014). The insoluble DF are made up of lignins, cellulose and hemicellulose while the soluble DF mostly consists of BG, which stands for 69% of the total soluble DF. This high amount of soluble fiber makes oat unique as other cereal grains usually contain more of insoluble than soluble fibers. Dietary fibres can be added to food product as loaf volumizers, bulking agents, fat replacers, emulsifiers and stabilizers. In recent years it has also become an additive for its promoting of health effects.

Beta-glucan is a linear unbranched polysaccharide (see Figure 2) that is built up of β-D-glucopyranosyl monomers that are connected through glycosidic bonds (Maheshwari *et al.*, 2017). In the whole oat groat about 3.8-6.1% consists of BG. The BG-polysaccharide is composed of around 70% β-(1,4) bonds and 30% β-(1,3) bonds (Hu *et al.*, 2015). It is due to the mixed-linkage of β-(1,3)/ (1,4) that makes BG water soluble. BG are known to reduce the risk of cardiovascular diseases, lower the blood glucose levels and the cholesterol levels (Benito-Román *et al.*, 2013). The lowering of LDL cholesterol, commonly referred to as the bad cholesterol, depends on the viscosity in the intestines (Wolever *et al.*, 2010). The viscosity in turn depends on the molecular weight and the concentration of BG (Tosh *et al.*, 2010).

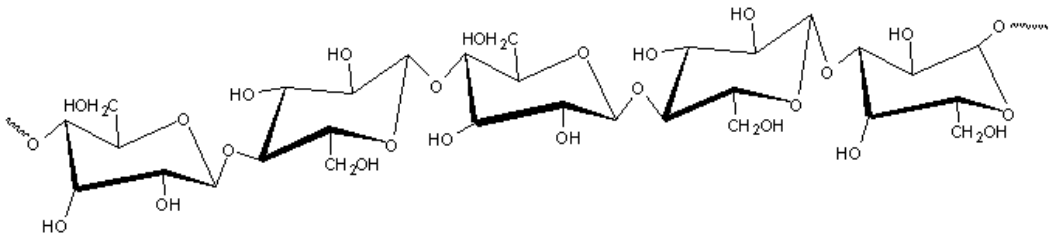

Figure 2. (1,3; 1,4) - β-D-glucan

2.2. Extraction of beta-glucan

In the endosperm cell walls the BG is tightly bound with starch, protein and pentosan (Yoo *et al.*, 2020). In the extractions of BG these therefore need to be separated. For this, different approaches exist and can broadly be divided into dry and wet techniques (see Figure 3).

2.2.1. Dry process

The whole oat grain is milled, and the bran is separated from the groats. The bran is then milled to a desired size and sieved to make sure that the particles are in right size (Vasanthan & Temelli, 2008). The milled parts have a variety of chemical compositions, starch, protein, BG, cellulose, lipids and minerals. They also vary in physical properties as shape, size and density. As most of the BG is concentrated in the endosperm the milled particles can be separated based on size to get a BG-enriched flour for further extractions. Some dry processes separate based on density.

2.2.2. Wet process

The wet process can be performed with acidic extraction, alkaline extraction, semi-alcoholic extraction, enzymatic extraction or aqueous extraction (Maheshwari *et al.*, 2017). The grain is firstly milled to a desired size and some processes use a preconditioning step to soak the bran to a certain moisture content before incubating. The general principle is to add the solution of choice to the milled bran and then incubate during a certain time, mostly 2-4h, and then centrifuge to separate the supernatant from the fibre residues (Aktas-Akyildiz *et al.*, 2018; Maheshwari *et al.*, 2017). As the BG is water soluble it will be found in the supernatant.

For the acidic extraction common acids are perchloric acid or citric acid and for the alkaline extraction NaOH or Na_2CO_3 is often used. The bran is often treated with ethanol independent of which of the processes that is used. That is to inactivate the β-glucanases that hydrolyses the BG. The ethanol also removes most of the lipids to which

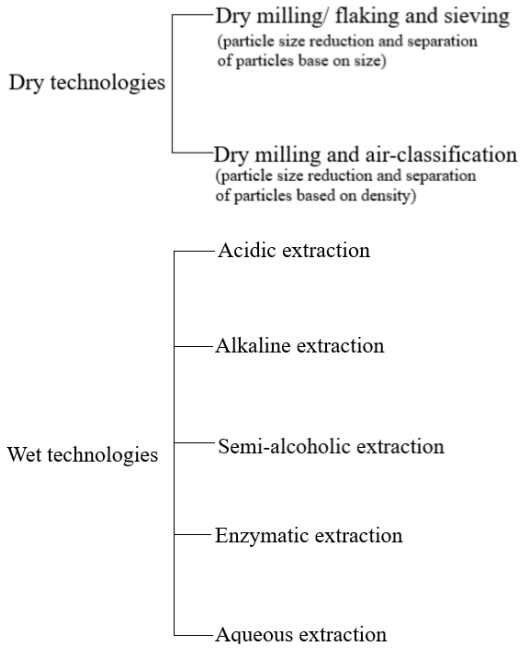

Figure 3. Overview of the most common dry- and wet techniques for extracting BG.

the BG is tightly bound. Often an α-amylase is used to hydrolyze the starch (Aktas-Akyildiz *et al.*, 2018). After incubation, the sample is centrifuged to separate the water-soluble components containing BG from the insoluble residues. Many procedures remove proteins and dextrins in further precipitation steps.

Ahmad *et al.* (2010) concluded that the extraction method giving the highest yield of BG and removes most of the starch, lipids and fat is the enzymatic extraction.

2.2.3. Subcritical water extraction

Subcritical water is water in liquid phase under high pressure which allows the temperature to rise above 100°C without boiling. With a subcritical water extraction (SWE) the temperatures can reach up to its critical temperature which is 374°C (Benito-Román *et al.*, 2013). Above that temperature the water losses its characteristics (Yoo *et al.*, 2020).

At subcritical conditions, the hydrogen bonds between the water molecules are disrupted and the changes of physical and chemical properties makes the water act more like an organic solvent and it can increase the solubility of different compounds. It has some advantages as short extraction time, cost-efficiency and high selectively and is widely used to extract phenolic compounds, essential oils and polysaccharides. At the same time, a high temperature can disrupt the BG, reduce the molecular weight and start an undesirable Maillard reaction.

2.3. Analytical methods

2.3.1. Particle size determination

The particle size can be determined using a light microscope. Many microscopes are equipped with a camera that is linked to a computer where the photography is used to determine particle size (Exbrayat, 2016). One method is to have a digital image which is converted to a binary image. The threshold can be adjusted manually or with an automatic adjustment. The threshold is used to separate the background or too small pieces from the particles of interest. The particle sizes can then be determined using a software, for this study ImageJ was used. The software can analyze cell count, total area of the cells and mean size of the cells (Igathinathane *et al.*, 2008). There are a few limitations when analyzing particle sizes; the particles need to be separated well and that is time consuming. Also, many replicates need to be analyzed to give a value representing the whole sample, and due to a small area analyzed large particles may be underestimated.

2.3.2. Study of cell walls of beta-glucan

To study the structure of the oat cell wall a common method is to stain the sample to see the different components in a fluorescence microscope (Exbrayat, 2016). The principle is to use light of a specific wavelength to excite molecules that are naturally present in the sample or that have been added. What is observed is the emitted light from excited molecules. To succeed the light used to illuminate the sample must be prevent from being observed in the microscope. This is performed by using filters that limits which light reaches the sample. This can control that only certain wavelengths passes and which light can then be observed (R. Spring, 2020).

Many samples need to be embedded or fixed to be studied. Fixation is commonly done with glutaraldehyde or formaldehyde which stabilizes mainly proteins (Dornez *et al.*, 2011). Samples are subsequently dehydrated by replacing water with ethanol. The dehydrated samples can then be embedded by replacing ethanol with plastic which is hardened. The samples can then be sectioned which allows staining and observation.

There are different staining techniques and some usual ones to detect BG are Calcofluor staining and immunolabeling of BG using monoclonal antibodies (Dornez *et al.*, 2011). The easiest technique is staining with Calcofluor to stain the BG that can be visualized as blue light and with Acid Fuchsin that visualize the proteins as red. As it is stated by Dornez *et. al* (2011) the calcofluor staining technique has an advantage as it stains several components in the cell walls at the same time, although it can be a disadvantage in this case as it is not specific for just the cell walls of BG.

2.3.3. Beta-glucan content

BG is a well-studied component and there are many ways of determining the BG content of a sample. A common technique that has been evaluated by AOAC International is the Method 995.16. The principles are that the samples are hydrated in a buffer solution of pH 6.5 and then incubated with lichenase which hydrolysis the (1,4)-β-D-glycosidic linkages in BG from polysaccharides to oligosaccharides (Megazyme, 2018). The oligosaccharides produced are then hydrolyzed by β-glucosidase to glucose and the amount of D-glucose is measured using glucose oxidase/ peroxidase reagent (McCleary *et al.*, 1988) and it is measured in a spectrophotometer. The samples are then compared to a D-glucose standard and to a standardized oat flour control.

2.3.4. Viscosity measurements and molecular weight

The viscosity is defined as the ratio of shear stress to shear rate (Cui *et al.*, 2013). The viscosity is the internal friction in a material or sample when subjected to deformation and it is in many cases dependent on the shear rate. This means that a material can be shear thinning and it is therefore important to take into account at what shear rate the viscosity is measured at.

Molecular weight, structure and the conformation of the polymer in a solution affect the viscosity (Mäkelä *et al.*, 2020). It has been stated that a high viscosity and a high molecular weight has a greater impact on the lowering of LDL cholesterol (Tosh *et al.*, 2010). A high molecular weight of BG is usually above 2 million g/ mol while a medium-molecular weight is around 500 000-800 000 g/ mol. A low-molecular weight is around 200 000 g/ mol or lower. Tosh (2010) showed a relationship between the viscosity and the molecular weight if the concentration of BG is known (see Equation 1). M_p is the peak molecular weight (kg/ mol), c_e is the concentration of BG in (g/ L) and the η is the viscosity measured in mPa·s at $30s^{-1}$.

Equation 1. Shows how to calculate the molecular weight (M_p) in kg/ mol when knowing the concentration (c_e) in g/ L and the viscosity (η) measured in mPa·s at $30s^{-1}$.

$$\eta = 5.5\ x\ 10^{-4}(M_p\ x\ c_e)^{1.58}$$

3. Materials and methods

3.1. Materials

Oat bran, calcium chloride 36% and an enzyme solution with Thermamyl was provided by Lantmännen Oats. The oat bran was a mix of the species *Kerstin* and *Galant*, which had not been heat treated to inactivate enzymes. Four samples of wet milled oat bran, taken out from different times in the beta-glucan process, were obtained from Lantmännen's plant in Kimstad. Equipment was provided by Swedish University of Agricultural Sciences (SLU, Ultuna) and Research Institutes of Sweden (RISE, Ultuna) provided their warehouse for milling. Mixed-linkage beta-glucan assay kit was from Megazyme (Bray).

3.2. Methods

3.2.1. Experimental design

Oat bran was milled in 4 different particle sizes: 0.5, 0.7, 1.0 and 1.5 mm. For the 0.5 mm a Retsch mill ZM 200 was used, and for the other particle sizes a Brabender mill was used. The different particle sizes including unground oat bran were vacuum sealed and stored in a freezer at -21°C until use.

For this project three replicates in all particle sizes were used for the extraction in the water bath (see Figure 4). The SWE at 95°C, 150°C and 170°C was performed in duplicates[1] with the particle sizes of 0.5 mm and unground oat bran.

The ratio of oat bran: enzyme solution for the water extraction and the SWE was 2:50. The solution for the extractions consisted of 3.25 L deionized water, 0.25mL enzyme solution with α-amylase and 1.35mL $CaCl_2$ 36%.

For the light microscope oat bran in all particle sizes were used (0.5-, 0.7-, 1.0-, 1.5 mm and unground oat bran).

[1] Except the SWE for 0.5 mm at 150°C which were performed in triplicate.

The dispersion viscosity (Pa·s) was measured for the supernatants both from the water bath extraction and from the SWE. The supernatants were also used to determine the BG content with the Megazyme mixed-linkage beta-glucan assay kit.

An independent part of this project was to evaluate the effect of the wet mills at Lantmännen's plant in Kimstad producing BG. For this fluorescence microscopy was performed. Four samples of oat bran slurry obtained from Lantmännen's plant in Kimstad were analyzed. Sample 1 was taken out before the wet milling, sample 2 was taken out after the first wet grinding mill, sample 3 after the second wet grinding mill and sample 4 after the third wet grinding mill.

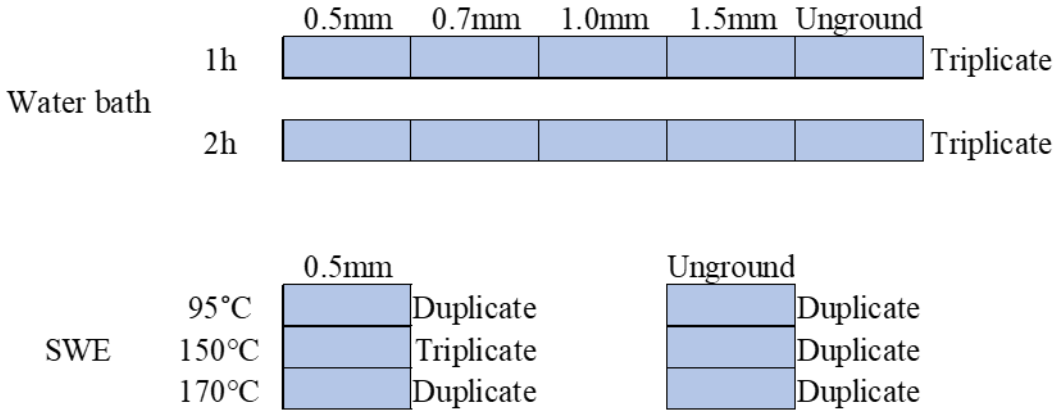

Figure 4. Figure of the experimental design for the extraction in water bath and the SWE.

3.2.2. Particle size determination

A weight of 20 mg oat bran was put into Eppendorf tubes from the different particle sizes: 0.5, 0.7, 1.0, 1.5 mm and unground oat bran. The sample was dispersed in paraffine oil and the volume was adjusted to 1mL. The process was performed in three replicates for all particle sizes except the unground that was performed in one set. The tube was stirred on a vortex mixer and 4 drops were applied to a glass slide and a coverslip was placed on top. A light microscope was used with a 4x (NA 0.2) objective and 4 micrographs were taken with Nikon Eclipse Ni-U microscope that was equipped with a Nikon Digital Sight DS-Fi2 camera. The four micrographs were overlapped to one with Nikon Software. The micrograph was made 8-bit in the software Fiji ImageJ. An auto-threshold was applied, and the micrograph was inverted. It was then made binary and holes were filled before the particles were analyzed. From the analysis of the particle sizes an estimation was made that the particles were spherical, and an estimated particle diameter was calculated. The particle sizes distribution was presented as cumulative area in % of total area

projected by particles. In Figure 5 the first image is the 4 overlapped images of the size 1.0 mm. Figure 6 is the image processed in ImageJ with numbers for each particle seen.

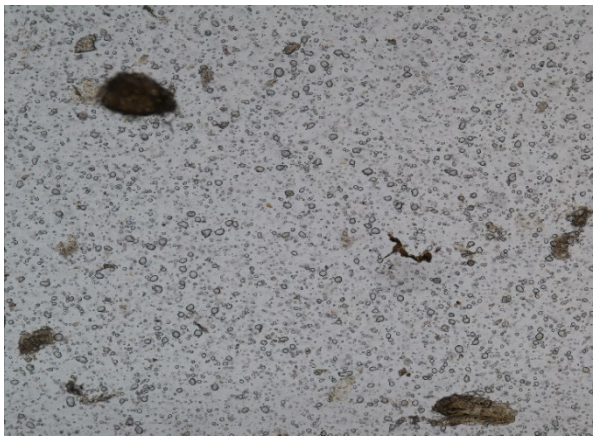

Figure 5. An example of four pictures taken and overlapped together. The size of the milled oat bran is 1.0 mm.

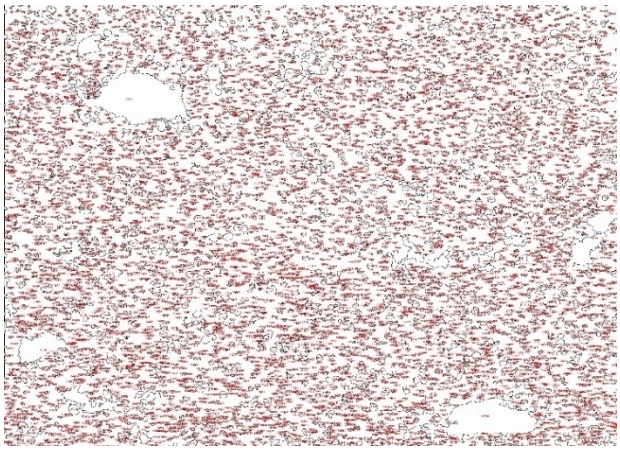

Figure 6. The same picture that has been converted to 8-bit. Auto-threshold and made binary has been applied and the holes have been filled.

3.2.3. Analysis of cell walls

From the 4 samples of wet milled oat bran provided from Lantmännen's plant in Kimstad 5 g from each sample was diluted with 5 mL deionized water. To the sample 1 mL of glutaraldehyde 25% was added to give a glutaraldehyde concentration of 2.5% and the sample was mixed on a vortex. The samples were sealed with parafilm and left in the fridge overnight.

An agar solution was prepared in a beaker by mixing 1 g of Agar Noble with 25 mL deionized water. The beaker was heated to 85°C in a water bath to mix the ingredients before decreasing the temperature to 45°C and kept there until use. 3 mL of agar solution and 3 mL of the sample were mixed and distributed in molds and let cool down.

The solidified agar with sample was cut in pieces of 2x2x2 mm and dehydrated in series of ethanol of increasing concentration: 30%, 50%, 70%, 90%, 95% and 100% ethanol. The dehydration process continued overnight in an Automated Tissue Processor Leica EM TP. This was followed by infiltration of the samples with Technovit and hardener I.

Two polypropylene vials were filled with10 mL respectively 15 mL of Technovit and hardener I. The samples were taken out from the Leica EM TP and put in the first vial for 1h. The samples were taken out, wiped dry and transferred to new molds.

From hardener II 1 mL was added to vial 2 and the solutions were mixed. The mixture was added with an amount only to cover the samples. The molds were left in a fume hood during the weekend to harden.

The hardened plastic samples were cut to 1 μm thin sections with a glass knife by using an ultramicrotome, Leica ARTOS 3D. The sheet was put on a glass slide with a distilled water drop on it and the glass slide was let to dry. One drop of acid fuchsin was added for 1 min before it was washed away with distilled water. One drop of calcofluor was added and washed away after another 1 minute. A couple of distilled water drops were added to cover the sample and a cover slip was placed on top. The cell walls were observed in a fluorescence microscope with a mercury lamp and the filter used for the calcofluor had an excitation of 340-380 nm and an emission of 435-485 nm. The filter for acid fuchsin had an excitation of 512-552 nm and an of emission 565-615 nm.

3.2.4. Extraction in water bath

The experiments were performed both for 1 hour and for 2 hours. Both the raw unground oat bran and the 4 milled particle sizes were used (0.5, 0.7, 1.0 and 1.5 mm). 6.6 g oat bran was placed in a reagent bottle and 10 solid-glass beads with a diameter of 6 mm were put into the bottle. To each bottle 154 mL enzyme solution was added and the bottles were immediately incubated in a shaking water bath at speed 9 at 95°C for 1 and 2 hours, respectively. To stop the extraction the bottles were taken out and put in a cold-water bath for 20 minutes. From the bottle 20 mL was transferred to a Falcon tube and the tubes were centrifuged at 5000G for 15 min at 20°C. The supernatant was transferred to new Falcon tubes for viscosity analyses by using a DHR3 Rheometer (TA instruments Ltd., Newcastle, DE, U.S.A.). Also 3 mL of the supernatant was put in a freezer for further analyses of the BG content.

3.2.5. Subcritical water extraction

A pressure cell was mounted on the rheometer, and 1.07 g of oat bran was added to the chamber. The experiments were performed at three different temperatures: 95°C, 150°C and 170°C. The two sizes of oat bran used were 0.5 mm and unground oat bran. The temperature for the pressure cell was set to 50°C and the soak time was 1 min. The procedure was timed to 3 minutes from adding the enzyme solution until the experiment started. The amount of enzyme solution added was 25 mL.

The session started at 50°C and increased with 10°C/ min up to 95°C where it was held for 30 minutes. Then it increased to 150°C during 5 min or to 170°C during 7 min. For the experiments at 150°C and 170°C the temperature was held for 30 minutes at the highest temperature until it decreased to 50°C in the end. The hold time for the sample at 95°C was set to 82 min. From the achieved temperature

the decreasing time to reach 50°C again took 34 min from 95°C, 40 min from 150°C and 42 min from 170°C. The total time for the extractions were 2h at 95°C, 1h 49min at 150°C and 1h 53min at 170°C.

When reaching 50 °C the chamber was taken out and cooled down in cold water for 10 minutes before the slurry was poured into a Falcon tube and centrifuged at 5000G for 15 min at 20°C. From the supernatant 10 mL was transferred to a new Falcon tube for measurement of viscosity and 3 mL was transferred to a glass tube that was put in the freezer for further BG analysis.

3.2.6. Beta-glucan analysis

The BG content was determined using a mixed-linkage BG assay kit (Megazyme, Ireland). Buffers prepared was sodium phosphate buffer (20 mM, pH 6.5) that was made with 3.12 g sodium dihydrogen orthophosphate dihydrate dissolved in 900 mL distilled water with the pH adjusted to 6.5 by adding 100 mM sodium hydroxide (4 g/L). The volume was adjusted to 1 L and 0.2 g sodium azide was added.

A sodium acetate buffer (50 mM, pH 4.0) was prepared by adding 2.9 mL glacial acetic acid to 900 mL distilled water. The pH was adjusted to 4.0 by adding 1 M sodium hydroxide solution. The volume was adjusted to 1 L and 0.2 g sodium azide was added.

For the sodium acetate buffer (200 mM, pH 4.0) 11.6 mL glacial acetic acid was added to 900 mL distilled water and the pH was adjusted to 4.0 by adding 1 M sodium hydroxide solution. The volume was adjusted to 1 L and 0.2 g sodium azide was added.

The lichenase solution was made up by diluting the supplied bottle of lichenase in 20.0 mL sodium phosphate buffer (20 mM, pH 6.5). For the β-glucosidase the entire bottle supplied was diluted in 20 mL sodium acetate buffer (50 mM, pH 4.0). The glucose oxidase/ peroxidase (GOPOD) reagent was prepared by diluting one bottle of GOPOD reagent buffer in 1 L distilled water. The bottle of GOPOD reagent enzymes was dissolved in the previous solution.

For each running of the set of BG determination a reagent blank and a glucose standard were made up. The reagent blank comprised 0.1 mL distilled water, 0.1 mL sodium acetate buffer (50 mM, pH 4.0) and 3.0 mL GOPOD. The glucose standard was prepared by 0.1 mL D-glucose standard, 0.1 mL sodium acetate buffer (50 mM, pH 4.0) and 3.0 mL GOPOD.

For each set of determinations an oat standard was made by weighing in 100 mg and adding 0.2 mL aqueous ethanol (50% v/v) and 4.0 mL sodium phosphate buffer (20 mM, pH 6.5). The content was stirred on a vortex mixer and put in a boiling water bath for 2 min. The tube was taken out and stirred on a vortex mixer and then incubated in the water bath for another 4 min. The tube was let cooling to room temperature and then followed the same steps as for the liquid supernatant with the common step starting from incubating in 50°C for 10 min and further steps.

The supernatant from the extraction containing 3 mL was taken out from the freezer and was allowed to adjust to room temperature. The supernatant was transferred to a centrifuge tube that was incubated in boiling water for 10 minutes and let cool to room temperature. 3 mL of 95% aqueous ethanol was added, and the tube was stirred on a vortex. Another 5 mL of 95% aqueous ethanol was added, and the tube were stirred on a vortex. The tube was centrifuged for 10 min at 1800 G and the supernatant was discarded. The pellet was resuspended in 8 mL 50% v/v aqueous ethanol. The tube was stirred on a vortex and centrifuged for 10 min at 1800 G again and the supernatant was discarded. The pellet was resuspended in sodium phosphate buffer (20 mM, pH 6.5) and the volume was adjusted so the total volume was 4 mL.

The tube as well as the oat standard was incubated at 50°C for 10 min. The tubes were taken out and 0.2 mL lichenase was added before the tubes were stirred on a vortex mixer and sealed with parafilm. The tubes were incubated at 50°C for 1h with 4 vigorous stirrings on a vortex mixer during the incubation time. After incubation 5 mL of sodium acetate buffer (200 mM, pH 4.0) was added and the tubes were stirred on a vortex and left for equilibration to room temperature for 10 minutes followed by centrifugation at 1000 G for 10 min. Carefully 0.1 mL of the supernatant was transferred to three glass tubes. 0.1 mL glucosidase was added to two of them and to the third (the reaction blank) 0.1 mL acetate buffer (50 mM, pH 4.0) was added. The tubes were incubated for 10 min at 50°C. To each tube 3 mL GOPOD reagent was added and the tubes were incubated at 50°C for 20 min. The absorbance of the samples was read on a spectrophotometer at 510 nm within 1 h.

3.2.7. Viscosity

The dispersion viscosity (Pa·s) was measured with a DHR3 Rheometer (TA instruments Ltd., Newcastle, DE, U.S.A.). The geometry used for the supernatants from the water bath was a 40 mm stainless steel cone peltier plate. For the supernatants from the SWE a 40 mm aluminium steel parallel peltier plate was used.[2] The supernatants were measured directly after centrifugation after the extractions. The sample was presheard for 1 min at a shear rate of 100 s^{-1}. An equilibration time of 300 s was implemented before the flow sweep started. The shear rates were from 0.1-100 s^{-1} and the temperature was set to 20°C. From the supernatants 700 µL was placed at the rheometer. The software used was Trios.

[2] The switch of geometry was due to damage to the first geometry which gave different results within the same sample. The second geometry was calibrated against the first one and the same results were obtained with both geometries.

3.2.8. Statistical analysis

The analyses were conducted with two or three replicates in a randomized order. The statistical analyses were conducted with Minitab®18 using a General Linear Model analyzing if there was an interaction between the two factors (time/ particle size for water bath and temperature/ particle size for SWE). If the effects were significant for both or any of the parameters, a pairwise comparisons with correction for multiple testing according to the Tukey method were made for the significant factors, or in the case of interaction, between all samples. 95% significance level was used for all the tests. Raw data from the statistical analysis can be seen in Appendix. All other calculations were made in Microsoft Excel 2016.

4. Results and discussion

4.1. Particle size and cell walls

4.1.1. Particle size determination

A cumulative area fraction was calculated (see Figure 7) and for the 0.7 mm almost all the particles were smaller than 100 µm. For the samples 0.5 mm and 1.0 mm the particles have pretty much the same size distribution and that may be due to that the 0.5 mm particles were ground in a different mill compared to the others. The particles were screened through a size excluder when milled but they were not sieved after milling. It might have been fractions of larger sizes that contaminated the samples which can explain the variation of particles within one sample.

For the larger sizes: 1.5 mm and unground oat bran, particles below 100 µm constituted 60% of the total particle area, but it had a larger range of different particle sizes within the samples.

Furthermore, this method of particle size determination may not be sufficient to distinguish 0.5, 0.7, 1.0, 1.5 and unground particles from each other. Also, the small number of replicates and the small amount of sample analyzed did not give a representative sample of particles.

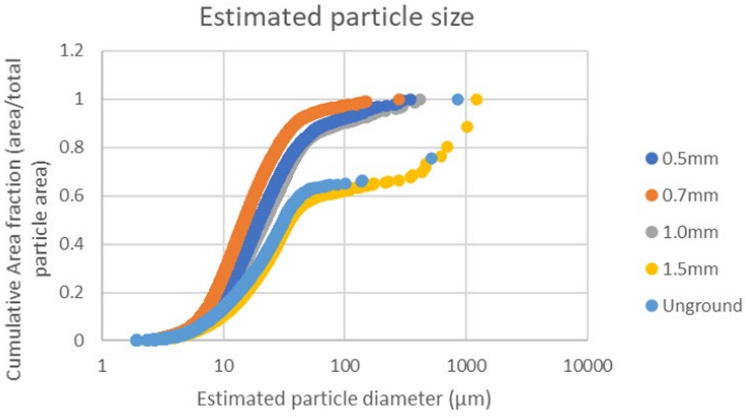

Figure 7. The particle size distribution for oat bran milled in 0.5, 0.7, 1.0 and 1.5 mm including unground oat bran. The three smallest sizes have a similar size distribution and the two largest have a similar size distribution.

4.1.2. Analysis of cell walls

This was an independent part of the project to evaluate the effect of Lantmännen's wet mills on the structure of the oat bran.

The fluorescence micrographs in Figure 8 shows the protein in red color while the blue color shows the cell walls. Micrograph 1 shows the unground sample and the aleurone layer is aligned with residues of starchy endosperm which can clearly be seen by the blue cell walls. Compared to the three other pictures all of them have their endosperm removed. The grains are still intact as the kernel is not crushed, but the endosperm is scraped off. The image 2, 3 and 4, from the samples that have been wet milled, the outer layer is the aleurone layer containing BG.

It is hard to distinguish differences between picture 2, 3 and 4. The aleurone layers seems to be intact and the endosperms is removed which confirm that the wet grinding mills fulfill their missions to open up the bran and make the BG-rich aleurone layer available for contact with enzymes. To compare the effect of the grinding mills further research is needed.

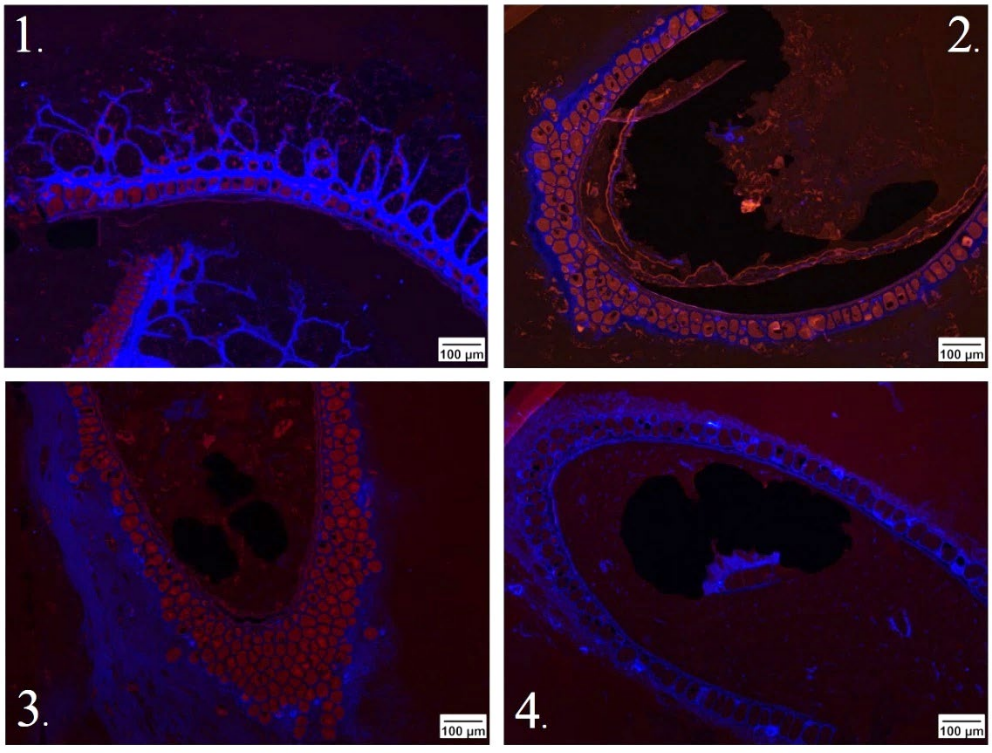

Figure 8. Four images of oat bran slurry. Image 1 is before the wet mill, image 2 after the first wet milling, image 3 after the second wet milling and image 4 after the third wet milling. The protein can be seen as red and the cell walls can be seen as blue.

4.2. Beta-glucan content

4.2.1. Water bath

Both the time and the particle size individually had significant effect on the BG yield, but there were no interactions between them.

The extraction in water bath showed that with a decreasing particle size the BG extracted increased. That is also stated by Maheshwari *et al.* (2017) and Johansson *et al.* (2018). Comparing the extractions in water bath for 1h and 2h (see Figure 9) the yield of BG was higher for 2h than for 1h, and this could be seen for all the particle sizes, which agrees with the studies of Benito-Román *et al.* (2011).

Probably the particle size play roll as grounded samples have a larger area in contact with the solution and therefore more BG can be extracted. As seen in the fluorescence microscope (see Figure 8) it seems that when grounding oat bran, more of the BG-rich aleurone layer and endosperm layer is exposed for the enzymes and therefore more BG is available for extraction. The same principle is for the dry milled oat bran in this case and with longer extraction time more BG is dissolved in the solution and can be extracted.

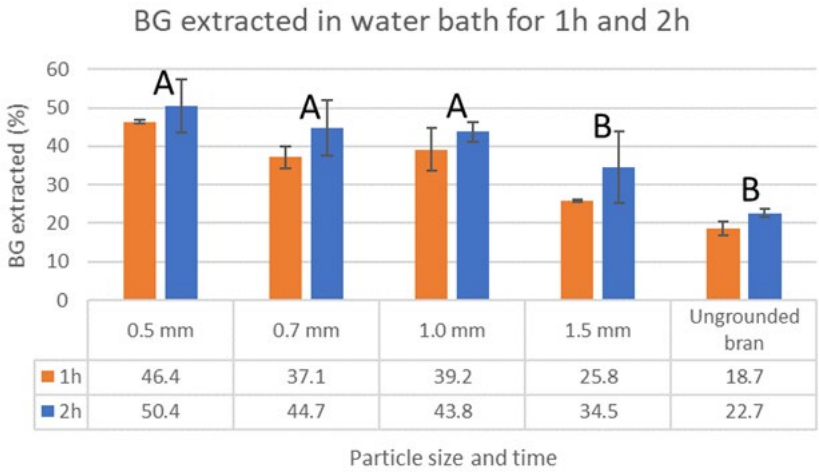

Figure 9. Average BG yield extracted with WB. Highest yield was obtained for 0.5 mm at 2h and the yield is decreasing both with increasing particle size and with shorter extraction time. Both time and particle size had a significant effect on the BG extracted. The letters show the significant differences regarding particle sizes. Means that do not share letter are significantly different.

It can be seen in Figure 9 that there is no significant difference between the sizes 0.5, 0.7 and 1.0 mm. This is probably because the sizes are similar to each other. Visually these particle sizes looked more like a flour than 1.5 mm and unground oat bran which had a larger variety of sizes. There are significant differences between 0.5, 0.7 and 1.0 mm compared to 1.5 mm and unground bran. That is also in line with the particle size determination which showed that the three smallest

particle sizes had a similar size distribution, and the two largest had a similar size distribution (see Figure 7).

4.2.2. Subcritical water extraction

With SWE the highest yields of BG were obtained with higher temperatures at 150°C and 170°C (see Figure 10). The particle size of 0.5 mm had a higher BG yield than the unground oat bran, at all temperatures. Both the temperature and the particle size individually had significant effects on the yield, but there were no interactions between them.

As the BG is tightly bound in the bran the high temperature and the pressure in the chamber gives a higher yield compared to extraction in water bath. That may be due to the weakening of the bonds in the polysaccharide of BG which release the BG into the solvent. This is similar to previous studies where the BG yield was twofold higher with SWE compared to water extractions (Yoo *et al.*, 2020). The lower yield obtained with SWE at 95°C may have been higher with a longer extraction time, which is also stated by Benito-Román *et al.* (2013).

No significant differences in BG extracted were found between 150°C and 170°C (see Figure 10). But there is a significant effect between 95°C and the two others. This shows that a higher BG yield is extracted with higher temperature.

Yoo *et al.* (2020) stated that broken endosperm fractions containing a lot of BG in contact with the solvent may affect the high BG extracted. The current results with higher BG yields with 0.5 mm are in line with previous studies

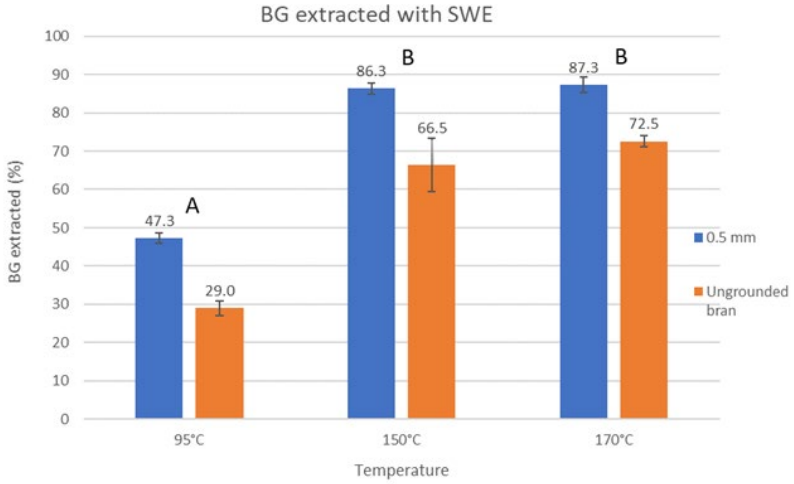

Figure 10. Average BG yield extracted with SWE. It shows that higher temperatures give a higher yield and with a decreasing particle size more BG is extracted. Both temperature and particle size had significant effects on the BG yield. The letters show the significant differences between the temperatures without regard to particle size. Means that do not share letter are significantly different.

4.3. Viscosity measurements and molecular weight

4.3.1. Water bath

Viscosity

Both the time and particle size individually had significant effects on the viscosity. The statistical analysis showed that there was an interaction between the time and the particle size which means that they are both contributing to a significant effect on the viscosity.

The viscosity of the supernatants from the water bath was highest for the particle size 0.5 mm with an extraction for 2h (see Figure 11). The viscosity trend is similar to the trend for BG extracted. This may be explained that BG is very water absorbing and gel building and with a higher content of BG the viscosity increases (Mäkelä *et al.*, 2020). The viscosity is decreasing with increasing particle sizes and the lowest viscosity is seen for unground oat bran. The viscosity is in all cases higher for the 2h than for the 1h extraction within each particle size.

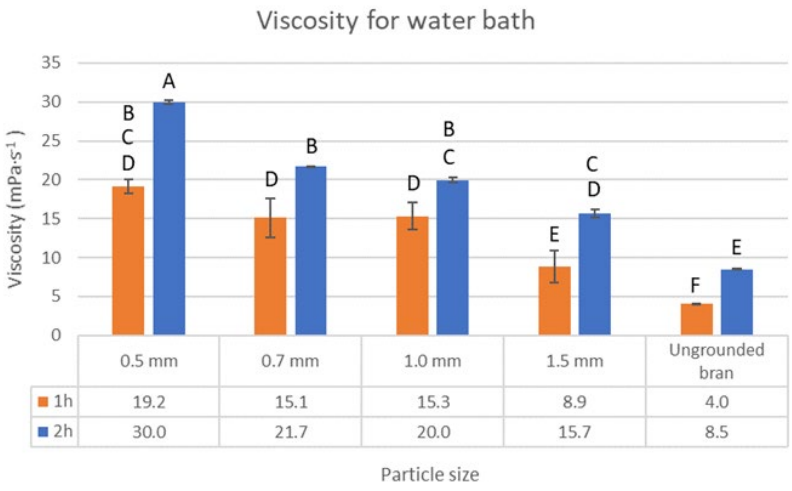

Figure 11. The mean viscosity of the supernatants from extraction in water bath. It is highest for the particle size 0.5 mm and then decreasing with increasing particle size. Within each particle size the viscosity is higher for 2h than for 1h. The letters show the interactions between extraction time and particle size. Means that do not share letter are significantly different.

Probably it is a combination of the small particle sizes contributing to a larger area exposed and the BG precipitating more during longer time of extraction which contribute to a higher BG yield which increases the viscosity. This agrees with what Yoo *et al.* (2020) stated.

What is interesting is that the highest viscosity was obtained with the size 0.5 mm although it did not show any differences for the particle size distribution compared to 0.7 mm and 1.0 mm (Figure 7).

Molecular weight

The molecular weight is calculated based on the viscosity and the concentration of BG based on a relationship from the literature (see Equation 1). Therefore, it is an estimated molecular weight that is obtained.

For all particle sizes the molecular weight was higher for the 2h extraction than for the 1h extraction (see Figure 12). Only the time had significant effects on the molecular weight. The effect of particle size was not significant for the molecular weight and that is why an average molecular weight for time is shown in Figure 12, with no regards to particle size.

According to Wolever (2010) the molecular weight needs to be medium or high to have a positive impact on lowering the cholesterol. In their study they are referring to a medium-molecular weight around 500-800 kg/ mol, and the molecular weight for all the samples in this case are within that interval, ranging from around 600-700 kg/ mol. As the molecular weight is based on literature data for the relationship between viscosity and concentration, it preferably needs further studies to surely determine the actual molecular weight of the BG.

It seems that extractions at 95°C for 2h are close to an optimum time for this extraction method, as a longer time can degrade the BG even more which gives a lower molecular weight (Aktas-Akyildiz *et al.*, 2018).

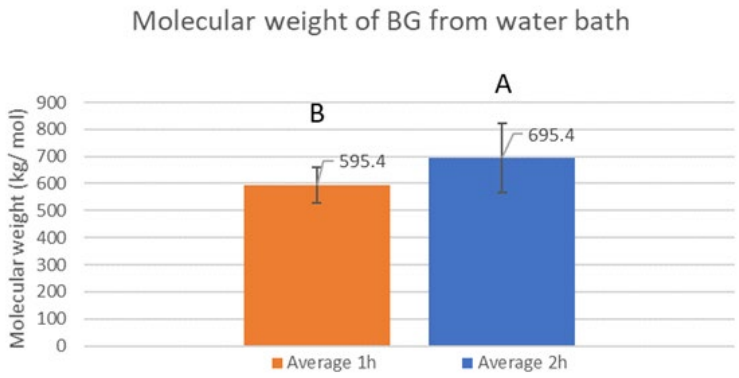

Figure 12. The molecular weight is depending on the equation knowing the viscosity and the concentration. Only time had significant effects on the molecular weight. Shown is an estimated molecular weight for BG extracted in water bath with an average for 1h and 2h, no regards to particle size. Means that do not share letter are significantly different.

4.3.2. Subcritical water extraction

Viscosity

Only the temperature had a significant effect on the viscosity but there is still an interaction between the temperature and the particle size. The viscosity is highest for the SWE at 95°C with 0.5 mm (see Figure 13). At 150°C the viscosity for unground oat bran is higher than the viscosity for 0.5 mm and at 170°C there is almost no differences between the two particle sizes.

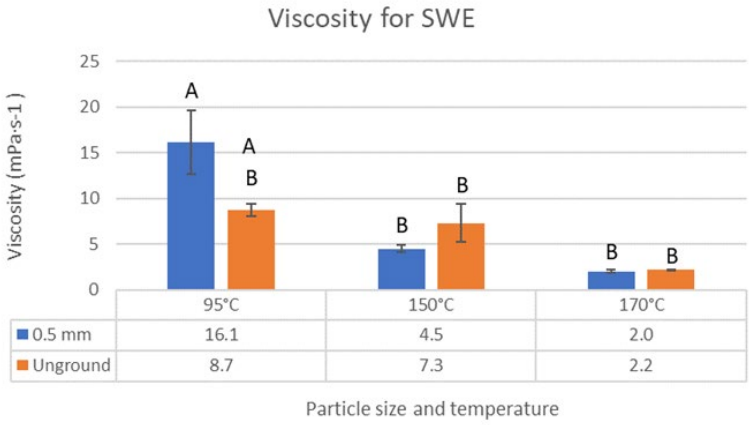

Figure 13. The mean viscosity of the supernatants for 0.5 mm and unground oat bran at 95-, 150- and 170 degrees. Only the temperature had a significant effect on the viscosity but there is an interaction between the temperature and particle size. The letters show the interaction between temperature and particle size and means that do not share letter are significantly different.

For the temperatures there is no significant difference for the viscosity at 150°C compared to 170°C, but there is a significant difference between 95° compared to the others, which also could be obtained both visually and physically when performing the extractions. In Figure 14 the supernatants can be seen, and visually the sample extracted at lowest temperature behaved more like a gel compared to the samples at higher temperatures that behaved more water-like.

Figure 14. Supernatants from SWE extraction of unground oat bran. From left to right: 95°C; 150°C; 170°C.

Comparing the viscosity for the water bath (WB) at 95°C with the viscosity for SWE at 95°C the viscosity is higher for the 0.5 mm in the WB (30,0 $mPa \cdot s^{-1}$ vs. 16.1 $mPa \cdot s^{-1)}$. Comparing the viscosity for unground oat bran in 95°C WB with SWE at 95°C there is almost no differences (8.5 $mPa \cdot s^{-1}$ in WB vs. 8.7 mPa·s-1 in SWE).

The differences in viscosity for 0.5 mm may be due to different stirring advices used for WB versus SWE. The samples of 0.5 mm had a very lumpy texture after extraction with SWE. Maybe the stirring advice for SWE was not efficient enough for the small particle size to get a proper stirring. It can also be due to a smaller chamber used for the SWE than the reagent bottle used for the WB. The small chamber used for SWE may not be enough and the samples were concentrated in the bottom of the chamber for 0.5 mm. A larger container would improve the stirring and soaking the samples better.

Also, the longer time for warming up the samples in SWE may cause degrade in BG. For the WB, the temperature was already set to 95°C when the extraction started, while the temperature went from 50°C to 95°C with the SWE.

Molecular weight

The estimated molecular weight of BG extracted from SWE showed that the highest molecular weight was obtained from the extraction at 95°C both for the 0.5 mm and for the unground oat bran. Only temperature had a significant effect on the molecular weight while there was no effect for the particle size. The molecular weight at 95°C was significantly different compared to the molecular weight at 150°C and 170°C while there were no significant differences on the molecular weight between 150°C and 170°C (see Figure 15).

The molecular weight dramatically decreases when comparing 95°C to 150- and 170°C. This may be due to higher temperatures disrupting the molecule structure and degrading the BG which is also stated by Yoo *et. al* (2020). From a health perspective a high-molecular weight or a medium-molecular weight is desirable (Wolever *et al.*, 2010). In this case only the BG at 95°C achieved that, as the molecular weight at 150- and 170°C for both particle sizes had a low-molecular weight between 70-200 kg/ mol.

Another observation is that the BG content was lowest at 95°C with SWE, but on the other hand the viscosity and molecular weight was highest. This may also be due to BG with a higher molecular weight is more water absorbing and can more easily form gels.

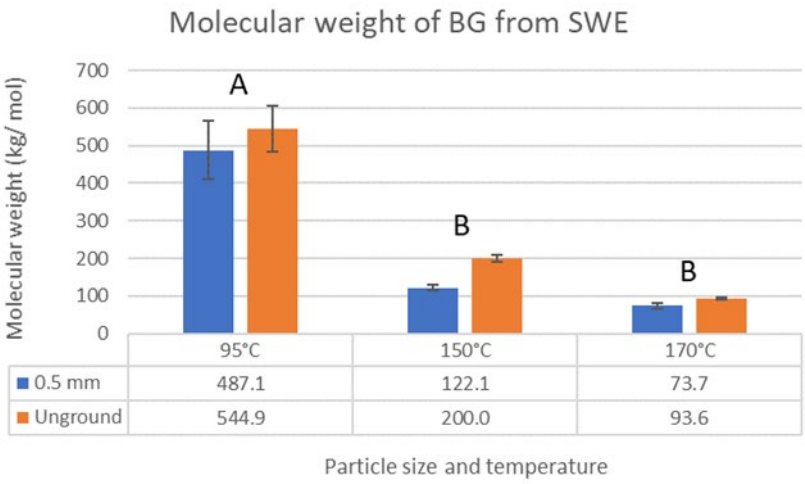

Figure 15. Showing the mean molecular weight of BG from SWE. The molecular weight at 95°C are of medium-molecular weight while the molecular weight at 150- and 170°C are of low-molecular weight. Only the temperature had a significant effect on the molecular weight and the significant differences are stated with letters. Means that do not share letter are significantly different.

5. Conclusion

The purpose of this project was to investigate how the particle size, the time and the temperature used when extracting BG can affect the viscosity, the BG yield and the molecular weight. Two different methods were used, both extraction in WB and extraction with SWE.

The results from the WB showed that with a decreasing particle size and with a longer extraction time, an increase in viscosity and BG yield was obtained. The highest yield was obtained with a particle size of 0.5 mm and an extraction time of 2h. The molecular weight determined from the water bath showed that the highest molecular weight was obtained with a 2h extraction, and the particle size had no effect.

For the SWE only temperature had a significant effect on the viscosity and the molecular weight. The viscosity and molecular weight were highest at 95°C and decreased a lot with higher temperatures. For the BG extracted with SWE the yield was almost two-fold higher with higher temperatures. This shows that with SWE a high BG yield can be obtained with high temperatures, but the molecular weight is decreasing dramatically to a low-molecular weight around 70-200 kg/ mol.

The conclusion is that a SWE is very advantageous method when extracting BG if yield is the sole focus but not concerning the health effects. However, further research needs to investigate how a high yield of BG can be obtained, but still with a high- or medium-molecular weight to be health promoting. Preferrable with investigations of at which temperatures and at which time the molecular weight changes from a medium-molecular weight to a low-molecular weight.

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Appendix 1 Raw data from statistical analysis for subcritical water extraction

General Linear ANOVA: Extracted BG % versus Temperature and Particle size. Comparisons with Tukey pairwise comparisons.

General Linear Model: BG % versus Temp., Particle size

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Temp.	Fixed	3	150, 170, 95
Particle size	Fixed	2	0,5, unground

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temp.	2	4395.44	2197.72	122.95	0.000
Particle size	1	986.24	986.24	55.17	0.000
Temp.*Particle size	2	14.39	7.19	0.40	0.683
Error	7	125.13	17.88		
Total	12	5791.52			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.22792	97.84%	96.30%	91.54%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	64.82	1.19	54.65	0.000	
Temp.					
150	11.57	1.63	7.11	0.000	1.32
170	15.10	1.70	8.87	0.000	1.30
Particle size					
0,5	8.81	1.19	7.43	0.000	1.02
Temp.*Particle size					
150 0,5	1.11	1.63	0.68	0.517	1.32
170 0,5	-1.43	1.70	-0.84	0.430	1.30

Regression Equation

BG % = 64.82 + 11.57 Temp._150 + 15.10 Temp._170 - 26.68 Temp._95 + 8.81 Particle size_0,5
- 8.81 Particle size_unground + 1.11 Temp.*Particle size_150 0,5
- 1.11 Temp.*Particle size_150 unground - 1.43 Temp.*Particle size_170 0,5
+ 1.43 Temp.*Particle size_170 unground + 0.32 Temp.*Particle size_95 0,5
- 0.32 Temp.*Particle size_95 unground

Fits and Diagnostics for Unusual Observations

Obs	BG %	Fit	Resid	Std Resid	
10	59.53	66.47	-6.94	-2.32	R
11	73.41	66.47	6.94	2.32	R

R Large residual

Comparisons for BG %

Tukey Pairwise Comparisons: Temp.

Grouping Information Using the Tukey Method and 95% Confidence

Temp.	N	Mean	Grouping
170	4	79.9196	A
150	5	76.3911	A
95	4	38.1394	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Particle size

Grouping Information Using the Tukey Method and 95% Confidence

Particle size	N	Mean	Grouping
0,5	7	73.6270	A
unground	6	56.0064	B

Means that do not share a letter are significantly different.

General Linear ANOVA: Viscosity versus Temperature and Particle size.
Comparisons with Tukey pairwise comparisons.

General Linear Model: Viscosity mPa.s-1 versus Temp., Particle size

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Temp.	Fixed	3	150, 170, 95
Particle size	Fixed	2	0,5, unground

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temp.	2	217.764	108.882	22.31	0.001
Particle size	1	6.893	6.893	1.41	0.273
Temp.*Particle size	2	59.892	29.946	6.14	0.029
Error	7	34.161	4.880		
Total	12	319.203			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.20909	89.30%	81.65%	57.48%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.824	0.620	11.01	0.000	
Temp.					
150	-0.903	0.850	-1.06	0.323	1.32
170	-4.699	0.889	-5.28	0.001	1.30
Particle size					
0,5	0.737	0.620	1.19	0.273	1.02
Temp.*Particle size					
150 0,5	-2.152	0.850	-2.53	0.039	1.32
170 0,5	-0.817	0.889	-0.92	0.389	1.30

Regression Equation

Viscosity mPa.s-1 = 6.824 - 0.903 Temp._150 - 4.699 Temp._170 + 5.603 Temp._95
+ 0.737 Particle size_0,5 - 0.737 Particle size_unground
- 2.152 Temp.*Particle size_150 0,5 + 2.152 Temp.*Particle size_150
unground - 0.817 Temp.*Particle size_170 0,5
+ 0.817 Temp.*Particle size_170 unground + 2.968 Temp.*Particle size_95
0,5 - 2.968 Temp.*Particle size_95 unground

Fits and Diagnostics for Unusual Observations

Obs	Viscosity mPa.s-1	Fit	Resid	Std Resid	
1	12.69	16.13	-3.45	-2.21	R
2	19.58	16.13	3.45	2.21	R

R Large residual

Comparisons for Viscosity mPa.s-1

Tukey Pairwise Comparisons: Temp.

Grouping Information Using the Tukey Method and 95% Confidence

Temp.	N	Mean	Grouping	
95	4	12.4270	A	
150	5	5.9212		B
170	4	2.1251		B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Particle size

Grouping Information Using the Tukey Method and 95% Confidence

Particle size	N	Mean	Grouping
0,5	7	7.56093	A
unground	6	6.08788	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Temp.*Particle size

Grouping Information Using the Tukey Method and 95% Confidence

Temp.*Particle size	N	Mean	Grouping	
95 0,5	2	16.1315	A	
95 unground	2	8.7224	A	B
150 unground	2	7.3362		B
150 0,5	3	4.5062		B
170 unground	2	2.2051		B
170 0,5	2	2.0451		B

Means that do not share a letter are significantly different.

General Linear ANOVA: Molecular weight versus Temperature and Particle size. Comparisons with Tukey pairwise comparisons.

General Linear Model: MW versus Temp., Particle size

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Temp.	Fixed	3	150, 170, 95
Particle size	Fixed	2	0,5, unground

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temp.	2	431761	215880	75.80	0.000
Particle size	1	8549	8549	3.00	0.127
Temp.*Particle size	2	1859	929	0.33	0.732
Error	7	19935	2848		
Total	12	471039			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
53.3658	95.77%	92.74%	83.13%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	253.6	15.0	16.94	0.000	
Temp.					
150	-92.6	20.5	-4.51	0.003	1.32
170	-169.9	21.5	-7.91	0.000	1.30
Particle size					
0,5	-25.9	15.0	-1.73	0.127	1.02
Temp.*Particle size					
150 0,5	-13.0	20.5	-0.63	0.547	1.32
170 0,5	16.0	21.5	0.74	0.481	1.30

Regression Equation

MW = 253.6 - 92.6 Temp._150 - 169.9 Temp._170 + 262.5 Temp._95 - 25.9 Particle size_0,5
+ 25.9 Particle size_unground - 13.0 Temp.*Particle size_150 0,5
+ 13.0 Temp.*Particle size_150 unground + 16.0 Temp.*Particle size_170 0,5
- 16.0 Temp.*Particle size_170 unground - 3.0 Temp.*Particle size_95 0,5
+ 3.0 Temp.*Particle size_95 unground

Fits and Diagnostics for Unusual Observations

Obs	MW	Fit	Resid	Std Resid	
1	409.6	487.1	-77.6	-2.06	R
2	564.7	487.1	77.6	2.06	R

R Large residual

Comparisons for MW

Tukey Pairwise Comparisons: Temp.

Grouping Information Using the Tukey Method and 95% Confidence

Temp.	N	Mean	Grouping	
95	4	516.039	A	
150	5	161.013		B
170	4	83.637		B

Means that do not share a letter are significantly different.

Appendix 2 Raw data from statistical analysis for water bath

General Linear ANOVA: Extracted BG % versus Time and Particle size. Comparisons with Tukey pairwise comparisons.

General Linear Model: % BG versus Time, Particle size

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Time	Fixed	2	1h, 2h
Particle size	Fixed	5	0.5, 0.7, 1.0, 1.5, Unground

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	1	247.47	247.467	6.98	0.016
Particle size	4	2856.19	714.048	20.13	0.000
Time*Particle size	4	28.51	7.127	0.20	0.935
Error	20	709.46	35.473		
Total	29	3841.63			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5.95594	81.53%	73.22%	58.45%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	36.32	1.09	33.41	0.000	
Time					
1h	-2.87	1.09	-2.64	0.016	1.00
Particle size					
0.5	12.07	2.17	5.55	0.000	1.60
0.7	4.59	2.17	2.11	0.048	1.60
1.0	5.15	2.17	2.37	0.028	1.60
1.5	-6.17	2.17	-2.84	0.010	1.60
Time*Particle size					
1h 0.5	0.88	2.17	0.41	0.690	1.60
1h 0.7	-0.90	2.17	-0.41	0.684	1.60
1h 1.0	0.58	2.17	0.27	0.791	1.60
1h 1.5	-1.44	2.17	-0.66	0.516	1.60

Regression Equation

% BG = 36.32 - 2.87 Time_1h + 2.87 Time_2h + 12.07 Particle size_0.5 + 4.59 Particle size_0.7
+ 5.15 Particle size_1.0 - 6.17 Particle size_1.5 - 15.64 Particle size_Unground
+ 0.88 Time*Particle size_1h 0.5 - 0.90 Time*Particle size_1h 0.7
+ 0.58 Time*Particle size_1h 1.0 - 1.44 Time*Particle size_1h 1.5
+ 0.87 Time*Particle size_1h Unground - 0.88 Time*Particle size_2h 0.5
+ 0.90 Time*Particle size_2h 0.7 - 0.58 Time*Particle size_2h 1.0
+ 1.44 Time*Particle size_2h 1.5 - 0.87 Time*Particle size_2h Unground

Fits and Diagnostics for Unusual Observations

Obs	% BG	Fit	Resid	Std Resid	
10	45.57	34.47	11.10	2.28	R
12	22.78	34.47	-11.68	-2.40	R

R Large residual

Comparisons for % BG

Tukey Pairwise Comparisons: Time

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
2h	15	39.1970	A
1h	15	33.4529	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Particle size

Grouping Information Using the Tukey Method and 95% Confidence

Particle size	N	Mean	Grouping
0.5	6	48.3940	A
1.0	6	41.4795	A
0.7	6	40.9116	A
1.5	6	30.1590	B
Unground	6	20.6807	B

Means that do not share a letter are significantly different.

General Linear ANOVA: Viscosity versus Time and Particle size.
Comparisons with Tukey pairwise comparisons.

General Linear Model: Viscosity mPa.s-1 versus Time, Particle size

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Time	Fixed	2	1h, 2h
Particle size	Fixed	5	0.5, 0.7, 1.0, 1.5, Unground

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	1	332.54	332.543	151.82	0.000
Particle size	4	1144.09	286.023	130.59	0.000
Time*Particle size	4	38.89	9.723	4.44	0.010
Error	20	43.81	2.190		
Total	29	1559.33			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.47997	97.19%	95.93%	93.68%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	15.829	0.270	58.58	0.000	
Time					
1h	-3.329	0.270	-12.32	0.000	1.00
Particle size					
0.5	8.744	0.540	16.18	0.000	1.60
0.7	2.582	0.540	4.78	0.000	1.60
1.0	1.805	0.540	3.34	0.003	1.60
1.5	-3.558	0.540	-6.58	0.000	1.60
Time*Particle size					
1h 0.5	-2.063	0.540	-3.82	0.001	1.60
1h 0.7	0.050	0.540	0.09	0.927	1.60
1h 1.0	1.000	0.540	1.85	0.079	1.60
1h 1.5	-0.089	0.540	-0.16	0.872	1.60

Regression Equation

Viscosity mPa.s-1 = 15.829 - 3.329 Time_1h + 3.329 Time_2h + 8.744 Particle size_0.5
+ 2.582 Particle size_0.7 + 1.805 Particle size_1.0
- 3.558 Particle size_1.5 - 9.573 Particle size_Unground
- 2.063 Time*Particle size_1h 0.5 + 0.050 Time*Particle size_1h 0.7
+ 1.000 Time*Particle size_1h 1.0 - 0.089 Time*Particle size_1h 1.5
+ 1.102 Time*Particle size_1h Unground + 2.063 Time*Particle size_2h 0.5
- 0.050 Time*Particle size_2h 0.7 - 1.000 Time*Particle size_2h 1.0
+ 0.089 Time*Particle size_2h 1.5 - 1.102 Time*Particle size_2h Unground

Fits and Diagnostics for Unusual Observations

Obs	Viscosity mPa.s-1	Fit	Resid	Std Resid	
19	18.642	15.132	3.509	2.90	R
24	12.862	15.304	-2.442	-2.02	R
25	11.729	8.853	2.875	2.38	R

R Large residual

Comparisons for Viscosity mPa.s-1

Tukey Pairwise Comparisons: Time

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
2h	15	19.1586	A
1h	15	12.4998	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Particle size

Grouping Information Using the Tukey Method and 95% Confidence

Particle size	N	Mean	Grouping
0.5	6	24.5730	A
0.7	6	18.4115	B
1.0	6	17.6341	B
1.5	6	12.2711	C
Unground	6	6.2562	D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Time*Particle size

Grouping Information Using the Tukey Method and 95% Confidence

Time*Particle size	N	Mean	Grouping
2h 0.5	3	29.9657	A
2h 0.7	3	21.6908	B
2h 1.0	3	19.9638	B C
1h 0.5	3	19.1803	B C D
2h 1.5	3	15.6890	C D
1h 1.0	3	15.3043	D
1h 0.7	3	15.1323	D
1h 1.5	3	8.8532	E
2h Unground	3	8.4834	E
1h Unground	3	4.0290	F

Means that do not share a letter are significantly different.

General Linear ANOVA: Molecular weight versus Time and Particle size. Comparisons with Tukey pairwise comparisons.

General Linear Model: MW versus Time, Particle size

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Time	Fixed	2	1h, 2h
Particle size	Fixed	5	0.5, 0.7, 1.0, 1.5, Unground

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	1	75000	75000	5.66	0.027
Particle size	4	20834	5208	0.39	0.811
Time*Particle size	4	20996	5249	0.40	0.809
Error	20	265119	13256		
Total	29	381949			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
115.135	30.59%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	645.4	21.0	30.70	0.000	
Time					
1h	-50.0	21.0	-2.38	0.027	1.00
Particle size					
0.5	4.6	42.0	0.11	0.914	1.60
0.7	-1.4	42.0	-0.03	0.974	1.60
1.0	-29.2	42.0	-0.70	0.495	1.60
1.5	46.6	42.0	1.11	0.281	1.60
Time*Particle size					
1h 0.5	-20.3	42.0	-0.48	0.634	1.60
1h 0.7	26.7	42.0	0.63	0.533	1.60
1h 1.0	34.2	42.0	0.81	0.426	1.60
1h 1.5	-6.3	42.0	-0.15	0.882	1.60

Regression Equation

MW = 645.4 - 50.0 Time_1h + 50.0 Time_2h + 4.6 Particle size_0.5 - 1.4 Particle size_0.7
- 29.2 Particle size_1.0 + 46.6 Particle size_1.5 - 20.6 Particle size_Unground
- 20.3 Time*Particle size_1h 0.5 + 26.7 Time*Particle size_1h 0.7
+ 34.2 Time*Particle size_1h 1.0 - 6.3 Time*Particle size_1h 1.5
- 34.2 Time*Particle size_1h Unground + 20.3 Time*Particle size_2h 0.5
- 26.7 Time*Particle size_2h 0.7 - 34.2 Time*Particle size_2h 1.0
+ 6.3 Time*Particle size_2h 1.5 + 34.2 Time*Particle size_2h Unground

Fits and Diagnostics for Unusual Observations

Obs	MW	Fit	Resid	Std Resid	
10	505.0	748.3	-243.3	-2.59	R
12	1053.0	748.3	304.7	3.24	R

R Large residual

Comparisons for MW

Tukey Pairwise Comparisons: Time

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
2h	15	695.4	A
1h	15	595.4	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Particle size

Grouping Information Using the Tukey Method and 95% Confidence

Particle size	N	Mean	Grouping
1.5	6	692.000	A
0.5	6	650.000	A
0.7	6	644.000	A
Unground	6	624.833	A
1.0	6	616.167	A

Means that do not share a letter are significantly different.

Appendix 3 Visualisation of the different particle sizes of oat bran

The oat bran seen are milled in the sizes of 0.5 mm, 0.7 mm, 1.0 mm, 1.5 mm and unground oat bran.

Appendix 4 Popular science summary

Introduction

An increasing number of people have unhealthy eating habits with a too high energy intake but low in nutritional value. This contributes to the increasing number of people suffering from lifestyle related diseases. At the same time the population is increasing, so there is a need to utilize the food resources we already have. One way to address both issues is to use the whole grains instead of throwing away edible parts.

Background

Oat along with wheat, barley and rye are the four most cultivated crops in Sweden. Oat consists of an unpalatable husk and when it is removed the three mayor parts are the oat bran, the starchy endosperm and the germ in the center. Oat bran is a by-product in the production of oat products, although it is full of nutrients as minerals, vitamins and it is a good source of dietary fibres. The recommendations of a fibre intake are 25-35 grams per day. That is not achieved by many and therefore easier ways to achieve the daily recommended dose of dietary fibre needs to be available.

The main dietary fibre in oat bran is beta-glucan and it make up about 5-20% of the oat bran. A daily intake of beta-glucan is known to reduce the risk of cardiovascular diseases and to lower both the blood glucose- and the cholesterol levels. To have a positive health effect the molecular weight of beta-glucan needs to have a medium or high molecular weight. It is possible to extract beta-glucan from oat bran, which results in a product rich in beta-glucan that can be added to juices or yoghurts e.g. to easily increase the daily amount of an intake of dietary fibres.

Performance of the study

This master's thesis was performed at the Swedish University of Agricultural Sciences in a collaboration with Lantmännen. The purpose of this study was to determine how the extraction time, the extraction temperature and different particles sizes of milled oat bran can affect the beta-glucan yield and the molecular weight of beta-glucan.

Oat bran was milled in five different sizes and two different extraction methods were used: one with a general water bath were the milled oat bran was soaked in an enzyme solution. The bottles were put into a 95°C water bath for 1 or 2 hours.

The other method was extraction in subcritical water, which is water under high pressure which allows the temperature to rise above 100°C without boiling. The temperatures used were 95°C, 150°C and 170°C.

Results

For the extraction in water bath the highest yield was obtained with milled oat bran in the smallest size, 0.5 mm, and with an extraction time for two hours. Around 50% of the available beta-glucan in the oat bran was extracted. The yield decreased with larger sizes of oat bran and with shorter extraction times. For the molecular weight all the samples extracted in water bath had a medium-molecular weight, but the weight was slightly higher for two hours than for one hour.

With extraction in subcritical water the beta-glucan yield was almost two-fold higher with the higher temperatures 150°C and 170°C compared to 95°C. The highest yield which was 87.3% beta-glucan was obtained with the particle size 0.5 mm at 170°C. For the molecular weight it was highest at 95°C and decreased dramatically with the higher temperatures. It changed from a medium-molecular weight at 95°C to a low-molecular weight at 150°C and 170°C.

Conclusions

Extractions in water bath showed that with a decreasing particle size and with a longer extraction time the yield is increasing, and the molecular weight has a medium weight which has proved to have positive health impacts.

For the extractions in subcritical water the higher temperatures increased the yield significantly, but the extract obtained had a low-molecular weight.

Subcritical water extraction can be very advantageous according yield, time consumption and cost-efficiency but, under conditions in this study, not very useful concerning the health effects. Further studies are needed to investigate at which time and at which temperature the molecular weight changes from medium to low, to extract as much beta-glucan as possible that still contribute with health benefits.