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Accumulation of oat nutritive compounds during grain filling with an emphasis on beta-glucan

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Abstract

The health benefits of oat cereals are linked to its high nutritional compounds, mixed-linkage (1→3)(1→4)-β-D-glucans (commonly referred to as β-glucans) in particular. This thesis was intended to focus on the accumulation features of oat nutritional compounds with an emphasis on β-glucans. Four Norwegian oat varieties (Odal, Haga, Vinger and Belinda) were grown at Vollebekk experiment farm of Norwegian University of Life Sciences (NMBU) in the growing season 2013. The sample collection was started fifteen days after anthesis (DAA), and nine harvesting dates were performed in whole growing season. The samples were analyzed for total β-glucans content (including water-soluble and water-insoluble β-glucans analysis for a variety Odal), total starch content and protein content in addition to gelatinization properties of oat starch using Differential scanning calorimetry (DSC). The result showed a significant increasing trend for each nutritional compound per groat during grain filling period. The average content (in %) of total beta-glucans increased from 0.3% at 15-DAA to 4.2% at 41-DAA, and the sharp increases occurred in the 10 days period from 20-DAA to 30-DAA.The statistical analysis showed that the accumulation features of those nutritional compounds and the gelatinization parameters of oat starch are significantly affected by varieties and the different harvesting date. Overall, Belinda will be the best choice for food because of the higher content of β-glucans content compared to other varieties.

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1. General Introduction

Over the last few decades, oat has become a popular food cereal and an attractive scientific subject due to its multifunctional characteristics and nutritional benefits to human beings. Oat is nutritionally unique among the cereals, owing to the higher content of dietary fiber, Mixed-linkage (1→3)(1→4)-β-D-glucans (hereafter referred to as β-glucans) in particular, small granules of starch, high quality of protein and other important nutritive compounds such as vitamins, minerals, and antioxidants.

Because of the importance for potential health benefits, many studies have focused on the nutritional components of oat cereal. The functional characteristics and nutritional profile of β-glucans (Andersson & Börjesdotter, 2011; Brennan & Cleary 2005; Gajdošová et al., 2007; Johansson, Tuomainen, Ylinen, Ekholm, & Virkki, 2004; McMullen, 2000; Saastamoinen, Hietaniemi, & Pihlava, 2004; Welch, Brown, & Leggett, 2000; Wood, 2011), physicochemical properties of starch(Hartunian-Sowa & White, 1992; R. Hoover & Vasanthan, 1992; Hoover, Smith, Zhou, & Ratnayake, 2003; Sayar & White, 2011; Wang & White, 1994a; Wang & White, 1994b; Zhou., Robards., Glennie-Holmes., & Helliwell., 1998) and protein quality(Zarkadas,Yu, &Burrows, 1995a; Zarkadas, Yu, & Burrows, 1995b) have been very well documented during the last decades.

β-glucans is the most studied soluble dietary fiber found in the cell walls of both

aleurone layer and endosperm in oat grain (Brennan & Cleary 2005; Wood, 2011). β-glucans is believed to have many positive health effects to human, and researchers have documented that the adequate daily intake of β-glucans (3g per day) has a significant effect on lowering cholesterol level, balancing blood glucose, avoiding immune system disorders and preventing people from coronary artery diseases (Brennan & Cleary 2005; Butt, Tahir-Nadeem, Khan, & Shabir, 2008; Gajdošová et al., 2007; Johansson et al., 2004; Tiwari & Cummins, 2008; Wood, 2011). Moreover, the water-soluble and water-insoluble part of β-glucans is related to physiological effects of β-glucans as well (Butt et al., 2008; Gajdošová et al., 2007).

There are many studies indicating that the content of β-glucans in oat is affected by the factors such as genotype, environment and agronomic practices(Brennan & Cleary 2005; Redaelli et al., 2013; Saastamoinen et al., 2004; Wood, 2011). However, the studies mentioned above mainly focused on the final mature oat grain, and none of them have paid attention on the accumulation features of β-glucans in during grain filling. Even if oat products are based on the mature grain, more knowledge to the synthesis and accumulation features during grain filling may contribute to the understanding of environmental factors that affect β-glucan content. It is also essential for receiving the largest health benefits from oat cereal.

1.1. Statement of the problem

There is an increasing interest also in Norway to utilize oats as food, and there is a need for more knowledge on β-glucans contents in the Norwegian oats and particularly in the commercial varieties that are grown predominantly. In a recent research project in Norway, β-glucans content of the main varieties was characterized. However, there is continuously new varieties being released, and there is a need to analyse β-glucans also in newly released varieties. So far, characterization of β-glucans is not done as a routine in the testing of new oat varieties in Norway.

Therefore, in the purpose of understanding more about the β-glucans content in main oat varieties grown in Norway and newly released and promising oat varieties, this thesis will specifically focus on the accumulation features of β-glucans during grain filling period. Besides, some other important quality parameters in the aspect of oat food are considered, such as starch and protein content.

1.2. Objectives of study

There is a need for more knowledge on the content of β-glucan in Norwegian grown oats, and how this may relate to other quality traits as the physical grain characteristics and chemical composition. There are newly released and promising oat varieties that are not characterized for β-glucans content. Furthermore, the variation in β-glucans content due to environmental factors is important, but scarcely understood. To investigate the accumulation of β-glucans and the other main chemical compounds during grain filling may contribute to better understanding of the variation in β-glucans content in the mature grains.

Therefore the objectives of this thesis are:

- To study the β-glucans content as well as other nutritive compounds and the physical grain characteristics of four Norwegian oat varieties including two newly released varieties (not characterized for β-glucans before);
- To study how β-glucans as well as the other main chemical compounds (starch and protein) of oats are accumulated during grain filling period and maturation;
- To study starch gelatinization properties at different development stages to see how it changes in grain filling period

2. Literature Study

2.1. Oat plant

2.1.1. Oat production

Oat is unique among the cereals due to its multifunctional properties and nutritional benefits to human being. During last thirty years, oat has become world sixth cereal in production quanta after wheat, maize, rice, barley, and sorghum, and the greatest production occurs in Russian Federation accounting for 30% of world total production (McMullen, 2000). The other leading oat-producing countries include Canada, United State, Australia, Germany, Poland and Finland (Marshall et al., 2013). It is generally believed that oat plant originated from the Asia Minor region, and now it is mostly grown in the world temperate areas between northern latitude of 35-50°and southern latitude of 20-40° (McMullen, 2000). The most widely cultivated oat nowadays is *Avena sativa.* (Valentine, Cowan, & Marshall, 2011).

2.1.2.Oat for food

Oat has become a more popular food during the last thirty years due to its unique nutritional value to human diet. Oat products are usually high in dietary fiber especially β-glucans, which has many potential functional properties as a food component. The sufficient amount of oat β-glucans consumption reduces serum cholesterol level and moderates blood sugar metabolism, and hence resulting in

reduced cardiovascular diseases, coronary heart diseases, immune system disorder, diabetes and obesity in consumers (Butt et al., 2008). In general, oat is normally consumed as whole grain therefore oat also has significant contribution to mineral nutrition of human diets, as the minerals are mainly distributed in the oat bran (McMullen, 2000). Additionally, oat is also a good source of vitamins for human, thiamine and pantothenic in particular (McMullen, 2000). However, the health benefits from oat food are only visible when it is consumed for longer time in sufficient amount.

2.2.Chemical composition of oat grain

The mature oat grain is composed of a soft kernel and hull. As a food for human, the oat grain should be de-hulled before use. After de-hulling, the oat kernel approximately accounts for 65-75% of the whole grain, and the hull content of the grain is about 25-30% (Butt et al., 2008). Oat groat consists of the fruit and seed coat layers (pericarp and testa), the endosperm including the aleuron layer and the starchy endosperm, and the embryo (Figure 1). Oat groat contains high amounts of valuable nutrients such as starch, dietary fibers particularly β-glucans, proteins, unsaturated fatty acids, vitamins, minerals and phytochemicals. The hull includes cellulose, hemicellulose and lignin. However, the ratio of oat kernel and hull content varies depending on genotypes and environment conditions.

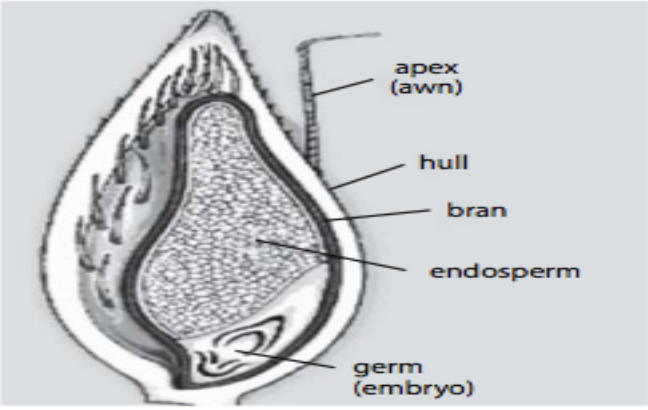


Figure 1. Cross section of oat grain. (Encyclopedia Britannica Inc., copyright 1996.)

2.2.1. Oat starch

Occurrence and structure of oat starch

Starch is an important energy source for human diet, and is the major constituent of the endosperm in cereal grains. Oat groat contains high levels of starch, which is found in small starch granules in the endosperm (Figure2). It has been approved that the oat starch content varies depending on the extraction techniques, and by wet milling, the starch content ranges between 43% -61% (McMullen, 2000). The main carbohydrates of oat starch are two different polysaccharides: amylose and amylopectin. The amylose is linear molecule of (1→4) linked α-D-glucopyranosyl units with only some slightly 1→6 linkage-branched molecules, while the amylopectin is highly branched by 1→6 linkages, which averagely appears once every 20-25 straight chain residues (Sayar & White, 2011). The ratio of these two polysaccharides depends largely on their botanical origin in other cereals such as barley, however waxy or high amylose types are not known in oats (Zhou. et al.,

1998). Moreover, it has been approved that the ratio of amylose and amylopectin and the frequency and spacing point in amylopectin molecules affect the physical and functional properties of oat starch together with genotype and environmental factors (Sayar & White, 2011).

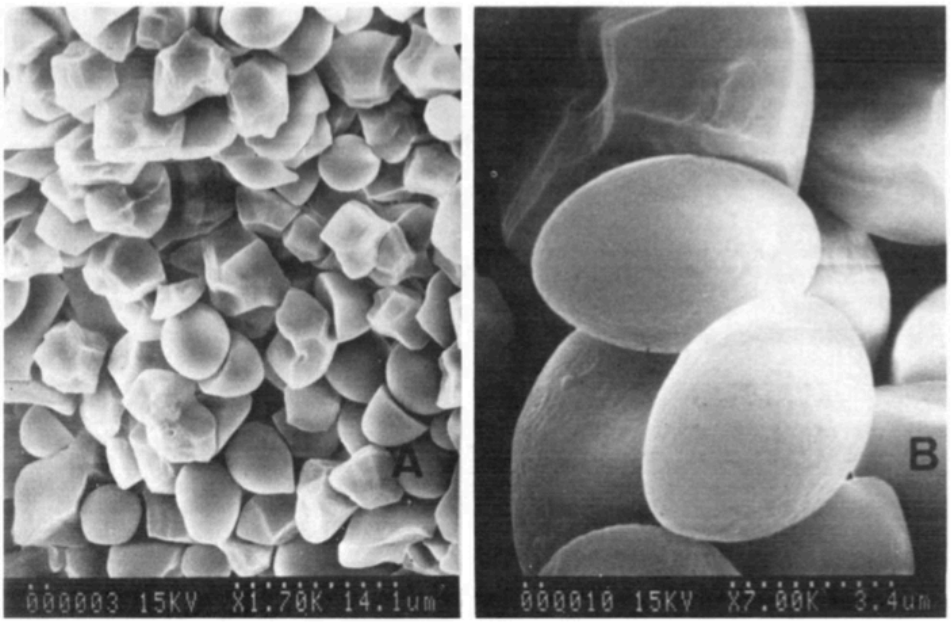


Figure2. Scanning electron photomicrographs at different magnifications of native oat starch granules: (A) 1700×;(B) 7000×. (Hoover & Vasanthan, 1992)

In oat starch, there are two types of amylose: the apparent amylose, which is defined as the amylose containing some lipid complex, accounting for 16.7-22% of oat starch, and the total amylose, which is the amylose free from lipid, is in the range of 19.4-33.6% (Sayar & White, 2011). However, the variances of the content mainly result from the genotype and extraction methodology (Wang & White, 1994b). The important properties of oat starch are not only dependent on the structure of the main carbohydrates and the architecture of granules, but also dependent on the

non-carbohydrate of the oat starch such as lipid, protein, inorganic materials and phosphorus, which are the minor constituents of the starch (Sayar & White, 2011). Oat starch has relatively higher amount of lipid in contrast to that of other cereals, because of the appearance of higher amount of amylose-lipid complex in oat starch. Researches have classified the starch lipid into three groups: internal lipid, starch surface lipid and non-starch lipid, depending on the location of the lipid in the oat starch(Zhou. et al., 1998).

In addition to lipid, there are also small amount of protein that is contained in the oat starch ranging from 0.3-0.9% of total oat starch, which is variable between varieties (Sayar & White, 2011). The protein appeared in starch includes real protein, peptides, amides, amino acids, enzymes, and nucleic acids (Swinkels, 1985). Furthermore, oat starch contains higher concentration of phosphorus in comparison to that of other cereal starch. Paton (1977) indicated that the reason of higher amount of phosphorus in oat starch is the appearance of phospholipid in the oat starch. Later, others found that there is a negligible little amount of phosphorus in lipid-free starch, which further explained that phosphorus in the starch is mostly bound with lipid (Hartunian-Sowa & White, 1992).

Gelatinization properties of oat starch

Gelatinization is the most important properties of oat starch in terms of processing,

and it shows the disruption of native starch crystalline in the process of heating in excess water. At the gelatinization temperature, the starch loses the crystalline order and some of the amylose are also dissolved irreversibly(Sayar & White, 2011).

There are many different techniques that have been used in the studies of gelatinization properties, among which differential scanning calorimetry (DSC) is one of the most popular technique used nowadays. DSC is a technique in which the difference in energy inputs into a substance and a reference is measured as a function of temperature or time, while the substance and reference are subjected to a controlled temperature program. The main application field of DSC includes the determination of thermal effects, melting behavior, crystallization, purity, polymorphism, glass transition, oxidative stability, chemical reactions, reaction kinetics and specific heat. DSC is most used in the field of polymers, pharmaceuticals, foodstuffs, chemical and industrial products. An example of DSC thermogram on native oat starch is shown below (Figure3). The explanation of the thermogram is given by (Sayar & White, 2011): The data obtained from the DSC thermogram is the enthalpy of oat starch gelatinization and the various temperatures related to the gelatinization phenomena. There are two endothermic transitions obtained in the curve of first-run oat starch, the low-temperature endotherm (M_1) expresses the melting of starch crystallites, and the high-temperature endotherm (M_2) expresses the melting of amylose-lipid complex. In addition, T_o refers to onset gelatinization temperature; T_p refers to peak gelatinization

temperature; *Tc* refers to conclusion gelatinization temperature. However, in the curve of re-run starch sample, only the high-temperature endotherm is found. Therefore, it is indicated that the melting of oat starch crystallites is irreversible, whereas the formation of amylose-lipid complex is reversible.

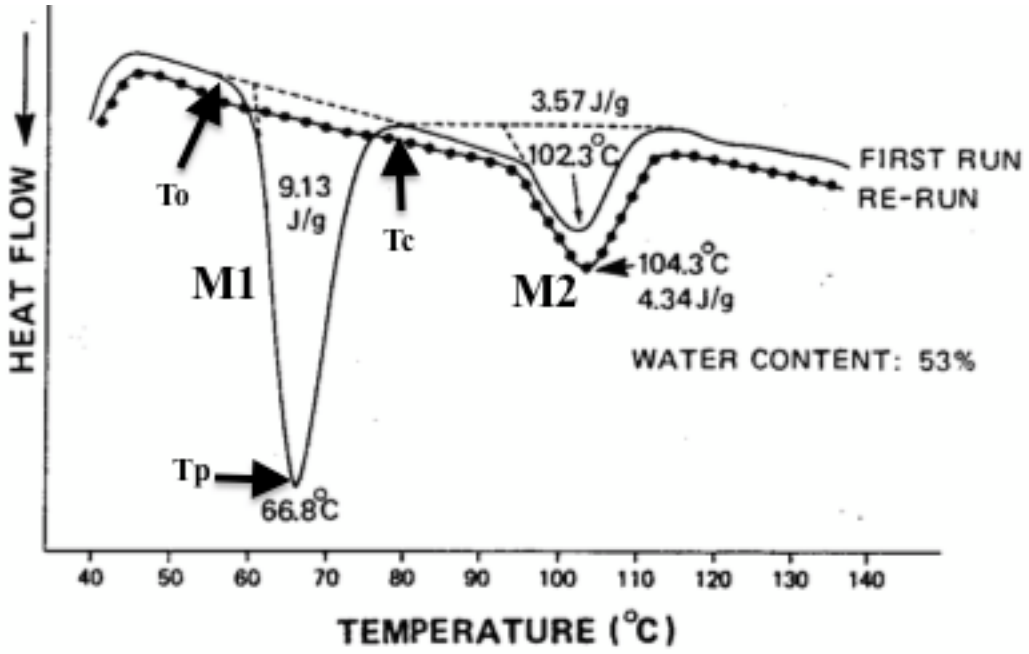


Figure3. DSC thermogram of native oat starch. (Zhou. et al., 1998)

Application of oat starch

Because of its appearance in small granules and containing relatively higher amount of lipid, oat starch shows some industrial purposes on paper sizing, pharmaceuticals, and infant food. In addition to that, oat starch is useful to cosmetic industry to replace talcum powder, and also using as dusting powder for surgical gloves(Sayar & White, 2011). In the future, to increase the utilization of oat starch, more potential modification can be studied in both food and nonfood application.

2.2.2. Oat β-glucans

Dietary fiber

In comparison with other cereals, oat has significant high levels of dietary fibers, β-glucans in particuler. The official definition of dietary fibers is proposed by the AACC Dietary Fiber Technical Committee (2001): “Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation”.

Dietary fibers can be classified as soluble fiber and insoluble fiber (Brennan & Cleary 2005). Soluble fibers contain gums, mucilage, pectin and some hemicelluloses, whereas insoluble fiber includes cellulose, lignin and rest of the hemicelluloses(Butt et al., 2008). It is clear that soluble fiber dissolves in water, while insoluble fiber dose not. However, both types of fibers positively influence human health in their special ways. Soluble fibers slow down glucose absorption, and reduce plasma cholesterol concentrations. In comparison to soluble fibers, insoluble fibers increase fecal bulk and softness, as well as the frequency of defecation. Additionally, it also reduces the intestinal transit times (Dewettinck et al., 2008). Behind those benefits of dietary fibers, there is an attractive composition of water-soluble fibers called β-glucan, which extensively enhances the nutritional value of oat.

Occurrence and structure of β-glucans

β-glucans are non-starchy polysaccharides (NSP), and mostly found in the cell walls of both aleurone layer and endosperm in oat grain together with other NSP like cellulose, arabinoxylan, glucomannan and the cytoplasmic fructans (Brennan & Cleary 2005). Oats β-glucans are composed of linear, unbranched polysaccharide molecules with about 30% (1→3)-linkeges and 70% (1→4)-linkeges of β-D-glucans (Figure4), and it is present more in the cell wall of starchy endosperm than the aleurone cell wall (Wood, 2011).

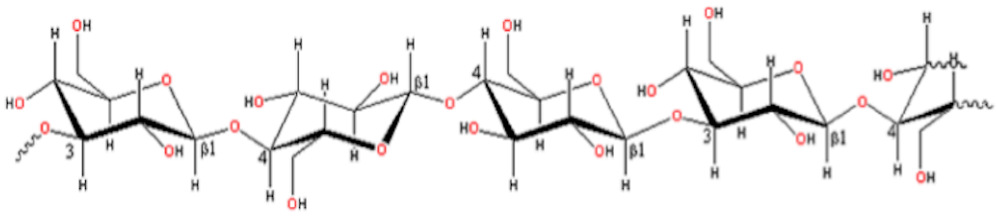


Figure4. Linear structure of β-glucans in oat.

It is recently indicated by an European project that the genetic background is the most important factor to determine the β-glucans content in oat, and statistically the content ranges from 2.85% to 6.77% under dry weight basis (Redaelli et al., 2013). The variation in β-glucans content due to environmental factors is also important, but scarcely understood. To investigate the accumulation of β-glucans and the other main chemical compounds during grain filling may contribute to better understanding of the variation in β-glucans content in the mature grains. Moreover, the water

availability during maturation is found to have a major effect on the levels of β-glucans in the grain, and it has been shown that the increased irrigation results in a decreased β-glucans content in the grain (Brennan & Cleary 2005). In some other studies, the higher amount of β-glucans appears in the grain that met heat stress before harvest(Bendelow, 1975), whereas the β-glucans content tends to decrease when the dry condition occurs during grain filling stages (Savin & Nicolas, 1996; Savin, Stone, Nicolas, & Wardlaw, 1997). It is also believed that β-glucans content has a positive relationship with the grain weight (Brennan & Cleary 2005). Additionally, the relation between other chemical compositions of the grain and the β-glucans content has been mentioned in some studies, and the nitrogen fertilizer application also showed a positive correlation with the β-glucans content of whole grain (Brennan & Cleary 2005).

Analysis of oat β-glucans

To analyze the β-glucans, the extraction is the first step to avoid starch interference by using reagents such as hydrazine, perchloric acid and 4% sodium hydroxide to get a quantitative amount of β-glucans. The nature of extraction procedure has a significant effect on molecule weight (MW), which further reflects on the functional properties of the β-glucans(Wood, 2011).

According toBrennan and Cleary (2005), the extraction of β-glucans from cereal grain includes three steps: (1) inactivation of endogenous enzymes; (2) extraction of

β-glucans; (3) precipitation of β-glucans. During last few decades, many extraction approaches have been developed, but the most widely used method is the AOAC official method 995.16, which is also the AACC method 32-33 (Wood, 2011). The method first uses lichenase, (1→3)(1→4)-β-D-glucan-4-glucanohydrolase, to catalyze the main β-glucans chain to release oligosaccharides, and then uses a purified β-glucosidase to yield glucose from the oligosaccharides. At the end, the glucose oxidase reagent is used to quantify the β-glucans (Figure5). In addition, the soluble and insoluble β-glucans can also be analyzed separately, but no sharp distinction has been found, and the ratio of those two fractions is mainly related to the extraction method (Wood, 2011).

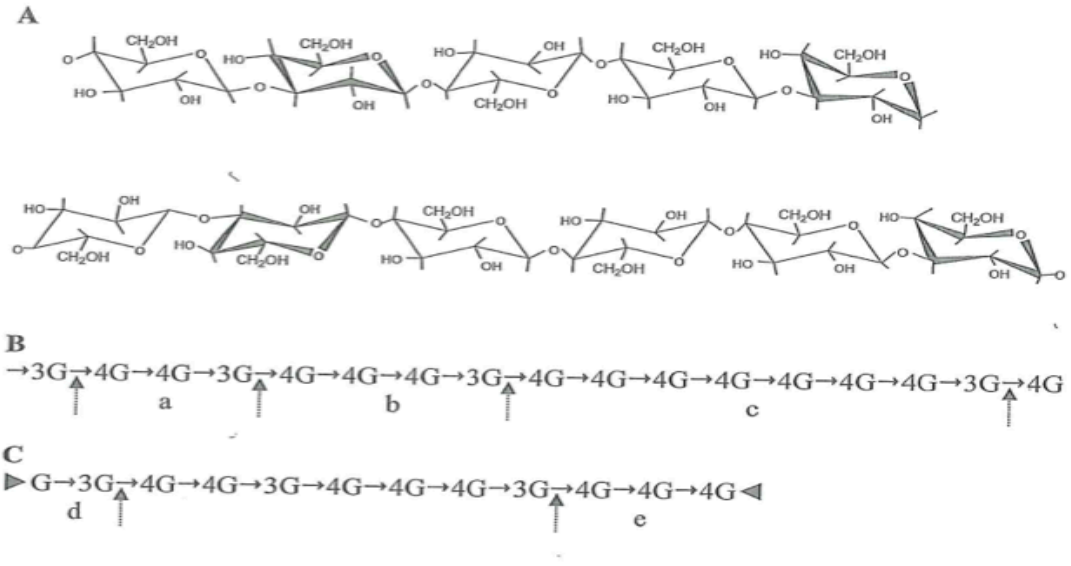


Figure5. Illustration of extraction method oat β-glucans. A: main chain of the β-glucans, β-(1→3)-linked cellotriosyl and cellotetraosly units. B: schematic of main chain showing oligosaccharides produced by action lichenase, a=3-O-β-cellotetraosly-D-glucose, b=3-O-β-cellotetraosly-D-glucose, c=cellodextrin with 3-linked glucose reducing end unit. C: schematic of lichenase action at chain ends; d=laminaribiose, e=celloteriose. ↑ =sites of lichenase cleavage.

Physiological effects of β-glucans

β-glucans is reported to have many positive effects to human health. Many clinical studies have been conducted on rats in the purpose of finding out how oats β-glucans influence human health. There are certain considerable results that have been documented: in gastrointestinal studies, oats β-glucans delayed gastric emptying and increased intestinal transit time; in glycemic response studies, oats β-glucans showed a significant effect on postprandial blood glucose concentration; also in serum cholesterol studies, a reduced serum cholesterol concentration was found after treatment of oatmeal; in immune function studies, rats consumed oats β-glucans had increased resistance to infection(Butt et al., 2008; Wood, 2011). As a consequence, nutritionists suggested that the adequate daily intake of β-glucans (3g per day) has a significant effect on lowering cholesterol level, balancing blood glucose, avoiding immune system disorders and preventing people from coronary artery diseases (Brennan & Cleary 2005; Butt et al., 2008; Gajdošová et al., 2007; Johansson et al., 2004; Tiwari & Cummins, 2008; Wood, 2011).

2.2.3.Oat proteins

Oat grain has higher amount of protein content, and it typically accounts for 15-20% of the groat (McMullen, 2000). Oat proteins are the important nutritional compounds to human in terms of essential amino acids. According to Food and Agriculture Organization (FAO) reference, oat proteins surpassed the requirements for all

essential amino acid except lysine and threonine(Welch & Yong, 1980). It has been also indicated that the total protein content and the ratio of amino acid in oat protein fluctuate with the development stages (David, 2011). In addition, the oat protein content varies depending on genotype, climate condition and fertilizer application. It is reported that the late application of nitrogen fertilizer results in higher amount of groat protein than the early application, and the double application (early and late application) had no effects on the protein content. (Zarkadas et al., 1995a).

2.2.4.Others

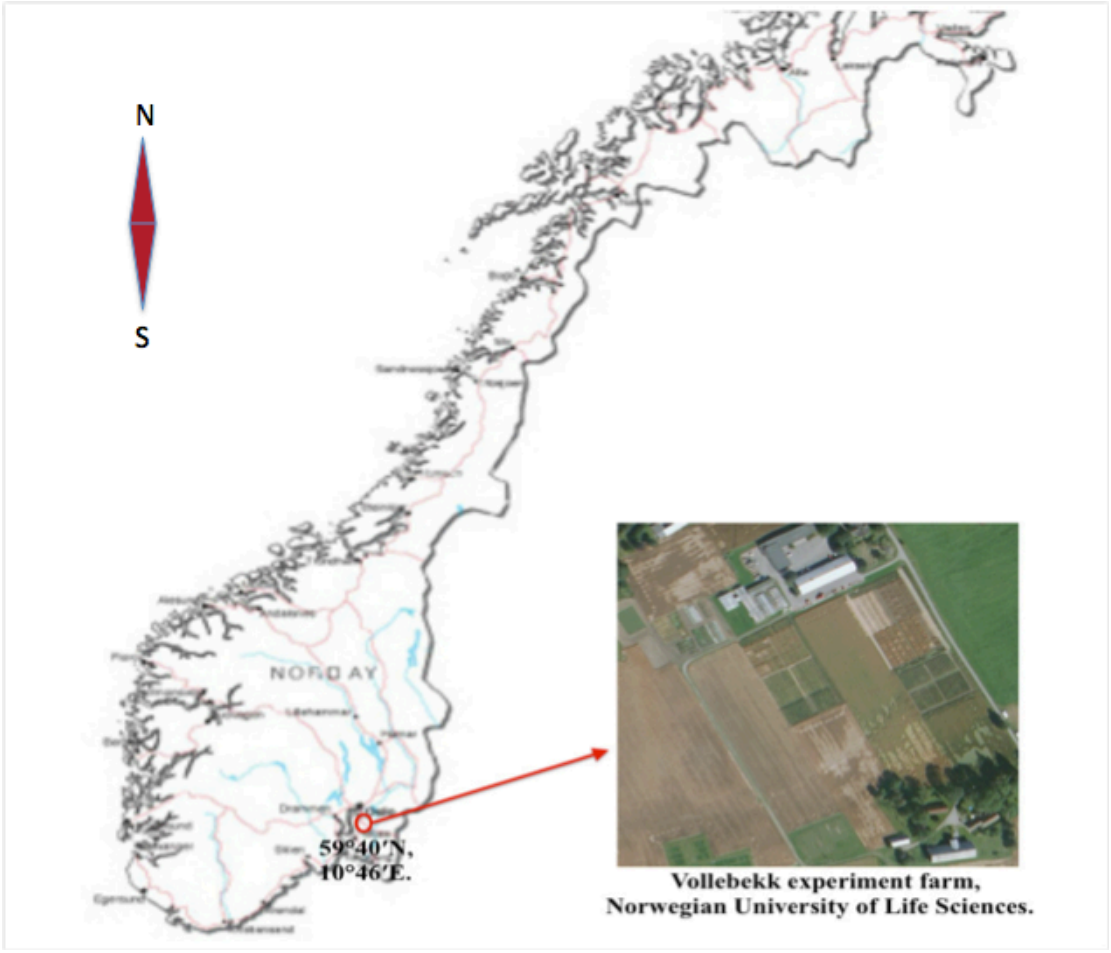
Except those nutritional parameters that are briefly mentioned above, oat grain has the highest lipid content in comparison to other cereals. Oat lipid is distributed throughout the oat kernel, and is mainly composed by unsaturated fatty acids (Butt et al., 2008). Lipid in oat grain also contributes to the functional properties of oat starch because of its appearance in amylose-lipids complex if the starch. In general, it is believed that the different amount of lipid appearance in starch has different effects on structure and physiochemical properties of oat starches (Wang & White, 1994b).

In addition to lipids, oat also contains other healthy components such as phenolic acids, B complex vitamins and minerals. The properties of those components cannot be neglected when it comes to functional characteristics of oat towards human health.

3. Material and Methods

3.1.Plant Materials

Four Norwegian oat varieties were selected for this thesis that were grown in a field experiemnt in growing season 2013 at Vollebekk experiment farm in Norwegian University of Life Sciences (NMBU) (59°40′N, 10°46′E) (Map1). The field trials were laid out as a block experiment with two biological replicates (Figure6). The oats were sown on 19/05/2013, and harvested by combiner on 28/08/2013. The information of the practical management during growing season is shown in Table1.



Map1. Map of the field trail location.

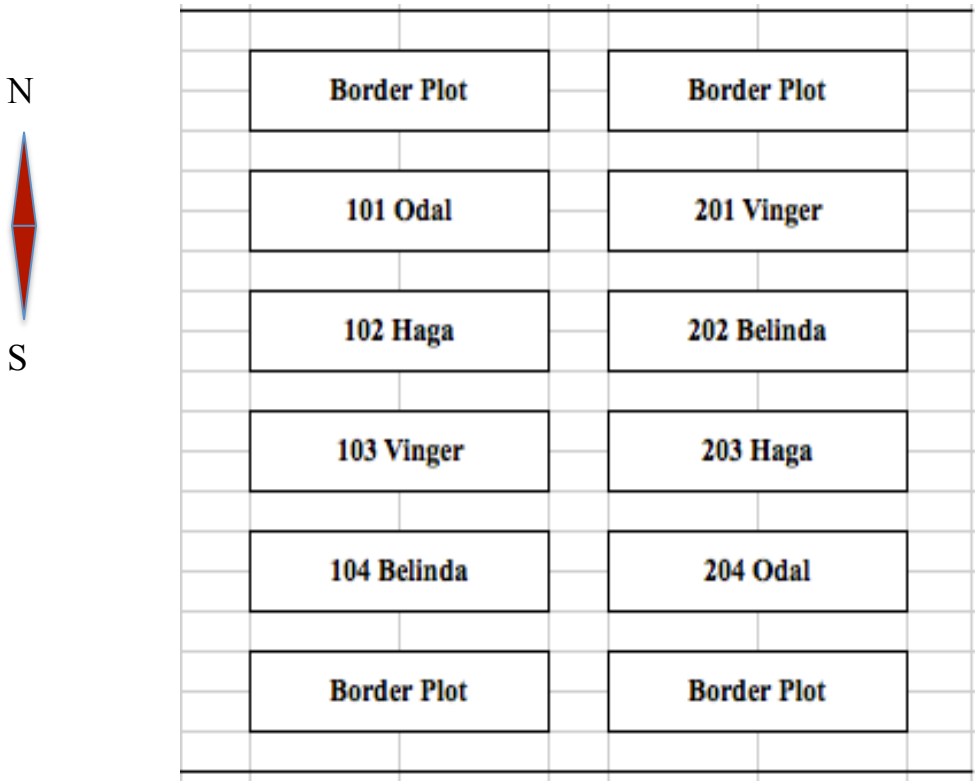


Figure6. Plot design of the field trails.

Table1. The information of the practical management in the field trails

	Fertilizer (N:K:P) Application	Herbicide Application	Fungicide and pesticide	
			1st Application	2nd Application
Date	18/05/2013	08/06/2013	12/07/2013	17/07/2013
Chemicals	22%N-3%P-10%K	Areane S in 250 liter water	Delaro and 200 g/ha Karate in 250 liter water	Proline and 200 g/ha Karate in 250 liter water
Amount	555 kg/ha	2220 ml/ha	800 ml/ha	800 ml/ha

3.2. Recording of plant development and sample harvesting

The heading date, anthesis date and date for yellow ripening of each oat plot was recorded to support the sampling time. Heading date was specified as the date when half of the ears in one plot were completely out of the flag leaf sheath, whereas anthesis was specified as the date when half of the florets in the randomly chosen panicles from one plot had anthers turned yellow. Yellow ripening was specified as the date when half of the plants in one plot are turned yellow.

During whole growing season, the nine harvesting dates were performed from fifteen days after anthesis (DAA) until maturation: five times before yellow ripening, harvested in 5-days intervals (15-DAA, 20-DAA, 25-DAA, 30-DAA, 35-DAA), and three times after yellow ripening, harvested in 3-days intervals (38-DAA, 41-DAA, 44-DAA). At each harvesting time, 10 panicles were randomly collected from each oat plot individually., All kernels from the panicles were de-hulled by hand, and counted, and the fresh weight was weighed before frozen under -20℃ in freezer. In addition to the harvesting the whole plots were harvested by the combiner at maturity (47DAA). For combiner-harvested oat samples, cleaning, sorting and de-hulling were done by machine before storing them in freezer at -20℃. There were 72 samples (64 panicle samples and 8 combiner-harvested samples) in total.

3.3. Analysis of Physical Characters

Physical characters of oat grain such as moisture content, average dry weight per kernel, test weight, thousand groat weight and hull content were measured at the grain laboratory at Vollebekk. Milling was performed after freeze-dried the samples at Nofima Food at Ås.

3.3.1. Moisture Content and Average Dry Weight Per Kernel

The moisture content of all 72 samples were measured individually while the sample was harvested by drying at 105°C for 16 hours in a drying chamber. The average dry weight per kernel was also calculated according to fresh weight and moisture content of each sample.

3.3.2. Test Weight, Thousand Groat Weight and Hull Content

The combiner-harvested samples were cleaned by Perten Grain Cleaner 5110 (Perten Instruments AB, Sweden). The samples were also sorted slightly by Perten Grain Cleaner 5110 (Perten Instruments AB, Sweden) to remove the very light and shriveled kernels to prpare the samples for dehulling. Test weight, thousand groat weight and hull content of combiner harvested eight samples were recordedat both cleaning and

sorting stage. Here, cleaning was defined as the step to separate oat grains from branch residue, whereas sorting was defined as the step to standardize the grain size.

3.3.3. Milling

Milling was completed on Stein Laboratory Mill (manufactured by Fred Stein Laboratories, INC. Atchison, Kansas) at Vollebekk. The panicle samples were freeze-dried before milling. Moisture content of all oat flour samples was also measured by drying at 105°C for 16 hours in a drying chamber to support the calculation of chemical analysis under dry weight basis.

3.4. Analysis of Chemical Composition

3.4.1. Total Beta- glucans Analysis

The total beta-glucans was analyzed by streamline method using Megazyme Assay kit －Mixed-Linkage Beta-Glucans (Megazyme International Ireland Ltd., Wicklow, Ireland), approved by the AACC Method 32-23 and the AOAC Method 995.16(McCleary & Codd, 1991). The analysis was carried out on all four varieties of oat flour including their biological replicates. The samples from 15, 20, 25, 30, 35, 41 DAA and combiner-harvested stage were chosen, and two technical replicates were performed for each sample.

3.4.2. Soluble and Insoluble Beta-glucans Analysis in Odal

Soluble and insoluble beta-glucans were only determined on the variety Odal with its two biological replicates. The analysis was done on the samples from 15, 20, 25, 30, 35-DAA and combiner-harvested stage, and two technical replicates were included for each sample. For insoluble beta-glucans analysis, the hot water extraction was used to separate the soluble part in the supernatant from the insoluble part. The pellet from the extraction was dried at 65℃ overnight, and analyzed for its beta-glucans content using the same method descried in total beta-glucans analysis above. The soluble beta-glucans was calculated by subtracting the insoluble beta-glucans from total beta-glucans.

3.4.3. Total Starch Analysis

Total starch was analyzed using Megazyme Assay kit - Total Starch (Megazyme International Ireland Ltd., Wicklow, Irelan), approved by AOAC Method 996.11 and AACC Method 76.13. The four varieties of oat samples from 15, 30, 41 days after anthesis and mature stage were selected for the analysis, and two biological replicates and two technical replicates were included.

3.4.4. Gelatinization properties of oat starch

Gelatinization properties of oat starch were detected by Differential scanning calorimeter (DSC 823e, METTLER SOFTWARE STAR SW 9.01). The samples used in total starch analysis were tested, and each sample had two technical replicates. For

each test, 30mg milled sample was weighed into the bottem part of the steel vial, and sealed tightly with the top part of the vial after adding 60µl distilled water.

3.4.5. Protein Analysis

Protein analysis was done at IPM laboratory of NMBU. For measuring protein content, first the total nitrogen (N) amount of all 72 oat flour samples was tested according to the Dumas method(Bremner & Mulvaney, 1982), and adjusted again to the protein content by the equation N*6.25.

3.5. Statistical Analysis

All row data were recorded and calculated using Microsoft Excel 2011 for Mac, and all results related to chemical composition were adjusted to dry weight basis.

Analysis of variance (ANOVA) was performed using General Linear Modal (GLM) on Minitab software with significance level set at $P<0.05$, and the model response = varieties + harvesting time + varieties * harvesting time was used. In addition, two-way ANOVA was applied on results from the combiner-harvested samples. In addition, the Least Significant Difference (LSD) was also calculated for some of the experimental data.

4. Result

4.1. Physical characteristics of oat grain

4.1.1.Moisture content, fresh weight and dry weight of oat groat during grain filling

In order to assess how the water content of oat groat is changed and how the oat groat is filled with the nutritive compounds, the grain filling was characterized by recording moisture content and the fresh and dry weight of the groats for the eight development stages, from fifteen days after anthesis (15-DAA) until 44-DAA, and for the combiner-harvested stage as well. The analysis of variances using general linear model (GML) showed that, during grain filling, both varieties and different development stages affected the moisture content, fresh and dry weight of oat groat significantly ($p<0.001$). However, no significant interaction was found between the varieties and different development stages (Table2.)

Table2. P-values from statistical analysis using general linear model (GML)

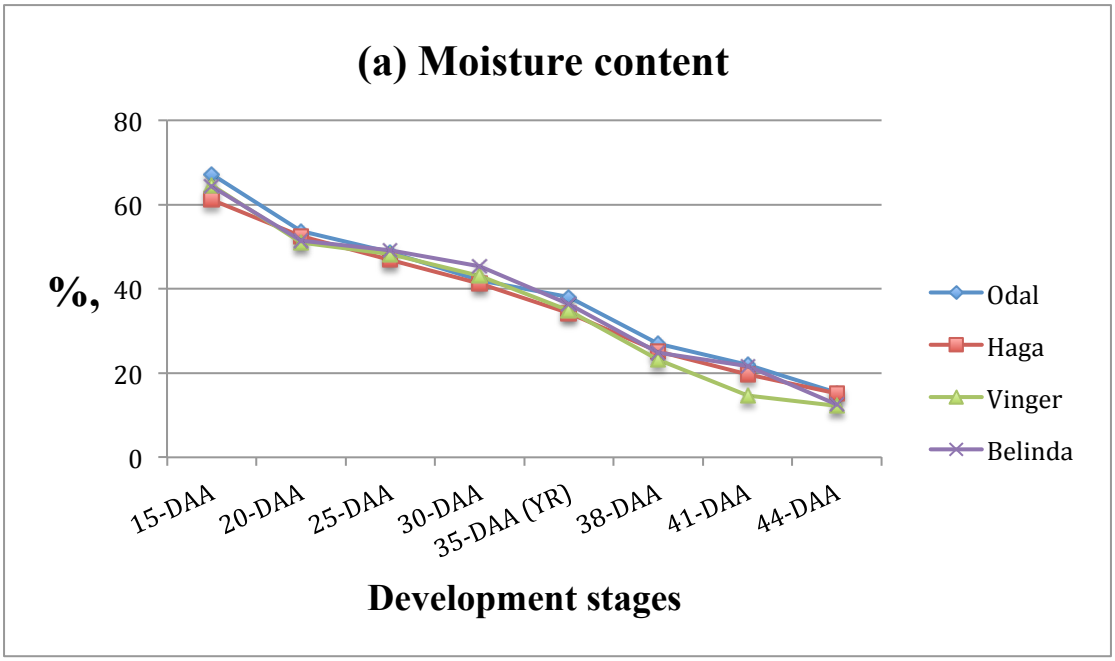
	Moisture content	**Fresh weight per groat**	**Dry weight per groat**
Varieties	P＜0. 001	P＜0. 001	P＜0. 001
Development stages	P＜0. 001	P＜0. 001	P＜0. 001
Varieties * Development stages	P=0. 068	P=0. 554	P=0. 947

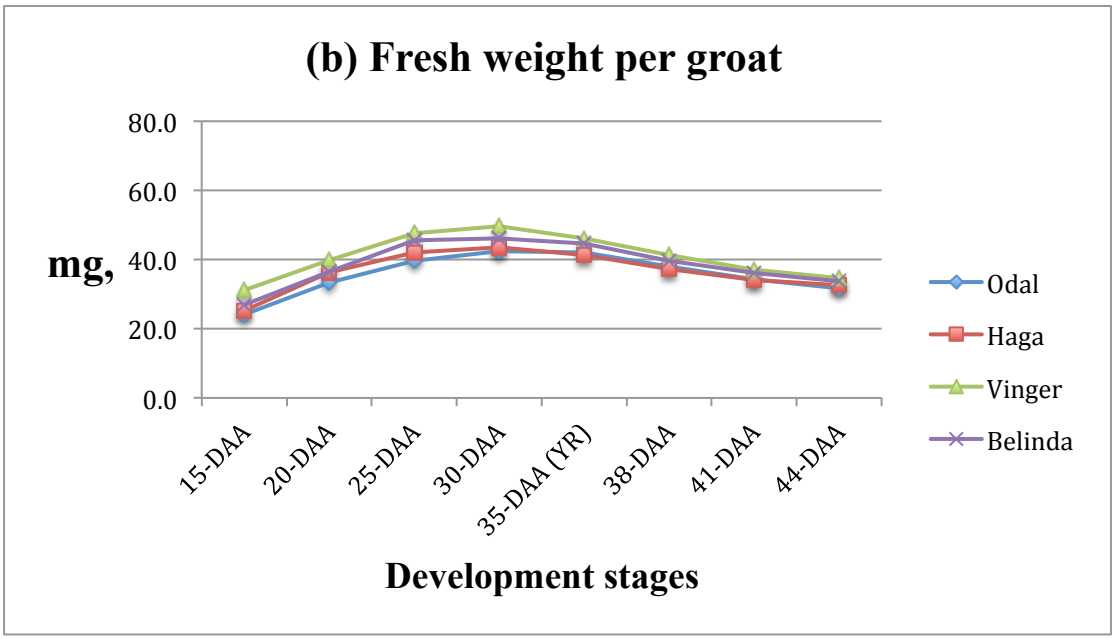
As shown in Figure7-(a), the moisture content of four oat varieties was steady decreased during the development stages from 15-DAA to 44-DAA, averagely from

64.4% to 13.8%. At 35- DAA the moisture content ranged from 34.3% – 38.1% for the varieties, and this stage were identified as the yellow ripening (YR) stage for Odal, whereas the other varieties had just passed yellow ripening at 35-DAA. Among the varieties, Vinger turned to contain less water than other three varieties after yellow ripeness.

In the case of fresh weight per groat shown in Figure7- (b), the average amount of four oat varieties increased sharply from the development stage 15-DAA to 30-DAA, increasing from 26.8mg to 45.4mg, and then the fresh weight slowly decreased to 33.1 mg at 44-DAA. Among the four varieties, Vinger always had the highest fresh weight per groat in the eight development stages, while Odal had the lowest fresh weight per groat until 30-DAA, and then it shared a similar trend with Haga until 44-DAA. Additionally, the average fresh weight per groat in four oat varieties at combiner-harvested stage was 33.8 mg.

As for dry weight per groat shown in Figure7- (c), it is clear that the average dry weight of the groats increased from 9.6 mg at 15-DAA to 28.6 mg at 44-DAA. A slight decrease in dry weight was seen in Belinda from 38-DAA to 41-DAA. For combiner-harvested stage, the average dry weight per groat of four oat varieties reached a point 31.0 mg. Interestingly, during whole grain filling period including both hand-harvesting and combiner-harvesting stage, the highest dry weight per groat always occurred in Vinger, whereas the lowest amount appeared in Odal.


(a) Moisture content
80
60
40
20
0
%,
15-DAA
20-DAA
25-DAA
30-DAA
35-DAA (YR)
38-DAA
41-DAA
44-DAA
Development stages
Odal
Haga
Vinger
Belinda


(b) Fresh weight per groat
80.0
60.0
40.0
20.0
0.0
mg,
15-DAA
20-DAA
25-DAA
30-DAA
35-DAA (YR)
38-DAA
41-DAA
44-DAA
Development stages
Odal
Haga
Vinger
Belinda

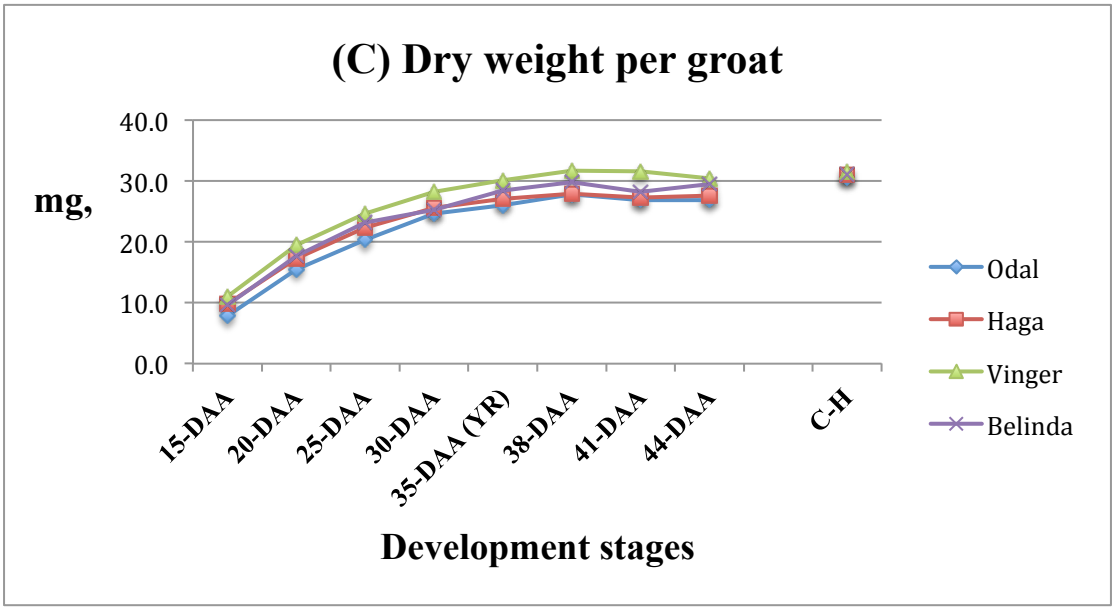


Figure7. Physical parameters of four selected oat varieties during grain filling. (a) moisture content; (b) fresh weight per groat; (c) dry weight per groat. 'DAA': days after anthesis; YR: yellow ripening; C-H: combiner-harvesting stage.

4.1.2. Physical characteristics of combiner-harvested oats

The physical characteristics of combiner-harvested samples were shown in Table3. It can be seen that test weight of four oat varieties were in the range of 54.2 kg/hl and 54.5kg/hl, and no significant differences between the varieties were found. As for thousand groat weight, Vinger had the highest value (34.4g) followed by Belinda (33.9g) and Haga (33.2), while it was the lowest for Odal (32.1g). In addition to that, the groat and hull accounted 75.0-77.0% and 23.0-25.0% of the whole oat in the four varieties, respectively, and no significant differences were found between the varieties.

Table3. Test weight, thousand groat weight and hull content of four oat varieties

Varieties	Test Weight, (Kg/hl)	Thousand Groat, Weight, (g)	Groats, (%)	Hull Content, (%)
Odal	54.4 ^{a}	32.1 ^{b}	76.2 ^{a}	23.8 ^{a}
Haga	54.2 ^{a}	33.2 ^{ab}	77.0 ^{a}	23.0 ^{a}
Vinger	54.5 ^{a}	34.4 ^{a}	76.9 ^{a}	23.1 ^{a}
Belinda	54.4 ^{a}	33.9 ^{ab}	75.0 ^{a}	25.0 ^{a}

TKW: thousand kernel weight; Values in the same column with different superscripts (a-b) are significantly different (p <0.05).

4.2. Beta-glucans

4.2.1. Total beta-glucans per kernel and the total β-glucans content of dry weight in oat during grain filling

The total β-glucans per groat and the total β-glucans content (as % of dry weight) of the four oat varieties at different developing stages during grain filling are presented in Table5, and the average accumulation patterns are shown in Figure8. The analysis of variances showed that there was a significant interaction between varieties and different harvesting stages ($p<0.05$), and the effects of those two factors on both the total β-glucans per groat and the total β-glucans content were significant ($p<0.001$) as well (Table4).

Table4. P-values from statistical analysis using general linear model (GML)

	Total β-glucan per groat (mg)	Total β-glucan content (%)
Varieties	P＜0. 001	P＜0. 001
Development stages	P＜0. 001	P＜0. 001
Varieties * Development stages	P=0. 015	P=0. 020

During the development stages, the overall average of total β-glucans increased from 0.03mg to 1.2mg per groat, from the stage15-DAA to 41-DAA. The variety Vinger had the highest amount at every hand-harvesting stage except at 20-DAA, while the lowest amount occurred in variety Odal from 15-DAA to 25-DAA, and then in Haga from 30-DAA to 41-DAA. As for combiner-harvested sample, the average total β-glucans per groat of four oat varieties was 1.37mg, with the highest amount of 1.52mg in variety Belinda and the lowest amount of 1.19mg in variety Haga.

The average content (in %) of total β-glucans increased from 0.3% at 15-DAA to 4.2% at 41-DAA. Sharp increases in β-glucans content occurred from 20-DAA to 30-DAA and more than half of the β-glucans were accumulated in this 10 days period. Interestingly, changes in β-glucans content was found also atfter yellow ripening. The content of total β-glucans decreased in variety Odal (from 4.4% to 4%), while it increased in Haga (from 3.5% to 3.6%) and Belinda (from 4.1% to 4.7%), and had no change in Vinger with 4.6%. As for the total β-glucans content in combiner-harvested samples, it can be seen that the variety Belinda had the highest

value (4.9%) followed by Vinger (4.5%) and Odal (4.4%), while it was the lowest for Haga (3.9%).

Table5. The total β-glucans per groat and the total β-glucans content (as % of dry weight) of four oat varieties during grain filling.

Specification	Development stages						
	15-DAA	20-DAA	25-DAA	30-DAA	35-DAA (YR)	41-DAA	C-H
Total β-glucan per groat, (mg)							
Odal	0.02	0.10	0.36	0.83	1.14	1.06	1.35[ab]
Haga	0.03	0.13	0.45	0.8	0.95	0.98	1.19[b]
Vinger	0.03	0.14	0.59	1.16	1.39	1.45	1.43[a]
Belinda	0.03	0.17	0.43	0.81	1.15	1.34	1.52[a]
Total β-glucan content, (as %* of dry weight*)							
Odal	0.3	0.6	1.8	3.4	4.4	4.0	4.4[a]
Haga	0.3	0.8	2.0	3.1	3.5	3.6	3.9[b]
Vinger	0.3	0.7	2.4	4.1	4.6	4.6	4.5[a]
Belinda	0.3	1.0	1.9	3.2	4.1	4.7	4.9[a]

15-DAA, 20-DAA, 25-DAA, 30-DAA, 35-DAA (YR) and 41-DAA are the hand-harvesting stages. D=days after anthesis, YR= yellow ripeness. And, C-H=combiner-harvested stage. Values in the C-H column with different superscripts (a,b) are significantly different (p <0.05).

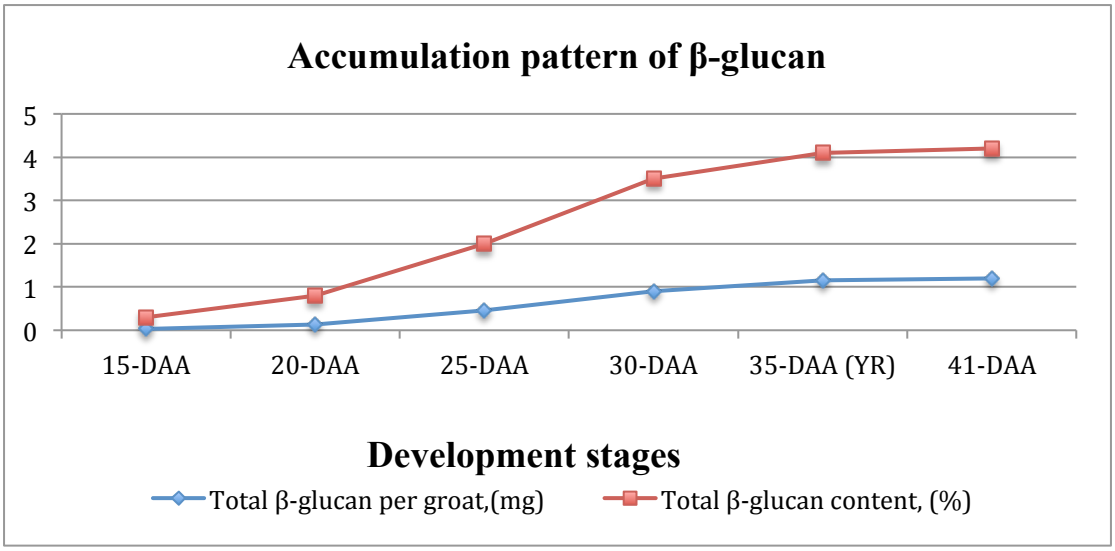


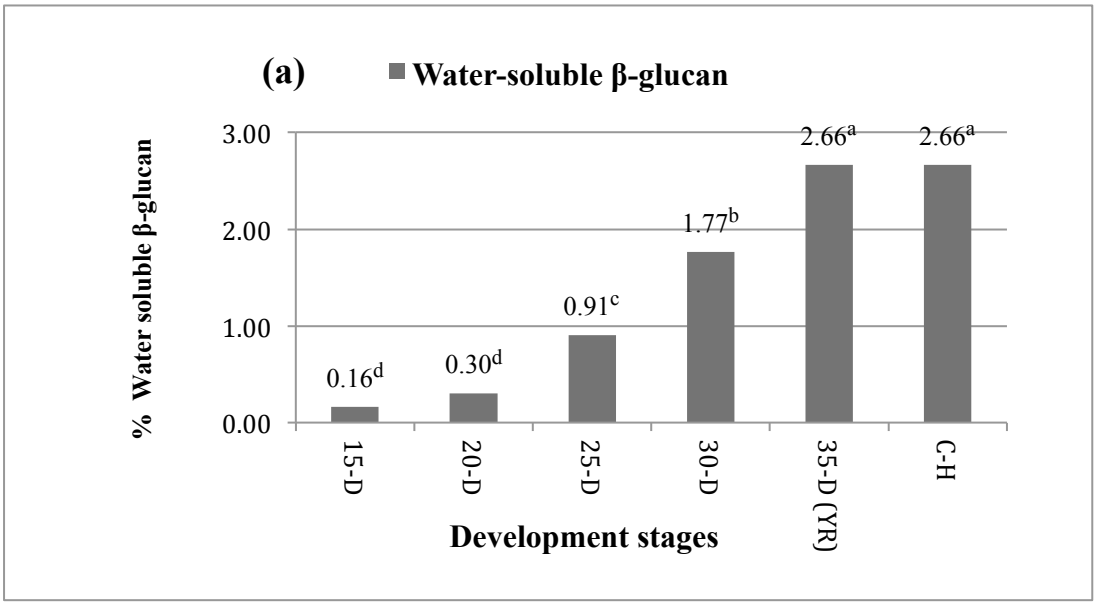
Figure8. Accumulation patter of β-glucan during grain filling; 15-DAA, 20-DAA, 25-DAA, 30-DAA, 35-DAA (YR) and 41-DAA are the development stages. DAA=days after anthesis, YR= yellow ripeness.

4.2.2. Water-soluble beta-glucans in Odal

The water-soluble and water-insoluble β-glucans contents (as % of dry weight) in variety Odal were shown in Figure9 (a) and (b). It is clear that, in the hand-harvesting stages from 15-DAA to yellow ripeness (35-DAA), the water-soluble and water-insoluble β-glucans contents significantly ($p<0.001$) increased from 0.16% to 2.66% and from 0.11% to 1.74%, respectively. However, in combiner-harvested sample, the content of water-soluble and water-insoluble β-glucans was alomost the same with that of yellow ripeness.

Additionally, the relative amount of water-soluble and water-insoluble β-glucans as percentage of the total β-glucans in variety Odal in different harvesting stages are shown in Figure10. It can be seen that the water-soluble fraction ranged 48-61%

during hand-harvesting stages, in which the amount was higher at 15-DAA (59%) and yellow ripeness (61%) than that of other hand-harvesting stages. And, in combiner-harvested sample, the ratio was the same with that of yellow ripeness.



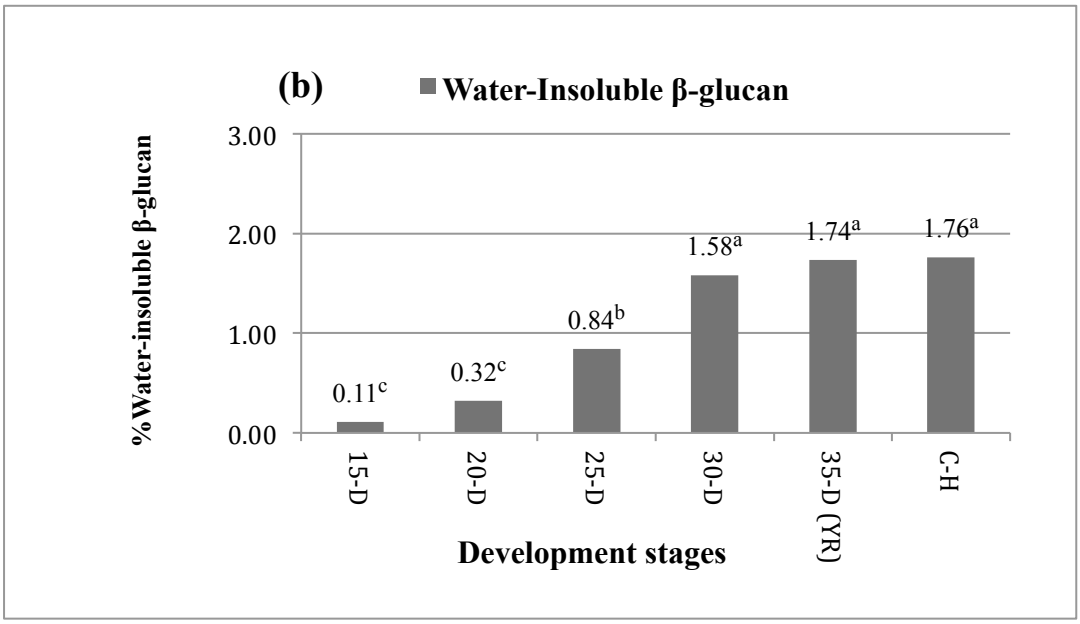


Figure9. Average content of water- soluble and water-insoluble β-glucan as percentage of dry matter for variety Odal diring different harvesting stages. (a) water-soluble β-glucan, (b) water-insoluble β-glucan. Values with different superscripts (a-c) are significantly different (*p* <0.05).

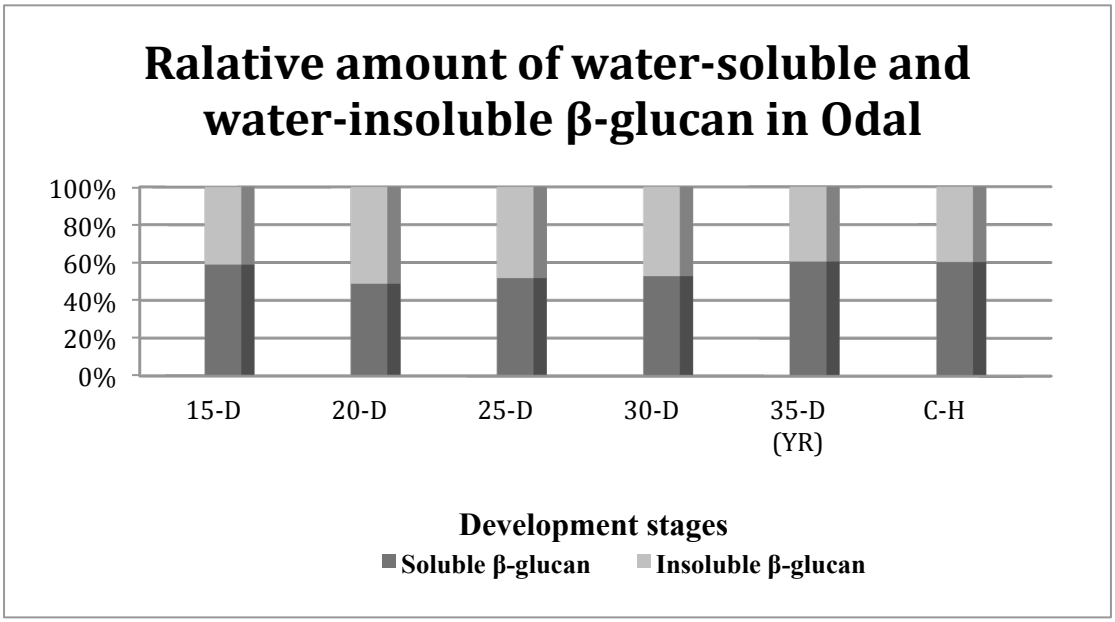


Figure10.The amount of water-insoluble and water-soluble β-glucans as percentage of the total β-glucan content of Odal at different hand-harvesting stages. 'D': days after anthesis; 'YR': yellow ripeness, C-H: combiner-harvested large samples.

4.3. Starch

4.3.1. Total starch per kernel and the total starch content of dry weight in oats during grain filling

Table7 showed the total starch per groat and the total starch content (as % of dry weight) of four oat varieties at different developing stages, and the average accumulation patterns are shown in Figure11. It was found from the analysis of variance that the total starch per groat of oat was significantly affected by both varieties and different harvesting stages ($p < 0.001 =$, but there was no interaction between those two factors (Table6). During the development stages, the total starch

per groat of four varieties averagely increased from 5.2mg (at 15-DAA) to 16.3mg (at 41-DAA), and among the varieties, Vinger contained the highest amount of total starch per groat, whereas the lowest amount appeared in Odal at every development stage. For combiner-harvested samples, the total starch per groat of four oat varieties ranged between 17.6mg – 18.8mg, and there was no significant differences between the varieties.

Table6. P-values from statistical analysis using general linear model (GML) - Total starch.

Source	Total starch per kernel, (mg)	Total starch content, (as % of dry weight)
Varieties	P＜0.001	P=0.058
Development stages	P＜0.001	P＜0.05
Varieties * Development stages	P=0.879	P=0.407

Statistically (Table6), the differences between the varieties were not significant in the starch content, whereas the effect of different development stages to the content was significant ($p<0.05$). It can be seen from Table7 that the starch proportion of four oat varieties during development stages ranged between 47.8 – 60.8%, in which the sample from Odal at 15-DAA had the lowest content, while Belinda at 30-DAA had the highest content. In combiner-harvested samples, the starch content of four oat varieties was in range 51.7%-59.4%, and no significant differences were found between the varieties.

Table7. The total starch per kernel and the total starch content (as % of dry weight) of four oat varieties during grain filling.

Specification	Development stages			
	15-DAA	30-DAA	41-DAA	C-H
Total starch per kernel, (mg)				
Odal	3.7	14.2	15.0	17.6[a]
Haga	5.6	15.4	16.2	18.8[a]
Vinger	6.4	17.0	17.8	18.7[a]
Belinda	5.0	14.3	16.3	18.5[a]
***Total starch content, (as %* of dry weight)**				
Odal	47.8	56.5	57.9	51.7[a]
Haga	57.7	60.1	60.2	56.5[a]
Vinger	55.9	59.3	56.5	57.9[a]
Belinda	57.7	60.8	59.3	59.4[a]

15-DAA, 30-DAA and 41-DAA are the hand-harvesting stages. D=days after anthesis. And, C-H= combiner-harvested stage. Values in the C-H column with different superscripts are significantly different (p <0.05).

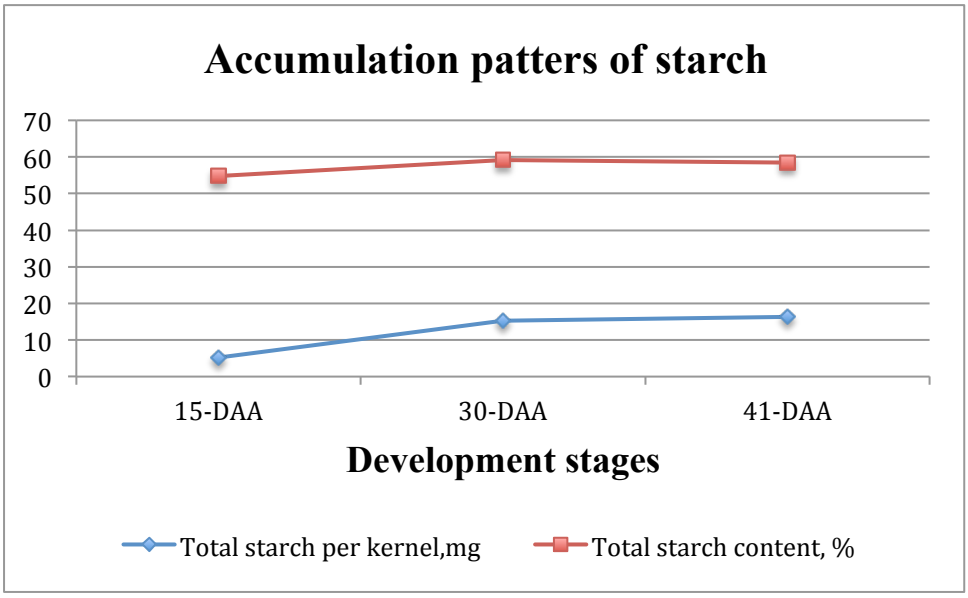


Figure11.Accumulation patterns of starch. 15-DAA, 30-DAA and 41-DAA are the hand-harvesting stages. DAA=days after anthesis.

4.3.2. Gelatinization properties of oat starch

In differential scanning calorimetry (DSC) analysis, two endotherms, first endotherm (M_1) and second endotherm (M_2), were found for all samples that were tested. The statistical analysis of GML showed that the gelatinization temperatures of two endotherms were significantly affected by both varieties and development stages, except the peak temperature of second endotherm. For gelatinization enthalpy of two endotherms, the varieties showed no significant effects, but the effect of different development stages in first endotherm was significant ($p<0.05$)(Table8 and9).

Table8. P-values from statistical analysis using general linear model (GML) – First endotherm

Source	Onset T(℃)	Peak T (℃)	Endset T (℃)	ΔH_{M1}
Varieties	P<0.001	P<0.001	P<0. 05	P=0. 592
Development stages	P<0.001	P<0.001	P<0.001	P<0. 05
Varieties * Development stages	P=0. 976	P=0. 765	P=0. 159	P=0. 773

T= temperature; ΔH_{M1}= the gelatinization enthalpy of first endotherm.

Table9. P-values from statistical analysis using general linear model (GML) – Second endotherm

Source	Onset T (℃)	Peak T (℃)	Endset T (℃)	ΔH_{M2}
Varieties	P<0.001	0. 992	0. 228	P=0. 550
Development stages	P<0. 05	P<0.001	P<0. 05	P=0. 127
Varieties * Development stages	P<0. 05	P=0, 686	P=0. 563	P=0. 437

T= temperature; ΔH_{M2}= the gelatinization enthalpy of second endotherm.

The gelatinization parameters of first endotherm found in four oat varieties during

different development stages are shown in Table10. The gelatinization onset temperature, peak temperature and endset temperature decreased in all four varieties along the hand-harvesting stages from 15-DAA to 41-DAA. Among them, the overall average onset temperature of four varieties decreased from 62.0℃ to 54.4℃, peak temperature from 69.2℃ to 62.2℃, and endset temperature from 77.3℃ to 73.2℃. However, the average gelatinization enthalpy of first endotherm (ΔH_{M1}) increased from 6.59 J/g to 7.54 J/g from development stage 15-DAA to 41-DAA. As for combiner-harvested samples, four oat varieties had the average gelatinization onset temperature with 53.9, peak with 61.9, endset with 72.8, and the average ΔH_{M1} with 7.36 J/g.

Table11 showed the gelatinization parameters of second endotherm for the oat varieties from different development stages. During the development stages from 15-DAA to 41-DAA, the gelatinization onset temperature, peak temperature and endset temperature decreased in all four varieties with the average variation 86.2-85.4 ℃, 97.9-96.5 ℃ and 107.3-105.7 ℃, respectively. However, the gelatinization enthalpy ΔH_{M2} was unpredictable during the varieties and in different development stages as well. The ΔH_{M2} had a variation from 1.87J/g to 2.99 J/g, in which the samples from 41-DAA in Belinda had the lowest value, while the highest occurred in Haga at 15-DAA. For combiner-harvested samples, the overall average gelatinization onset temperature, peak temperature and endset temperature of four oat varieties was 85.0℃, 96.5℃ and 105.5℃, respectively. The ΔH_{M2} of the varieties was ranged between 1.77 - 2.31 J/g.

Table10. DSC gelatinization properties of oat flour – first endotherm

Specification	Development stages			
	15-DAA	30-DAA	41-DAA	C-H
Onset T (℃)				
Odal	64.4	57.6	56.5	55.9[a]
Haga	60.3	53.8	52.8	52.7[c]
Vinger	61.1	54.4	53.5	53.0[c]
Belinda	62.2	56.0	54.8	54.1[b]
Average	***62.0***	***55.4***	***54.4***	***53.9***
Peak T (℃)				
Odal	70.9	64.4	63.6	63.3[a]
Haga	68.4	62.0	61.1	60.8[c]
Vinger	68.2	62.3	61.7	61.4[bc]
Belinda	69.3	63.4	62.5	61.9[b]
Average	***69.2***	***63.0***	***62.2***	***61.9***
Endset T (℃)				
Odal	78.9	74.1	73.7	73.6[a]
Haga	76.9	73.2	72.8	72.3[a]
Vinger	76.2	73.2	73.0	72.8[a]
Belinda	77.3	73.7	73.2	72.6[a]
Average	***77.3***	***73.5***	***73.2***	***72.8***
ΔH_{M1}(J/g) of first endotherms, (dry weight basis)				
Odal	6.98	7.48	7.64	7.36[a]
Haga	6.60	7.18	7.52	7.39[a]
Vinger	6.21	7.16	7.69	7.49[a]
Belinda	6.56	7.56	7.29	7.19[a]
Average	***6.59***	***7.35***	***7.54***	***7.36***

15-DAA, 30-DAA and 41-DAA are the hand-harvesting stages. DAA=days after anthesis.

And, C-H= combiner-harvested stage. ΔH_{M1}= the gelatinization enthalpy of first endotherm.

T=temperature. Values in the C-H column with different superscripts (a-c) are significantly different (p <0.05).

Table11. DSC gelatinization properties of oat flour – second endotherm

Specification	Development stages			
	15-DAA	30-DAA	41-DAA	C-H
Onset T （℃）				
Odal	87.4	86.4	86.1	85.6[a]
Haga	85.7	85.4	84.5	83.9[b]
Vinger	85.9	85.8	85.9	85.4[a]
Belinda	85.9	86.2	85.0	85.0[ab]
Average	***86.2***	***86.0***	***85.4***	***85.0***
Peak T （℃）				
Odal	97.7	97.3	96.5	96.7[a]
Haga	98.0	97.0	96.7	96.7[a]
Vinger	98.2	96.9	96.3	96.0[a]
Belinda	97.8	97.2	96.4	96.4[a]
Average	***97.9***	***97.1***	***96.5***	***96.5***
Endset T （℃）				
Odal	106.3	107.2	105.4	105.2[b]
Haga	107.7	107.3	105.7	105.8[a]
Vinger	108.5	106.9	106.5	105.7[a]
Belinda	106.7	107.2	105.0	105.3[b]
Average	***107.3***	***107.2***	***105.7***	***105.5***
ΔH_{M2} *(J/g) of* **second endotherm** *(dry weight basis)*				
Odal	2.19	2.73	2.20	1.77[a]
Haga	2.99	2.97	2.26	2.31[a]
Vinger	2.88	2.45	2.64	1.99[a]
Belinda	2.71	2.94	1.87	1.80[a]
Average	***2.69***	***2.77***	***2.24***	***1.97***

15-DAA, 30-DAA and 41-DAA are the hand-harvesting stages. DAA=days after anthesis.

And, C-H= combiner-harvested stage. ΔH_{M2}= the gelatinization enthalpy of second endotherm.

Values in the C-H column with different superscripts (a-c) are significantly different (p <0.05).

4.4. Protein

The protein per groat and the protein content (as % of dry weight) of four oat varieties at different harvesting stages are presented in Table13, and the average accumulation patterns are shown in Figure12. The analysis of variance showed that both varieties and harvesting stages significantly ($p<0.001$) affected the protein per groat and the protein content in oats, but no interaction was found between these two factors (Table12).

Table12.P-values from statistical analysis using general linear model (GML) - Total starch.

Source	Protein per groat, (mg)	Protein content, (as % of dry weight)
Varieties	P<0.001	P<0.001
Development stages	P<0.001	P<0.001
Varieties * Development stages	P=0. 931	P=0. 458

During the development stages, the average protein per groat of four oat varieties increased from 15-DAA (1.0 mg) to 41-DAA (3.0 mg), and at 44-DAA, the average amount was the same with that of 41-DAA. For combiner-harvested samples, the protein per groat was in the range of 3.1-3.4 mg.

From Table 13, it also can be seen that the protein content of four oat varieties during development stages had a variation from 8.9% to 11.7%. The lowest proportion

occurred in the samples of Haga at 20-DAA and 25-DAA, while the sample in Odal at15-DAA had the highest content of protein. What is interesting in the table was that the protein content of each variety at 15-DAA was the highest in comparison to that of other stages. As for combiner-harvested oat samples, the protein content was in range 9.3-10.3%.

Table 13. The protein per kernel and the protein content (as % of dry weight) of four oat varieties during grain filling.

	Development stages								
	15-DAA	**20-DAA**	**25-DAA**	**30-DAA**	**35-DAA (YR)**	**38-DAA**	**41-DAA**	**44-DAA**	**C-H**
Protein per groat, (mg)									
Odal	0.9	1.6	2.1	2.4	2.7	2.9	2.8	2.9	3.3[a]
Haga	1.0	1.6	2.0	2.4	2.5	2.7	2.8	2.8	3.1[b]
Vinger	1.1	1.9	2.3	2.9	3.2	3.1	3.2	3.2	3.2[ab]
Belinda	1.0	1.8	2.2	2.5	2.9	2.9	3.0	3.1	3.4[a]
Average	***1.0***	***1.7***	***2.2***	***2.6***	***2.8***	***2.9***	***3.0***	***3.0***	***3.2***
Protein content, (as %* of dry weight*)									
Odal	11.7	10.0	10.0	9.5	10.2	10.1	10.1	10.5	10.3[a]
Haga	9.9	8.9	8.9	9.2	9.1	9.4	9.8	9.6	9.3[b]
Vinger	10.3	9.5	9.3	10.1	10.2	9.6	10.0	10.2	9.5[b]
Belinda	10.4	9.8	9.1	9.8	9.8	9.5	10.4	10.3	10.2[a]
Average	***10.6***	***9.5***	***9.3***	***9.6***	***9.8***	***9.7***	***10.1***	***10.1***	***9.8***

15-DAA, 20-DAA, 25-DAA, 30-DAA, 35-DAA (YR), 38-DAA, 41-DAA and 44-DAA are the development stages. DAA=days after anthesis, YR= yellow ripeness. And, C-H=combiner-harvested stage. Values in the C-H column with different superscripts (a,b) are significantly different (p <0.05).

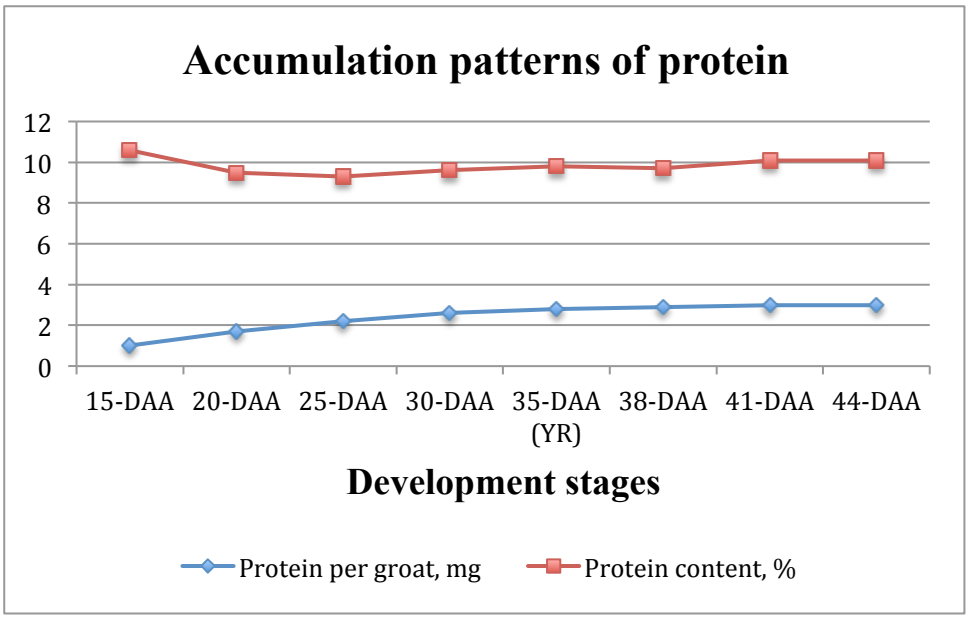


Figure12. Accumulation patters of protein;15-DAA, 20-DAA, 25-DAA, 30-DAA, 35-DAA (YR), 38-DAA, 41-DAA and 44-DAA are the hand-harvesting stages.DAA=days after anthesis, YR= yellow ripeness.

5. Discussion

5.1.Physical characteristics of grains during development

One of the objectives of this thesis was to study the physical characteristics of the selected oat varieties at maturity as well as during development. To fulfill the objective, physical characteristics such as moisture content, fresh and dry weight of oat groat was recorded in different plant development stages, and the test weight, thousand groat weight, groat percentage and hull content of the oat grain was tested for combiner-harvested oat samples.

The moisture content of the groat was linearly decreased in all oat varieties with the plant development stages. According to development principles of cereal plant, the cereal grain from early grain filling stage has the highest moisture content, whereas the grain development and maturation result in moisture loss. In this study, the moisture content of oat averagely declined from 64.4-13.8% during the period from 15-D to 44-D. Studies investigating the grain development in other cereals have shown similar decreases in moisture, however, the ranges of moisture may vary according to the selected sampling times during development. Adams and Rinne (1980) found that the moisture content decreases from 68% to 10% in wheat, and from 84-23% in corn. It is observed for cereals that both fresh weight and dry weight

of grain rapidly increases after pollination, but fresh weight is remarkably decreased at maturation, while the accumulation of dry weight is slowly continued and stopped at the end(Adams & Rinne, 1980). These changes were obviously seen in the result of this thesis. The increasing trend in both fresh weight and dry weight before yellow ripening (YR) stage in the result chapter could also be explained by the active biosynthesis of subcellular organelles such as starch granules, protein and lipid bodies. However, the decreases in fresh weight after YR may be related to net loss of water content in the grain (Adams & Rinne, 1980).

For the combiner-harvested samples, the overall average test weight in this study was in the range 44.0–55.2kg/hl reported by Buerstmayr, Krenn, Stephan, Grausgruber, and Zechner (2007). Researchers have earlier demonstrated that the test weight of oat grain is highly related to groat percentage (Doehlert, McMullen, & Baumann, 1999). However, more precisely determination has been done recently by the same research group saying that there are two factors affecting test weight in oat cereals, which are kernel density and packing factor (Doehlert, McMullen, & Jannink, 2006). In further, it relates to kernel mass and volume of the individual oat grains, as well as the space between kernels in measurement container. Additionally, they assumed that the tightly fitting hulls may also contribute to the higher test weight (Doehlert et al., 2006).

The thousand groat weight in the present study was somewhat higher than in

the study of McMullen (2000), ranging from (16.7-22.7g), however it was within the range of 23.6-38.2g reported by Buerstmayr et al. (2007). As for hull content, the result of thesis was lower than the range 25-35% reported elsewhere (McMullen, 2000). It also has been indicated that the groat weight and hull content of oats are differed by both genotype and environment (McMullen, 2000). Therefore, the higher range of groat weight and the lower range of hull content of the present study could be explained by a relatively low temperature during grain filling in Norway compared to elsewhere. In addition, the varieties showed similar proportions of both groat and hull, and the only difference found were for thousand-kernel weight where Vinger were slightly higher than other varieties.

5.2. Accumulation of chemical compounds during oat development stages

The main objective of the thesis was to study the accumulation features of oat chemical compounds with the emphasis on β-glucans. For β-glucans, the accumulation of total β-glucans during oat development stages was studied, and the variation of water-soluble and water-insoluble β-glucans during the plant development was also studied. Some other important quality parameters in the aspect of oat food are also examined, such as starch and protein content.

5.2.1. Accumulation of β-glucan

The result of the thesis showed that the both total β-glucans content per kernel and total β-glucans concentration increased sharply in the period from 20-DAA to approximately 30-DAA, and more than half of the β-glucans were accumulated in these 10 days. Further increases, but less sharp, were found after 30- DAA. This indicated that the β-glucans synthesis became very active approximately twenty days after anthesis. In the past, there was few, if any result in the study regarding the accumulation features of oat β-glucans during different development stages in grain filling. However, in barley, it was mentioned that the concentration of β-glucans increased during kernel development, and leveling off or decrease at later maturation stage(Åman, Graham, & Tilly, 1989). In addition, Brennan and Cleary (2005) indicated dry condition before harvest result in a high β-glucan level, whereas the heat stress during grain filing causes a reduction in β-glucan level. Tiwari and Cummins (2008) found that the initial level of β-glucans has a major effect on final β-glucans level in harvested barley. However, more studies have to be done for further confirmation.

The variation in total β-glucans content from combiner-harvested final samples in this thesis was within the range of earlier studies (Demirbas, 2005; Redaelli et al., 2013; Tiwari & Cummins, 2009), and slightly higher than that of other studies (Andersson & Börjesdotter, 2011; Papageorgioua, Lakhdarab, Lazaridouc, Biliaderisd, &

Izydorczyke, 2005). However, the highest content of β-glucans in oats appeared in wild species as reported by Welch et al. (2000). Many studies have confirmed that both genotype and environment affect the β-glucans content in oat, and it is difficult to point out which one affects the most. Demirbas (2005) listed out a table of average β-glucans content of cereals grown in different countries, in which the higher oat β-glucans content was found in USA(6.6%), Turkey(5.7%) and Australia (5.4%) where the growing season is longer and warmer, whereas the relatively lower content was found in Holland(4.6%) and Sweden(3.2%) where the growing season is shorter and cooler. In addition, a study (Saastamoinen et al., 2004), presenting the β-glucans content of six different oat varieties from three consecutive growing seasons in Finland, found an average β-glucans content ranging 4.8-5.1%, which is still lower than that of the countries with longer and warmer growing season. This indicates that the environment is an important factor which influences β-glucans content in oat plant. Besides, the result of the present thesis found a significant difference in total β-glucans content between different oat varieties during different development stages, where Haga always had a relatively lower amount of β-glucans compared to other varieties. Redaelli et al. (2013) have evaluated the effect of genotype to β-glucans content using eleven standard oat cultivars from two consecutive years, and reported a statistically significant result. The same result also found in other study (Doehlert, 2001). Moreover, in some other cereals grain such as in barely, the genetic factor is considered to be more important than environment factor as a final determinant of β-glucans level (ÖZkara, Basman, Köksel, & ÇElík, 1998). However, it is difficult to

make a direst conclusion as there are still some other factors influencing the β-glucans content in cereals.

Except genotype and environment, the agronomic practices such as nitrogen application, sowing date and germination time are also believed to affect the β-glucans level in oat. Brunner and Freed (1994) demonstrated that the increased level of nitrogen application result in the increased level of β-glucans in oat. However, the nitrogen fertilization had no significant effect on the content of β-glucans in oat resulting from a three year's trial study in Finland (Saastamoinen et al., 2004). A simulation study on the factors affecting β-glucans level in oat cultivation suggested that the sowing date is also a major factor influencing the content of β-glucans. They reported that the delayed sowing positively affects the final β-glucans level (Tiwari & Cummins, 2009), and this effect has also been demonstrated elsewhere(Humphreys, Smith, & Mather, 1994). Tiwari and Cummins (2009) also indicated that germination time has a negative effect on harvested oat β-glucans content. However, Saastamoinen et al. (2004) had a conclusion for their study indicating that there was no significant differences between traditional and organic cultivations towards oat β-glucans level.

Overall, oat β-glucans content can be influenced by many factors, but the result from this present study could only support the effect of genotype, since the varieties were grown in one environment. However, this thesis indicated that β-glucans synthesis is slightly started at 15-DAA, which were much later than for starch and protein, and

occurred at high rates from 20DAA-30-DAA. Among the varieties, Belinda will be the best choice for food because of the higher content of β-glucans content, whereas Haga may the good choice for feed where the lower β-glucans content is desirable.

5.2.2.Changes in water-soluble and water-insoluble β-glucan

The present study showed significant increases in both water-soluble and water-insoluble β-glucan content (dry weight basis) in oat during grain filling. The content of water-soluble β-glucan was higher than that of water-insoluble β-glucans in early development (15-D) and also at the later maturation stages (30-D to maturity). In between the amounts of soluble and insoluble β-glucan retain somewhat equally, indicating that the ratio of water-soluble and water-insoluble β-glucan is varied during grain filling in oat grain. There is no comparable result from other studies to make an agreement with the findings of this thesis. The water-soluble percentage of total β-glucans from combiner-harvested final sample of this experiment was in the range (50.7-85.2%) reported elsewhere(Gajdošová et al., 2007; Redaelli et al., 2013). The water-insoluble part of β-glucans found in this experiment was higher than the range (5.15-33.73%) reported by Gajdošová et al. (2007). The variation in the water-soluble and water-insoluble β-glucans content and the changes of their ratio in oat grain can be explained by different factors such as extraction methods.

Redaelli et al. (2013) reported that both genotype and environment affected the water-soluble fraction in oat, and the major effect was resulted from genotype

according to their statistical analysis. However, this experiment only focused on one variety during different development stages, and found the changes in water-soluble and water-insoluble ratio. The changes could be linked to the accumulation features and structural modifications of β-glucans during grain development stages(Hoover et al., 2003). In addition, others pointed out that the water-soluble and water-insoluble fraction of β-glucans is varied depending on the extraction condition(Sundgren, Åssveen, & Stabbetorp, 2013).

5.2.3. Accumulation of starch

As mentioned earlier in the result chapter, the starch concentration of oat accumulated sharply in the early stages of grain filling, and there was no significant increasing trend during late maturation stage (from 30-D to maturity). For cereals, the starch is believed to be a major reserve of oat grain (Hoover et al., 2003; McMullen, 2000; Sayar & White, 2011; Valentine et al., 2011; Zhou. et al., 1998). The present study found that the variation of starch content during grain filling was within the range (43-61%) reported elsewhere (McMullen, 2000; Sayar & White, 2011). This indicated that the starch synthesis are started early as a main component of oat grain, and the concentration is less affected as large increases in both grain size and starch content in the grains are increasing with development stage. Moreover, the starch content reported in this experiment was differed between the varieties and different development stages. These differences could be explained by the variation in the

content of other chemical compounds, especially crude protein. Because the protein content found in this experiment also showed some fluctuations during grain filling stages, whereas the β-glucan content was linearly increased. However, the exact statistical analysis is needed here since the supporting literatures are relatively few regarding the specific correlations of important chemical compounds in oat grain. Furthermore, the starch content could be affected by the chemical composition of oat starch itself, especially by the non-starch component of starch granules such as proteins and lipid. Zhou. et al. (1998) indicted that the most significant proteins effecting the oat starch behavior are enzymes, which relates to some properties and stability of oat starch. Hartunian-Sowa and White (1992) also found a positive correlation between lipid content in groat and the amylose content isolated oat starch. Furthermore, the extraction methods also can be a factor to influence the starch content in oat. Many studies have demonstrated that oat starch cannot be easily extracted due to the appearance of lipid in starchy endosperm(Hoover et al., 2003; Sayar & White, 2011; Wang & White, 1994b; Zhou. et al., 1998). As a result of this, the oat starch only has a little interest to cereal chemists, but understanding the accumulation features and functional properties of oat starch is still essential to achieve the maximum use of the oat cereal.

5.2.4.Gelatinization properties of oat starch

It is extensively observed that the oat starch differed from other cereal starches by its

physicochemical properties such as gelatinization properties, viscosity, paste properties, swelling power and amylose leaching extent (Hoover et al., 2003; Sayar & White, 2011; Zhou. et al., 1998). Paton (1987) studied the gelatinization properties of oat starch using DSC, and found that there is two endotherms, one around 66°C which corresponds to the melting of starch crystallites and a second at 102-104 °C which corresponds to the melting of an amylose-lipid complex. This is consistent with the result of the present study. The present result also revealed that both varieties and different development stages during grain filling significantly affected the gelatinization parameters of oat starch, especially the different development stages showed a major effect on both gelatinization temperatures and gelatinization enthalpy. Many studies have confirmed that the gelatinization properties differed between cultivars(Hoover et al., 2003; Sayar & White, 2011; Wang & White, 1994a; Zarkadas et al., 1995b). And, the researchers explained the fact that the effects of cultivars to the physicochemical properties of oat starches are related to the moisture availability, growth temperature and day length(Hoover et al., 2003). But, there is few, if any result in the literatures regarding the effects of different development stages on the gelatinization properties of oat starch.

Others reported the range of gelantinization temperatures and enthalphy of the melting of starch crystallites in oats as 56.0-74.0°C and 12.4-14.6 J/g, respectively (Hoover et al., 2003; Šubaric´, Babic´, Lalic´, Ačkar, & Kopjar, 2011), is relatively higher than that of the finding in the present study. However, higher gelatinization

temperatures were found from the early development stages in this experiment in comparison to that of final mature oat starch presented in the studies mentioned above. In earlier study, researchers demonstrated that the starch gelatinization properties are significantly affected by the molecular structure of amylopectin, but not by the amylose-to-amylopectin ration (Noda et al., 1988). Additionally, Hoover et al. (2003) reported that the lower gelatinization temperatures may result from the presence of abundant short amylopectin chains, while increases in the amylopectin branch length may result in an increased gelatinization enthalpy. Therefore, according to this present study, it could be assumed that the presence of short amylopectin chains become more abundant and the amylopectin branch length are increased in oat starch along the development stages during grain filling.

5.2.5. Accumulation of protein

The result of this thesis indicated that the protein content of per oat groat is accumulated steady along the development stages during grain filling period, and it is hard to find a similar study elsewhere to make a comparison with this findings. However, it could be somehow explained by the steady dry weight increases per oat kernel. The finding also indicated that there was no big fluctuation in the protein percentage of oat groat during grain filling period, but the variation was lower than the range (15-20%) reported on the typical mature oat grain (Biel, Bobko, & Maciorowski, 2009; McMullen, 2000). This may be explaind by the fact that protein content may vary widely in different regions. Moreover, the present study

found that both protein concentration and protein content are significantly affected by the varieties and different development stages, where Odal and Vinger always showed a relatively higher content than Haga and Belinda. This result was similar to earlier variety test in Norwegian trials 2010-2012 (Sundgren et al., 2013). Researchers indicated that the differences of protein content existed between the varieties, but the environmental factors could maximize the differences (Shewry, 2007). It has also been reviewed by David (2011) that climatic factors such as moisture, temperature, photoperiod, and incident radiation are believed to affect the protein concentration in oat. Furthermore, many studies have reported that the protein accumulation can be increased by adequate nitrogen application (David, 2011; Shewry, 2007; Welch & Yong, 1980). Welch and Yong (1980) also found that the late application of nitrogen increased the protein content more than the early application. However, in this experiment, the fertilizer (N:P:K=22:3:10) was only given once before sowing, therefore the protein content of early development stages was higher than that of final combiner-harvested stage. In addition, the plant diseases might affect the protein content, but this study was not in the focus on that specific area, despite the there are some researchers have reported a positive relationship between the protein content and the disease infection on oat grain (David, 2011; Sundgren et al., 2013). Overall, since the quantity and quality of oat protein is adequate for the most utilization purpose, the study of the accumulation features during grain filling could be encouraged, and also to be able to avoid any unfavorable degradation of protein content in oat grain.

6.Conclusion

In this study, the result from combiner-harvested oat sample suggested that, Norwegian oat variety Belinda, Odal and Vinger, all are the good choise for food, but Belinda is the best because of the relatively higher amount of β-glucans than others. However, Haga showed a significantly lower amount of β-glucans in this study, and might be a good choice for feed where the lower β-glucans content is desirable. In addition, there was a valuable observation in this study showing that more than half of the β-glucans were accumulated in 10 days between 20-30 days after antheis. It indicated that the β-glucans synthesis is most active during 20-30 days after antheis, but the reason was unknown. Consequently, more studies on the accumulation features of oat β-glucans during grain filling is expected in the purpose of understanding more about the variation in β-glucans content during grain filling for achieving maximum use of oat cereal as a healthy diet for human.

7.References

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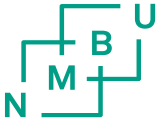

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