Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Molecular Sciences

Optimization of β-glucan levels in different oat fractions by milling and air classification

Optimering av β-glukanhalt i olika havrefraktioner med malning och vindsiktning

Sumaiya Majid

Department of Molecular Sciences
Master Thesis • 30 hec • Second cycle, A2E
Agriculture Programme – Food Science
Molecular Sciences, 2018:12
Uppsala, 2018

Optimization of β-glucan levels in different oat fractions by milling and air classification

Optimering av β-glukanhalt i olika havrefraktioner med malning och vindsiktning

Sumaiya Majid

Supervisor: Roger Andersson, Swedish University of Agricultural Sciences, Department of Molecular Sciences

Assistant supervisor: Christian Malmberg, Lantmännen

Examiner: Annica Andersson, Swedish University of Agricultural Sciences, Department of Molecular Sciences

Credits: 30 credits
Level: Second cycle, A2E
Course title: Independent project /degree project in Food Science - Master's thesis
Course code: EX0425
Programme/education: Agricultural Programme - Food Science

Place of publication: Uppsala
Year of publication: 2018
Title of series Molecular Sciences
Part number 2018:12
Online publication: https://stud.epsilon.slu.se

Keywords: optimization, oat, β-glucan, air classification, milling, sieve analysis

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Department of Molecular Sciences

Abstract

Oat (*Avena Sativa*) is an important crop worldwide which is mostly due to its various characteristics and nutritional profile. The recent progression in food nutrition have shown the importance of the different components in oats. Many health benefits of oats are attributed to β-glucan which have been demonstrated and well-studied over the years. This investigation is part of a project commissioned by Lantmännen that has for a long time pursued plant breeding of oats and invested in research and development. The aim of this project was to investigate if there is a potential to obtain a fraction from oat with high levels of β-glucan. Air classification and sieve analysis were utilized for β-glucan enrichment from a pre-fractionated oat fraction with 20 % β-glucan. Effects of speed of the classifying wheel, size of particle and different milling settings on the β-glucan separation efficiency were evaluated. To better understand the separation efficiency between the fine and coarse fractions, both particle size distribution analysis and scanning electron microscopy were performed. Through optimization of milling and air-classification parameters, a β-glucan content up to 26.9 % could be obtained. Results indicate that air classification is a good method to use for enrichment of β-glucan from oats and prevents breakdown of the β-glucan molecule. The ability to enrich β-glucan level can increase the economic value of oats and possibility to tailoring fraction for specific markets in the food industry and lead to new product development. It can be concluded that in the near future there will be health-promoting products in the market where β-glucan content is increased.

Keywords: optimization, oat, β-glucan, air classification, milling, sieve analysis

Sammanfattning

Havre (*Avena Sativa*) är en viktig gröda världen över, vilket främst beror på dess olika egenskaper och näringsprofil. De senaste framstegen inom nutrition har visat vilken betydelse de olika komponenterna havre har. Många av havrens hälsofördelar kan tillskrivas β-glukan som har påvisats och studerats väl genom åren. Denna studie är en del av ett projekt från Lantmännen som länge har drivit odling av havre och investerat mycket i forskning och utveckling. Syftet med detta projekt var att studera om det fanns potential att erhålla en havrefraktion med hög halt av β-glukan. Vindsiktning och siktanalys användes för anrikning av β-glukan från en tidigare fraktionerad havrefraktion med 20 % β-glukan. Effekten av hastigheten hos klassificeringshjulet, partikelstorlek och olika malningsinställningar för separationseffektiviteten av β-glukan utvärderades. För att bättre förstå separationseffektiviteten mellan de fina och grova fraktionerna genomfördes både analys av partikelstorleksfördelning och svepelektronmikroskopi. Genom optimering av malning-och vinsiktsparametrar, kunde en β-glukan halt på upp till 26.9 % erhållas. Resultaten visar att vinsiktning är en bra metod att använda för anrikning av β-glukan från havre utan nedbrytning av β-glukanmolekylen. Möjligheten att kunna anrika β-glukan kan öka det ekonomiska värdet hos havre och möjliggöra skräddarsydda fraktioner för specifika marknader inom livsmedelsindustrin och leda till ny produktutveckling. Genom resultaten från detta projekt kan slutsatsen dras att inom den närmaste framtiden kommer det finnas allt fler hälsofrämjande produkter på marknaden med ökad β-glukanhalt.

Nyckelord: optimering, havre, β-glukan, vindsiktning, malning, siktanalys

Table of contents

List of tables

List of figures

1 Introduction

Oat (*Avena Sativa*) is an important crop worldwide which is mostly due to its various characteristics and nutritional profile. The recent progression in food and nutrition have shown the importance of the different components in oats. This has especially been demonstrated for β-glucan, a dietary fiber found in the oat bran (Butt *et al.*, 2008).

The interest of oats has increased primarily because of the beneficial health effects that has been demonstrated and well-studied over the years. The primary health benefits of oat are principally attributed to water-soluble fiber where β-glucan is the major component. The dietary fiber in oats has been shown to have various physiological effects in which cholesterol-lowering and postprandial hyperglycemia are the most well-known (Liangli *et al.*, 2012). The beneficial health effects related to oats have increased consumers awareness of it. Today, the health claims approved both by FDA (2003; 1997) and EFSA (2011a; 2011b) have encouraged the consumption of oat foods. The European Union permits food companies and producers to use health claims such as reduction of blood cholesterol concentrations and postprandial glycemic responses, if products contain 1 g β-glucan per portion (EFSA, 2011a)**.** The significance of β-glucan content in oat milling is greatly understood by all contributors in the value chain since it is the source for various well documented health benefits (Beck *et al.*, 2009b; Andersson *et al.*, 2002; Beer *et al.*, 1995). The consumer identifies the β-glucan by labels with health claims on the products and the marketing of these claims are carried out by milling and food processing companies. It is therefore important for companies to work closely with oat producers and breeders to communicate these requirements.

This thesis is part of a project commissioned by Lantmännen. Lantmännen has for a long time pursued plant breeding of oats, invested in research and development to take advantage of the favourable qualities. Findings with favourable health effects can affect the economical outcome but also new product development. They have especially investigated the refining and developing of new food products that today exist on the market. Nevertheless, new areas have yet to be explored and knowledge

to be gained. For this project, Lantmännen are interested to investigate the possibilities to further improve the qualities of one oat variety. Air classification and sieve analysis were utilized for β-glucan enrichment from a pre-fractionated oat fraction. The effect of the speed of the classifying wheel, size of the particle as well as different milling settings on the beta-glucan separation efficiency was evaluated.

1.1 Aim

The aim of this project is to see if there is a potential to obtain a fraction from oat with high levels of β-glucan by using milling and air classification,

2 Litterature review

In this thesis the literature was obtained from various databases such as FSTA, Google Scholar and Web of sciences. Primarily, published research articles from various journals have been used in this literature review.

2.1 Oat

Oat (*Avena Sativa)* belongs to the *Poaceae* family and is one of the most important cultivated crops worldwide (Butt *et al.*, 2008). Oats are a vital source of livestock feed both as a nutritious grain and as a forage. Nevertheless, oats are likewise used in production of various human food products due to its nutritional profile (Strychar *et al.*, 2011). Oat has been and still is a staple component for those two matters and has also played an important role in agricultural and cultural development worldwide. The diversity of form within oat allows plant breeders to develop and cultivators to exploit its genetic potential to their economic benefit.

2.1.1 Kernel structure

The oat kernel is long and elliptical in shape and covered with fine hairs (Welch, 1995b). The outer layer of an oat kernel is called the hull and is primarily composed of cellulose and hemicellulose (Welch, 1995b; Welch *et al.*, 1983). The hull takes up approximately 25-33 % of the total weight of an oat kernel. It is usually not used in food, however, new processes have been developed commercially where insoluble cellulosic fiber is used as food ingredient (Stevenson *et al.*, 2011).
The hull has a very low content of protein demonstrated in various studies. (Welch *et al.*, 1983)

Bran is the term that is used for the outer part of the dehulled oat kernel. The oat bran is referred to the milling fraction and according to AACC (Anon, 1989) the oat

bran is produced upon grinding "and separating a bran fraction that is not more than 50 % of the starting material and has a total β-glucan content of at least 5,5 % of dry weight and a total dietary fibre content of at least 16% of dry weight with the result that at least one-third of the total fibre is soluble". Generally, oat bran can be produced by grinding oat groats and thereafter sieve to get rid of flour containing starchy endosperm. This will result in an oat fraction enhanced with β-glucan and can be further processed to meet the oat bran definition (Kulp, 2000).

The oat bran consists of different layers. Starting from the outer part the layers included are the pericarp, the testa, also known as seed coat, the nucellus, the aleurone and a large part of the subaleurone starchy endosperm. The aleurone layer in oats is not separated distinctly as it is with wheat for example. Though the aleurone layer is a part of the outer layers but through processing it is botanically a part of the starchy endosperm (Miller & Fulcher, 2011).

The major tissue in oat grains is starchy endosperm (Youngs, 1972). The endosperm is located inside the cell wall layers of the kernel and can contribute up to 70 % of the mature kernel depending on the oat variety. It is the starchy endosperm that is the primary storage location of lipid, protein, starch and β-glucan. Protein endosperm in the highest concentration is found towards the periphery and decreases near the interior of the oat kernel, while starch is most concentrated in the endosperm centre (Miller & Fulcher, 2011). The endosperm accounts the majority, up to 90 %, of the total lipid content in oats (Youngs *et al.*, 1977).

The germ is where a new plant starts to sprout and contains high amounts of protein and lipid, however small amount of starch. The protein amount in the germ can be up to 29-38 % compared to the bran, which is 23 % and 12 % in starchy endosperm. The lipid content is higher in the germ (15-24 %) compared to any other groat tissues (Youngs, 1972).

2.1.2 Composition

The nutritional composition of oats can be considerably changed by different processing methods. This could include the depletion of fiber-rich bran layers along with associated vitamins and minerals, in addition to fats, salt, sugar and other ingredients, as well as enrichment of vitamins and minerals for fortification. However, the composition of protein, starch and lipid is extremely reliant on the oat variety and therefore the composition stated is a generalization.

The main constituent in the oat grain is carbohydrate (42-65 %) with starch content comprising the majority of the carbohydrate reserve. Nevertheless, oat grains also comprise significant amounts of non-starch polysaccharides and small amounts of simple sugars (Welch, 1995a). The size of the starch granule range between 2-15 µm and have a polynormal form (Jane *et al.*, 1994).

Oat grains have naturally high amounts of protein (11-15 %). The protein content can be as high as 12.4-24.5 % if the hull, with high cellulose and low protein, is removed. This makes oat the cereal with the highest amount of protein among the most consumed cereal grains (Lásztity, 1998).

2.2 β-Glucan in oats

The β-glucan in oat is included in a large family of mixed-linkage β-glucan molecules that are found in plants, fungi and microorganisms (Kale *et al.*, 2014). Oat β-glucan ((1→3), (1→4)-β-D- glucan) is a linear polysaccharide composed of β-D-glucose units. The β-(1→3) linkages prevents the molecule to pack close and therefore makes the molecule partly water-soluble (Daou & Zhang, 2012). β-Glucan has established important functional and nutritional properties demonstrating high viscosities at relatively low concentrations.

β-Glucan in oats was first described in oat kernels by Morris (1942). Today several studies have investigated and confirmed that β-glucan are mainly found in the cell walls of starchy endosperm and the bran, concentrated in the sub aleurone layer (Miller *et al.*, 1995; Wood *et al.*, 1983; Wood & Fulcher, 1978). However, smaller amounts have been found in the oat germ, although there are no indication that β-glucan exists in the hull (Wood, 2011).

There are multiple oat varieties that exist, where growing conditions differ among season and region hence the characteristics of oat β-glucan vary (Wood, 2011). Although, the β-glucan content in oat groats differ vastly if it is influenced by a combination of both environment and genetics, the latter is the more dominant factor (Miller *et al.*, 1993b). Cho and White (1993) demonstrated that the majority of oat cultivars showed a β-glucan content ranging between 4.5-5.5 %. In a study by Miller *et al.* (1993a), it was reported that the β-glucan level ranged between 1.8 – 5.5% of oats from 18 different species. In another study by Peterson (1991) some cultivars conveyed as much as 8.5 % of β-glucan. Andersson and Börjesdotter (2011) found that oat β-glucan content ranging between 2.3 and 3.2 % was influenced by a genotype to a larger extent than growing conditions. The β-glucan level can be significantly increased by processing such as milling and sieving (Wood, 1991). Enrichment of β-glucan content can be accomplished further by defatting oat groats or sieving in aqueous ethanol.

β-Glucan molecules are polysaccharides and they do not occur as a single distinct degree of polymerization (DP), but instead as a distribution of molecular weights (MW). For this reason, they are described as weight averages of the weight (M_w), which is the most common way to define. The distribution of the molecular weight

is a vital characteristic of polysaccharides and important, especially for β-glucan in oats. This is mainly due to the fact that different rheological behaviour, related to physiological function, might be observed depending on MW (Wood, 2011). The M_w of β-glucan has been reported to vary between 6.5×10^4 and 3.1×10^6 Da, and to partially be described by environmental and genotypic factors. It was demonstrated in a study where four oat varieties, grown in 11 different environments had a more limited M_w range for β-glucan where the range was between $1.73 – 2.02\times10^6$ Da (Andersson & Börjesdotter, 2011). There are various methods to measure M_w that have been reported. Nevertheless, HPSEC analysis with fluorescence detection is mostly useful for measuring β-glucan in cereals due to the selective dye binding reagent of Calcofluor to β-glucan (Rimsten *et al.*, 2003), which gives a precise and quantitative detection of β-glucan without interfering with other components (Wood *et al.*, 1983).

Soluble β-glucan can be measured and extracted with a range of different methods. Depending on the analytical procedures and sample source, the β-glucan solubility will vary (Welch, 1995a). Carr *et al.* (1990) demonstrated an analysis of retail oat products which estimated to have β-glucan solubility ranged between 41-57 %.

2.3 Processing of oats

Oats are a challenging cereal to process due to its high content of lipids (Brown & Craddock, 1972; Brown *et al.*, 1966). The difficulty with lipids when milling oats is when the material stick on the surface of the processing equipment. Lehtinen (2003) states that lipids might cause sensory problems in the final products due to enzymatic hydrolysis of acylglycerols and non-enzymatic oxidation of the unsaturated fatty acid moieties, which is acylated to polar lipids. These reactions can easily cause rancidity and products with off flavours.

The oat hull accounts up to 25 % of the total grain and are rich in crude fiber, which is not suitable for human consumption. For oat milling the aims are for that reason to, firstly, eliminate the hull from the kernel and clean the kernels from other unnecessary parts. Secondly, it is important to achieve an attractive appearance of the finished products, good digestibility, pleasant taste and lastly, a quality that can be maintained. Therefore, it is vital that the raw material is of good quality and by using processes to achieve that it will yield a product which meets the demands (Ganßmann & Vorwerck, 1995).

2.3.1 Cleaning, grading and dehulling

The first technological step in oat processing is cleaning. The aim of this step is to remove unwanted parts such as straw particles and loose husks. Oat grains for instance pin oats, light oats or double oats, which are unsuitable for milling, are separated in this step as well (Salisbury & Wichser, 1971).

The main reason for the grading process of oats is to separate clean oats into two or four fractions based on density and weight (Girardet & Webster, 2011). Oat consist of a mixture of grains in various sizes which is due to the biological structure of oats. It is important that the oat mixture is graded for size so the process can be closely matched to the different behaviour of each grain stream. The grading process is mainly grounded on either grain width or weight (Ganßmann & Vorwerck, 1995).

Oat is one cereal that provides a challenge for millers. When the oat kernel is harvested it is encased in a hull which consist of an outer and inner layer called lemma and palea, respectively. Before processing the grain any further, the hull must be removed. Opportunely the hull and kernel are not attached together as it could be in other grains, such as barley and rice, and therefore the removal cause less damage of the kernel tissue. The dehulling operation can vary but there are mainly two different methods, namely using stone or impact dehullers. However, today impact dehullers is the most common system. The primary difference between the two methods is that with stone dehullers the kilning process occurs before dehulling, while with impact dehullers it is performed after. The hull in the impact system is removed by impact action and abrasion. Oats are fed through the centre of a high-speed rotator, which throws the oats against an impact ring that is fixed to the outer exterior of the machine. The rim can me made of steel or plastic, however, with a steel ring there could be more breakage and damage to the groats (Ganßmann & Vorwerck, 1995). The main kernel characteristics that are vital for the influence of dehulling efficiency are moisture content, groat percentage and kernel weight.

2.3.2 Kilning and flaking

After dehulling the oats, the next step in the process is kiln drying. This stage is a key step to the production of high-quality oat products. Principally it is performed to stabilize the oat groat by inactivate enzymes in oats and make them susceptible to enzymic rancidity. Compared to other grains oat have the highest fat content, which range from 6-8 % and as well plenty of lipases. If oats are not treated they will quickly develop off-flavours due the action of lipase, lipoxygenase and peroxidase (Biermann & Grosch, 1979; Kazi & Cahill, 1969). When there is disruption of the oat kernel, the enzyme system will rapidly be activated, leading to oxidative breakdown. Consequently, inadequate inactivation of oat lipases, lipoxygenases and

peroxidases will result in this and therefore high-quality oat product with decent shelf life requires correct kilning. The enzymes in grains are heat labile and therefore enzyme activity can be prevented by heat treatment. There are many methods of heat treatment that has been applied through the years (Girardet & Webster, 2011). However, the most effective inactivation of these enzymes can be reached by heating 90-100 °C with a product moisture content at 12 %. The kiln dryers used today are called radiator kilns, which consist of series of heating and cooling sections on top of each other. The sections have louvres and piping where steam and air movement comes in and flows through the kiln. The heating section on top gives indirect heat whereas the bottom sections induce air through the oat steam to make the product cool and remove excess moisture. The retention time usually ranges from 90 to 120 min (Ganßmann & Vorwerck, 1995). It is significant to have a proper control of the time-temperature-moisture profile along with the kilning for the consistency of flavour and nutritional quality of the final product. Other than extending shelf-life and inactivate the enzyme system, kilning serves other purposes. The kilning process is accountable for giving oat products the traditional nutty, browned, toasted-oat flavour and aroma characteristics. Additionally, the process reduces bacteria and fungi that have contaminated the groat surface (Girardet & Webster, 2011). The conventional end-products for human consumption are primarily rolled or flaked oats and oat flour. Oat flakes establishes the main consumption and are produced by flattening or cut groats between roller under heavy pressure. Flaking can occur immediately after drying phase in kilning process. Usually, an additional steaming or tempering step is applied to the dried groats after kilning in order to toughen the oat groats. This is done in order to reduce the production of fines during the flaking process. The moisture content after the kilning step is between 9-12 %, which makes the groats delicate. After the moisture is assessed, the groats are equilibrated in a tempering chamber for 20-30 min. When tempering the oat groats, the temperature is increased up to 95-102 °C. The flaking process is thereafter performed, which is when the tempered groats pass through flaking rolls. The flakes can have different thickness and sizes depending on the end-products usage. After being rolled, the flakes are then cooled down immediately to reduce the moisture and temperature. This is done to ensure an acceptable shelf-life (Girardet & Webster, 2011; Ganßmann & Vorwerck, 1995).

2.3.3 Milling and bran separation

Milling of grains has been practiced for centuries, where the earliest milling processes were very simple with simply using rocks as resource. As technology have been developed and new knowledge gained, the milling process has evolved. This progression has given us the efficient mills that today produce the refined and

quality flours we nowadays use (Girardet & Webster, 2011). Oat flour can be milled from either groats or flakes. The grinding step can be performed by a pin disc mill, roller mill or hammer. Due to oats high fat content, it is essential to draw out exhaust air via the mill to keep the mill screen perforations open. By doing this it avoids the fine flour to stick inside the mill and prevent overheating of the machine.

Oat bran has mainly a high content of soluble dietary fiber and β-glucan. Extraction of oat bran was primary described by Gould *et al*. (1980), where the oat bran was separated from the flour by numerous grinding and sieving processes. It is most common that oat groats, flakes and cut groats are produced as previously described and thereafter milled. The milled oats are then sieved, which gives a coarse fraction (bran) and fine (flour) fraction. The foremost parts of the cell wall will be found in the coarse bran fraction while the fine fraction has mainly starch and protein content from the original kernel (Girardet & Webster, 2011; Gould *et al.*, 1980).

2.3.4 Dry fractionation and air classification

Both dry and wet fractionation methods have been established to make it possible for separation of fiber-rich cell walls, starch or protein from each other. A majority of these fractionation methods have been used for enrichment of cell walls with high content of β-glucan. Particle size is an important factor in air classification of cereal flours and ought to be sufficiently small so that cell component can be separated (King & Dietz, 1987). Compared to wet fractionation, dry fractionation is a much better choice when it comes to developing ingredients for food products. Generally, it is more economical to use dry fractionation since there are no need for drying steps that requires immense amount of energy. Furthermore, the mass yield obtained of the fraction are usually much higher than in wet extraction (Ganßmann & Vorwerck, 1995).

Air classification is a method used to separate particles of a size range with centrifugal force in form of air streams (Figure 1). The main objective of air classification is to achieve a separation of a material into fine and coarse fraction. The milled material is transported by a feeding screw where the air will drive the particles towards the classifier wheel. The speed of the classifier wheel determines if the particles are small enough to pass through the wheel. If the particles are too large they will instead fall down by gravity and be obtained in the coarse fraction, while the finer particles are more likely to be carried along the air and fall down into a separate container. Gracza (1960) was one of the first that developed a commercial air classification process with an aim to separate finely ground flours into fractions. Nowadays it is a common method for enrichment of β-glucan, principally using impact and roller milling followed by air classification that separates the starchy finer particles from larger, coarser fiber particles (Stevenson *et al.*, 2011).

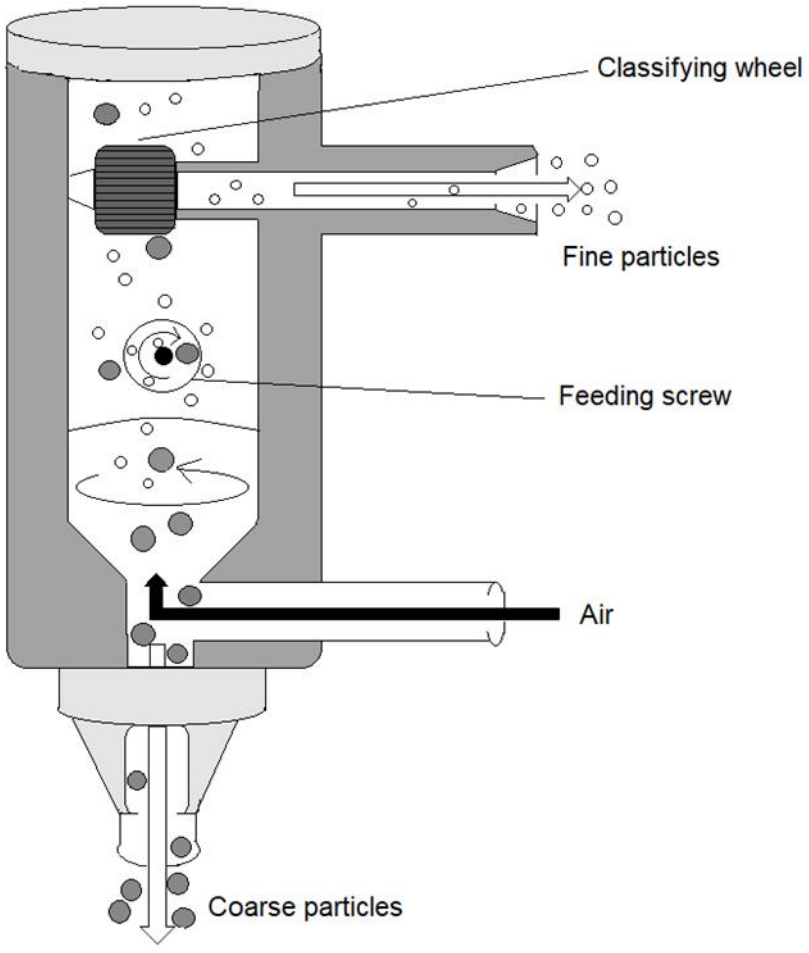

Figure 1. Schematic figure of an air classifier. The oat flour is fed into the feeding screw. The particles are separated by centrifugal force and gravity by the air streams. Coarse and fine particles are then separated and led to separate containers.

2.4 Particle size distribution and analysis

Particle size play a significant role throughout particle formation and processing as they unswervingly affect the quality in the final food product. Hence, proper monitoring and control of these processes and intermediate products are required. Laser diffraction is an established and one of the most efficient light scattering method for particle size analysis. Compared to other particle size techniques, laser diffraction

has a great advantage due to its high speed, good reliability and high reproducibility (Ma *et al.*, 2001).

Measuring particle size distribution of cereal flour is complex due to the fact that it is a heterogenous mixture of particles with different densities, shapes and sizes. Cereal flour comprises endosperm cells, broken cells and cell debris containing detached starch granules and fragments of proteins in between (Stevens, 1963). To better understand particle size, sieving or air classification is applied for a more thorough understanding.

2.5 Oats and it's health benefits

As mentioned previously, oat has a unique nutritional composition and several studies have demonstrated a cholesterol-lowering effect. Additionally, further research has showed other favourable health benefits and may extend the knowledge we already possess. Research on health benefits regarding oats have already led to some health claims on oat products. This has increased the interest to develop a better understanding of oats in general. From breeding the finest oat cultivar, the processing and quality, as well as nutritional research on oats and its health benefits. However, there are challenges and the task to improve the quality of oats does not only concern the yield, but a combination of three factors – yield, the percentage of groats and β-glucan content (Kris-Etherton *et al.*, 2013) .

Oat β-glucan has been proved to be the primary component for serum cholesterol-lowering effect (Braaten *et al.*, 1994). Although the mechanism is not entirely understood, there are two main mechanisms that have been proposed to explain the cholesterol-lowering effect with oat soluble fiber. One mechanism that could explain the cholesterol-lowering effect is the interference with absorption of bile acids and fats, which increases bile acid synthesis from serum cholesterol. The other major mechanism could be inhibition of endogenous cholesterol synthesis (Liangli *et al.*, 2012; Marlett *et al.*, 1994).

Anderson was one of the first who through several studies, demonstrated the potential cholesterol-lowering effect oat had (Anderson *et al.*, 1991; Anderson *et al.*, 1990; Anderson *et al.*, 1984). In the studies he showed that oat bran with β-glucan content was able to reduce the total serum cholesterol in subjects suffering from hypercholesteremia as much as 23 %. Additionally, there were no changes in the high-density lipoprotein (HDL). Since then several studies have investigated and published similar results.

Oat β-glucan have also showed gastrointestinal effects. Mälkki and Virtanen (2001) reviewed that in stomach and small intestine, the soluble oat β-glucan mainly acts via increasing the viscosity of the gastric and intestinal contents. This is mediated through the neo-hormonal systems which involve both endocrinal and gastrointestinal hormones. Vital factors of β-glucan viscosity are mainly molecular weight and degree of solubilisation, hence the behaviour throughout the gastrointestinal transit. The physicochemical properties of oat β-glucan are underlying basis for the physiological functions. They affect the gastrointestinal transit by swelling and their water binding capacities, viscosity in solutions and their ability to ferment. There are studies that have shown positive effects of β-glucan, namely that they lower or slow down the digestion and absorption of nutrients, thereby promoting satiety (Beck *et al.*, 2009a). However, there are also studies that showed no significant effect (Hlebowicz *et al.*, 2008; Kim, 2006).

3 Material and methods

3.1 Material

The oat fraction was provided by Lantmännen with a β-glucan content of 19.9 %. The fraction originates from defatted oat flakes that have been milled once and air classified with 1400 RPM and thereafter air classified a second time with the same speed. This subsequently gave a coarse fraction that is used as starting material in this project.

3.2 Methods

In this project, all analyses were made in duplicates. The reported results are therefore an average value based on dry matter content determined by drying at 105 °C for 16 h.

This project was divided into two parts. Milling, sieving and β-glucan analysis of the starting material were performed at the laboratory facilities at the Department of Molecular Sciences, SLU. The second part was carried out at VTT Technical Research Centre of Finland and analysis of those materials were made at the laboratory facilities in SLU. The same starting material was used in both trials.

3.3 Milling and sieving

Approximately 60 g of the starting material was milled in several rounds using a small scale Retsch ZM (Hann, Germany). The material was milled at 18000 RPM.

Sieving was performed both on the starting material and the milled starting material. This was done using a Retsch Analytical Sieve Shaker type AS200 (Haan, Germany). The sizes used for the sieving were 425 µm, 250 µm, 150 µm and 75 µm. A bottom collector was also used if the material passed through 75 µm. The sieve shaker was set on 3 min and thereafter the sieves were weighed to note the amount of material that was collected in each fraction. Sieving was performed multiple times and each fraction was pooled together with its respective size.

3.4 Milling and air classification

The trial for milling and air classification was performed at VTT Technical Research Centre of Finland.

The pre-fractionated starting material was milled by using a 100UPZ (Hosokawa Alpine AG) pin disc mill using three different grinding speeds; 17800, 12000 or 8000 rpm. The milling was performed twice of the same material and for each setting. This resulted in three different fractions that were used for air classification. The air classifications were performed using a Minisplit air classifier (British Rema Ltd) with an airflow of 220 m^3/h. The speed of the classifier wheel was between 1500-10000 rpm. For material milled at 17800 rpm and air classified at 4500 rpm, the air classification was performed in replicates and therefore statistical analysis was performed for only this setting (Figure 2).

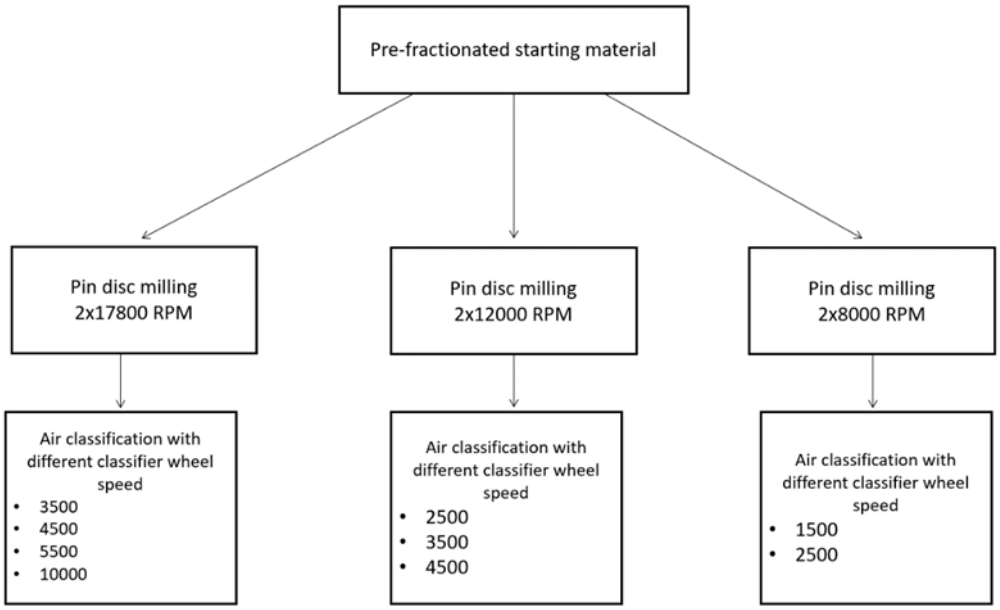

Figure 2. Schematic presentation of the milling and air classification procedure. Air flow of the classifier wheel was 50 m^3/h in all of the classifications.

3.5 β-Glucan analysis

The β-glucan analysis was performed enzymatically by using Megazyme Mixed-Linkage Beta-Glucan Assay Procedure (McCleary & Codd, 1991) K-BGLU 05/15, according to the manufacturer's instructions, with some modifications. The amount of the sample was decreased from 100 mg to 30 mg since the expected β-glucan content would be high. For the analysis, a spectrophotometer was used to measure absorbance. The duplicates were analysed at two different occasions. Dry matter for all samples were determined by drying at 105 °C for 16 h. The samples were taken out and put in a desiccator for 2 h and thereafter weighed.

3.6 Molecular weight determination of β-glucan

The molecular weight distribution of β-glucan and β-glucan content were analysed using high performance size exclusion chromatography (HPSEC) with fluorescence detection principally according to Rimsten et al. (2003) with some adjustments. For the analysis 0.1 M $NaNO_3$ with 0.02 % NaN_3, 50 % ethanol, calcium chloride

dihydrate and 25 mg/l calcofluor in 0.1 M Tris buffer were required. The latter solution was put in a dark bottle and inserted into the HPSEC together with $NaNO_3$ (0.1 M) with 0.02 % NaN_3.

Extraction of the oat flour and standard with α-amylase was done in replicates. Each sample was weighed approximately to 15 mg (± 5 mg) and mixed with 7.5 ml 50 % ethanol. The standard sample was weighed to 100 mg (± 5 mg). The samples were placed in a boiling water bath for 15 minutes and thereafter cooled down. Another 5 ml of aqueous ethanol (50%) were added and the samples were mixed and centrifuged (1000×g, 20 min). The supernatant was decanted and another 10 ml 50 % ethanol was added. The samples were then again mixed and centrifuged (1000 ×g, 20 min). The supernatant was discarded and the test-tubes were put upside down on a paper towel for 5 minutes. The samples were then stored in the fridge overnight. To the samples 20 ml distilled water with 0.30 mg/ml $CaCl_2$ were added, as well as α-amylase (50 µl). The samples were mixed and placed directly in a boiling water bath for 6 h. The samples were mixed every 30 min during this time. The tubes were cooled down in a water bath and thereafter mixed and centrifuged (1000×g, 15 min). The supernatants were filtrated (0,45 µm) into vials and injected into the HPSEC that ran overnight. Calculation of molecular weight was performed in Matlab and calculation of the area of the peak was made in Chromelion. The content of extractable β-glucan content was then calculated by using the area of the peak.

3.7 Particle size distribution

The particle size analysis was measured at Sympatec Gmbh Nordic office in Vimmerby. The system that was used for this measurement was a modular combination of HELOS laser diffraction and RODOS dry dispersion system. The vibratory feeder, VIBRI, was used as a feeder for the sample to constantly flow. All the samples were measured with similar settings, with a pressure of 1.5 bar. Different lenses to measure the particle size were used depending on the sample and their approximate size range. For the coarse fraction R3 and R5 lenses were used, whereas for the fine fractions R2 and R4 lenses were applied. Each sample was measured twice.

3.8 Scanning electron microscopy

Scanning electron microscopy (SEM) was performed for the raw material, the coarse and fine fraction from the material that was milled at 17800 rpm and air classified at 4500 rpm (Figure 2). The three samples were mounted on a circular stub with a conductive carbon tab which was divided into three parts, one for each sample. The SEM was performed using a Hitachi TM-1000 Tabletop Scanning Electron Microscope (Tokyo, Japan) with magnification ranging between ×1000-10000.

3.9 Statistical analysis

To demonstrate the statistical significance, an analysis of variance (ANOVA) and Tukeys pairwise comparison test was performed too observe the relation between the replicates of the sieving and air classification procedure. The statistical analysis was performed using MiniTab (Version 18 Statistical Software) and the level of significance was set at 99 %.

4 Results

4.1 Milling and sieving

Sieving of the non-milled and milled starting material was performed several times and then pooled together using Retsch Analytical Sieve Shaker type AS200 (Haan, Germany). The results of the obtained material and mass yield is presented in Table 1 and 2. These results present the average mean values from all sievings that were carried out.

The highest amount from the non-milled starting material could be found in 250 µm, while the lowest quantity was obtained in 75 µm (Table 1). It can also be observed that the majority of the material had a particle size between 250-150 µm.

Sieving of the milled starting material, showed that there was not a great difference between 250 µm and 150 µm. It can also be noted that a very small amount of material was obtained in 425 µm fraction, whereas significantly higher amount of material is observed in 75 µm compared to the non-milled material of the same sieve size. This indicates that a majority of this material particle size was 250 µm and below for both non-milled and milled fractions.

Table 1. *Result of the average values from sieving of the non-milled and milled (M) starting material. Milling was performed with 18000 rpm*

Sample	Sieve size (µm)	Material (g)	Mass yield (%)
Non-milled	425	3.1	11.3[c]
	250	16.1	57.9[a]
	150	7.6	27.4[b]
	75	0.9	3.1[d]
	Bottom collector	0	0
Milled	M425	0.3	1.2[c]
	M250	12	43.8[a]
	M150	11.9	43.1[a]
	M75	3.5	11.8[b]
	Bottom collector	0	0

Means that do not share a letter are significantly different from each other.

4.2 Milling and air classification

The starting material was pin disc milled with three different settings and also milled twice with each setting (2x17800, 2x12000 and 2x8000 rpm), which thereafter was subjected to air classifications with different speed settings (Figure 1). The air classification resulted in one fine and one coarse fractions where the mass yields were calculated (Table 2). For 2x8000/1500 rpm all material went to fine fraction and therefore no further analysis was made. Mass yields of both fine and coarse fractions after air classification were compared visually, assuming that enrichment of β-glucan resulted in darker colour of the coarse fraction flour.

Different milling settings applied lead to visually prominent differences in the particle sizes of the raw material. As can be seen, the fractionation efficiency was observed to be dependent on the milling conditions (Table 2). When applying the most effectively milling condition, 2x17800 rpm, the yield for the coarse fractions ranged between 54.7 and 64.8 %.

The lowest yields of the coarse fraction were obtained when the air classifier wheel was on the speeds of 3500 rpm (55.5 %) and 10000 rpm (54.7 %) for 17800 rpm milled material. The classification setting between those, 4500 and 5500 rpm, gave higher coarse fraction yields of 63.5 and 67.7 %. It can be observed that higher coarse fraction yields up to 78 % were achieved when milling settings were 2x8000 and 2x12000 rpm.

The 2x12000 milled raw material allowed production of coarse fractions with mass yields of 60.4 and 78.4 %. Milling 2x800 resulted in production of coarse fraction with a mass yield of 78.8 %.

Table 2. *Mass yields of fine and coarse fractions produced from air classification with differently milled starting material. Air flow of the classifier wheel was 50 m^3/h in all classifications. Replicates were only performed for fraction milled at 17800 rpm and air classified at 4500 rpm.*

Pin disc milling parameter (RPM)	2 x 17800				2 x 12000			2 x 8000	
Air classification setting (RPM)	3500	4500	5500	10000	2500	3500	4500	1500	2500
Coarse fraction mass yield (%)	55.5	63.5[a]	64.8	54.7	60.4	75.8	78.4	0	78.4
Fine fraction mass yield (%)	44.3	35.3[b]	36.3	43.5	42.9	25.9	22.3	100 (All to fine fraction)	23.5
Difference (%)	-0.2	-1.2	+1.1	-1.9	+3.4	+1.7	+0.6	0	+1.9

Means that do not share a letter are significantly different from each other.

4.3 β -Glucan analysis

4.3.1 Milled and sieved fractions

In the milled and sieved fractions, it was shown that there was an evident trend with lower total amount of β-glucan in larger particles (Table 3). An exception is for the sieve size 150 µm, where the total amount of β-glucan is slightly higher than 75 µm. Upon milling it was found that the β-glucan contents are higher in all particle sizes except for 75 µm, where the total amount of β-glucan is evidently lower compared to the non-milled fraction of the same sieve size. The accuracy of the present β-glucan content was based on β-glucan content determined for a barley standard (Golf VK-95 milled 090831). The same standard was used in all β-glucan analyses.

Table 3. *Average mean value of total amount of β-glucan content and β-glucan yield from the non-milled and milled (M) sieved fractions. β-glucan solubility are presented in parenthesis*

Sample	Sieve size (µm)	β-glucan content dry weight (%)	β-glucan yield (% of starting material)
Non-	425	16.8 (25.2)	9.7
milled	250	19.2 (12.3)	57.2
	150	20.6 (18.3)	29.2
	75	19.9 (14.6)	3.3
Milled	M425	16.9 (34.2)	0.9
	M250	19.7 (26.0)	42.4
	M150	21.9 (24.2)	46.8
	M75	15.8 (33.8)	9.9

4.3.2 Milled and air classified fractions

All milled fractions had lower β-glucan content before air classification, where fraction milled at 17800 rpm had the lowest content (Table 4). The greatest enrichment of β-glucan was in the fraction that was milled at 2x17800 rpm and air classified at 3500 rpm, which contained 26.9 % of β-glucan. It can also be observed that all coarse fraction milled at 2x17800 rpm ranged between 25.7-26.9 % in β-glucan content with different air classification parameters applied (Table 5). The lowest β-glucan content was observed in fraction 12000/2500. The least effective milling parameter, 8000 rpm, showed to have 21 % β-glucan content.

Table 4. *Average mean value of total amount of β-glucan content and yield after pin disc milling with different revolutions per minute (rpm)*

Fraction (rpm milling)	β-Glucan content (% of dry weight)
178000	15.9
12000	18.9
8000	18.9

Table 5. *Average mean value of total amount of β-glucan content and yield after pin disc milling and air classification with different parameters. β-Glucan solubility are presented in parenthesis*

Fraction (rpm milling/rpm air classification	β-Glucan content (% of dry weight)		β-Glucan yield (% of starting material)	
	Coarse	Fine	Coarse	Fine
17800/10000	26.3 (72.1)	9.6	72.5	27.4
17800/5500	26.9 (75.0)	5.9	81.4	18.5
17800/4500	26.3 (80.1)	8.7	75.6	24.7
17800/3500	25.7 (48.7)	9.9	72.0	27.9
12000/4500	23.4 (64.1)	5.7	79.5	20.5
12000/3500	20.9 (58.3)	7.9	72.9	27.1
12000/2500	20.1 (71.1)	15.4	57.6	42.4
8000/2500	21.0 (20.1)	13.1	61.5	38.5

4.4 Molecular weight determination of β-glucan

Molecular weight determination was performed for both sieved and air classified fractions. Figure 3 illustrates the molecular weight distributions obtained from the analysis. A represents molecular weights of all air classified fractions whereas B shows molecular weight for all the sieved fractions. Both distributions demonstrate similar molecular weight, approximately over 10^6 g/mol. The sieved fractions (B) shows a slightly larger variation in distributions compared to the air classified fractions.

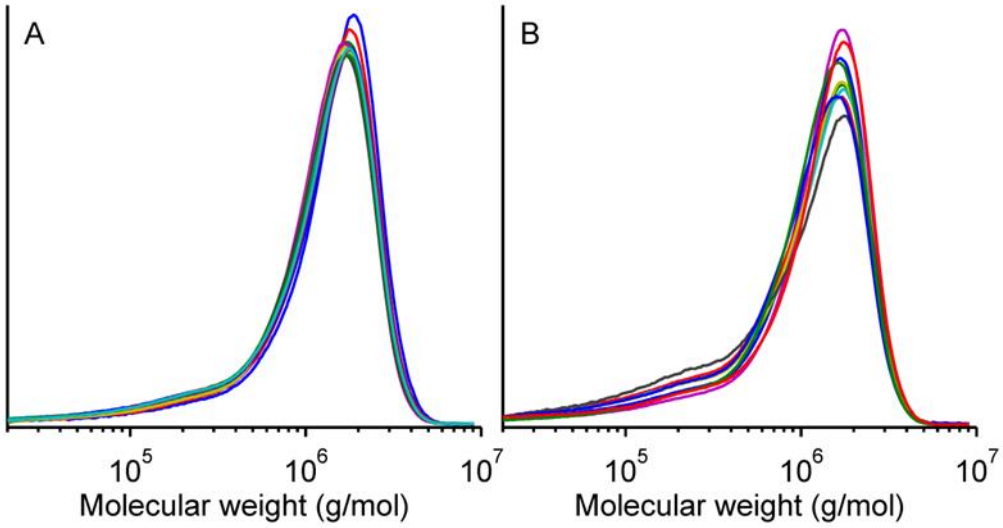

Figure 3. Molecular weight distribution of β-glucan for air classified and sieved fractions. A = All air classified fractions and B = All sieved fractions.

4.5 Particle size distribution

Particle size distribution was obtained for both sieved and air classified fractions, which is illustrated in Figure 4 and 5, respectively. For all air classified fractions, a similar structure can be observed in all milling parameters. All milled and fine fractions have a bimodal distribution, whereas the coarse fraction has a unimodal distribution. This applies for all air classified fractions, however, the distribution density varies depending on air classification parameter. This is mostly evident for the fine fractions, where the fraction milled at 2x8000 had a higher peak whereas fraction milled at 2x17800 had the lowest peak. It can also be observed that B has a small peak for material from sieve size 75 µm whereas A does not (Figure 4).

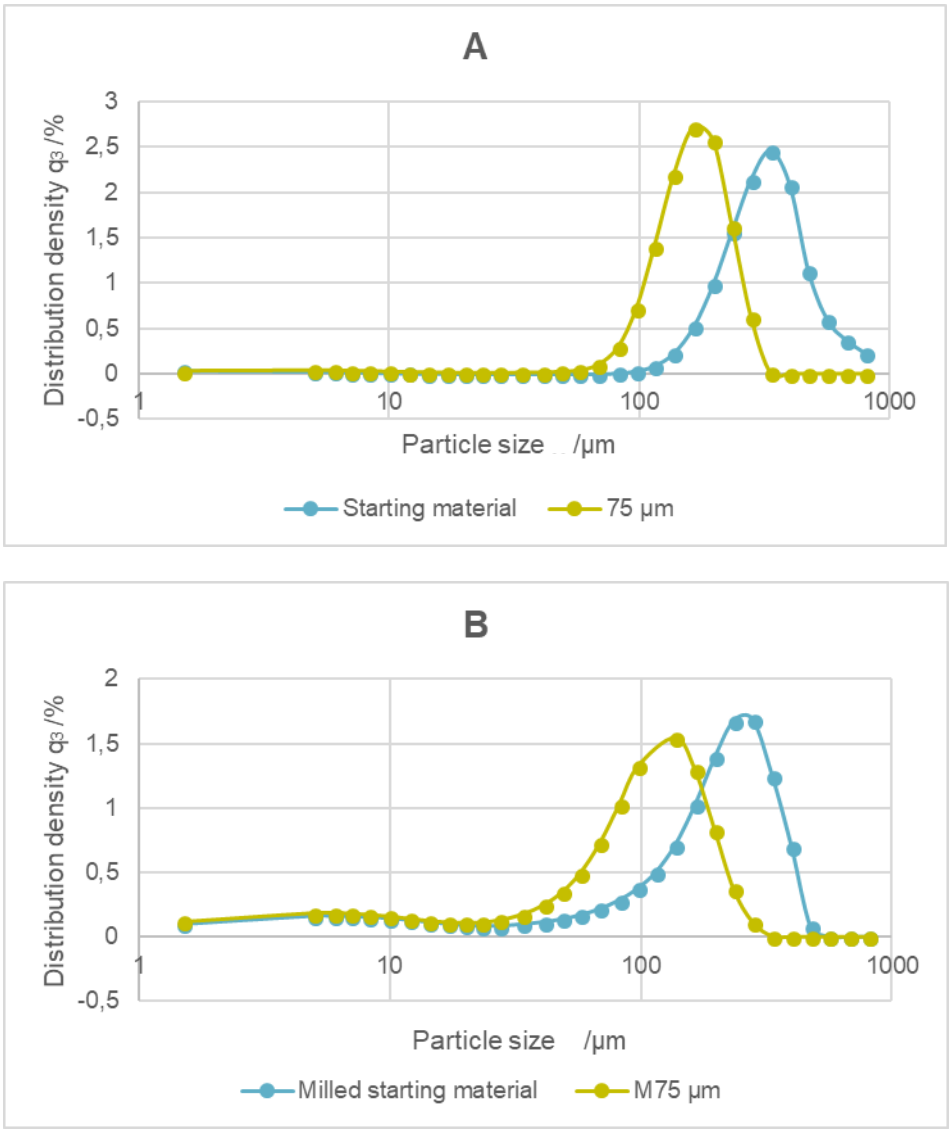

Figure 4. Particle size distribution (obtained by laser diffraction) for starting material and material obtained from sieve size 75 µ. (A) non-milled fractions ;(B) milled fractions.

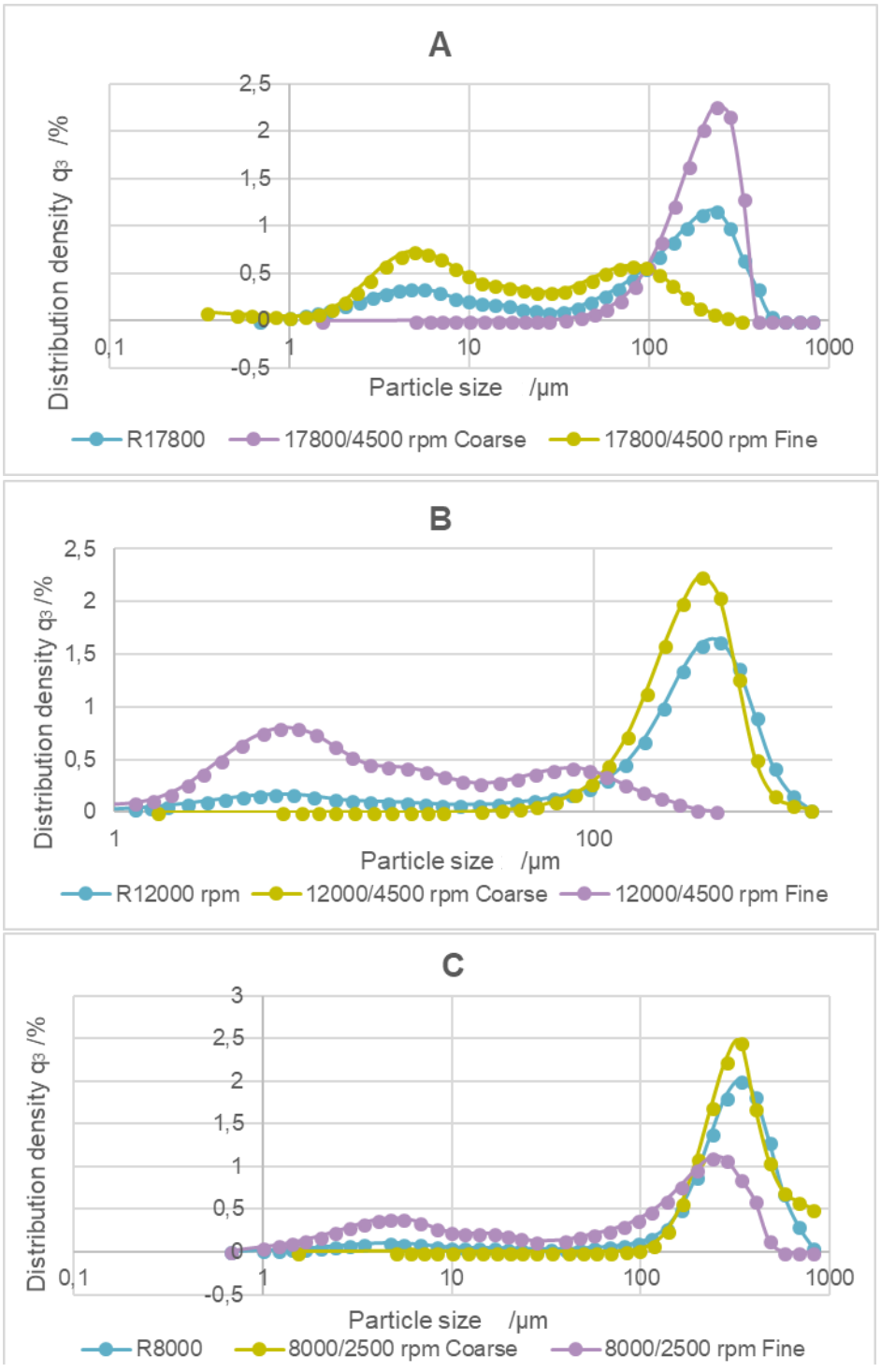

Figure 5. Particle size distribution (obtained by laser diffraction) for milled starting material and air classified fractions with different milling and air classification parameters. (A) Milled at 17800 rpm and air classified at 4500 rpm; (B) Milled at 12000 rpm and air classified at 4500 rpm; (C) Milled at 8000 rpm and air classified at 2500 rpm.

4.6 Scanning electron microscopy

Scanning electron micrographs were taken of 2x178000 rpm milled pre-fractionated starting material and the air classified fractions for 4500 rpm. A separation of smaller and larger particles can be observed in both milled starting material and fine fraction (Figure 6 and 7). It can be observed that those fractions have more of the smaller particles with less than 5 µm in size. Some larger particles can be observed as well. In the coarse fraction, however, the majority of the particles are around 30 - 40µm (Figure 8) and very few smaller particles can be noticed. All the micrographs presented below have a magnification of ×1500 and a scale bar of 50 µm.

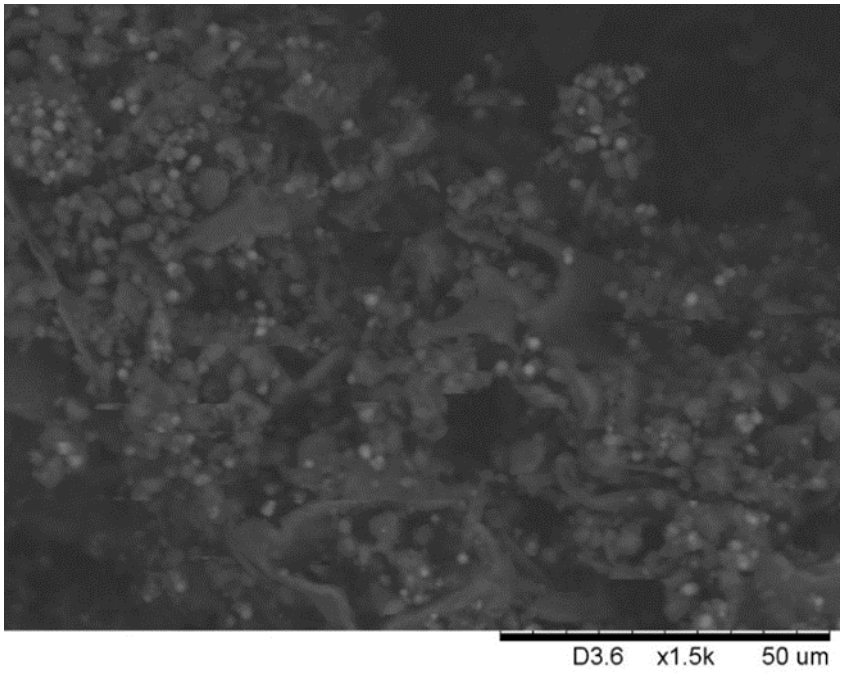

Figure 6. Scanning electronic microscopic picture of milled (17800 rpm) starting material.

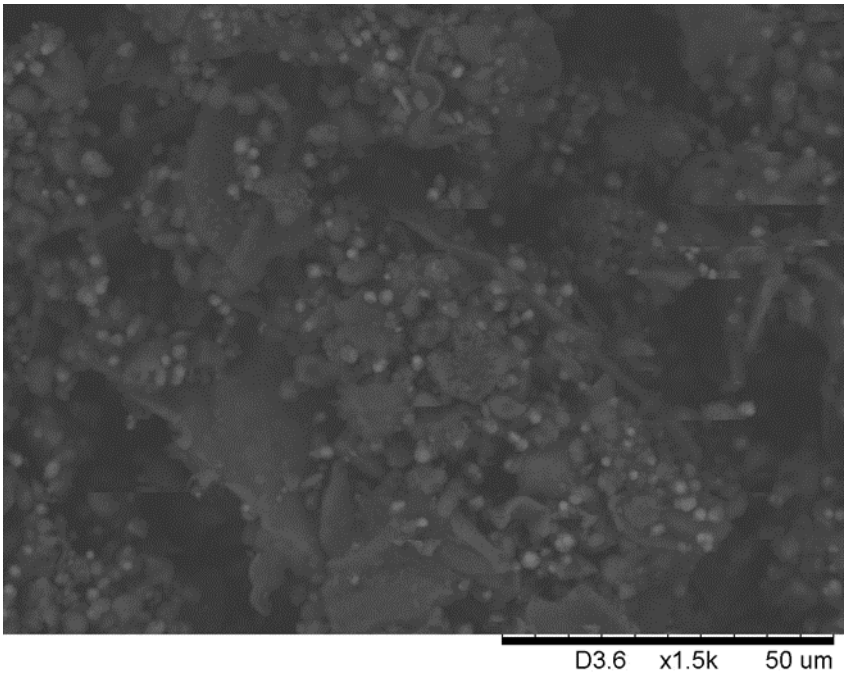

Figure 7. Scanning electronic microscopic picture of fine fraction (milled at 178000 and air classified 4500 rpm).

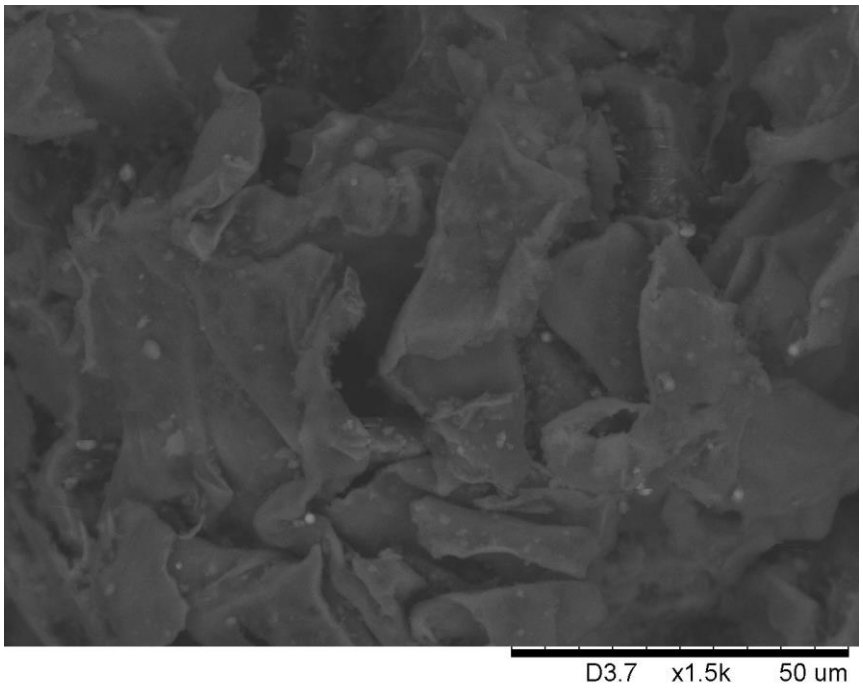

Figure 8. Scanning electronic microscopic picture of coarse fraction (milled at 178000 rpm and air classified at 4500 rpm).

5 Discussion

5.1 Dry fractionation

Milling is an important step of dry fractionation since it determines how well the different grain components separates from each other. Due to the fact that oat has a high lipid content, which particularly holds bran components together, it can make the milling step aggravating (Miller & Fulcher, 2011). Pin disc milling is one of the most efficient methods for oats and the milling effect of the pin disc appears to be superior compared to other grinding methods. A great advantage with pin-disc milling is that material does not tend to easily stick to the pins, so there is minimal loss of material when milling (Sibakov *et al.*, 2014). Advantage of using air classification as fractionation method is that the procedure can simply be scaled up for commercial purposes, without having fine particles clogging and therefore loose yield (Wu & Doehlert, 2002). For this investigation, however, the starting material was defatted which made the processing procedure easier.

The results for the air classification trial is presented in table 2. The different milling condition showed visible difference in the particle sizes of the raw material, which was to be expected. Also, the fractionation efficiency was observed to be dependent on the milling condition. As the result showed, when the starting material was milled at 2x17800 rpm, the coarse fraction mass yield varied between 56.7 and 64.8 %. The changes in mass yields did not, however, follow the changes as clearly in the fractionation parameters. As an example, the lowest mass yields (55.5 and 54.7 %) were obtained with air classification wheel speeds of 3500 and 10000 respectively. The two classification wheel speeds (4500 and 5500) between these resulted in a higher mass yield of 63.5 and 64.7 %. This phenomenon cannot be unambiguously described, however, it proposes that air classification of 2x17800 milled material permits only separation of coarse fractions with mass yields of 54.7-64.8 %. Furthermore, it has also been demonstrated in this project that the

differences in mass yields does not significantly affect the β-glucan content. The highest coarse fraction mass yields with 78 % were only obtained when milling of the material was gentler (2x12000 or 2x8000). This designates that the raw material was not as efficiently milled as it was with the higher milling speed.

In air classification it is generally evident that the finer the material is, the less sample will be obtained in the coarse fraction when applying same fractionation parameters. This was prominent in this trial where air classification with 3500 rpm resulted in a mass yield of 55.5 % for the coarse fraction with the most finely milled raw material 2x17800 rpm, whereas the yield (75.8 %) of the coarse fraction was higher for the raw material milled with 2x12000 rpm. Milling with 2x8000 parameter produced only one coarse fraction with mass yield at 78.4 % and was produced with lower air classifier wheel speed (2500 rpm) compared to material milled with 2x12000, which gave 75.8 % (3500 rpm). This indicates that the particle size of the milled material with 2x12000 rpm was reduced more than that of 2x8000 rpm milled material and hence it was easier to separate finer particles from the raw material. The reason for why only one coarse fraction was produced for 2x8000 rpm milling parameter was because lower speed of the classifying wheel speed resulted in only one fine fraction. Possibly a coarse fraction could have been obtained if the air classifier speed ranged between 1500 and 2500 rpm. However, this would have resulted in a lower mass yield and is therefore not relevant in this investigation.

When combining mass yields of the fine and coarse fractions there were for some air classifications either less or more than 100 % (Table 2). This is partially due to the attachment of the starting material to the classifier chamber during air classification, or the detachment of the material that was attached for previous air classifications trials of the material.

The fraction that was used in milling and air classification trial at VTT was obtained from a previous dry fractionation process. This could mean there is a possibility for loss of β-glucan content in that process, which therefore could have limited the fractionation efficiency of the raw material used in this project.

The statistical analysis showed that the yields were significantly different from each other, both for sieved and air classified fractions. However, milled material obtained from the sieve sizes 250 and 150 µm were not significantly different from each other, which mostly is due to the efficient milling that was applied.

5.2 β-Glucan content

5.2.1 Sieve analysis

For the sieve analysis the β-glucan content ranged between 16.8-20.6 % whereas for the milled material sieved in same condition had a range between 15.8-21.9 % (Table 3). The difference between milled and non-milled seems to not have a great impact on β-glucan content. There are, however, some small differences that can be observed. For example, it shows that the larger particle size has less β-glucan content. This is most evident in the non-milled fractions. For both of the sieve analyses the particles obtained with 150 µm sieve, resulted in the highest β-glucan content. The lowest β-glucan content was obtained with 75 µm sieve, which also was the smallest sieve that was used in this project. Though applying milling, it seems not to have affected the β-glucan content immensely.

5.2.2 Milling and air classification

In this project it was shown that oat milled at 2x217800 rpm had a β-glucan content of 26.9 %, which is a significant increasement from the pre-fractionated starting material (Table 5). The β-glucan content range within fractions milled at 2x17800 rpm were between 25.7-26.9 %, where the highest enrichment could be found in the 5500 rpm air classified fraction. The highest β-glucan yield, 81.4 %, was obtained in the fraction that was milled at 2x17800 rpm and air classified with 4500 rpm. However, the differences between the air classification parameters (3500-10000 rpm) did not differentiate expressively if they had the same milling parameter. This indicates that the milling step, and therefore the particle size of the material, is more of an important factor than air classification. This is evidently demonstrated for the milling parameter 2x12000 and 2x8000 rpm. Both of these are gentler milling and will therefore give rise to larger particle sizes. This is also why the mass yield in the coarse fractions for these milling parameters are higher compared to 2x17800 rpm. What also can be noticed is that the higher mass yield obtained in the trials gave lower β-glucan contents (Table 2 and 5).

Air classification of barley (Wu *et al.*, 1994) and oat groats (Wu & Stringfellow, 1995) for enrichment of β-glucan have previously been reported. Wu and Doehlert (2002) have also demonstrated enrichment of β-glucan of oats bran which showed that enriched β-glucan was obtained in good yield. However, none of these studies

can be compared to the current investigation since different method procedures were applied.

5.3 Particle size and scanning electron microscopy

As stated above, for enrichment of a dietary fiber it is important to separate particles rich in protein and starch from particles rich in cell walls. The bran layers consist of fibrous cell wall polymers such as β-glucan, while the endosperm mainly consists of starch, protein and lipids (Miller & Fulcher, 2011; Lim *et al.*, 1992). The lipids in oats and proteins are typically bound to each other, which makes it difficult to separate them. Nevertheless, the oat used in this project was defatted and therefore the separation should be carried out easier. To better understand the separation efficiency between the fine and coarse fractions, both particle size distribution and scanning electron microscopy were performed.

For the non-milled and milled sieved fraction the particle size distribution appeared to be very similar (Figure 4). A difference that, however, can be noticed is when comparing non-milled and respectively milled material obtained from sieve size 75 µm (A and B) (Figure 4). B appears to first have a low peak as well as a second sharp peak whereas A µm only have one sharp peak. This phenomenon is most likely because of the milling procedure. The milling applied for the sieved material and air classification were not under the same condition. It seems therefore that the small scale Retsch ZM (Hann, Germany) is a more efficient mill due to the fact that it can mill smaller particles.

The particle size distribution of fine fractions and milled starting materials were bimodal, distributed in two peaks (Figure 5). The explanation for this is that in the first peak, starch or protein that are smaller in particle size could be found. In the other peak, with larger particle sizes, β-glucan can be obtained. The bimodal peaks can be observed in milled starting material, before air classification is performed. The reason for this is because we have both cell wall and cell content, which is illustrated in the peaks. This can also be observed in scanning electron microscopy images (Figure 6). Observing the fine fraction there are more of the white particles, presumably starch granules, and what seems to appear as protein structures (Figure 7). There are a few larger particles that can be observed, which possibly is cell wall structure that have not succeeded in being separated into the coarse fraction. The coarse fraction has some cell wall structure, which is the larger particles that can be seen on the image (Figure 8). There are a few white particles which most likely is starch granules. This indicates that separation was successful. The microscopy

images of the fine and coarse fractions also showed that the structures are partly broken due to the efficient milling.

5.4 Molecular weight of β-glucan

The molecular weight distribution shows relatively sharp peaks for both sieved and air classified fractions. Figure 3 illustrates both fractionation methods and it can be observed that all A fractions have very similar molecular weight. The same applies to all B fractions. Comparing the two there are no significant differences, which shows that both A and B fractions give rise to same molecular weight of β-glucan. The A fractions are slightly more similar to each other than B fraction, which most likely is due to the implementation of the analysis. The molecular weight determination was performed first on the sieved fractions (B) and because of inexperience of the analysis it might have affected the results. However, overall results showed that milling and air classification did not change the molecular weight of β-glucan, which indicates that the method does not break down the β-glucan during processing. This is an important factor when processing oat food products. It is therefore important that the processes used for manufacturing high β-glucan content products and for incorporation in other foodstuff, the process should be designed to prevent breakdown of the molecule.

6 Conclusion

This small-scale investigation demonstrated that through optimization of milling and air-classification parameters, β-glucan content up to 26.9 % with a coarse fraction yield of 81.4 % could be obtained. Although the optimization of β-glucan showed promising results there could be possibilities to improve the fractionation process and therefore to simplify the usability of β-glucan in food products. It can however be concluded that in the near future there will be health-promoting products in the market where β-glucan content is increased. It will then be possible to achieve good levels of intake without having to consume large amount of the product. What should be taken in consideration is that the β-glucan content of oats varies by cultivar and is affected by the environment, as well as isolation, purification, and detection methods.

It has also been demonstrated in this project that air classification is a good method to use for enrichment of β-glucan from oats and prevents breakdown of the β-glucan molecule. This is an important factor when manufacturing products with high levels of β-glucan. The ability to enrich β-glucan level can increase the economic value of oats and possibility to tailoring fraction for specific markets in the food industry and lead to new product development. Findings in this project can also give a better understanding of how this particular method would apply in an industrial scale. There could be possibilities to optimize further when carrying this to a larger scale up but it will however require some additional studies.

Acknowledgements

I would firstly like to thank my assistant supervisor, Christian Malmberg from Lantmännen R&D. Thank you for giving me the opportunity to carry out this project and helping me with everything I needed for this project to work. I have very much enjoyed working on this project.

I would also like to thank my supervisor Roger Andersson, professor at the Department of Molecular Sciences, SLU. I am grateful for all your advice and help during this project.

References

Anderson, J.W., Gilinsky, N.H., Deakins, D.A., Smith, S.F., O'Neal, D.S., Dillon, D.W. & Oeltgen, P.R. (1991). Lipid responses of hypercholesterolemia men to oat-bran and wheat-bran intake. *The American Journal of Clinical Nutrition,* 54(4), pp. 678-683.

Anderson, J.W., Spencer, D.B., Hamilton, C.C., Smith, S.F., Tietyen, J., Bryant, C.A. & Oeltgen, P. (1990). Oat-bran cereal lowers serum total and LDL cholesterol in hypercholesterolemic men. *The American Journal of Clinical Nutrition,* 52(3), pp. 495-499.

Anderson, J.W., Story, L., Sieling, B., Chen, W.J.L., Petro, M.S. & Story, J. (1984). Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. *The American Journal of Clinical Nutrition,* 40(6), pp. 1146-1155.

Andersson, A.A.M. & Börjesdotter, D. (2011). Effects of environment and variety on content and molecular weight of β-glucan in oats. *Journal of Cereal Science,* 54(1), pp. 122-128.

Andersson, M., Ellegård, L. & Andersson, H. (2002). Oat bran stimulates bile acid synthesis within 8 h as measured by 7α-hydroxy-4-cholesten-3-one. *The American Journal of Clinical Nutrition,* 76(5), pp. 1111-1116.

Anon (1989). AACC Committee adopts oat bran definition. *Cereal Foods World*(34), pp. 1033–4.

Beck, E.J., Tapsell, L.C., Batterham, M.J., Tosh, S.M. & Huang, X.-F. (2009a). Increases in peptide Y-Y levels following oat β-glucan ingestion are dose-dependent in overweight adults. *Nutrition Research,* 29(10), pp. 705-709.

Beck, E.J., Tosh, S.M., Batterham, M.J., Tapsell, L.C. & Huang, X.F. (2009b). Oat β-glucan increases postprandial cholecystokinin levels, decreases insulin response and extends subjective satiety in overweight subjects. *Molecular Nutrition & Food Research,* 53(10), pp. 1343-1351.

Beer, M.U., Arrigoni, E. & Amadò, R. (1995). Effects of oat gum on blood cholesterol levels in healthy young men. *European journal of clinical nutrition,* 49(7), pp. 517-522.

Biermann, U. & Grosch, W. (1979). Bitter-tasting monoglycerides from stored oat flour. *Zeitschrift für Lebensmittel-Untersuchung und Forschung,* 169(1), pp. 22-26.

Braaten, J., Wood, P., Scott, F., Wolynetz, M., Lowe, M., Bradley-White, P. & Collins, M. (1994). Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *European journal of clinical nutrition,* 48(7), pp. 465-474.

Brown, C.M., Alexander, D.E. & Carmer, S.G. (1966). Variation in Oil Content and Its Relation to Other Characters in Oats (Avena sativa L.) 1. *Crop science,* 6(2), pp. 190-191.

Brown, C.M. & Craddock, J.C. (1972). Oil Content and Groat Weight of Entries in the World Oat Collection 1. *Crop science,* 12(4), pp. 514-515.

Butt, M.S., Tahir-Nadeem, M., Khan, M.K.I., Shabir, R. & Butt, M.S. (2008). Oat: unique among the cereals. *European Journal of Nutrition,* 47(2), pp. 68-79.

Carr, J., Glatter, S., Jeraci, J. & Lewis, B. (1990). Enzymatic determination of beta-glucan in cereal-based food products. *Cereal Chemistry,* 67(3), pp. 226-229.

Cho, K.C. & White, P.J. (1993). Enzymatic analysis of b-glucan content in different oat genotypes. *Cereal Chem,* 70(5), pp. 539-542.

Daou, C. & Zhang, H. (2012). Oat Beta-Glucan: Its Role in Health Promotion and Prevention of Diseases. *Comprehensive Reviews in Food Science and Food Safety,* 11(4), pp. 355-365.

EFSA (2011a). 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 1924/20061. *EFSA Journal*, 9(6), pp. 2207, 1-15.

EFSA (2011b). Scientific Opinion on the substantiation of health claims related to oat and barley grain fibre and increase in faecal bulk (ID 819, 822) pursuant to Article 13 (1) of Regulation (EC) No 1924/2006. *EFSA Journal*, 9(6), pp. 2249, 1-13.

FDA (1997). Food labelling: Health claims; oats and coronary heart disease; final rule. *Federal Register, 62*, 3584–3601

FDA (2003). Food labelling: Health claims; soluble dietary fiber from certain foods and coronary heart disease. *Federal Register, 68*, 44207–44209.

Ganßmann, W. & Vorwerck, K. (1995). Oat milling, processing and storage. In: Welch, R.W. (ed. *The Oat Crop: Production and Utilization*. Dordrecht: Springer Netherlands, pp. 369-408. Available from: https://doi.org/10.1007/978-94-011-0015-1_12.

Girardet, N. & Webster, F.H. (2011). Oat Milling: Specifications, Storage, and Processing. In: *Oats: Chemistry and Technology* AACC International, Inc., pp. 301-319. Available from: https://doi.org/10.1094/9781891127649.014 [2018/04/09].

Gould, M.R., Anderson, J.W., O'Mahony, S., Inglett, G. & Munck, L. (1980). Biofunctional properties of oats. *Cereals for food and beverages. Recent progress in cereal chemistry and technology*, pp. 447-460.

Gracza, R. (1960). The subsieve-size fractions of a hard red spring wheat flour produced by air classification. *Cereal Chem*, 37, p. 579.

Hlebowicz, J., Darwiche, G., Björgell, O. & Almér, L.-O. (2008). Effect of Muesli with 4 g Oat β-Glucan on Postprandial Blood Glucose, Gastric Emptying and Satiety in Healthy Subjects: A Randomized Crossover Trial. *Journal of the American College of Nutrition*, 27(4), pp. 470-475.

Jane, J.L., Kasemsuwan, T., Leas, S., Zobel, H. & Robyt, J.F. (1994). Anthology of starch granule morphology by scanning electron microscopy. *Starch-Stärke,* 46(4), pp. 121-129.

Kale, M., Hamaker, B. & Bordenave, N. (2014). Oat β-Glucans: Physicochemistry and Nutritional Properties. *Oats Nutrition and Technology*, pp. 123-169.

Kazi, T. & Cahill, T. (1969). A rapid method for the detection of residual lipase activity in oat products. *Analyst*, 94(1118), pp. 417-417.

Kim, H. (2006). Short-term satiety and glycemic response after consumption of whole grains with various amounts of beta-glucan. *Cereal Foods World*, 51(1), pp. 29-33.

King, R. & Dietz, H. (1987). Air classification of rapeseed meal. *Cereal Chem*, 64(41), pp. 1-413.

Kris-Etherton, P., Khoo, C.S. & Chu, Y. (2013). Introduction: Oat Nutrition, Health, and the Potential Threat of a Declining Production on Consumption. In: *Oats Nutrition and Technology* John Wiley & Sons Ltd, pp. 1-6. Available from: http://dx.doi.org/10.1002/9781118354100.ch1.

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

Lásztity, R. (1998). Oat grain—a wonderful reservoir of natural nutrients and biologically active substances. *Food Reviews International*, 14(1), pp. 99-119.

Lehtinen, P. (2003). *Reactivity of lipids during cereal processing*: Helsinki University of Technology.

Liangli, L.Y., Tsao, R. & Shahidi, F. (2012). *Cereals and pulses: nutraceutical properties and health benefits*: John Wiley & Sons.

Lim, W., Liang, Y., Seib, P. & Rao, C. (1992). Isolation of oat starch from oat flour. *Cereal Chem*, 69(3), pp. 233-236.

Ma, Z., Merkus, H.G., Veen, H.G.v.d., Wong, M. & Scarlett, B. (2001). On-line Measurement of Particle Size and Shape using Laser Diffraction. *Particle & Particle Systems Characterization*, 18(5-6), pp. 243-247.

Marlett, J.A., Hosig, K.B., Vollendorf, N.W., Shinnick, F.L., Haack, V.S. & Story, J.A. (1994). Mechanism of serum cholesterol reduction by oat bran. *Hepatology*, 20(6), pp. 1450-1457.

McCleary, B. & Codd, R. (1991). Measurement of (1 → 3),(1 → 4)-β-D-glucan in barley and oats: A streamlined enzymic procedure. *Journal of the Science of Food and Agriculture,* 55(2), pp. 303-312.
Miller, S. & Fulcher, R. (2011). Microstructure and chemistry of the oat kernel. In: *Oats (Second Edition)* Elsevier, pp. 77-94.
Miller, S., Fulcher, R., Sen, A. & Arnason, J. (1995). Oat Endosperm Cell Walls: I. Isolation, Composition, and Comparison with Other Tissues. *Cereal Chem,* 72(5), pp. 421-427.
Miller, S., Wood, P., Pietrzak, L. & Fulcher, R. (1993a). Mixed Linkage -Glucan, Protein Content, and Kernel Weight in Avena Species. *Cereal Chemistry,* 70, pp. 231-231.
Miller, S.S., Fulcher, R.G., Vincent, D.J. & Weisz, J. (1993b). Oat β-glucans: An evaluation of eastern Canadian cultivars and unregistered lines. *Canadian Journal of Plant Science,* 73(2), pp. 429-436.
Morris, D.L. (1942). Lichenin and araban in oats (Avena sativa). *Journal of biological chemistry,* 142, pp. 881-891.
Mälkki, Y. & Virtanen, E. (2001). Gastrointestinal Effects of Oat Bran and Oat Gum: A Review. *LWT - Food Science and Technology,* 34(6), pp. 337-347.
Peterson, D. (1991). Genotype and environment effects on oat beta-glucan concentration. *Crop science,* 31(6), pp. 1517-1520.
Rimsten, L., Stenberg, T., Andersson, R., Andersson, A. & Åman, P. (2003). Determination of β-glucan molecular weight using SEC with calcofluor detection in cereal extracts. *Cereal Chemistry,* 80(4), pp. 485-490.
Salisbury, D. & Wichser, W. (1971). Oat milling systems and products. *Bulletin Association Operative Millers*(1971), pp. 3242-3247.
Sibakov, J., Abecassis, J., Barron, C. & Poutanen, K. (2014). Electrostatic separation combined with ultra-fine grinding to produce β-glucan enriched ingredients from oat bran. *Innovative Food Science & Emerging Technologies,* 26, pp. 445-455.
Stevens, D.J. (1963). Measurement of the sub-sieve particle size distribution of flour. *Journal of the Science of Food and Agriculture,* 14(6), pp. 405-411.
Stevenson, D.G., Inglett, G.E., Webster, F. & Wood, P. (2011). Oat dietary fiber: commercial processes and functional attributes. *Oats: Chemistry and Technology,* pp. 321-328.
Strychar, R., Webster, F. & Wood, P. (2011). World oat production, trade, and usage. *Oats: Chemistry and Technology,* pp. 77-94.
Welch, R.W. (1995a). The chemical composition of oats. In: Welch, R.W. (ed. *The Oat Crop: Production and Utilization*. Dordrecht: Springer Netherlands, pp. 279-320. Available from: https://doi.org/10.1007/978-94-011-0015-1_10.
Welch, R.W. (1995b). The chemical composition of oats. In: *The oat crop* Springer, pp. 279-320.
Welch, R.W., Hayward, M.V. & Jones, D.I.H. (1983). The composition of oat husk and its variation due to genetic and other factors. *Journal of the Science of Food and Agriculture,* 34(5), pp. 417-426.
Wood, P. (1991). Potential for β-glucan enrichment in brans derived from oat (Avena sativa L.) cultivars of different (1-> 3),(1-> 4)-β-D-glucan concentrations. *Cereal Chem.,* 68, pp. 48-51.
Wood, P. & Fulcher, R. (1978). Interaction of some dyes with cereal beta-glucans. *Cereal Chemistry,* 6(55), pp. 952-966.
Wood, P.J. (2011). Oat β-Glucan: Properties and Function. In: *Oats: Chemistry and Technology, 2nd edition*. St. Paul, MN: AACC International Press, pp. 219-254.
Wood, P.J., Fulcher, R.G. & Stone, B.A. (1983). Studies on the specificity of interaction of cereal cell wall components with Congo Red and Calcofluor. Specific detection and histochemistry of (1→3),(1→4),-β-D-glucan. *Journal of Cereal Science,* 1(2), pp. 95-110.
Wu, Y., Stringfellow, A. & Inglett, G. (1994). Main content area Protein-and beta-glucan enriched fractions from high-protein, high beta-glucan barleys by sieving and air classification. *Cereal Chemistry,* 71(3), pp. 220-223.
Wu, Y.V. & Doehlert, D. (2002). Enrichment of β-glucan in oat bran by fine grinding and air classification. *LWT-Food Science and Technology,* 35(1), pp. 30-33.
Wu, Y.V. & Stringfellow, A. (1995). Enriched protein and β-glucan fractions from high-protein oats by air classification. *Cereal Chem,* 72(1), pp. 132-134.
Youngs, V. (1972). Protein distribution in the oat kernel. *Cereal Chemistry,* 49, pp. 407-411.

Youngs, V., Puskulcu, M. & Smith, R. (1977). Oat lipids. I. Composition and distribution of lipid components in two oat cultivars. *Cereal Chemistry*.

Appendix 1 - Popular scientific summary

Optimization of β-glucan levels from different oat fraction by milling and air classification

Oat is an important crop worldwide which is mostly due to its various characteristics and nutritional profile. The recent progression in food and nutrition have shown the importance of the different components in oats. It has especially been demonstrated for β-glucan which is a dietary fiber found mostly in the oat bran and in cell walls of the endosperm. The interest of oats has increased primarily because of the beneficial health effects that has been demonstrated and well-studied over the years. β-Glucan in oats has been shown to have various physiological effects of which cholesterol-lowering and postprandial hyperglycemia are the most well-known. The beneficial health effects related to oats have increased consumers awareness and sparked interest in developing a greater understanding of oats, from breeding for the best oat cultivar, processing and nutrition research. The health claims approved both by FDA and EFSA have also encouraged the consumption of oat foods. Plant breeding and investing in research and development of oats gives the possibility to take advantage of the favorable qualities. Findings that have favorable health effects can affect the economical outcome but also give rise to new product development.

This study was an investigation to see the possibilities to further improve qualities of a pre-fractionated oat fraction. The aim of this project was to investigate if there was a potential to obtain a fraction from oat with high levels of β-glucan by using milling and air classification. Air classification is a method that is used to separate particles of a size range with centrifugal force in form of air streams where the main objective is to achieve a separation of a material into fine and coarse fractions. Therefore, for this project, effect of speed of the classifying wheel, particle size and also different milling parameters on the separation efficiency of β-glucan were evaluated. Content and molecular weight of β-glucan was analysed in the different fractions. To further understand the separation efficiency, particle size distribution and scanning electron microscopy (SEM) were performed as well.

The result showed that it was possible to enrich β-glucan even further from a pre-fractionated fraction through milling and air classification. The molecular weight analysis showed that β-glucan molecule had similar size when applying different processing parameters. This indicates that air classification is a good method that prevents breakdown of the β-glucan molecule. This is an important factor when developing processing methods for manufacturing food products with enriched β-glucan content. Particle size distribution and SEM demonstrated that the separation efficiency between the fractions were succeeded which was most evident for the coarse fractions, where the enriched β-glucan can be obtained. The results also gave valuable information about the fractionation possibilities and that there could be potentials to optimize even further for some fractions. Findings in this investigation gave a better understanding of how this method would apply in an industrial scale. There could be possibilities to optimize more when carrying this out to a larger scale but it will however require some further studies.

Although the optimization of β-glucan showed promising results there could be possibilities improve the fractionation process and therefore to simplify the usability of β-glucan in food products. It can however be concluded that studies like this will bring us closer to have health-promoting products in the market where β-glucan content is increased but also it will be possible to achieve good levels of β-glucan intake without having to eat large amount of the product.