Quality of Norwegian barley (*Hordeum vulgare*) for food

Effects of variety and growth environment on food quality characteristics with emphasis on total β-glucan content

Kvalitetsegenskaper i norsk bygg (*Hordeum vulgare*) til mat

Betydning av sort og dyrkningsmiljø for matkvalitet med hovedfokus på totalt innhold av β-glukan

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With this master thesis I complete my five years as a student of Plant Science at the University of Life Science (UMB) in Ås. The thesis was a part of the project "Polysaccharides in barley and oats - adaptation for food and for feed". A more closely description is found in the introduction.

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Abstract

Barley (*Hordeum vulgare*) as a food crop has been a subject to increased focus over the last decades. This interest may be explained by new knowledge towards the health benefits of barley, especially linked to the amount of the dietary fibre (1→3),(1→4)-β-D-glucan in the grain, hereafter referred to as β-glucan.

This thesis has studied the content of total β-glucan in nine varieties of barley (Edel, Tiril, Heder, Helium, Marigold, Skaun, Olve, Karmosé and Magdalena) grown in different environments. The selected varieties included both varieties that are commonly used in arable farming in Norway today and varieties that obtained special qualities in the regards of starch composition and total β-glucan content. All the varieties were grown at six different locations in Norway (Jæren, Sarpsborg, Romerike, Namdalen, Apelsvoll and Vågå) and one location in Wohlde, Germany. All the data in is from 2009.

The main focus was to investigate how the content of total β-glucan changed over different growth environments. All samples were analyzed for total content of β-glucan with Near Infrared Spectroscopy (NIR), in addition some samples were analyzed by the Megazyme streamlined method to evaluate the NIR analyses. These results were later used to improve the NIR calibration. In addition, the samples were analyzed for content of protein and starch, yield, thousand grain weight and test weight.

Total β-glucan and the other parameters were compared with climate data gathered for all locations to study possible correlations between climate data and total content of β-glucan. Comparisons between total β-glucan and the other parameters were also performed to explore if there was any relationships between total content of β-glucan and the other quality and agronomic parameters. The results showed clearly differences between locations for all parameters, including β-glucan, but it was hard to explain this variation from the collected climate data. No correlations between total β-glucan and climate data were found, except for a positive correlation between the minimum temperature in the grain filling phase and total content of β-glucan. However, the summer of 2009 was wetter and colder than average from the last 5 years, and was an untypical summer for most of the locations.

Variety was found to be the most important parameter to influence the total β-glucan content (counted for 57.9% of the variation) in this study. The location counted for almost 40% of the variation.

The special starch varieties, Karmosé and Magdalena together with Olve, had the highest β-glucan content. Olve matured earlier than the other two, and yielded better than both Magdalena and Karmosé. Based on the results of this study Olve was recommended as the best variety for food. Heder and Marigold showed lowest content of β-glucan, subsequently these two varieties were recommended for animal feed, especially for poultry. Jæren was outstanding as the location with the highest average content of β-glucan for almost all varieties, but with a low yield. The field trials will be performed at least one more season and hopefully giving data with more variation in growth conditions. This is expected to be necessary to relate the variation in total β-glucan content to specific environmental conditions.

The results showed that the variation in β-glucan content within the Norwegian varieties was large, and thus a better selection between barley to food and feed should be considered by the industry.

Sammendrag

Bygg (*Hordeum vulgare*) til mat har kommet mer og mer i fokus de siste tiåra, særlig siden det har kommet ny kunnskap rundt helseeffektene av bygg. Disse helseeffektene er forbundet med innholdet av kostfiberet (1→3),(1→4)-β-D-glukan, senere omtalt som β-glukan.

Denne oppgava har tatt for seg β-glukan innholdet i ni sorter bygg (Edel, Tiril, Heder, Helium, Marigold, Skaun, Olve, Karmosé og Magdalena) dyrka under ulike forhold. Halvparten av sortene er utbredt i praktisk dyrkning i Norge i dag. De resterende sortene er spesialsorter med spesiell stivelsesoppbygning og høyere β-glukannivå. Alle sortene ble dyrka på seks ulike lokaliteter i Norge (Jæren, Sarpsborg, Romerike, Apelsvoll, Vågå og Namdalen) og en i Wohlde, Tyskland. Dataene i denne oppgava er basert på vekstsesongen 2009.

Hovedfokuset i oppgaven var å se på hvordan innholdet av β-glukan endra seg med forskjellig dyrkningsklima. Alle sortene ble analysert for innhold av β-glukan ved hjelp av Near Infrared Spectroscopy (NIR) og i tillegg ble noen analysert ved Megazyme Streamlined metoden. Dette ble gjort for å kontrollere resultatene fra NIR analysene. Resultatene ble senere brukt til å forbedre NIR kalibreringa. Sortene ble også analysert for protein- og stivelsesinnhold, samt avling, tusenkornvekt og hektolitervekt.

Total mengde β-glukan og de andre parameterne ble sammenlignet med værdata samlet inn for feltene, for å studere mulige korrelasjoner mellom klimadata og totalt innhold av β-glukan. Det ble også gjort sammenligninger mellom totalt β-glukan og de kvalitetsmessige og agronomiske parameterne for å se etter sammenhenger mellom disse og totalt innhold av β-glukan. Det var betydelig variasjon mellom stedene for alle parameterne, inkludert β-glukan, men det var vanskelig å forklare dette ut fra de valgte klimaparameterne. Det ble ikke funnet noen sammenhenger med været, annet enn en positiv sammenheng mellom minimumstemperaturen i kornfyllingsfasen og innholdet av totalt β-glukan. Sommeren 2009 var våt og kald i store deler av Norge, og var i så måte ikke en typisk sommer de fleste stedene.

I dette forsøket var sort den komponenten som hadde størst innvirkning på totalt β-glukan innhold (ca 58% av variasjonen) mens sted sto for ca 40% av variasjonen.

Om det fokuseres på høyt innhold av totalt β-glukan pekte spesialstivelsessortene Magdalena og Karmosé seg ut sammen med Olve. Olve var tidligst moden av de tre og ga høyere avling enn både Magdalena og Karmosé og er, med bakgrunn i denne oppgava, den sorten som bør anbefales som matbygg dersom høyt β-glukan innhold ønskes. Heder og Marigold var de to sortene med lavest innhold av β-glukan og kan dermed anbefales til dyrefôr, spesielt til fjørfe. Stedsmessig pekte Jæren seg ut som den lokaliteten med det høyeste innholdet av totalt β-glukan for nesten samtlige sorter. Forsøket skal utføres i minst en sesong til og dette vil gi et større datamateriale fra flere dyrkningsmiljø. Dette er trolig nødvendig for å kunne relatere variasjon i totalt β-glukaninnhold til spesielle miljøbetingelser.

Resultatene viser at variasjonen i totalt β-glukaninnhold mellom de norske sortene er stor og at sortering hos industrien av bygg til mat og fôr bør utføres i enda større grad enn i dag.

Table of contents

1. Introduction

During the last decades, studies have documented that barley has greater beneficial nutritional effects compared to wheat (reviews by (Brennan & Cleary 2005; Wood 2007)). These effects are due to the high content of dietary fibres, especially (1→3),(1→4)-β-glucans (hereafter referred to as β-glucans). β-glucan is the major component in the endosperm cell walls of barley, and accounts for about 75% of the total cell wall polysaccharides (Fincher 1975). The effects of lowering blood cholesterol levels and to attenuate the postprandial glycemic and insulinemic response are the most well documented effects (Frost et al. 1999; Gallagher et al. 1993; German et al. 1996; Newman et al. 1989). Diets that include barley (and oat) have shown preventing and rehabilitate effects on important life style diseases, in particular coronary heart disease and diabetes. These effects are linked to dietary fibres, especially β-glucan.

Over the last few years the Norwegian food industry has shown an increasing interest in barley for food. In this regard it is important to emphasize the quality characteristics important for food consumption. However, over the last decades Norwegian barley breeders have mainly focused on agronomic parameters and feed quality, making a wide range of the Norwegian barley varieties better suited for feed than food. A high content of β-glucan is desirable for human consumption and thus regarded as a food quality characteristic. In contrast, this is opposite to what is desired for feed purposes, especially when barley is used for non-ruminant animals like poultry.

International studies have previously shown that the β-glucan content varies with growing environments (Ehrenbergerova et al. 2008; Pérez-Vendrell et al. 1996; Zhang et al. 2001; Özkara et al. 1998). It is reasonable to expect similar effects in Norway as well, where the climate conditions vary widely among the different cereal cultivation areas. The composition of non-starch polysaccharides (including β-glucan) in some Norwegian barley varieties have been characterized and documented in a couple of studies the recent years (Holtekjølen et al. 2006b; Holtekjølen et al. 2008b). In addition, Anker-Nilssen et al. (2008) performed experiments using Norwegian varieties grown under controlled temperature in growth chambers. From these studies the variety Olve showed an especially high content of β-glucan (Holtekjølen et al. 2006b; Holtekjølen et al. 2008b), and it was observed a significant increase in soluble β-glucan with increasing temperatures (Anker-Nilssen et al. 2008).

However, there is a lack of information regarding possible variations in total β-glucan content with different climatic conditions and thus, different growth locations within Norway. Information regarding effects of temperature and precipitation on β-glucan levels and information on stability of different barley varieties will be valuable for both the industry and breeding companies.

This thesis will therefore focus on the variation in total β-glucan content among different Norwegian barley varieties and examine effects of the growing environment and climate on the content of β-glucan. A special attention was given to the variety Olve, to see if it still showed a high β-glucan content when grown at different locations in Norway, and if the content of β-glucan correlated with growth temperature. Other quality aspects important for the use of barley to food was included, like starch and protein content and physical quality parameters like test weight and kernel weight and degree of pre-harvest sprouting. The relationships between the different quality parameters are also studied.

This master thesis was part of an ongoing, four-year research project (2009-2012) “Polysaccharides in barley and oats - adaptation for food and for feed”. Only data from the first year were used in this thesis. The project was founded by the Foundation for Research Levy on Agricultural Products, The Norwegian Agricultural Purchasing and Marketing Co-operation's joint venture for the development of feed products for livestock (Felleskjøpet Fôrutvikling), Lantmännen Cerealia, Norgesmøllene, Graminor, Strand Unikorn/Norgesfôr, Ottadalen Mølle and The Norwegian Agricultural Extension Service.

The project is managed by Bioforsk Øst Apelsvoll in close cooperation with the Department of Plant and Environmental Sciences, the Department of Animal and Aquacultural Sciences at University of Life Sciences (UMB) and Nofima Mat. The principal objective with the project was to obtain new knowledge for the grain industry on the effects of genotype, climate parameters and processing methods on the grain quality of starch and cereal fibres in barley and oats.

2. Research aims

The aims of this master thesis were:

- Study the grain content of total β-glucan in commonly used Norwegian barley varieties from a range of different growing climates
- Study the content of other important quality and agronomic parameters (such as yield, test weight, thousand grain weight, protein and starch) to see if there was any relation to total β-glucan content.
- Examine weather data from the different locations to see if there was any relation between the weather data and total content of β-glucan.

3. Literature study

3.1. The barley crop

Domesticated barley (*Hordeum vulgare*) belongs to the family Poaceae (grass family) and the tribe Triticeae. It is a widely accepted theory that barley originated from the Fertile Crescent of Middle East, as many of the other commonly cropped cereals. However, controversy is connected to this theory as other sites of origin are also being proposed and discussed. Other origins suggested are claimed to be in: Morocco/Western Mediterranean (Molina-Cano et al. 1987; Molina-Cano et al. 1999), Ethiopia (Bekele 1983) and Tibet (Xu 1982). More recently Molina-Cano et al. (2005) proposed a polyphyletic origin for barley, which means that there has been several events of domestication at different cites (Fertile Crescent, Ethiopia and Western Mediterranean) and not only at one place.

H. vulgare is one of the most genetically diverse cereal species. Barley can be classified as two- or six-rowed, hulled or hulless, winter or spring type or after the composition of starch (waxy, high-amylose and normal) (Baik & Ullrich 2008; Hockett 2000). The spike of barley is made up of spikelets attached to nodes of a flat, zigzag rachis (main axis of the spike). A spikelet is single-flowered and is build up of two glumes and a floret. Each node has three spiklets attached, and they alternate from side to side in the whole length of the rachis (Figure 1).

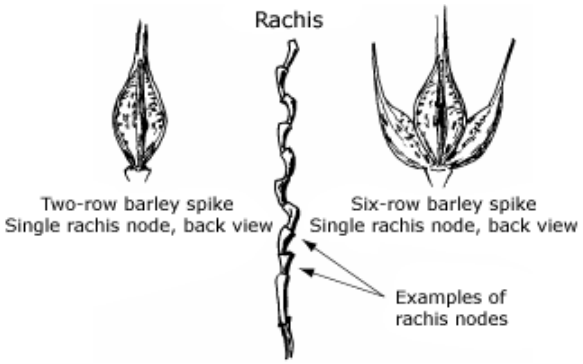

Figure 1: Morphological description of the two- and six-rowed barley ear. Adapted from http://www.brewingtechniques.com/bmg/graphics/rachis.gif

In two-rowed barley only the middle spiklets of the triplet is fertile, where in six-rowed barley all three spiklets are fertile. In six-rowed barley 2/3 of the kernel are twisted because of insufficient space for symmetrically growth. Especially in the lower part of the kernel the twist is most distinct. Barley is diploid (2n = 2x-14), and it has a small number of chromosomes, which are relatively large (Newman & Newman 2008; Reid 1985). Figure 2 shows a barley kernel with the main morphological parts, the fruit-and seed coat (pericarp and testa), endosperm, scutellum and the germ. The hull (consisting of the palea and lemma) is for barley tightly attached to the grain, and is usually removed by pearling when used for food. In naked types the hull is loosely attached to the grain, and usually falls off during harvesting.

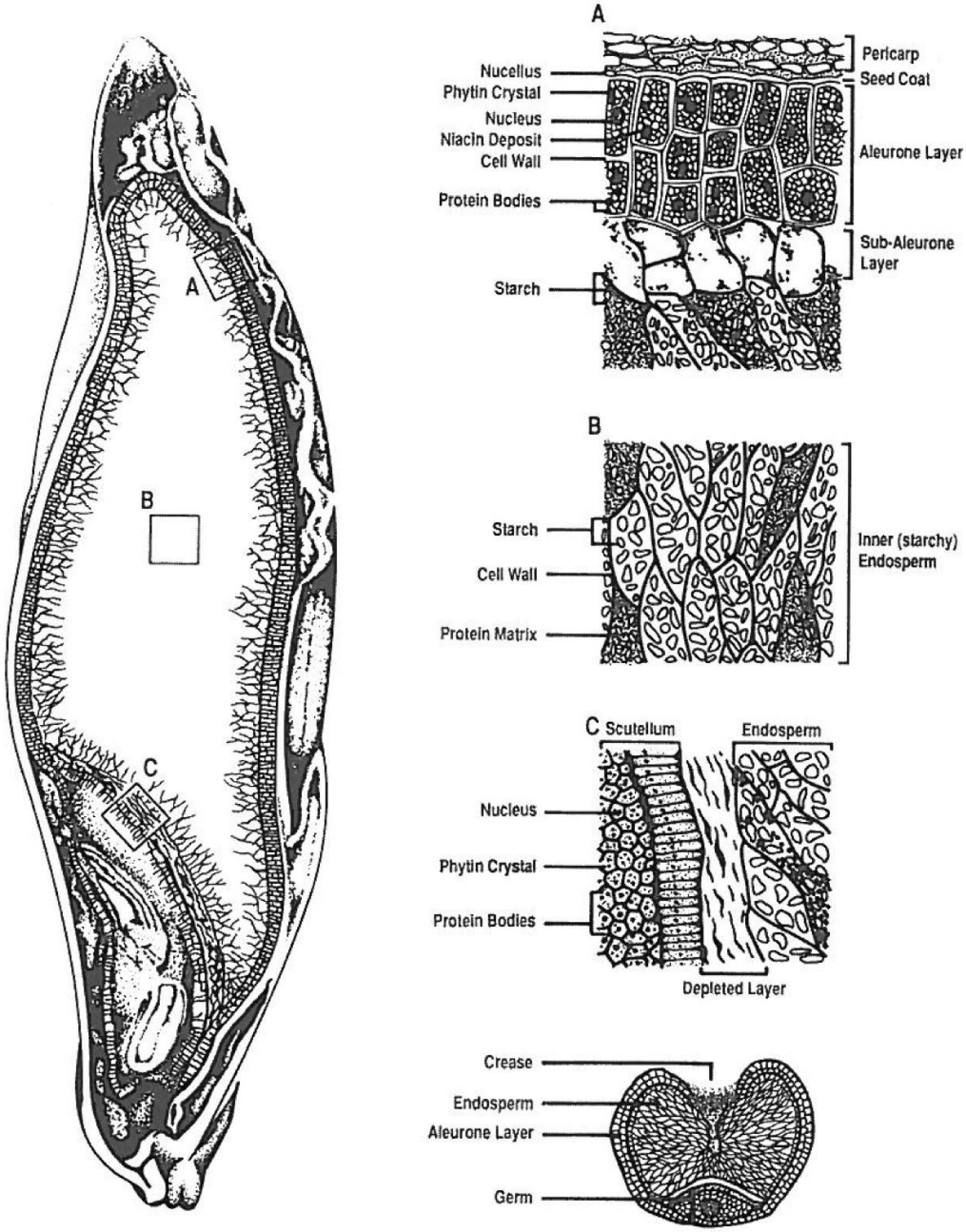

Figure 2: Morphological structures of the mature barley grain as viewed both longitudinally and transvertically. The enlarged sections cover the outer layers (A), the inner endosperm (B) and the scutellum-endosperm border (C). Adapted from (Newman & Newman 2008).

3.1.1. Agronomy and production

Barley is the most widely geographically grown cereal crop in the world. It has an ability to mature earlier than other cereals, if the right variety is chosen. It can also be grown on broader latitudes and higher altitudes than any other cereal crops, as well as further into deserts than any other cereal (Baik & Ullrich 2008; Hockett 2000). Barley is the fourth most produced cereal commodity in the world following maize, rice and wheat (Food and Agriculture Organization of the United Nations 2009b). Russia is producing

the most barley (approximately 23 million tons) in the world, and Ukraine, France, Germany, Canada and Spain is the next five, with a production about 12 million tons (Food and Agriculture Organization of the United Nations 2009a). The global use of barley today is approximately two-third to animal feed, one-third to malting and only 2% to food consumption directly (Baik & Ullrich 2008).

The barley acreage in Norway is approximately half of the total cereal and oil seed acreage (Statistics Norway 2009a), and the production is around 600 000 tons (Figure 3). The traditions of using barley for food in Norway goes back to the Neolithic Age, and was known as the most important cereal crop at that time (Mikkelsen 1979). Barley is well adapted for the Norwegian climate, which might explain why as much as half of the cereal acreage is cropped with barley. From the 1900 food consumption of barley in Norway was relatively quickly replaced, mainly by wheat. Thus, today only 0.3-0.7% of the barley produced in Norway is used for food, and this production (3000 tons) covers all the barley needed for food in Norway (Norwegian Agricultural Authority 2009; Statistics Norway 2009b).

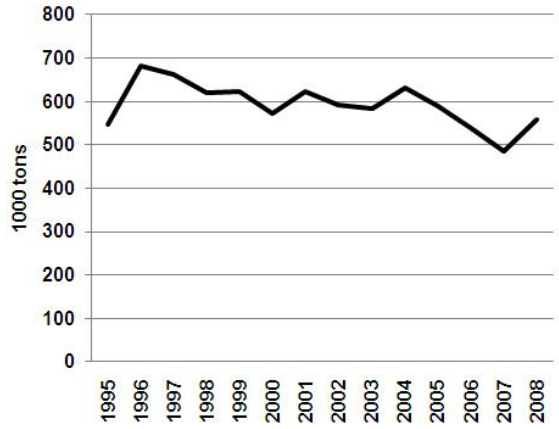

Figure 3: Barley production in Norway from 1995 to 2008 in 1000 metric tons (Statistics Norway 2009b).

The Scandinavian Feed Unit (SFU), in Norway called “feed units lactation” (FE_m) was from the year 1916 related to 1 kg barley as basis for the unit (Sundstøl 1993) and this states the importance of barley for feed (especially to ruminants) in Scandinavia the last century. Today the definition is 1 FE_m = 6900 kJ NE_l (net energy lactation) = 1 kg barley with 87% dry matter (Harstad 2009).

Barley develops best under cool and relatively dry conditions, similar to the conditions often found in Scandinavia. High humidity may result in problems with different diseases. Winter types of barley is less winter hardy than wheat and rye, but more hardy than oat (Hockett 2000). In Norway, we grow winter varieties of wheat and rye, but not of barley and oats. Winter barley is grown to large extents in Southern Sweden and Denmark. Experimental cultivation of winter barley was done in Norway from 1993-96 (Åssveen et al. 1997), and the conclusion drawn was that cultivation of winter barley in Norway was too risky with the present varieties. Spring barley would give a better and more stable yield over years. It has not been done any further work either with more experimental cultivation or breeding in Norway since 1996, even though the climate might have changed in the more favourable direction for cropping of winter barley.

Another negative aspect with winter barley is the higher pathogen pressure, especially from powdery mildew (*Blumeria graiminis*) and rust diseases (*Puccinia spp*). Pathogens survive on winter barley during winter, and cause an earlier and stronger infection pressure in the spring barley. This again will increase the use of pesticides. This was the argument behind the 13 year ban (from 1967 to 1980) of winter barley in Denmark (Stabbetorp 1995).

When it comes to soil conditions, barley prefers a well-drained fertile loam or light clay as sandy soils will not hold enough water. In heavier clays barley will easily get problems with water logging. Barley is the most tolerant cereal to alkaline conditions, and the most sensitive to acid soil. It is known as one of the more drought resistant cereal, but this can also be explained by its faster maturation. The most critical stage for water supply is during in the late boot-heading stage (Hockett 2000). In Norway rye is considered as the most drought resistant cereal, and is the best performing cereal on drought-sensitive sandy soil.

Planting of barley should be done as early in spring as possible, when the soil is dry enough for planting and treatment by machinery. In Norway the recommended drilling depth are 3 cm and it is recommended 400-450 viable seeds per m^2, which gives a seed rate of 15-25 kg/daa (Flaa 2009).

3.2. Chemical composition of barley

The barley grain contains starch (60%), fibre (20%), proteins (10%) and has a low content of fat (3%) and sugars (2%) (Table 1). Barley is an excellent source of a range of vitamin Bs like vitamin B1 (0.57 mg/100 g), B2 (0.22 mg/100 g) and B6 (0.33 mg/100 g), niacin (6.4 mg/100 g) and pantothenic acid (0.73 mg/100 g) (Hockett 2000). Barley has got eight naturally occurring tocopherols (vitamin E) (Morrison 1993). It is also a good source of minerals as P, K, Mg, Ca, Na, Fe, Zn, Mn, and Cu (Liu et al. 1974).

The major component of a barley kernel is carbohydrates, which comprise approximately 80% of the total dry matter. Carbohydrates are composed of carbon, hydrogen and oxygen, and are grouped into mono-, di-, oligo-, and polysaccharides after degree of complexity. Polysaccharides, the most complex carbohydrates, make up the bulk of grain carbohydrates. In barley, as in other cereals the polysaccharides are usually classified as starch and non-starch polysaccharides (NSP) (Newman & Newman 2008).

Mixed-linked (1→3),(1→4)-β-D-glucans (β-glucans), arabinoxylans and cellulose ((1→4)-β-D-glucan) are the three major non-starch polysaccharides found in barley. Total dietary fibre (TDF) includes in addition glucomannan and (1→3)-β-glucan. Lignin is closely associated with cellulose, and is therefore often included in the carbohydrate complex (even though it is not a carbohydrate) and in the dietary fibres (Newman & Newman 2008).

Table 1: Variation in chemical composition (given in % w/w dry basis) of grain of 2-rowed (n=81) and 6-rowed (n=11) barley cultivars grown in Sweden (Åman et al. 1985).

Chemical constituent	Mean value	Standard deviation	Minimum value	Maximum value	Coefficient of variation (%)
2-row barley					
Glucose	0·3	0·1	0·1	0·8	57·5
Fructose	0·1	0·05	Traces	0·4	46·2
Sucrose	1·6	0·7	0·6	3·1	41·3
Fructosans	0·4	0·2	Traces	0·8	57·4
Starch	62·2	2·0	55·9	66·6	3·2
Crude protein ($n \times 6{\cdot}25$)	10·7	1·0	8·6	13·4	9·7
Crude fat	3·0	0·2	2·7	3·3	5·9
Ash	2·4	0·2	1·8	2·9	9·4
Total fibre	19·3	1·9	14·0	24·7	9·7
Crude fibre	3·2	0·5	2·5	5·2	14·7
6-row barley					
Glucose	0·6	0·5	0·2	1·4	80·7
Fructose	0·2	0·1	0·1	0·5	66·8
Sucrose	1·9	0·7	1·1	3·9	40·1
Fructosans	0·3	0·3	Traces	1·0	95·9
Starch	58·9	3·5	52·9	64·1	6·0
Crude protein ($n \times 6{\cdot}25$	11·5	1·7	8·9	14·0	15·2
Crude fat	3·3	0·3	2·8	3·7	8·3
Ash	2·4	0·2	2·2	2·7	7·4
Total fibre	20·9	2·0	17·8	23·8	9·4
Crude fibre	4·2	1·0	3·1	6·0	24·2

Åman et al. (1985) conducted a comprehensive study of the carbohydrate composition in Swedish barley varieties (Table 1). The study included 92 cultivars grown on 16 locations from 66°N to 56°N. They found only small differences between 6- and 2-rowed barleys. 2-rowed barley had slightly higher content of starch, while 6-rowed barley had a higher content of crude protein, total fibre and crude fibre. In another study including Norwegian barley varieties, no significant differences were found between Norwegian 6- and 2-rowed barley varieties for starch and TDF contents (Holtekjølen et al. 2006b). However, significantly higher protein contents were seen in the Norwegian 2-rowed barley varieties compared to the 6-rowed varieties.

3.2.1. Starch

In barley starch is the major source of energy when used for food and feed, as well as for growth of the new plant after germination. As the major component, it is also the component that has the largest variation of content in the grain (Newman & Newman 2008). Åman et al. (1985) found a variation from 52-66% in starch content in the study of Swedish barleys presented in Table 1. The starch level among the Norwegian barley varieties is reported between 51-62 % (Holtekjølen et al. 2006b). However, a range of barley genotypes having mutations in starch synthesizing enzymes are identified, some with a very low starch content (less than 30% starch) (Munck et al. 2004).

Starch is only found in the endosperm of mature kernels, but the distribution is not uniform within the endosperm. The most starch is found in the centrally located endosperm cells. The subaleurone and aleurone region (Figure 2A) contains most

protein (Duffus & Cochrane 1993; Newman & Newman 2008). Type and quantity of starch in the barley could give different effects on nutritional quality, processing characteristics, and end-product utilization. Milling of high β-glucan barley, with modified amylose content (either waxy or high amylose), gave a much lower flour yield than normal starch barley with the same β-glucan level. This suggest that the starch composition probably affects the milling process more than the β-glucan content (Izydorczyk et al. 2003). The volume and firmness of breads could vary with flour with different starch composition, and with different processing of the grain (Gill et al. 2002).

The starch is deposited in amyloplasts as granules, and in mature barley grain starch can be divided into two different types of granules. The large, lenticular granules, with a diameter from 15-25 μm, are often referred to as A-type granules. The smaller granules are less than 10 μm in diameter, and have an irregular shape. These are often referred to as B-type granules (MacGregor & Fincher 1993; Savin & Molina-Cano 2002). 95% of the starch is stored in the kernels during the 11-28 first days after ear emergence, and during this period the ratio of amylose to amylopectin increases to the final ratio (Hockett 2000). The A-type granules are the first one to appear after ear-emergence, only a few days after ear-emergence they are found in the endosperm (Briarty et al. 1979). Formation of the B-type granules occurs from 15 days after ear-emergence. When all starch is developed, there is normally a 1:10 ratio in number of granules between the A- and the B-type (Jenner et al. 1991). Even though the B-type (the smallest one) is completely outnumbered by the A-type, they only count for one third of the total starch weight (Evers 1973; Evers & Lindley 1977).

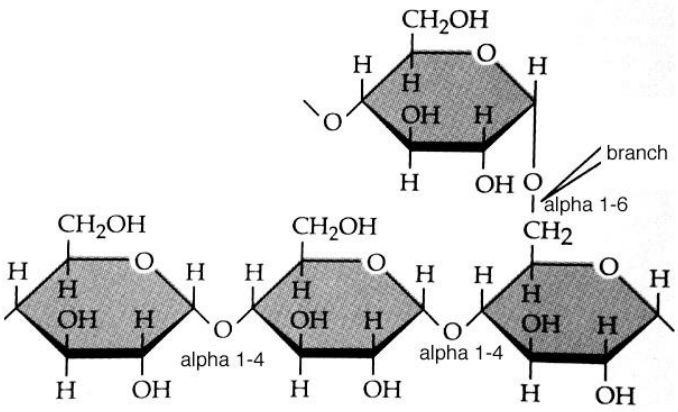

Figure 4: Structural formula of starch. Amylose and amylopectin structure with glucose molecules linked together with alpha-1-4 and alpha 1-6 linkages. Adapted from: http://academic.brooklyn.cuny.edu/biology/bio4fv/page/starch.html

Amylopectin and amylose are the two different structural types of barley starch. Amylopectin is branched while amylose has a low level of branching (much less than amylopectin) (MacGregor & Fincher 1993). Amylose is build up by glucose molecules linked together with alfa-1-4-bindings in relatively long, and very little branched chains (Figure 4). In amylopectin the glucose units are bound together with alpha-1-4 linkages, and these again are interconnected through alfa-1-6 linkages which results in a much more branched appearance as seen in Figure 5 (Aspinall & Greenwood 1962). The arrangement of amylose and amylopectin in the starch granule is still unknown (MacGregor & Fincher 1993).

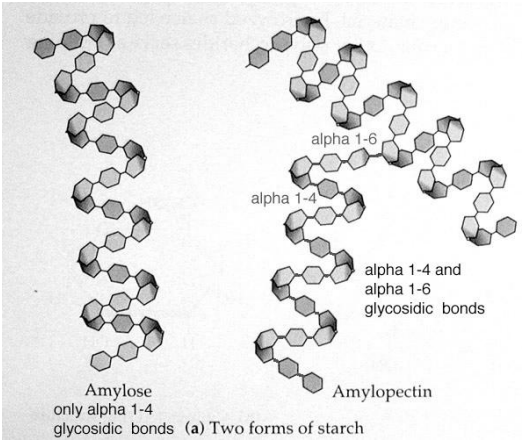

Figure 5: Schematic outline of amylose (to the left) and amylopectin (to the right) molecules. Adapted from: http://academic.brooklyn.cuny.edu/biology/bio4fv/page/starch.html

Amylopectin is the major molecule in most barley starches, giving a 3:1 ratio of amylopectin:amylose (AP:AM). Barley with the normal 3:1 ratio is named normal starch (or nonwaxy) barley. Waxy barley is used for varieties with high levels (95-100%) of amylopectin, and then low levels of amylose (0-5%). A third starch type is barley with a higher amount of amylose than normal (40-70% of total). These barley types are classified as high-amylose barley (Newman & Newman 2008). Waxy barley contains significantly less starch and a significantly higher content of total and soluble β-glucans than normal starch barleys (Gao et al. 2009; Holtekjølen et al. 2008a; Ullrich et al. 1986; Xue et al. 1997). High-amylose varieties also show higher β-glucan contents compared to normal starch barleys (Gao et al. 2009; Holtekjølen et al. 2006b). In Norway, normal starch varieties are dominant.

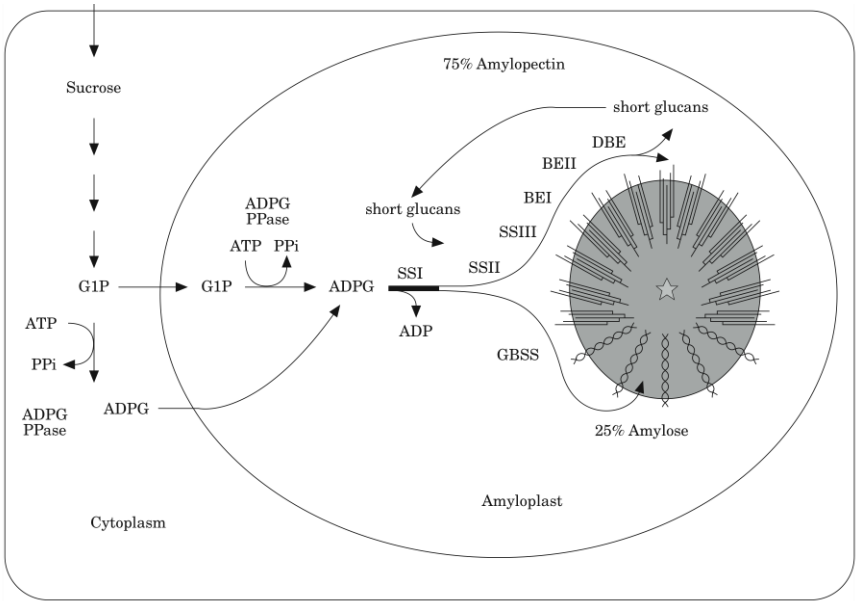

Figure 6: Starch synthesis in cereals, a general scheme. Adapted from Rahman et al. (2000).

There are at least four classes of starch synthases in cereal important for the starch synthesis. These four are: granule bound starch synthase (GBSS), starch synthase I, II and III (SSI, SSII, SSIII). As seen in Figure 6 GBSS is closely linked to the amylose

synthesis, and SSI, SSII, SSIII is linked to the amylopectin synthesis (Rahman et al. 2000). GBSS (known as the waxy protein) is critical for the synthesis of amylose, as it is the only enzyme that controls the amylose synthesis. This makes GBSS to the primary target when the aim is to reduce the content of amylose (and thereby increase the content of amylopectin) and produce waxy barleys. SSI, SSII and SSIII are all thought to have a predominant role in the synthesis of amylopectin (Rahman et al. 2000). There are several barley varieties today containing close to zero amylose (waxy barley), as only one enzyme is controlling the amylose synthesis. The opposite (barley with close to zero amylopectin) is not known. This is probably related to the higher number of enzymes influencing the amylopectin synthase (Rahman et al. 2000).

3.2.2. β-glucan

β-glucans are almost exclusively found in the cell walls of almost all members of the plant family Poaceae (Burton & Fincher 2009). It is an ongoing discussion why Poaceae has got this adaption and few other plant families have this adaption. Instead of β-glucan other plant families have got other principal microfibril cross-linking polymers (Buckeridge et al. 2004). The research has mainly focused on fundamental knowledge around β-glucans, and thus the present knowledge does not provide any clear answers. One hypothesis suggests that a rapid accumulation and hydrolysis of β-glucan will give more flexible cell wall architecture, which will be able to respond more rapidly on physiological signals for wall extensions under different conditions. This has been linked to the fact that many of the grasses are adapted to open areas where the light intensity is high, giving a high rate of photosynthesis and a need for rapid cell elongation. However, since the plants with the highest levels of β-glucan are the C3-plants (plants which the CO_2 is first fixed into a compound containing three carbon atoms before entering the Calvin cycle of photosynthesis), this theory is not so likely to be correct (Buckeridge et al. 2004).

The cold-season grasses are among the species that store fructan before starch. This preference demonstrates an apparent advantage for more easily metabolism of soluble and accessible molecules at cooler temperatures (Hendry 1993). Buckeridge et al. (2004) questioned if β-glucan *may be a more easily mobilized reserve material than starch at limiting temperatures and thereby will permit a more rapid germination response to appropriate environmental cues* (Buckeridge et al. 2004, p.125).

The highest amounts of β-glucan are found in barley and oats, but rye and wheat are as well sources for β-glucans in the human diet (Tiwari & Cummins 2009; Wood 2007). Levels for the different cereals are 2-10% for normal starch barley (Ehrenbergerova et al. 2008; Fincher & Stone 2004; Güler 2003; Lee et al. 1997; Wood 1994), 6-15% for waxy hulless barley (Fincher & Stone 2004), 3.8-6.1% for oats (Fincher & Stone 2004; Lee et al. 1997), 1-2% in rye (Ragaee et al. 2001) and less than 1% in wheat (Beresford & Stone 1983).

β-glucan is the major component in non-starch polysaccharides (NSP) in barley together with arabinoxylans and cellulose. Cellulose is primarily found in the hull, pericarp and testa (Fincher & Stone 1986). Only small amounts are found in the aleurone (Figure 2A) and starchy endosperm (Figure 2B) (MacGregor & Fincher 1993). Thus, during dehulling much of the cellulose disappears. MacLeod and Napier (1959)

found that only 4% of the cellulose is left after removing the hull. The two other components, β-glucan and arabinoxylans, are both integral components of the cell walls of the aleurone and starch endosperm. There are about similar amount of both of β-glucan and arabinoxylans, but they are inversely distributed in the aleurone and starchy endosperm cell walls (Table 2). The deposition of β-glucan in the cell walls mainly happens in the later stages of the grain filling, and in a trial with barley grown in Scotland (Swanston et al. 1997) the β-glucan content increases most rapidly from 300-600 degree-days after anthesis. Another study (Coles et al. 1991) found the β-glucan accumulation to happens most rapid from 17-30 days after anthesis, and this correspond with the period of rapid accumulation of dry matter in the grain.

Table 2: Distribution of arabinoxylan and β-glucan in barley grain (Fincher 1975; MacGregor & Fincher 1993)

	Aleurone cell walls	**Starchy endosperm cell walls**
β-glucan	25%	75%
Arabinoxylan	71%	20%

β-glucans can be seen as unbranched, linear polysaccharides of β-D-glucosyl, where approximately 30% is (1→3)-linkages and 70% is (1→4)-linkages. The β-glucan chain is normally built up with blocks of two or three contiguous β-(1→4)-linked units separated by a single β-(1→3)-linkage (Figure 7) (Woodward et al. 1983). There are not known blocks of two or more (1→3)-linkage (Edney et al. 1991; Woodward et al. 1983). It is not possible to show a single structure of β-glucan, since they belong to a group of polysaccharides heterogeneous in molecular size, solubility and molecular structure. It is the kinks made by β-(1→3)-linkages that causes the irregularities in the chain, and this influence the solubility (Bacic & Stone 1981; Edney et al. 1991).

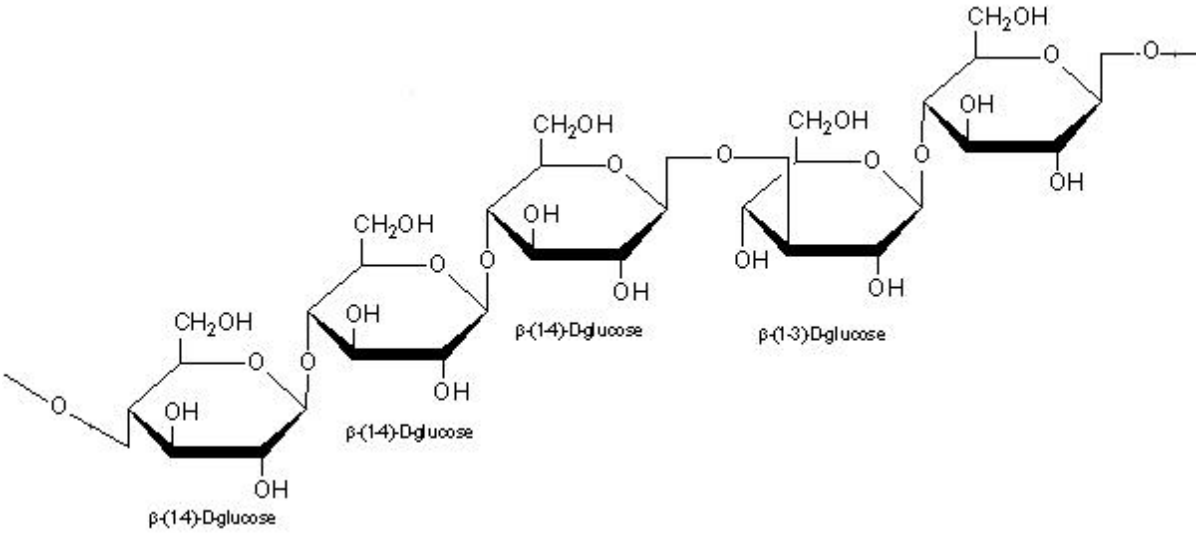

Figure 7: The chemical structure of cereal (1→3),(1→4)-β-D-glucans. Adapted (partly altered) from http://www.sigmaaldrich.com/etc/medialib/life-science/biochemicals/migrationbiochemicals1/cerial-beta-glucan.Par.0001.Image.599.gif

3.2.3. β-glucan in barley

β-glucan is found in the cell walls of both the aleurone layer and the starchy endosperm. In both cell walls types, it is found together with arabinoxylan, another large-molecular-weight polysaccharide. In the endosperm walls there is a greater amount of β-glucan compared to arabinoxylan (Newman & Newman 2008).

Åman and Graham (1987) found that it is possible to achieve higher β-glucan levels in barley than oats. Barley has a more even distribution of the β-glucan within the grain than oats. In oat most of the β-glucan is found in the outer position of the kernel where they are concentrated in the subaleurone layer (Figure 2A) as structural cell wall components (Miller & Fulcher 1994; Zheng et al. 2000). In a Finnish study (Lehtonen & Aikasalo 1987) it was shown that 2-rowed barley had generally higher content of β-glucan than 6-rowed barley.

Significantly higher values of β-glucan were found in varieties of waxy starch barleys than normal starch barleys (Ehrenbergerova et al. 2008; Fastnaught et al. 1996; Granfeldt et al. 1994; Holtekjølen et al. 2006b; Ullrich et al. 1986; Xue et al. 1997). The same was found for high-amylose barleys, with more β-glucan than normal starch barley (Granfeldt et al. 1994; Oscarsson et al. 1998). The waxy and high-amylose varieties have shown lower yield than the normal starch varieties (Oscarsson et al. 1998). In the US many of the waxy varieties also are hulless (Hang et al. 2007; Rey et al. 2009). The hulless has lower yield potential, but they give fewer processing operations after harvesting (Bhatty et al. 1975; Cavallero et al. 2004; Rey et al. 2009). At the moment barley is paid after amount, not quality. A different price system is needed if hulless, and lower yielding barley with interesting quality characteristics, should be cropped more widely. In barley most of the β-glucan (99%) is found the endosperm, so by dehulling the risk of losing β-glucan is low (Henry 1987).

3.2.4. Factors influencing β-glucan content

There is an ongoing discussion whether genetics or environment is the most important factor determining β-glucan content in barley. Both factors have been given importance, and Ehrenbergerova et al. (2008), Özkara et al. (1998) and Pérez-Vendrell et al. (1996) found that both environmental and genetic variation was important for β-glucan content. Other studies (Henry 1986; Molina-Cano et al. 1997; Stuart et al. 1988) found the genetic factor to be most important. Contrarily, Zhang et al. (2001) found environment to be the factor mainly influencing the β-glucan content.

Zhang et al (2001) concluded that conditions favouring endosperm development also increased the β-glucan accumulation in the grain. For the endosperm development, high precipitation is unfavourable and high temperatures shorten the grain filling period. This indicates that conditions providing a longer grain filling period might give a higher content of β-glucan than growing conditions giving a shorter grain filling period (Zhang et al. 2001). Fastnaught et al (1996) and Perez-Vendrell et al. (1996) found that warmer temperatures and high precipitation during grain filling lead to lower β-glucan content, while Wallwork et al. (1998) did not find any effect of high temperatures on β-glucan content. Ehrenbergerova et al. (2008), Swanston et al (1997) and Morgan and Riggs (1981) all found that total β-glucan content increased when barley was grown in hot and

dry conditions. Conflicting findings by Savin et al. (1997) showed that short periods with very high temperatures (30˚C) decreased the content of β-glucan in barley.

The timing of the heat and drought stress (Savin et al. 1997), as well the duration of the stress period (Morgan & Riggs 1981), effects the β-glucan level. Macnicol et al. (1993) found that drought stress late in the grain-filling period had no effect on the total β-glucan content. The same study found a 24% reduction in β-glucan content because of water stress 17 days after anthesis, but no reduction due to heat stress (same result as Savin et al. (1996)). This is also in agreement with the results of Coles et al. (1991) where the β-glucan content decreased with increasing drought stress. Other studies (Aastrup 1979) have on the other hand found water stress to increase content of β-glucan.

In a study from the Czech Republic; Ehrenbergerova et al. (2008) found that higher precipitation than average during flowering time and grain filling had negative impact on the content of β-glucan. This corresponds with results from Turkey (Güler 2003) showing decreased β-glucan content of the grain with increased irrigation. Colder temperatures are often linked to periods with rain, and Ehrenbergerova et al. (2008) found that lower temperatures during flowering time also decrease β-glucan content.

A number of studies (Güler 2003; Henry 1986; Oscarsson et al. 1998; Sørensen & Truelsen 1985; Truelsen 1987) have found that increased application of nitrogen significant increased the level of β-glucan. These results are in line with studies of oats (Baur & Geisler 1996; Brunner & Freed 1994). However, a study from central and north-central Montana (Jackson et al. 1994) found grain β-glucan content to be more related to environmental factors than to nitrogen application.

Few of the studies referred to in this chapter, have tried to isolate the effect of one environmental factor. This makes it difficult to study the importance of single environmental factors and to reveal the basic biological function (Anker-Nilssen et al. 2008).

There is no doubt that climate influences the content of β-glucan in barley grain, although it is hard to draw a clear conclusion from the studies mentioned above. Quite a few of the studies are conducted in the field making them hard to interpret, as there might be complex interactions as well as variation in other growth factors affecting the results. Therefore, it is not surprising that studies from field trials all over the world will give variable results.

Anyhow, the main features regarding β-glucan and factors important for total grain content seems to be temperature, water and nitrogen. High precipitation seems to lower the content of β-glucan, while dry conditions increases the content. There is certainly a need for more research on the impact of timing and duration of different types of environmental stress, especially linked to the grain filling period, as this seems to be of importance. Increasing the amount of nitrogen fertilization seems to have a positive impact on the amount of β-glucan.

3.3. Barley for food

Barley, together with wheat, was one of our earliest domesticated cereals. Traces of barley used for human consumption have been found in the Fertile Crescent of Middle East as early as 8000 BCE (Smith 1995).

After the twentieth century, the quantity of barley used in the human diet declined due to the awareness of its lower palatability, unfavourable baking quality and milling characteristics (Hockett 2000; Newman & Newman 2008). Meanwhile the consumption of other grains such, as wheat, rye and oats increased (Newman & Newman 2008).

The recent rise of the world market price of wheat and the newly discovered health benefit of barley has resulted in an increased interest in barley as food ingredient. It is important to produce barley that meets the quality demands from the food industry. Characteristics important for optimal quality would include different nutritional components (such as high content of starch, protein and β-glucan (Kasha et al. 1993)) with technological properties that is valuable for processing and product quality. For a baker, variations in β-glucan content will affect the baking properties of the flour. In a practical context this means that the water uptake of the flour will vary with a variation in β-glucan content, which can cause problems while baking (Holtekjølen et al. 2008a).

3.3.1. Health benefits of barley

The fact that β-glucan is regarded as a functional dietary fibres can explain a lot of the recent interest (Brennan & Cleary 2005). The official definition of dietary fibre is set by American Association of Cereal Chemist, (AACC), and it states:

"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." (AACC 2001) page 112.

The high β-glucan content is one of the reasons why barley is considered as a relatively rich source of dietary fibre (Brennan & Cleary 2005). Dietary fibres have shown a wide variety of potential health benefits including bowel transit time (Feldheim & Wisker 2000), prevention of constipation, colon (colorectal/large bowel) cancer (Bingham 1990; Hill 1997), lowering the blood cholesterol and regulation of glucose levels and thereby help controlling diabetes (Frost et al. 1999; Gallagher et al. 1993; German et al. 1996) and production of short-chain fatty acids which promote colonic health (Karppinen et al. 2000; Velazquez et al. 2000; Wisker et al. 2000). Dietary fibres promote the growth of beneficial gut microflora (Crittenden et al. 2002; Tungland 2003). Although, the positive effect against colon cancer is now considered doubtful. A review by Faivre and Bonithon-Kopp (1999) summarized that 12 case control studies support the effect from dietary fibre on colon cancer, nine studies found no significant effect, while two studies found an increased risk connected to high fibre intake. Newman et al. (1989) found that

a high β-glucan content is beneficial in human nutrition because it will restrict caloric intake, moderate hyper-cholesteremia and stabilize blood glucose for diabetics.

The US Food and Drug Administration approved a health claim for barley in 2006 (U. S. Food and Drug Administration 2009). To qualify for the health claim the barley food item must contain at least 0.75 g of soluble fibres per serving. The health claim was proposed based on the knowledge that the soluble fraction of the β-glucan fraction lower the total and low-density-lipoprotein (LDL) cholesterol levels (Brown et al. 1999; Pins & Kaur 2006) and a reduction in glycemic response among women (Kim et al. 2006). The study of Kim et al. (2006) concluded that a consumption of at least 2 g β-glucan per meal could reduce the risk factors for developing type II diabetes in overweight women.

A meta-analysis of 67 controlled trials done by Brown et al. (1999) suggested that 3 g soluble fibre from oats (corresponds to three servings of oatmeal with 28 g each) could decrease total and LDL cholesterol by 0.13 mmol/L.

3.4. Barley for feed

Corn, barley, oats and wheat are the four major feed grains in the world. Barley is used in feed to ruminants (as a source of energy and roughage), swine (major source of energy and protein) and laying hens (energy and to support egg production) (Albustany & Elwinger 1988; Arscott & Rose 1960). A high content of starch, protein and lipids are ideal to animal feed, while the β-glucan level should be low (Kasha et al. 1993). β-glucan is known cause digestive problems in broiler chickens. The chickens produce sticky, wet droppings that easily cause sanitary problems. To reduce these problems enzymes are added to the feed (Bhatty 1993; Hesselman & Aman 1986). For laying hens the problems are not so present, but too much barley can cause more dirty eggs (Jeroch & Dänicke 1995).

In poultry feed β-glucan acts like an indigestible barrier around the endosperm cells forming a viscous solution in the digestive tract, which easily capsule available nutrients. These two factors restrict the nutrients availability of barley for poultry and piglets (Hesselman & Aman 1986). As an attempt to reduce the effect of β-glucan, the degradable enzyme β-glucanase has been added to poultry feed that contains barley. This practice improved feed consumption, weight gain, higher feed efficiency and better cage cleanliness (Bhatty 1993). The best malting varieties are also often the best varieties used for poultry feed, as the malting barleys are commonly low in β-glucan content which is optimal for poultry feed. However, the protein content in barley should be low when used for malting while a high protein content is desired in poultry feed (Kasha et al. 1993).

Barley is rarely fed to animals as whole grains. There are a variety of methods used to improve the feed value: grinding, pelleting, flaking, cubing, rolling or micronizing. Removal of hull improves the feed value, especially for swine (Just 1982; Larsen & Oldfield 1961). Lysine is the main limiting amino acid for swine. High-lysine barley (discovered in 1970 of Munck et al. (1970)) fed to pigs, mice and rats improved the growth rate and increased the biological value of barley as feed (Bhatty 1993; Hockett 2000).

For ruminants barley is an excellent source of both protein and energy. Small ruminants like sheep and goats can be fed with non-processed barley without any problems. However, for larger ruminants some kind of processing is recommended (Barnes & Orskov 1982). Properly processed barley matches the feed value of maize, wheat, mixed concentrates and oats for ruminants (Tommervik & Waldern 1969). Cattle tend to bloat and tire more quickly of barley than other grains, so it should be fed in combination with other grains and grasses (Hockett 2000).

4. Materials and methods

4.1. Plant materials and field trial information

The selected barley varieties included some of the most commonly grown Norwegian varieties as well as some varieties possessing special qualities (waxy and high-amylose) (Table 3).

Table 3: The barley varieties used in the field trials, sorted after earliness.

Variety	Type	Breeder	Earliness	Year of approval	Special character
Tiril	6-rowed	Graminor, N	Early	2004	
Olve	2-rowed	Graminor, N	Medium early	1994	
Heder	6-rowed	Graminor, N	Medium early	2007	
Skaun	6-rowed	Graminor, N	Medium late	2009	
Edel	6-rowed	Graminor, N	Late	2002	
Helium	2-rowed	Pajbjergfonden, DK	Late	2004	
Marigold	2-rowed	Unisigma, FR	Late	2009	
Magdalena	2-rowed	Svalöf Weibull, S	Very late		Waxy
Karmosé	2-rowed	Svalöf Weibull, S	Very late		High-amylose

This study is based on data from the growing season of 2009. The field trials were located over a south-north axis (Figure 8), covering the actual producing area of cereals in Norway today. One location in Germany was also included (Figure 8). Local extension services have been responsible for the field trials with the exception of the one at Apelsvoll, located at Bioforsk Øst. An overview over soil type, weather conditions and plot size for each location can be found in Table 4.

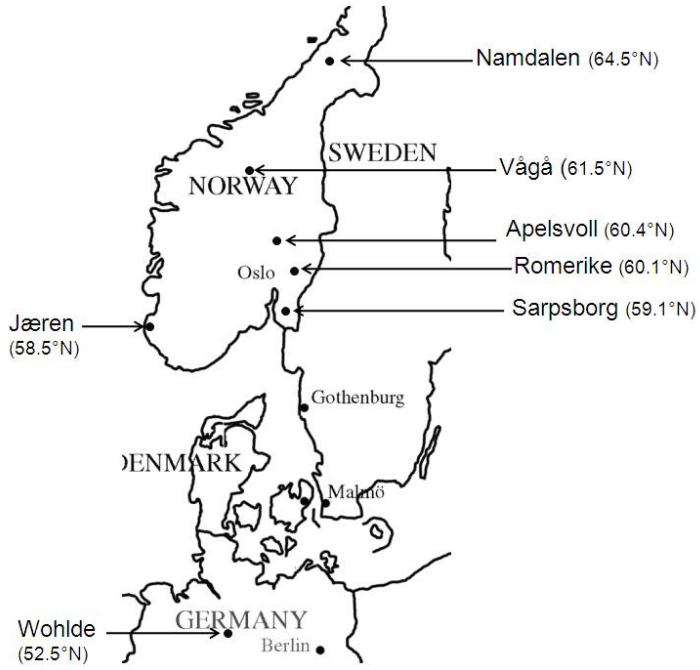

Figure 8: Map over the locations of the field trials.

Table 4: List over the field locations with sowing and harvesting dates for 2009, soil type (OM=organic matter) and average temperature and precipitation per day for the grain filling period (from heading to yellow ripening) and plot size for the different locations.

Location	Sowing date	Harvest date	Soil type	Average		Plot size (m²)	
				temperature	precipitation	Total	Harvested
Wohlde	07.04	21.07	Sandy loam	18.3˚C	2.2 mm	7.5	5.00
Jæren	02.05	20.08	Silty, medium sand	16.8˚C	5.9 mm	12.0	12.00
Sarpsborg	22.04	12.08	Silty loam	17.0˚C	3.7 mm	12.0	9.75
Romerike	12.05	31.08	Silty loam with high OM	15.1˚C	3.6 mm	12.0	9.75
Apelsvoll	11.05	01.09	Silty loam	14.9˚C	5.1 mm	12.0	8.40
Vågå	30.04	15.09	Silty, fine sand with OM	16.3˚C	2.4 mm	12.0	10.95
Namdalen	10.05	15.09	Silty, fine sand with OM	15.5˚C	3.0 mm	12.0	10.95

The barley variety trials were laid out as block trials (alpha design) with two replicates on selected farms in fields to be sown with barley. The fields were treated according to local cultivation practice concerning fertilizing (Table 5) and application of herbicides and insecticides. Neither growth regulators nor fungicides were applied. Tractor tracks and tracks for mobile irrigator were put orthogonal on the sowing direction of the plots, so all the plots in one replicate got the same strain. Yields and other main agronomic parameters (thousand grain weight, test weight and water content) were recorded.

Table 5: Type and amount of fertilizer used at the different locations. Dates of application are given in brackets.

Location	Main fertilization	Split fertilization	Total kg N/daa
Germany	60 kg Calcium ammonium nitrate		6.00
Jæren	5 ton slurry, pig[1] (2/5)		12.50
Sarpsborg	55 kg 21-4-10 (22/4)		11.55
Romerike	39 kg 25-2-6 (5/5) 4 kg OPTI-START NP 12-23-0 (5/5)		10.07
Apelsvoll	48 kg 19-4-12 (11/5)	11 kg OPTI-NS 27-0-0 (22/6)	11.90
Vågå	40 kg 22-2-12 (29/4)		8.64
Namdalen	45 kg 21-4-10 (18/5)		9.45

[1] According to Yara's Fertilizer handbook (Yara 2009) 1 ton of pig slurry contains 2.5 kg N/ton, 1.5 kg P/ton and 2.5 kg K/ton.

4.1.1. Climate data

Climate data was collected for each location using the closest Bioforsk agrometeorology station (http://lmt.bioforsk.no). For the location at Jæren data from the Norwegian Meteorological Institute (eklima.met.no) was used. For the location in Germany weather data was obtained from WetterOnline Meteorologische Dienstleistungen (www.wetteronline.de). Maximum temperature, average temperature, minimum temperature, relative humidity, and precipitation were collected for each location.

In Norway the main pattern for precipitation (Figure 9a) showed a drier May than normal (except for Jæren), a drier June, a much wetter July and a partly wetter August. Namdalen was unusual dry the whole summer, except for the extremely wet September. Jæren as well was much wetter than normal, especially in May and July.

The temperature followed the same pattern as the precipitation (Figure 9b), where May was a warmer month, and June and July were cooler months compared with the last four years. August was divided with warmer conditions at Jæren, Sarpsborg and Namdalen, and colder at Romerike, Apelsvoll and Vågå.

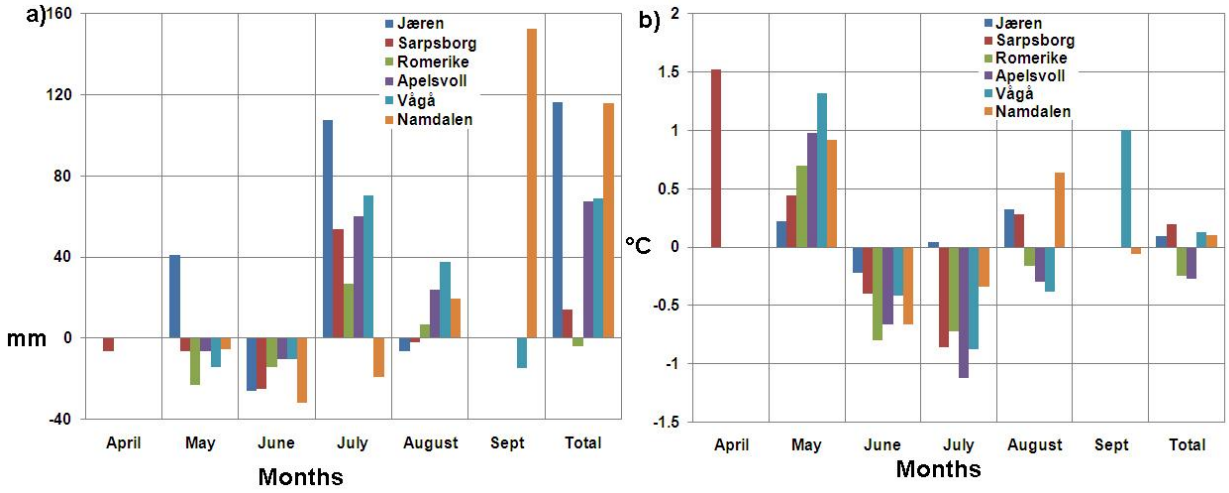

Figure 9 (a) The difference between total precipitation each month (April to September) in 2009 and average precipitation from 2005 to 2009. **(b)** The difference between average temperature each month (April to September) in 2009 and average temperature (label total) from 2005 to 2009.

4.1.2. Heading and yellow ripening

For the trials at Apelsvoll, Wohlde and Namdalen the dates of heading and yellow ripeness were recorded for each variety at each replicate (Table 6 and Table 7). For the trial in Sarpsborg only yellow ripening was recorded (Table 7). Heading was defined as the date when half of the ears in one plot are completely out of the flag leaf sheath. Yellow ripening was defined as the date when 50% of the plants in one plot had turned yellow, except for the uppermost node, which still is green. There were no recordings from Jæren, Romerike and Vågå.

For the trials with missing heading and yellow ripening data these were estimated based on the calculated degree-days from sowing to heading and from sowing to yellow ripeness obtained from the field trials where these data were recorded. In addition, data obtained from field trials with some of the same varieties located at Vollebekk, Ås were used.

Table 6: Average date (over replicates) of heading for all varieties in Germany, Apelsvoll, Namdalen and Vollebekk.

Variety	Germany	Apelsvoll	Namdalen	Vollebekk
Tiril	08.06	28.06	10.07	25.06
Olve	10.06	02.07	18.07	27.06
Heder	07.06	29.06	17.07	25.06
Skaun	11.06	30.06	13.07	23.06
Edel	11.06	02.07	16.07	25.06
Helium	15.06	06.07	23.07	30.06
Marigold	15.06	03.07	20.07	30.06
Magdalena	23.06	08.07	22.07	30.06
Karmosé	24.06	08.07	22.07	30.06

Table 7: Average date (over replicates) of yellow ripening for all varieties in Germany, Sarpsborg, Apelsvoll, Namdalen and Vollebekk.

Variety	Germany	Sarpsborg	Apelsvoll	Namdalen	Vollebekk
Tiril	12.07	27.07	15.08	12.08	
Olve	12.07	29.07	17.08	21.08	29.07
Heder	12.07	28.07	17.08	19.08	02.08
Skaun	13.07	01.08	17.08	18.08	
Edel	13.07	02.08	18.08	21.08	
Helium	20.07	04.08	21.08	27.08	03.08
Marigold	17.07	03.08	20.08	26.08	
Magdalena	20.07	07.08	24.08	25.08	03.08
Karmosé	20.07	10.08	23.08	29.08	04.08

Steps for the estimation:

1. Calculation of the degree-days from seeding to heading (the product of days between seeding and heading and average temperature) and from heading to yellow ripening (the product of days between heading to yellow ripening and average temperature) for each variety and replication at Apelsvoll, Vollebekk, Namdalen and Sarpsborg (yellow ripeness only).
2. Calculation the average degree-day for each variety by location.
3. Calculation of the average degree-day for each variety over location.
4. The degree-day value from step 3 was used to estimate the day of heading and yellow ripening for the locations were this information was missing.

For the locations at Romerike and Jæren an average from Vollebekk, Apelsvoll and Sarpsborg (only yellow ripening) was used for the estimation. Heading at Sarpsborg was calculated by the average from Vollebekk and Apelsvoll. The location in Vågå was estimated by using the average from Vollebekk, Apelsvoll, Sarpsborg and Namdalen.

4.2. Analysis

4.2.1. Protein content, starch content and physical grain characteristics

After winnowing the harvested grains, analyses of protein content, water content and test weight were performed at Bioforsk Øst, Apelsvoll by a Near-Infrared Transmission (NIT) machine (Foss Infratec™ 1241 Grain Analyzer, Foss Analytical A/S, Hillerød, Denmark).

Total starch content was analyzed at the Department of Animal and Aquacultural Sciences at University of Life Sciences by the method IHA-nrMSP 1159 (Svirhus 2002).

Thousand grain weight was counted and weighed at Vollebekk using at a Numerical Seed Counter (Tripette et Renaud, Paris, France). For each sample 251 – 419 kernels were counted.

Milling (0.5 mm) was carried out on a Perten Falling Number 3100 hammer mill (Perten Instruments AB, Huddinge, Sweden). Samples for milling and thousand grain weight determination were selected by using a sample divider to ensure representative samples.

4.2.2. Analysis for pre-harvest sprouting

Alpha-amylase activity was measured using a Megazyme assay kit (Megazyme International Ltd., Wicklow, Ireland) approved by the AACC (method 22-02), the AOAC (method 2002.01) and ICC standard no. 303. It was based on the ceralpha method (McCleary & Sheehan 1987).

All the samples were filtered through filter paper (no centrifugation) before measured in a spectrophotometer at 400 nm (Helios alfa, Unicam UV-Visble Spectrometer, Unicam Spectrometry, Cambrige, United Kingdom).

Alpha amylase activity was analysed for two varieties from each location with replicates to check for sprout damage. The earliest variety, Tiril, was chosen as it was considered the variety most likely to ripen first and therefore be subjected to pre-harvest sprouting. The other variety chosen was the later 6-rowed variety Edel. Edel has during the last five years been the most important barley variety in Norway, cultivated on 20-30% of the total Norwegian barley acreage (Åssveen et al. 2010).

Samples with ceralpha units over 0.4 were considered to have pre-harvest sprouting damage. For these samples the falling number test (AACC International 1999) were performed using a Falling number 1700 (Perten Instruments AB, Huddinge, Sweden).

4.2.3. Analysis for total β-glucan by NIR

The total β-glucan content were first estimated at a Near Infrared Spectroscopy (NIR) using a NIRSystems Model XDS Rapid Content Analyzer (Foss NIRSystems, Silver Spring, MD, USA) equipped with a quartz halogen lamp and a PbS detector at Nofima

Mat. The calibration used was created at Nofima Mat, and contained 61 samples from three field studies performed in different years. The samples are 39 samples of Norwegian, Swedish, Danish and Canadian barleys where 24 where grown in Norway, two in Sweden, two in Denmark and 11 in Canada (Holtekjølen et al. 2006b), 16 samples of Norwegian, Canadian and Danish barleys all grown in Norway (Holtekjølen et al. 2006a) and three Norwegian barleys grown at two different locations in Norway (Habereder 2009).

The spectra were collected in the reflectance mode with a ceramic reference standard over the spectral region of 400–2500 nm with a digital resolution of 0.5 nm. Each sample was divided into three replicates, and the spectra from the same sample were averaged for further use. The samples were acquired in a standard sample cup with a quartz window. Every spectrum is the average of 32 scans (Afseth 2010). All measurements were done in room temperature (22°C).

All NIR spectra were pre-processed using the same method prior to regression analysis. First, all replicate spectra were averaged. Then, the spectra were subjected to scatter correction based on the method of Extended Multiplicative Signal Correction (Martens & Næs 1989). Pre-processed NIR spectra covering the spectral region 400 – 2500 nm were used to develop multivariate regression models based on partial least-squares regression (PLSR) (Martens & Næs 1989).

4.2.4. Analysis for total β-glucan by the Megazyme streamline method

The content of total β-glucan was also analyzed by a Megazyme assay kit (Megazyme International Ltd., Wicklow, Ireland) (approved by the AACC (method 32-23) and the AOAC (method 995.16)) using a UV mini 1240 spectrophotometer (Shimadzu Corporation Kyoto, Japan) adjusted to 510 nm was used. For each sample two replicates were done. This kit is based on the McCleary method (the streamline method) (McCleary & Codd 1991).

The analysis was done on four selected varieties (Marigold, Helium, Magdalena and Karmosé) from all locations to validate the NIR data. They were selected due to their low (Marigold), medium (Helium) and high β-glucan contents and special starch characteristics (Magdalena and Karmosé). These results were then included in the NIR calibration and the results were recalculated.

4.3. Statistical methods

The field trials were set up as block trials (alpha design) with two replicates, with variety and field number as the two design parameters. All varieties were grown at all locations. Every sample was analyzed in duplicates (hereafter referred to as technical replicates), except for the starch content as these were analysed in one replicate (but with some extra controls). The NIR-analysis was performed with three replicates per sample. The samples were randomised during analysis. The data presented are means of biological and technical replicates.

Minitab (software release 15.1.0.0, Minitab Inc., State College, PA, USA, 2006) was the chosen statistical software. Two-way ANOVA, General Linear Model (GLM), Tukey-test and regression analysis were performed (Engstrand & Olsson 2003). The significance levels were set to 95%.

When performing the GLM all effects were considered as fixed, and the following model was used:
Response = variety + location + (variety * location) + residual

The scatter plots with regression lines were all drawn in Microsoft Excel 2008 for Mac version 12.2.4 (Microsoft Corporation, Redmond, WA, USA).

5. Results and discussion

5.1. Weather conditions in the growing season 2009

The growing season of 2009 was quite challenging and the locations in Norway differed compared to the last four years, especially regarding precipitation and temperature variations (Figure 9 and Figure 10). In the end of June, all locations experienced a period with very high temperatures, except for Jæren. In Sarpsborg and partly Vågå, the heat period came after heading, while at Romerike the high temperatures came before heading was finished (Figure 10).

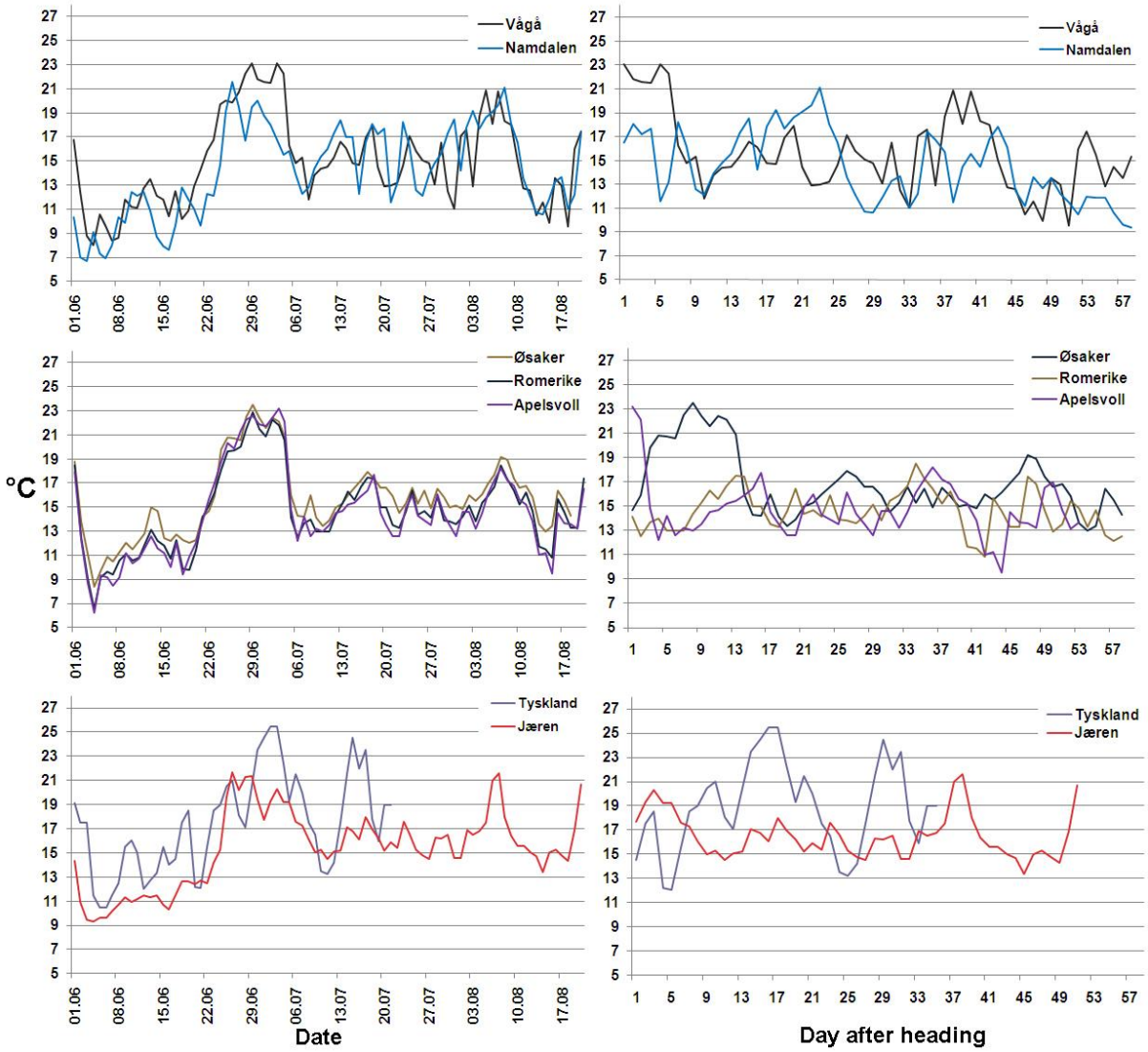

Figure 10: Average day temperature for each location from 1st of June till 18th of August (to the left), and average day temperature from the day of heading for each location (to the right).

5.2. Heading, yellow ripening and degree-days

Due to missing registrations of heading and yellow ripening for some of the fields, these were calculated based on the degree-days from sowing to heading and from heading to yellow ripening (Table 8 and Table 9). The shortest degree-day span from sowing to heading was found for Tiril at Apelsvoll (604.1 degrees) and the longest span for Karmosé in Germany (900.6 degrees). Apelsvoll showed the lowest degree-days from sowing, and Germany the highest.

Table 8: Accumulated degree-days for each variety from sowing to heading for Germany, Vollebekk, Apelsvoll, Namdalen, and two average values used for calculating the missing values at Jæren, Sarpsborg, Romerike and Vågå.

Variety	Germany	Vollebekk	Apelsvoll	Namdalen	Average[1] Ap, Vol	Average[2] Ap, Vol, Nam
Tiril	900.6	679.1	604.1	649.1	641.6	644.1
Heder	888.1	687.9	626.7	763.6	657.3	692.7
Skaun	947.1	648.9	648.6	700.8	648.8	666.1
Edel	947.1	698.6	692.7	747.1	695.6	712.8
Olve	932.1	728.7	692.7	781.7	710.7	734.4
Marigold	1000.7	773.5	715.9	816.6	744.7	768.7
Helium	1000.7	796.4	765.0	859.6	780.7	807.0
Karmosé	1142.5		791.8	841.4	791.8	816.6
Magdalena	1123.5	797.0	791.8	841.4	794.4	810.1
Average	**986.9**	**726.2**	**703.3**	**777.9**	**718.4**	**739.2**

[1] Average of Apelsvoll and Vollebekk used to calculate heading for Jæren, Sarpsborg and Romerike
[2] Average of Apelsvoll, Vollebekk and Namdalen, used to calculate heading for Vågå.

When it came to the time from sowing to yellow ripening, Tiril was again the variety with the lowest value (1188.2 degree-days) at Namdalen and Magdalena, Karmosé and Helium were all ranked as the latest with 1658.5 degree-days in Germany. Namdalen was the location requiring the lowest degree-days from sowing to yellow ripening, while Germany the highest, both for all varieties.

Table 9: Accumulated degree-days for each variety from sowing to yellow ripening for Germany, Sarpsborg, Vollebekk, Apelsvoll and Namdalen, and two average values used for calculating the missing values at Jæren, Romerike and Vågå.

Variety	Germany	Sarpsborg	Vollebekk	Apelsvoll	Namdalen	Average[1] Ap,Sarp,Vol	Average[2] Ap,Sarp, Vol,Nam
Tiril	1496.8	1324.4		1342.4	1188.2	1333.4	1285.0
Heder	1496.8	1340.9	1329.2	1370.6	1271.6	1346.9	1328.1
Olve	1496.8	1356.7	1269.2	1370.6	1305.7	1332.2	1325.6
Skaun	1514.3	1401.6		1370.6	1259.4	1386.1	1343.9
Edel	1514.3	1417.6		1384.2	1305.7	1400.9	1369.2
Marigold	1605.8	1433.1		1413.9	1377.5	1423.5	1408.2
Helium	1658.5	1449.2	1343.9	1430.9	1394.3	1408.0	1404.6
Magdalena	1658.5	1503.0	1343.9	1472.4	1363.0	1439.8	1420.6
Karmosé	1658.5	1555.9	1359.8	1472.4	1428.2	1462.7	1454.1
Average	**1566.7**	**1420.3**	**1329.2**	**1403.1**	**1321.5**	**1392.6**	**1371.0**

[1] Average of Apelsvoll, Sarpsborg and Vollebekk used to calculate heading for Jæren and Romerike
[2] Average of Apelsvoll, Sarpsborg, Vollebekk and Namdalen used to calculate heading for Vågå.

Quite a few potential errors may have influenced the calculated accumulated degree-days. First, different people recorded heading and yellow ripening both at the locations and between the locations. The recordings are based on visual judgement, and this will always differ slightly from person to person. Second, recording of yellow ripening in barley is challenging. Third, the impact of such a large quantity of missing data for many of the locations adds another element of uncertainty, as predicted values will not be as accurate as actual registrations.

Åssveen and Abrahamsen (1999) calculated the average degree-day sums for the most important varieties at that time. Our results are generally higher than the average degree-days calculated in the previously mentioned study, but it might be a result of the wet summer of 2009. It has previously been shown that 100 mm increase of the total precipitation increased the degree-day sum with 60-80 degree-days for barley (Strand 1969). Day length also influences the requirement for degree-days. One hour daylight more reduced the demand with 30 degree-days, this corresponded to 20 degree-days less per latitude (Strand 1969). This fits well with the data in this study, the difference in latitude is 7°N between Germany and Sarpsborg, and the degree-day difference were 146 degrees. The same relationship was found between all the other locations where heading and yellow ripening were registered. Taking all these considerations into account, the degree-day calculations look valid.

5.3. Yield and physical grain characteristics

5.3.1. Yield

The average yields obtained at the different locations (averaged over varieties) and for each variety (averaged over locations) are presented in Table 10, a and b respectively. The average yields varied greatly between locations, from 668 kg in Germany to 382 kg in Namdalen. By using the LSD value, the field trial in Germany gave significantly ($p<0.05$) higher yield than all other locations except for Vågå. Namdalen had significantly ($p<0.05$) lower yields than all the other locations.

The variation between varieties was lower than for locations, even though a variation of 200 kg/daa was observed ranging from the highest (606 kg, Edel) to the lowest (417 kg, Karmosé). Karmosé stood out as significantly lower ($p<0.05$) than all the other varieties while using the LSD value in Table 10b.

In official trials for testing the value for cultivation and use (VCU) for recommendation of varieties (Åssveen et al. 2010) Edel showed high yields. These results were further confirmed by this study.

In earlier VCU trials (Åssveen et al. 2010) the later varieties normally showed a greater yield, due to their longer period of assimilation. This study does not fully support this. The main reason was that the latest varieties in our study (Magdalena and Karmosé) were varieties with special starch characteristics (waxy and high-amylose). The special starch varieties were known from earlier studies to give lower yield than normal-starch varieties (Bhatty et al. 1975; Cavallero et al. 2004; Rey et al. 2009).

The statistical analysis showed significant ($p<0.001$) effects of both location and variety, and there was a significant interaction ($p<0.001$) between location and variety. The MS values from the variance analysis were used to calculate the variation explained by each factor. The interaction counted for 2.6%, location for 74.3% and variety for 21.8% of the variation (Table 11). The varieties were ranked more or less similar at all locations, except for Tiril that stood out from the rest with a slightly different pattern.

Table 10: Average yield and physical grain characteristic for **(a)** each location (as average of all varieties) and **(b)** each variety (as average of all locations).

(a)

Location	Yield, kg/daa	Thousand grain weight, g	Test weight, kg
Germany	668	41.2	68.9
Jæren	455	42.1	70.1
Sarpsborg	497	41.9	66.4
Romerike	587	38.6	64.0
Apelsvoll	594	43.6	65.3
Vågå	617	43.2	70.2
Namdalen	382	43.8	65.0
St.dev.	101	1.8	2.6
LSD	59	2.2	1.2

(b)

Variety	Yield, kg/daa	Thousand grain weight, g	Test weight, kg
Karmosé	417	42.9	64.6
Tiril	546	38.3	66.0
Magdalena	489	40.8	66.7
Skaun	590	40.1	67.1
Marigold	593	44.5	67.2
Heder	577	43.3	67.5
Olve	503	40.1	67.5
Helium	566	47.1	68.7
Edel	606	41.3	68.7
St.dev.	101	1.8	2.6
LSD	52	1.9	1.1

Table 11: Mean square values from General Linear Models and how much each factor (location, variety, location*variety and error) contributes to the total variance for yield, test weight and thousand grain weight.

	Yield		Test weight		Thousand grain weight	
	MS	%	MS	%	MS	%
Location	184349	74.3	57.7	27.2	118.6	80.6
Variety	54143	21.8	137.1	64.6	22.9	15.5
Location*variety	6483	2.6	13.2	6.2	4.4	3.0
Error	3044	1.2	4.1	2.0	1.3	0.9
Total	248019		212.1		147.2	

5.3.2. Thousand grain weight

The average thousand grain weights obtained at the different locations (averaged over varieties) and for each variety (averaged over locations) are presented in Table 10, a and b respectively. Except for Romerike that were clearly lower (38.6 g) than the other locations, the variation was quite low. The other locations showed a variation ranging from 41.15 g in Germany to 43.8 g in Namdalen (Table 10a).

The variation in variety was much larger than between locations. It showed a variation from 47.1 g (Helium) to 38.3 g (Tiril). Tiril and Helium were clearly in each end of the scale, while the rest of the varieties only varied from 40.1 to 44.5 g (Table 10a).

The very low thousand grain weights at Romerike were likely addressed to the fact that this location had a lot of lodging (up to 100% lodging for some of the replicates). The

other values were quite high which indicated a good grain development on all other locations than Romerike.

The statistical analysis showed significant ($p<0.001$) effect of both location and variety, and there was significant interaction ($p<0.001$) between location and variety. The MS values from the variance analysis were used to calculate the variation explained by each factor. The interaction counted for 2.0%, location for 27.2% and variety for 64.6% of the variation (Table 11). Regarding interaction, the picture was less clear than for the other parameters. There was a tendency that the same varieties were ranked either high or low, but this was not as apparent as for the other parameters.

5.3.3. Test weight

The average test weights obtained at the different locations (averaged over varieties) and for each variety (averaged over locations) are presented in Table 10, a and b respectively. There was a variation from 70.2 kg at Vågå to 64.0 kg at Romerike.

Karmosé showed the lowest values of test weights with 64.69 kg. The other varieties showed a relative small variation, from 68.7 kg (Edel) to 66.0 kg (Tiril) (Table 10b). The varieties which have been a part of the Norwegian VCU trials (Edel, Helium, Marigold, Tiril and Heder) were ranked almost the same in this study as in the VCU trials (Åssveen et al. 2010).

The low test weights at Romerike might again be due to the lodging problems, but not as much as for the thousand grain weight. It might as well have a connection with possible diseases occurring on the location or a drought period.

The statistical analysis showed significant ($p<0.001$) effect of both location and variety, and there was significant interaction ($p<0.001$) between location and variety. The MS values from the variance analysis were used to calculate the variation explained by each factor. The interaction counted for 3.0%, location for 80.6% and variety for 15.5% of the variation (Table 11). Regarding the interaction, varieties were ranked more or less the same from location to location. Karmosé and Magdalena behaved differently than the other varieties, especially at Romerike.

5.3.4. Pre-harvest sprouting

Pre-harvest sprouting was tested with both alpha-amylase activity and falling number. For all locations both replicates of the variety Edel and Tiril were tested for amylase activity. The results are shown in Figure 11. Values ranging above 0.4 were considered to have pre-harvest sprouting damage. As seen from the figure, Namdalen seemed to have a serious pre-harvest sprouting damage. The weeks before harvesting were very wet (up to the double of normal precipitation), so this was expected. The other locations had some high values, but the rest of the varieties were not suspected to have pre-harvest sprouting damage.

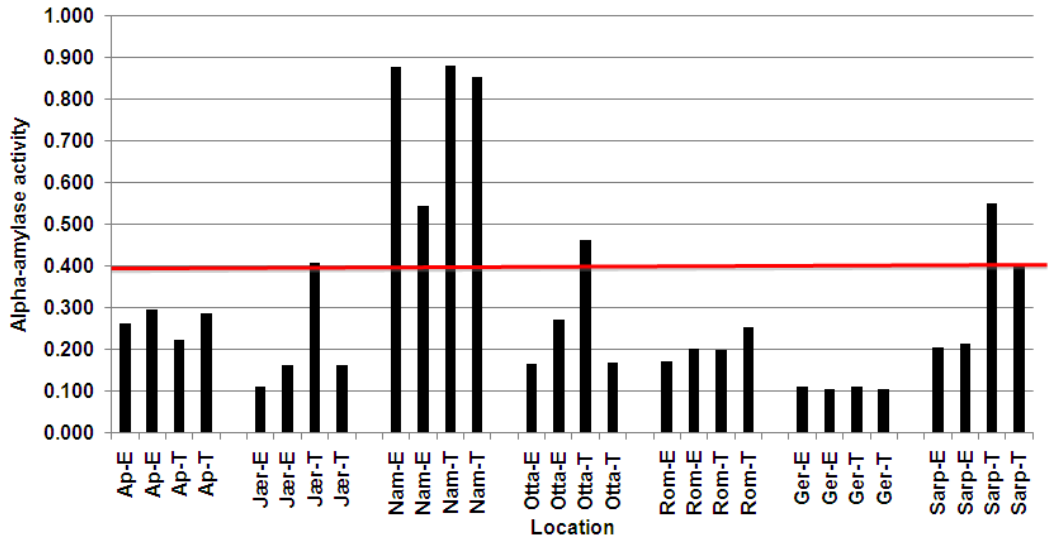

Figure 11: Alpha-amylase activity in Edel (E) and Tiril (T) for all the locations (Ap= Apelsvoll, Jær=Jæren, Ger=Germany, Rom=Romerike, Sarp=Sarpsborg).

All the locations that showed a tendency of pre-harvest sprouting damage were in addition tested with the falling number test (AACC International 1999) for a wider range of varieties (Figure 12). All the varieties tested from the field trial at Namdalen clearly showed pre-harvest sprouting damage. These results were confirmed in the falling number test. The samples with low falling numbers from Jæren, Vågå and Sarpsborg were the same samples as those showing pre-harvest sprouting damage in the alpha-amylase test. Both at Vågå and Sarpsborg the variety Skaun gave a low falling number, which might indicate more sprouting damage at these locations. Skaun was not included in the alpha-amylase test.

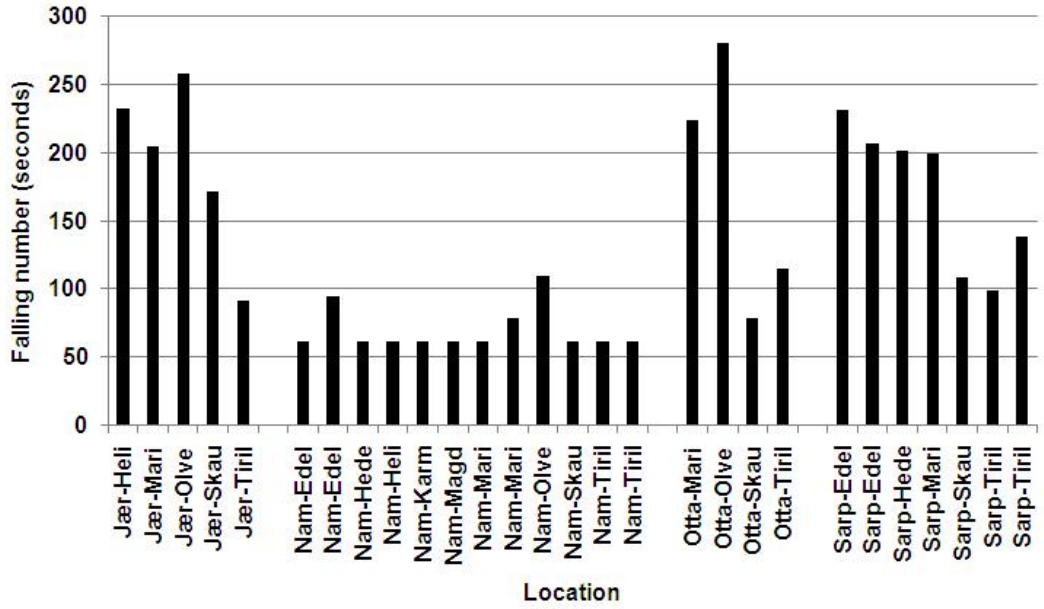

Figure 12: Falling number for different varieties from the locations with clear pre-harvest sprouting damage. Jær=Jæren, Nam=Namdalen, Sarp=Sarpsborg. Heli=Helium, Mari=Marigold, Skau=Skaun, Hede=Heder, Karm=Karmosé, Magd=Magdalena.

The alpha-amylase activity was plotted against falling number. A logarithmic regression line with an R^2-value of 0.915 (Figure 13) was obtained. This showed a good correlation between the alpha-amylase activity test and falling number test.

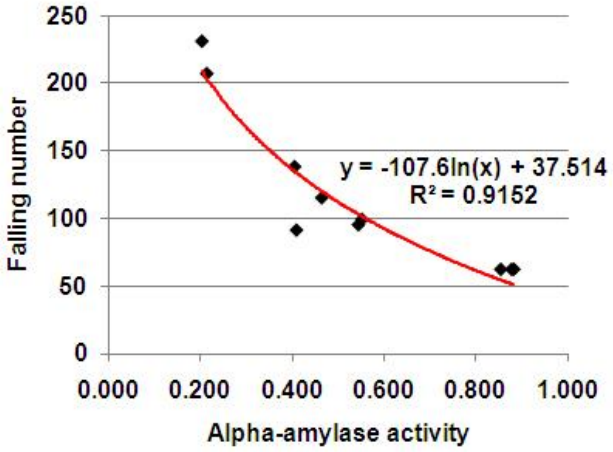

Figure 13: Scatter plot with a logarithmic trend line showing the relationship between falling number and alpha-amylase activity with equation and R^2-value**.**

Barley are not graded according to falling number in Norway, but this ought to be discussed when barley is used for food. An appropriate threshold value is required for using a grading system. In Norway both wheat and rye have a grading according to falling number, and the threshold values are 120 for rye and 200 for wheat. There is a need for more knowledge on this field, especially linked to different products and their minimum threshold values. It is highly valuable information knowing the different falling numbers of barley that can cause potential problems for processing and product quality.

For a more complete picture more samples should have been taken from more of the varieties and locations. The material was too small to draw any conclusion for feasible falling number for barley. One could expect a low falling number even though the alpha-amylase levels are under the limit for the waxy starch barley (Magdalena). This because waxy starch varieties of wheat are known to give a low falling number even though the alpha-amylase activity is low (Graybosch et al. 2000).

5.4. Protein and starch contents

5.4.1. Protein content

The average protein content in percent dry matter obtained at the different locations (averaged over varieties) and for each variety (averaged over locations) are presented in Table 12, a and b respectively. Within the locations Jæren had the highest protein content with 13.9% while Germany the lowest with 10.9%. As for the varieties, Olve had the highest protein content of all with 14.7 % and Edel the lowest with 11.4%.

The statistical analysis gave no interaction between location and variety, but significant effects ($p<0.001$) for both location and variety were seen. A Tukey comparison showed significantly lower ($p<0.001$) protein content at the location in Germany compared to the

other locations. The barley grown at Jæren had a significantly higher ($p<0.05$) protein content than the other locations, except for Vågå.

The Tukey test showed that Olve had the significantly highest ($p<0.05$) content of protein compared to the other varieties. Karmosé had a significantly higher protein level ($p<0.05$) than Skaun, Marigold, Heder and Edel. Edel had significantly lower levels ($p<0.05$) than Helium, Magdalena, Karmosé and Olve.

Compared to the Norwegian VCU trials from 2006-2008 Marigold and Edel showed the same low values for protein in this study as they did in the VCU trial (Åssveen et al. 2010). In a former study including 39 varieties of barley from the Nordic countries and Canada (Holtekjølen et al. 2006b) Olve was, out of the Nordic varieties, the variety with the highest content of protein. This is in agreement with this study.

A regression analysis between yield versus protein gave a negative correlation with R^2 =0.304. Conditions that increase yield for a given N application tend to give lower protein content, and vice versa.

The MS values from the variance analysis were used to calculate how much of the variance that was explained by each factor. Variety counted for 43.5% of the variation, location 53.7% of the variation and location*variety only for 1.2% of the total variation (Table 13). The interaction between variety and location was not significant. In this study location influenced the content of protein the most, but the influence of variety was as well important.

Table 12: Average protein and starch contents for **(a)** each location (as average of all varieties) and **(b)** each variety (as average of all locations).

(a)

Location	Protein, %	Starch, %
Germany	10.9	55.2
Jæren	13.9	54.6
Sarpsborg	13.0	54.5
Romerike	12.6	54.7
Apelsvoll	12.3	56.3
Vågå	13.1	55.7
Namdalen	12.4	54.9
St. dev.	0.9	0.7
LSD	0.7	1.3

(b)

Variety	Protein, %	Starch, %
Karmosé	13.3	52.3
Tiril	12.4	55.2
Magdalena	12.7	54.7
Skaun	12.0	55.3
Marigold	11.9	57.5
Heder	12.2	56.6
Olve	14.7	50.6
Helium	12.7	56.8
Edel	11.4	57.2
St.dev.	1.0	2.3
LSD	0.6	1.2

Table 13: Mean square values from general linear models and how much each factor (location, variety, location*variety and error) contributes to the total variance for protein, starch and total β-glucan contents.

	Protein		Starch		B-glucan	
	MS	%	MS	%	MS	%
Location	15.6	53.7	8.4	9.5	2.72	39.3
Variety	12.7	43.5	76.8	87.2	4.02	57.9
Location*variety	0.4	1.2	1.4	1.6	0.16	2.3
Error	0.5	1.6	1.5	1.7	0.03	0.5
Total	29.1		88.1		6.94	

5.4.2. Starch content

The average starch content in percent dry matter obtained at the different locations (averaged over varieties) and for each variety (averaged over locations) are presented in Table 12, a and b respectively. The starch content was highest at Apelsvoll (56.3%) and lowest in Sarpsborg (54.5%). The variation between locations was quite small for starch content.

The statistical analysis gave no interaction between location and variety, but significant effects ($p<0.001$) for both location and variety were seen. A Tukey comparison showed that the results from Apelsvoll were significant higher ($p<0.05$) than Jæren, Sarpsborg, Romerike and Namdalen. Vågå was significant higher ($p<0.05$) than Sarpsborg.

The variation in starch content was greater between varieties. Marigold had the highest value (57.5%) and Olve the lowest (50.6%) of all (Table 12b). The Tukey test showed that Olve had significantly ($p<0.05$) lower starch content than the other varieties. Karmosé and Magdalena, the two "special" varieties, were also significantly ($p<0.05$) lower than most of the varieties. Karmosé was significantly ($p<0.05$) lower than all others except Olve. Magdalena had a starch level significantly ($p<0.05$) lower than Edel, Heder, Helium and Marigold, and significantly ($p<0.05$) higher than Karmosé and Olve. These low values of starch for waxy and high-amylose varieties are known from earlier studies (Andersson et al. 1999; Holtekjølen et al. 2006b).

A regression analysis with protein versus starch gave negative correlation with a R^2=0.318. This indicates that when protein content is increasing the starch content will decrease. This makes sense since they both are competing for the same space in the endosperm, and if one increases, something else is likely to decrease.

In a former study with 39 varieties of barley originating from the Nordic countries and Canada (Holtekjølen et al. 2006b), Olve was the variety with the lowest content of starch. As in this study, Olve was even lower in starch content than the high amylose and waxy barleys that are known to have lower content of starch than normal starch varieties (Andersson et al. 1999; Holtekjølen et al. 2006b).

The MS values from the variance analysis were used to calculate the variation explained by each factor. Variety counted for 76.8% of the variation, location 8.4% of the variation and location*variety only for 1.6% of the total variation (Table 13). Interaction was not significant. In this study variety was the main factor influencing starch content.

5.5. β-glucan

5.5.1. Analysis by the Megazyme streamline method and improvements of the NIR calibration

The first screening of total β-glucan content of the samples was done by NIR. To validate these results, four of the varieties were further analyzed for total β-glucan content by the Megazyme streamlined method (McCleary & Codd 1991). These results

were compared, and a linear regression between NIR and the Megazyme method was calculated (Figure 14). The correlation obtained was lower than expected (R^2=0.55).

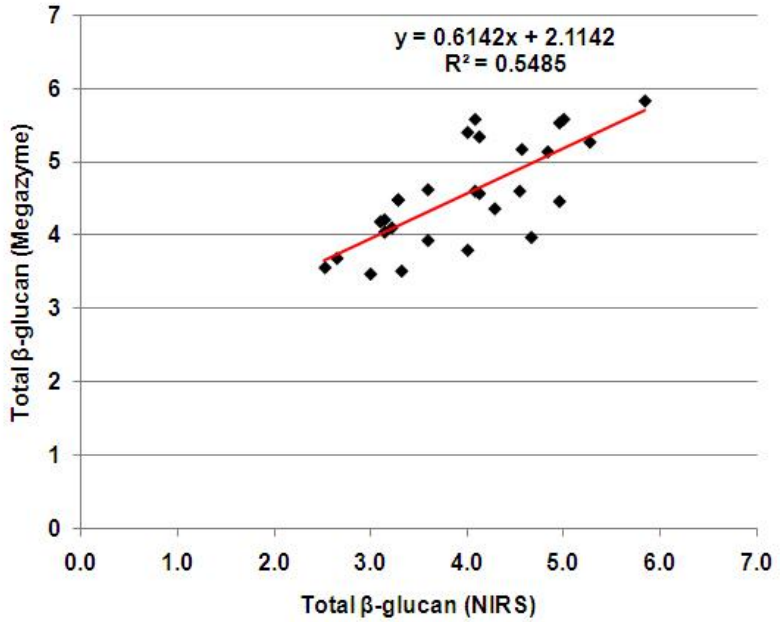

Figure 14: Scatter plot with a linear trend line showing the relationship between total β-glucan measured by NIR and the Megazyme streamlined method.

Therefore, the values from the Megazyme streamlined method (Table 14) were included in the NIR calibration set to improve the calibration. The old calibration contained 61 samples, and by adding the Megazyme analyzed samples to the calibration set, the new calibration contained 117 samples. The samples not analyzed by the Megazyme method were recalculated according to the new calibration.

Table 14: Average values for total β-glucan in percent analyzed by the Megazyme streamlined method, sorted internally for each variety after total β-glucan in % of dry weight.

Magdalena		Marigold		Helium		Karmosé	
Location	**%**	**Location**	**%**	**Location**	**%**	**Location**	**%**
Romerike	3.97	Romerike	3.47	Namdalen	3.79	Romerike	4.04
Apelsvoll	4.60	Namdalen	3.51	Romerike	3.93	Apelsvoll	4.48
Namdalen	5.14	Germany	3.56	Germany	4.21	Namdalen	4.62
Germany	5.17	Apelsvoll	3.68	Jæren	4.46	Vågå	5.34
Vågå	5.27	Vågå	4.10	Apelsvoll	4.48	Germany	5.40
Sarpsborg	5.58	Sarpsborg	4.18	Sarpsborg	4.57	Jæren	5.53
Jæren	5.83	Jæren	4.36	Vågå	4.60	Sarpsborg	5.58

Using the new calibration, the correlation between analysed values (by the Megazyme streamlined method) and the predicted values (from NIR) was improved (R^2=0.67). The new calibration was more robust, since the estimation error was reduced from 0.57 to 0.42. The NIR data recalculated from the newest calibration are used later in this thesis.

A R^2 around 0.7 is regarded as an acceptable value for a NIR calibration analysing β-glucan content. It is shown to be hard to achieve R^2 values much higher than 0.8 in calibrations for β-glucan. To improve the calibration a wider range of genetic variation in β-glucan content is needed. By adding more samples, especially from varieties with especially high and low β-glucan content, it could be possible to improve the calibration and make it more robust.

5.5.2. Total β-glucan content for all samples analysed by NIR

The average content of total β-glucan in percent dry matter obtained at the different locations (averaged over varieties) and for each variety (averaged over locations), are presented in Table 15, a and b respectively. The variation ranged from 5.07% at Jæren to 3.83% at Romerike (Table 15a). The LSD value showed that Jæren was significant higher ($p<0.05$) in total β-glucan than the other locations, while Romerike was significantly lower ($p<0.05$) than all the other locations.

Table 15: Average values for total β-glucan for **(a)** each location (as average of all varieties) and **(b)** each variety (as average of all locations), both sorted after content of total β-glucan in dry weight.

(a)

Location	Value, %
Romerike	3.83
Apelsvoll	4.11
Namdalen	4.34
Germany	4.36
Sarpsborg	4.47
Vågå	4.62
Jæren	5.07
St. dev.	0.39
LSD	0.19

(b)

Variety	Value, %
Heder	3.67
Marigold	3.84
Edel	4.03
Tiril	4.17
Helium	4.22
Skaun	4.56
Karmosé	5.00
Olve	5.02
Magdalena	5.08
St. dev.	0.54
LSD	0.17

The variation among varieties varied from 5.08% (Magdalena) to 3.67% (Heder) (Table 15b). There was larger variation among varieties than between locations. Using the LSD value Magdalena, Olve and Karmosé had significantly ($p<0.05$) higher content of total β-glucan than all other varieties. Heder and Marigold had had significantly ($p<0.05$) lower content of total β-glucan than all other varieties.

The variations of the different barley varieties at the different locations are seen in Table 16. Jæren was the location with the overall highest total β-glucan contents independent of variety (except for Helium and Karmosé). Sarpsborg had the largest variation in total β-glucan content, from the highest value among all locations for Karmosé (5.58) to the lowest of all for Tiril (3.52). Apelsvoll and Romerike had the smallest variation between varieties of all locations.

Table 16: Average values of total β-glucan for all varieties at all locations, all values are in % of dry weight.

	Heder	Edel	Tiril	Helium	Skaun	Olve	Karmosé	Marigold	Magdalena
Germany	3.66	3.83	3.91	4.21	4.26	5.25	5.40	3.56	5.17
Jæren	4.35	4.98	5.03	4.46	5.48	5.59	5.53	4.36	5.83
Sarpsborg	3.37	4.12	3.52	4.57	4.49	4.79	5.58	4.18	5.58
Romerike	3.31	3.43	3.87	3.93	4.03	4.44	4.04	3.47	3.97
Apelsvoll	3.55	3.67	4.19	3.99	4.10	4.68	4.48	3.70	4.60
Vågå	3.60	4.18	4.54	4.60	4.79	5.17	5.34	4.10	5.27
Namdalen	3.87	4.01	4.16	3.79	4.75	5.21	4.65	3.51	5.14
STD	0.35	0.49	0.49	0.33	0.50	0.39	0.61	0.37	0.62
LSD	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17

The high values of the special starch varieties (Karmosé and Magdalena) together with Olve were in line with earlier studies (Andersson et al. 1999; Holtekjølen et al. 2006b). The values of Olve found in this study corresponded well with the values found by Holtekjølen et al. (2006b). The results in this study gave slightly higher values for β-glucan content in Olve. The average value in this study was 5.02%, while it was 4.59% in the study by Holtekjølen et al. (2006).

The general linear model gave clear significance ($p<0.001$) for all variables (location, variety and location*variety). The MS values from the variance analysis were used to calculate the variation explained by each factor. Variety counted for 57.9% of the variation, location 39.3% of the variation and location*variety only for 2.3% of the total variation (Table 13). The varieties ranked more or less the same way when it came to varieties with high and low content of β-glucan (Figure 15). Sarpsborg and Namdalen were the two locations that differed most from the rest. This might be the reason for the interaction found. Most of the variation in total β-glucan content in this study can be related to variety. Location was also of importance to the total β-glucan content.

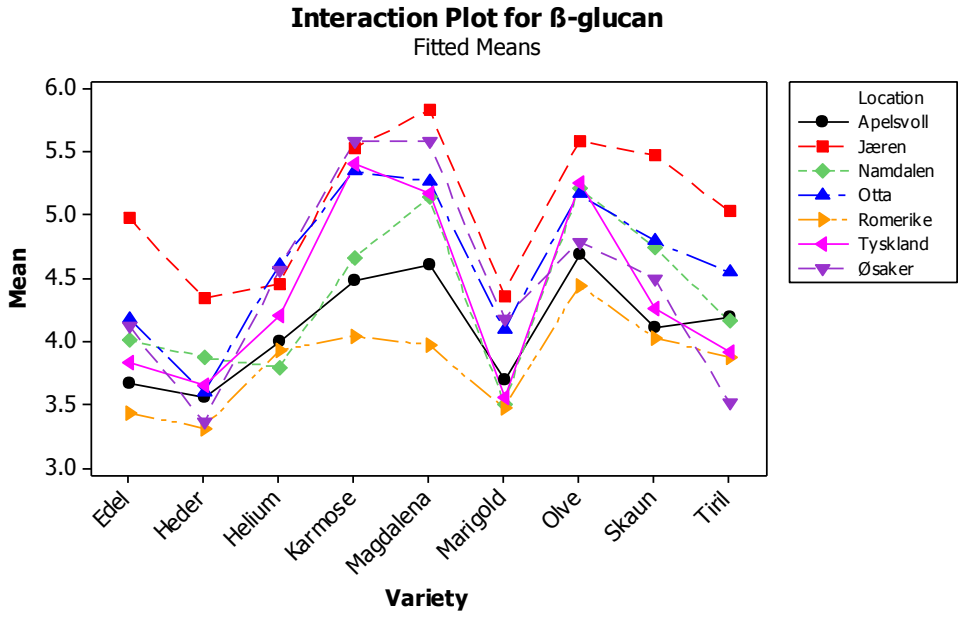

Figure 15: Interaction plot for total β-glucan content with factors variety and location.

5.5.3. Total β-glucan content versus the other parameters

A regression analysis was performed with total β-glucan content versus the other parameters (yield, test weight, thousand grain weight, protein and starch) to see if β-glucan related negatively or positively to the other parameters. Correlations, especially negative correlations might make it difficult to achieve improvements in all parameters when breeding new varieties. There was only a slightly positive correlation between total β-glucan content and protein content (R^2=0.234, Figure 16a) and a negative correlation between total β-glucan content and starch content (R^2=0.328. Figure 16b).

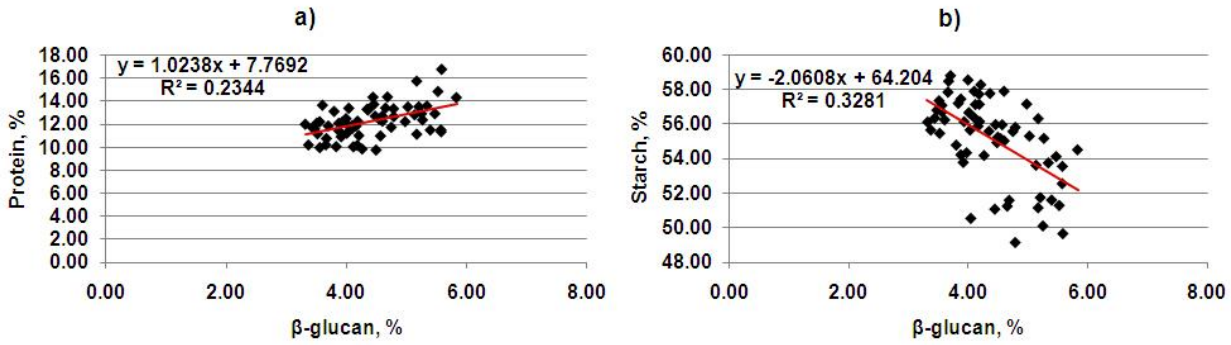

Figure 16: Linear regression of **(a)** total β-glucan content versus protein content and **(b)** total β-glucan content versus starch content.

Regression analyses were also carried out for total β-glucan content and starch content for each location. A negative, significant ($p<0.05$) correlation was found between these parameters at Apelsvoll (R^2=0.720). Negative, significant ($p<0.05$) correlations were as well found at Namdalen (R^2=0.581), Romerike (R^2=0.554), Jæren (R^2= 0.481) and Germany (R^2=0.458). Vågå and Sarpsborg gave no significant correlation. Varieties with high total content of β-glucan have in other studies showed a lower content of starch (Andersson et al. 1999; Hang et al. 2007; Holtekjølen et al. 2008a), since glucose is the basis of the synthesis of both β-glucan and starch. This negative correlation can vary from location to location.

A similar comparison was done for total β-glucan content versus protein content for each location. The results gave positive, significant ($p<0.05$) regressions for Apelsvoll (R^2=0.466) and Romerike (R^2=0.597), where a negative, significant ($p<0.05$) regression was found for Germany (R^2=0.596). The other locations gave no significant correlation.

The protein content in Germany was much lower than for the rest of the locations (Table 12a), and this might be caused by the amount of nitrogen applied. The yield in Germany was good, so it might be due to a lower nitrogen access during the grain filling period. This was not studied closely enough, and need to be given attention for a proper conclusion. Former studies (Güler 2003; Hang et al. 2007; Holtekjølen et al. 2006b) have found positive correlation between β-glucan and protein.

5.6. Chemical grain components versus weather data

Weather data was gathered and processed to study climatic influence on total β-glucan, starch and protein contents. The collected data were average temperature, minimum temperature, maximum temperature, precipitation and average temperature during the whole grain filling period and for eight degree-day periods, each of 100 degree-days during this period. The 100 degree-day intervals (Appendix 4) were obtained to investigate if there was any periods of the grain filling phase that were more important than other periods regarding the final content of total β-glucan. The intervals were made by summarizing the degree-days from heading to yellow ripening (grain filling phase), and thereby dividing this in intervals with 100 degree-days in each interval. The last interval was 7-800 degree-days for the latest varieties. The earliest varieties ended in the 5-600 degree-days period. For each interval the average temperature was calculated.

Table 17: Results from regression analysis for protein and total β-glucan content against temperature in the grain filling period (average, max, min and degree-day periods) and precipitation. Values are significant R^2-values ($p<0.05$) and ns=not significant**.**

	Protein	**β-glucan**
Average temp	0.075	0.089
Min. temp	0.178	0.263
Max. temp	ns	ns
0-100	ns	0.074
1-200	ns	ns
2-300	0.171	ns
3-400	0.136	ns
4-500	0.115	ns
5-600	ns	0.085
6-700	ns	ns
Average precipitation	0.126	ns

Regression analyses for total β-glucan, starch and protein contents versus weather data (average temperature, minimum temperature, maximum temperature, precipitation and average temperature for eight degree-day periods of 100 degree-days; all parameters for the grain filling phase) were conducted (Table 17).

Mainly, the regression analyses gave no clear answers regarding total β-glucan content. A small, positive and significant ($p<0.05$) correlation was found between total β-glucan content and the minimum temperature during grain filling (Table 17). In Figure 17 the correlation between β-glucan and minimum, average and maximum temperature is presented. From the figure an $R^2=0.263$ for minimum temperature can be observed. This might indicate that the content of β-glucan increased with higher minimum temperature, and that the minimum temperature is one of the limiting factors for total β-glucan content at some locations.

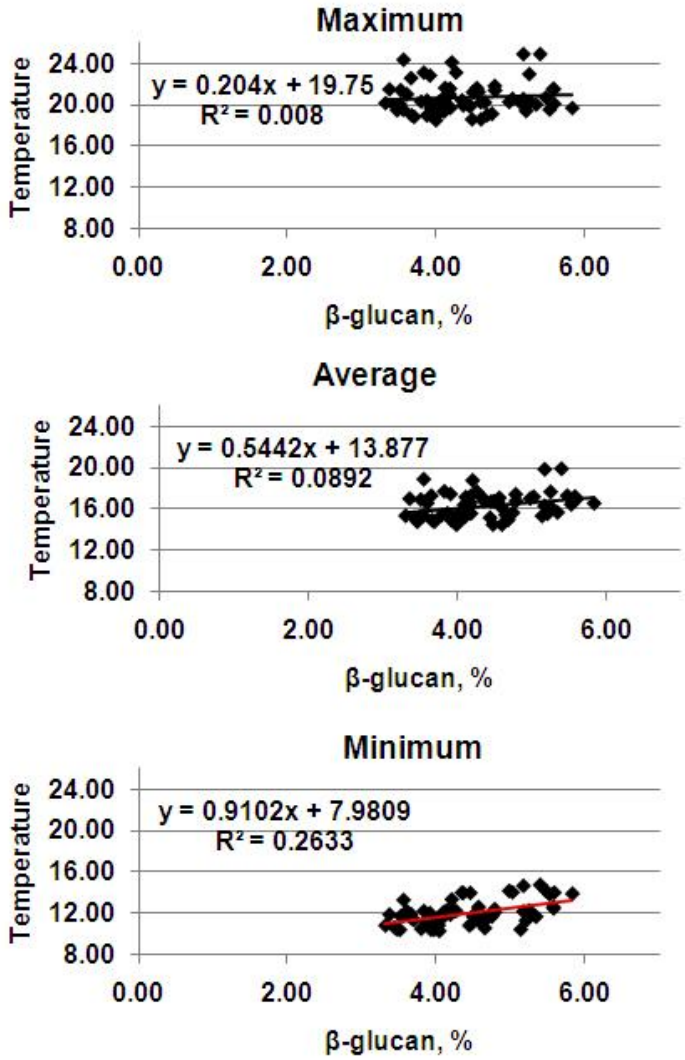

Figure 17: Linear correlation between total β-glucan content (in % dry weight) and the minimum temperature, average temperature and maximum temperature during the period of grain filling.

Protein content showed the same pattern for average, maximum and minimum temperatures as total β-glucan content. It gave significant effect of average (R^2=0.089) and minimum temperature (R^2=0.263). Starch showed no significant correlations with ant of the weather parameters. This suggested that other weather criteria or other environmental factors might influence the synthesis of starch. The variation in starch content between locations was as well quite small.

5.7. General discussion

An earlier study (Anker-Nilssen et al. 2008) found that higher temperature increased the content of total β-glucan. This was not seen in this study. The most southern location (Germany) was subjected to the highest average temperatures that resulted in medium total β-glucan content. Also, Jæren with the highest total β-glucan contents did not show any particular high or low temperatures in the grain filling phase. These results suggest that other environmental factors also influence the total β-glucan content. The results from this study showed that the minimum temperature during the grain filling phase was of relevance. It is possible that other factors not investigated in this study are of larger importance for total β-glucan content. It is not sure that one factor alone was the causing agent either; combinations of factors might as well be of importance. Interactions between climate and climate and soil can be complex, and more seasons and use of multivariate statistics can be necessary.

Data for heading and yellow ripening were only registered for some of the locations. Due to the lack of data, predicted values were compiled, and this might have resulted in more inaccurate values than if the registrations had been done for all fields. Anyhow, the predicted values were compared with previous data (Åssveen et al. 2010) and they looked reasonable, so this was not regarded as a main source of error.

Accumulation of β-glucan is shown to happen rapidly from 15-20 days after anthesis (Pérez-Vendrell et al. 1996), and it is reasonable to consider the weather in this period to be of greater impact than the periods earlier in the grain filling phase. This was the reason for dividing the grain filling period in 100 degree-day periods, to see if it could be found any effects of climate in the period with the most rapid β-glucan accumulation. This has been scarcely studied before, so the division in 100 degree-day periods was an attempt to study this.

Data gathered only from one season do not provide a complete picture, so data from more seasons are needed, as planned in this project. This might explain why it has been hard to find any clear and significant results. One of the reasons could be that the impact of the year is causing a lot of the variation, so when more years are added hopefully a more stable data material is collected.

As in previous studies, Olve stood out as a very interesting variety for the food industry. In this study more growing environments were included, and Olve performed well at each location. Only Magdalena had a higher, but not significantly higher, average value of total β-glucan content. Furthermore, Olve gave a better average yield than Magdalena, and is a much earlier variety than Magdalena. The varieties with highest total β-glucan contents (Magdalena, Olve and Karmosé) all had significantly ($p<0.05$) lower yields than all the other varieties in this study except for Tiril (Table 10a). If the industry is interested in barley with a higher content of total β-glucan, some kind of premium is probably needed to encourage farmers to grow high total β-glucan varieties.

One disadvantage with Olve is that it can be hard to harvest. In dry years it can be hard to remove the awn while threshing, and make the harvesting more labour-intensive (Borchsenius 2005). Also the fact that Olve, released on the market in 1994, is outyielded by the many, newer barley varieties has resulted in low demands, and seed grain of Olve is no longer available the Norwegian seed companies (Felleskjøpet and

Strand Unikorn). This fact underlines the need for an extra price incentive if high-β-glucan varieties are further requested.

The special starch varieties, Magdalena and Karmosé, were expected to have a higher content of total β-glucan compared to Olve, but this was not the case under the field conditions in 2009. Concerning the total β-glucan content, Olve should be the chosen variety in Norway because of the higher yield and earlier maturation than Magdalena and Karmosé. Karmosé has been evaluated and discussed by the industry, but it has the lowest yield and test weight compared with the other varieties. Karmosé further performed as one of the lowest in Germany as in southern parts of Norway. If Karmosé is the choice for growing high-amylose barley, a decrease in yield is inevitable. It is needed more testing of Karmosé and Magdalena for Norwegian conditions, since they have not been part of the VCU trials or other variety trials before.

Heder and Marigold was the two varieties with the lowest content of total β-glucan, and they will therefore probably be the best varieties for poultry feed and for malting. There is to this day no production of barley to malting in Norway, but this could be a potential market for Norwegian grown barley. For malting purposes not only β-glucan content, but also other characteristics are important, and Heder and Marigold are so far not tested for this use.

Zhang et al. (2001) found highly significant interaction between variety and location, and pointed on the importance of selection of proper varieties for particular locations. Also in the present study significant interaction between location and variety for most of the parameters were found, but the varieties ranked pretty consistently concerning amount of total β-glucan content independent of location. Olve, Magdalena and Karmosé seemed to be the best varieties if a high content of total β-glucan is requested, and Heder and Marigold if low content is in focus.

In this study, variety was found to be the most important parameter to influence the total β-glucan content (counted for 57.9% of the variation). Location counted for almost 40% of the variation. Experiments like this can easily be designed to give either variety or location the biggest influence on the statistical variance, dependent on the choice of type and number of varieties and locations. In this study we have chosen some of the most common barley varieties grown in Norway today and in addition the varieties Magdalena, Karmosé and Olve since they have got special interest from the milling industry. If the locations had been over a wider geographical range, the impact of location might have been stronger compared to variety. The main interest of this study was to see how different barley varieties performed under Norwegian growing conditions, and to study the magnitude of variation that might exist due to different growing conditions. In this regard the chosen locations provided sufficient information.

A field sown with barley, not to experimental use, surrounded the experimental areas. The nitrogen application for the experimental fields was done according to the fertilization of the barley field surrounding the experimental fields. This could be a source of variation that may explain some of the observed between the different locations. Several studies (Güler 2003; Henry 1986; Oscarsson et al. 1998; Sørensen & Truelsen 1985; Truelsen 1987) have showed that increased application of nitrogen increases the level of total β-glucan in the harvested grain. This implies that the level of nitrogen fertilizer applied could cause some of the variation seen between fields. The

nitrogen application for each location can be seen in Table 5. The variation in nitrogen application is as expected for fields from different locations in Norway, since they have different yield expectations and climate. Jæren was outstanding because of the use of animal manure, and this resulted in a much higher application of phosphorus and potassium than the other fields, although not a strong difference in nitrogen (Yara 2009). Animal manure may result in a different distribution of the nitrogen during the season compared to mineral fertilizer, and this might influence the end results.

Jæren was the location with the highest fertilizer level, but the second lowest average yield. The opposite was seen in Vågå, where the lowest fertilizer level was applied, but the second highest yield. Vågå and Jæren are the two locations with the highest content of total β-glucan. This clearly shows the complexity in studying environmental effects, where local environment, soil and other factors affect yield and other quality parameters. Based on the results from these two locations, however, it could look like nitrogen is of less influence.

Hulless, waxy varieties are often used in other studies (Hang et al. 2007; Rey et al. 2009), especially in North America. It is less focus on this in Norwegian research, but this might be something to consider for later studies. Hulless barley does not need dehulling, and therefore it is more easily used for processing. Hulless barley will give lower yields than hulled barley because of the lack of hull. If hulless barely is requested by the industry, there is a need for a different price system. Today all barley in Norway is paid per kilogram and some varieties with higher or lower feed quality are given 1-2 øre less or more per kilogram (Felleskjøpet 2009). This will not increase the production of varieties that give lower yield. Known problems with hulless barley are discolouration of the flour and lower germination percentage because of damages during harvesting.

If it is possible to shift the food barley production towards production of the varieties with higher content of total β-glucan the industry will get access to barley with higher content of total β-glucan and the possible health effects could increase. A shift from Helium to Olve would (from the results of this study) increase the content of total β-glucan with 19%. If recommended intake of barley per day is 75 g with a total β-glucan content like Helium in this study (4.22%), a shift to Olve (5.02%) means that the minimum recommended intake of barley could be lowered with 19% to 60.75 g. This would still require quite a bit of diet awareness, but it would be easier to obtain for more people.

6. Conclusion

There was found large variation in total β-glucan content between the different varieties in this study. The total β-glucan content varied from 3.31% in Heder at Romerike to 5.83% in Magdalena at Jæren. The ranking from highest to lowest content of total β-glucan for the varieties was more or less similar at all locations. Based on this study, Olve is the most suitable variety for the use for human consumption. This was due to its high content of total β-glucan and a higher yield than the two other high β-glucan varieties, and the earlier maturation.

Variety was found to be the most important parameter to influence the total β-glucan content (57.9% of the variation) in this study. The location counted for almost 40% of the variation. It was hard to relate the variation in total β-glucan content to climate parameters as precipitation and temperature. This study only found a small positive correlation between total β-glucan content and minimum temperature during the grain filling period. The impact of climate and other environmental parameters on the content of total β-glucan must be further studied by including more than one season.

There are good possibilities to find Norwegian barley varieties with qualities that fit the optimal requirements for food and feed. One challenge will be to do a proper sorting at the local grain receiving station.

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Appendix 1: Raw data

Appendix 1: Raw data from all analyses performed in this study, sorted after location.

Location	Variety	Replicate	Yield kg/daa	Test weight, kg	Thousand grain weight, g	Protein content % dry matter	Total β – glucan content % dry matter	Starch content % dry matter
Apelsvoll	Edel	1	643.90	66.22	43.49	10.45	3.73	57.7
Apelsvoll	Edel	2	627.74	65.53	41.57	11.12	3.60	59.3
Apelsvoll	Heder	1	655.52	66.92	48.49	11.99	3.60	57.9
Apelsvoll	Heder	2	618.06	66.95	45.30	12.51	3.50	56.3
Apelsvoll	Helium	1	604.08	65.88	51.92	12.25	4.01	58.1
Apelsvoll	Helium	2	604.58	65.51	47.85	12.79	3.97	59.1
Apelsvoll	Karmosé	1	402.81	65.30	35.18	12.65	4.35	57.7
Apelsvoll	Karmosé	2	482.21	60.86	42.53	12.82	4.61	52.2
Apelsvoll	Magdalena	1	481.63	62.00	36.05	11.96	4.55	55.2
Apelsvoll	Magdalena	2	518.50	64.01	39.57	12.64	4.65	54.9
Apelsvoll	Marigold	1	654.50	65.87	48.82	11.94	3.63	58.3
Apelsvoll	Marigold	2	665.90	65.15	49.09	11.82	3.77	59.4
Apelsvoll	Olve	1	528.62	64.25	42.77	14.11	4.52	52.3
Apelsvoll	Olve	2	533.99	66.03	43.67	14.71	4.85	50.9
Apelsvoll	Skaun	1	703.29	67.15	42.06	11.09	4.00	56.5
Apelsvoll	Skaun	2	669.02	65.95	41.18	12.27	4.20	56.2
Apelsvoll	Tiril	1	652.49	66.01	42.46	12.24	4.17	56.3
Apelsvoll	Tiril	2	650.96	66.20	43.26	12.34	4.21	56.1
Germany	Edel	1	686.35	68.82	36.43	10.09	3.85	57.9
Germany	Edel	2	752.73	70.60	39.98	10.07	3.81	56.6

Location	Variety	Replicate	Yield kg/daa	Test weight, kg	Thousand grain weight, g	Protein content % dry matter	Total β – glucan content % dry matter	Starch content % dry matter
Germany	Heder	1	649.32	69.76	36.93	10.24	3.78	57.8
Germany	Heder	2	634.60	70.65	41.78	10.23	3.53	57.9
Germany	Helium	1	623.19	70.26	50.07	11.09	4.17	56.8
Germany	Helium	2	833.96	70.99	49.63	11.01	4.24	59.8
Germany	Karmosé	1	594.99	65.71	45.51	11.50	5.57	51.8
Germany	Karmosé	2	625.92	64.74	45.43	11.54	5.22	51.4
Germany	Magdalena	1	676.23	70.79	40.73	10.96	5.10	56.9
Germany	Magdalena	2	711.33	68.25	41.97	11.37	5.25	55.8
Germany	Marigold	1	747.13	67.52	39.71	9.81	3.51	55.1
Germany	Marigold	2	833.13	69.67	48.94	10.19	3.60	58.4
Germany	Olve	1	613.48	71.40	41.85	12.54	5.24	50.4
Germany	Olve	2	682.94	71.08	41.53	13.38	5.27	49.8
Germany	Skaun	1	697.67	68.04	35.87	9.97	4.37	53.6
Germany	Skaun	2	725.80	69.64	38.75	9.78	4.16	54.8
Germany	Tiril	1	521.38	66.33	32.27	10.69	3.83	54.3
Germany	Tiril	2	414.00	65.94	33.40	11.19	3.99	54.2
Jæren	Edel	1	577.51	71.74	41.04	12.17	3.92	56.9
Jæren	Edel	2	593.63	70.19	40.61	12.34	4.09	57.5
Jæren	Heder	1	567.26	71.17	42.98	13.29	3.93	56.5
Jæren	Heder	2	552.02	69.26	44.06	13.40	3.81	54.7
Jæren	Helium	1	511.29	72.08	47.97	13.83	3.50	55.8
Jæren	Helium	2	378.46	72.06	47.96	13.69	4.09	56.1
Jæren	Karmosé	1	338.62	68.91	45.81	15.12	4.55	51.8
Jæren	Karmosé	2	238.10	65.81	45.75	14.64	4.76	50.8
Jæren	Magdalena	1	373.51	70.14	45.21	14.38	5.15	54.6
Jæren	Magdalena	2	346.48	71.44	44.00	14.32	5.13	54.4

Location	Variety	Replicate	Yield kg/daa	Test weight, kg	Thousand grain weight, g	Protein content % dry matter	Total β – glucan content % dry matter	Starch content % dry matter
Jæren	Marigold	1	473.84	70.46	42.99	13.01	3.38	57.2
Jæren	Marigold	2	432.66	69.96	42.89	13.53	3.64	58.4
Jæren	Olve	1	414.36	70.23	41.32	16.12	5.26	50.0
Jæren	Olve	2	364.84	70.76	39.65	17.50	5.16	49.3
Jæren	Skaun	1	562.84	70.12	38.33	12.95	4.63	55.1
Jæren	Skaun	2	456.83	70.24	39.09	12.94	4.87	53.1
Jæren	Tiril	1	538.09	69.14	36.32	13.49	4.04	56.1
Jæren	Tiril	2	471.56	68.13	31.72	13.59	4.28	54.5
Namdalen	Edel	1	460.59	67.32	46.28	10.61	4.19	56.6
Namdalen	Edel	2	329.98	67.18	42.57	11.89	4.16	56.7
Namdalen	Heder	1	397.80	65.21	43.69	10.87	3.52	57.4
Namdalen	Heder	2	372.61	66.08	44.18	12.06	3.68	57.6
Namdalen	Helium	1	438.89	65.81	46.41	11.80	4.67	57.6
Namdalen	Helium	2	360.27	64.34	48.29	14.50	4.53	52.0
Namdalen	Karmosé	1	370.55	62.75	47.19	12.62	5.35	51.2
Namdalen	Karmosé	2	240.79	63.71	46.68	14.28	5.33	51.4
Namdalen	Magdalena	1	457.14	66.10	45.92	12.34	5.41	54.6
Namdalen	Magdalena	2	319.13	66.45	43.98	13.37	5.13	52.7
Namdalen	Marigold	1	478.37	65.90	46.76	11.14	4.04	58.9
Namdalen	Marigold	2	370.78	64.05	45.23	13.12	4.16	55.9
Namdalen	Olve	1	399.53	66.19	43.68	13.43	5.04	52.7
Namdalen	Olve	2	327.85	66.26	43.13	13.69	5.31	50.8
Namdalen	Skaun	1	445.28	63.96	38.10	11.61	4.65	55.5
Namdalen	Skaun	2	328.33	62.91	40.02	11.92	4.93	55.7
Namdalen	Tiril	1	476.06	62.09	38.75	11.24	4.66	57.7
Namdalen	Tiril	2	303.78	62.91	36.85	12.50	4.43	53.2

Location	Variety	Replicate	Yield kg/daa	Test weight, kg	Thousand grain weight, g	Protein content % dry matter	Total β – glucan content % dry matter	Starch content % dry matter
Romerike	Edel	1	655.39	67.32	36.41	11.68	3.31	56.5
Romerike	Edel	2	632.15	67.68	40.90	11.72	3.56	56.3
Romerike	Heder	1	733.56	65.17	37.31	12.14	3.29	56.1
Romerike	Heder	2	706.51	66.17	41.32	12.03	3.34	56.2
Romerike	Helium	1	606.60	65.92	45.50	12.19	3.80	56.7
Romerike	Helium	2	615.41	66.42	46.43	12.20	4.05	55.6
Romerike	Karmosé	1	363.32	57.54	34.69	13.29	4.10	50.4
Romerike	Karmosé	2	371.01	56.29	35.76	13.42	3.98	50.7
Romerike	Magdalena	1	422.21	58.21	30.60	12.91	3.69	54.0
Romerike	Magdalena	2	499.29	62.38	33.32	12.20	4.25	54.7
Romerike	Marigold	1	512.17	64.83	38.89	12.81	3.34	56.7
Romerike	Marigold	2	610.79	63.68	44.30	11.62	3.60	56.9
Romerike	Olve	1	576.38	66.20	37.90	14.70	4.26	50.6
Romerike	Olve	2	528.63	64.30	40.69	14.39	4.62	51.6
Romerike	Skaun	1	691.64	64.21	38.33	12.26	3.84	56.3
Romerike	Skaun	2	680.85	66.40	40.95	11.93	4.22	55.0
Romerike	Tiril	1	657.51	63.07	32.91	12.84	3.71	54.6
Romerike	Tiril	2	698.34	65.88	38.91	12.11	4.04	55.7
Sarpsborg	Edel	1	600.65	68.23	39.75	12.02	4.24	56.8
Sarpsborg	Edel	2	538.72	67.52	40.97	11.52	3.99	57.5
Sarpsborg	Heder	1	543.23	66.29	40.76	13.25	3.59	55.5
Sarpsborg	Heder	2	494.63	63.55	40.69	11.78	3.15	55.8
Sarpsborg	Helium	1	481.48	68.23	47.02	14.18	4.71	54.4
Sarpsborg	Helium	2	521.80	68.59	49.13	12.13	4.43	57.6
Sarpsborg	Karmosé	1	394.44	67.11	44.70	14.08	5.64	52.0
Sarpsborg	Karmosé	2	402.48	65.44	47.07	12.93	5.51	53.2

Location	Variety	Replicate	Yield kg/daa	Test weight, kg	Thousand grain weight, g	Protein content % dry matter	Total β – glucan content % dry matter	Starch content % dry matter
Sarpsborg	Magdalena	1	439.59	67.88	40.85	14.03	5.75	53.2
Sarpsborg	Magdalena	2	473.52	67.43	38.56	12.18	5.41	54.0
Sarpsborg	Marigold	1	521.62	66.38	43.86	12.83	4.27	55.7
Sarpsborg	Marigold	2	592.71	66.51	43.78	11.19	4.10	58.6
Sarpsborg	Olve	1	453.51	66.24	41.14	15.74	5.13	48.3
Sarpsborg	Olve	2	460.67	64.64	39.55	13.78	4.44	50.0
Sarpsborg	Skaun	1	481.15	65.99	41.64	13.55	4.68	54.7
Sarpsborg	Skaun	2	569.41	65.17	40.22	11.93	4.31	55.8
Sarpsborg	Tiril	1	504.59	64.85	37.46	14.34	3.60	53.5
Sarpsborg	Tiril	2	473.32	64.90	37.28	12.59	3.44	54.1
Vågå	Edel	1	740.92	73.50	45.12	11.86	4.87	59.1
Vågå	Edel	2	641.51	69.87	44.81	12.60	5.09	56.4
Vågå	Heder	1	587.13	67.34	43.46	13.37	4.43	56.4
Vågå	Heder	2	567.31	70.88	43.26	14.00	4.26	56.1
Vågå	Helium	1	668.86	72.61	48.99	12.23	4.33	58.6
Vågå	Helium	2	676.72	72.57	45.71	13.25	4.58	57.3
Vågå	Karmosé	1	534.28	70.97	48.14	13.74	5.41	53.4
Vågå	Karmosé	2	472.19	69.07	40.43	13.50	5.65	54.1
Vågå	Magdalena	1	520.45	68.71	38.31	12.58	5.65	55.2
Vågå	Magdalena	2	604.19	69.74	39.97	12.22	6.02	55.2
Vågå	Marigold	1	711.20	71.03	48.32	11.66	4.33	58.2
Vågå	Marigold	2	699.34	69.96	46.65	12.18	4.39	57.7
Vågå	Olve	1	569.70	68.52	38.58	15.63	5.49	50.1
Vågå	Olve	2	587.37	69.34	39.56	15.94	5.69	52.2
Vågå	Skaun	1	721.36	69.35	40.63	12.83	5.39	55.9
Vågå	Skaun	2	531.12	70.67	41.88	12.57	5.56	55.7

Location	Variety	Replicate	Yield kg/daa	Test weight, kg	Thousand grain weight, g	Protein content % dry matter	Total β – glucan content % dry matter	Starch content % dry matter
Vågå	Tiril	1	722.19	70.58	42.77	11.87	5.08	55.9
Vågå	Tiril	2	559.04	68.00	40.27	13.08	4.97	55.9

Appendix 2: Average temperature 2005-2009

Appendix 2: Average temperature at the six Norwegian locations from 2005-2009, with average temperature for each month and for each season.

Month	2005	2006	2007	2008	2009	*Average*	*2009-average*
Jæren							
May	8.4	10.1	8.6	11.0	9.8	9.6	0.2
June	11.6	12.1	14.2	13.2	12.5	12.7	-0.2
July	14.8	16.9	13.7	16.8	15.6	15.6	0.0
August	13.5	16.2	14.0	15.1	15.1	14.8	0.3
May-Sept	12.1	13.8	12.6	14.0	13.3	13.2	0.1
Sarpsborg							
April	5.9	4.3	5.1	6.3	7.3	5.8	1.5
May	9.3	11.3	10.6	11.4	11.2	10.8	0.4
June	13.8	14.9	17.0	15.1	14.7	15.1	-0.4
July	17.8	18.6	16.3	17.2	16.4	17.3	-0.9
August	15.5	17.2	14.0	15.1	15.8	15.5	0.3
May-Sept	12.5	13.3	12.6	13.0	13.1	12.9	0.2
Romerike							
May	8.4	10.2	9.7	10.6	10.6	9.9	0.7
June	13.4	15.0	15.2	14.8	13.6	14.4	-0.8
July	16.9	18.1	14.8	15.8	15.5	16.2	-0.7
August	14.4	16.3	14.9	13.6	14.6	14.8	-0.2
May-Sept	13.3	14.9	13.7	13.7	13.6	13.8	-0.2
Apelsvoll							
May	8.2	9.4	9.7	10.2	10.6	9.6	1.0
June	12.9	15.3	15.4	14.1	13.6	14.3	-0.7
July	17.0	18.3	14.8	16.7	15.3	16.4	-1.1
August	14.2	16.2	14.7	13.6	14.3	14.6	-0.3
May-Sept	13.1	14.8	13.7	13.7	13.5	13.7	-0.3
Vågå							
May	7.7	8.8	9.8	9.9	10.7	9.4	1.3
June	12.5	15.5	16.1	14.0	14.0	14.4	-0.4
July	16.9	18.5	15.3	16.9	15.8	16.7	-0.9
August	14.0	16.0	14.7	13.6	14.1	14.5	-0.4
Sept	10.6	13.0	8.5	9.3	11.6	10.6	1.0
May-Sept	12.3	14.4	12.9	12.7	13.2	13.1	0.1
Namdalen							
May	6.5	9.4	8.5	8.6	9.4	8.5	0.9
June	11.4	12.2	14.0	13.3	11.9	12.6	-0.7
July	16.1	15.2	16.1	16.3	15.5	15.8	-0.3
August	13.2	17.5	13.6	13.3	15.2	14.6	0.6
Sept	9.7	12.1	8.2	9.5	9.8	9.9	-0.1
May-Sept	11.4	13.3	12.1	12.2	12.4	12.3	0.1

Appendix 3: Precipitation 2005-2009

Appendix 3: Precipitation per month at the six Norwegian locations from 2005-2009, with average total precipitation for each month and total precipitation for each season.

Month	2005	2006	2007	2008	2009	*Average*	2009-average
Jæren							
May	89.0	44.6	56.4	7.5	100.9	*59.7*	41.2
June	33.3	53.3	64.1	90.7	27.7	*53.8*	-26.1
July	47.2	58.0	183.6	97.8	230.9	*123.5*	107.4
August	132.7	157.1	142.7	151.9	138.3	*144.5*	-6.2
May-Aug	*302.2*	*313.0*	*446.8*	*347.9*	*497.8*	*381.5*	116.3
Sarpsborg							
April	25.0	56.9	7.1	52.6	27.3	*33.8*	-6.5
May	71.7	92.4	73.4	38.4	61.1	*67.4*	-6.3
June	47.1	60.2	81.6	102.5	41.9	*66.7*	-24.8
July	76.2	53.9	145.1	109.4	163.2	*109.6*	53.6
August	73.2	112.7	76.0	153.5	101.1	*103.3*	-2.2
April-Aug	*293.2*	*376.1*	*383.2*	*456.4*	*394.6*	*380.7*	13.9
Romerike							
May	70.3	87.7	69.0	42.1	38.4	*61.5*	-23.1
June	27.0	59.2	92.9	32.3	34.9	*49.3*	-14.4
July	79.3	102.2	83.5	46.5	111.6	*84.6*	27.0
August	97.8	155.0	41.1	55.5	95.7	*89.0*	6.7
May-Sept	*274.4*	*404.1*	*286.5*	*176.4*	*280.6*	*284.4*	-3.8
Apelsvoll							
May	44.3	66.7	48.9	64.2	48.1	*54.4*	-6.3
June	47.6	31.1	89.3	42.8	39.8	*50.1*	-10.3
July	86.4	30.1	117.8	82.1	154.4	*94.2*	60.2
August	58.8	130.9	77.4	140.2	131.6	*107.8*	23.8
May-Aug	*237.1*	*258.8*	*333.4*	*329.3*	*373.9*	*306.5*	67.4
Vågå							
May	68.1	94.3	60.1	69.3	55.2	*69.4*	-14.2
June	58.4	42.0	61.7	80.6	48.1	*58.2*	-10.1
July	67.6	110.4	100.5	44.9	168.6	*98.4*	70.2
August	88.0	146.5	103.6	104.0	157.4	*119.9*	37.5
September	38.3	69.2	63.4	63.1	40.3	*54.9*	-14.6
May-Sept	*320.4*	*462.4*	*389.3*	*361.9*	*469.6*	*400.7*	68.9
Namdalen							
May	114.8	73.4	98.0	67.8	82.0	*87.2*	-5.2
June	173.8	106.2	10.0	115.6	61.8	*93.5*	-31.7
July	48.0	111.0	94.2	33.8	47.6	*66.9*	-19.3
August	151.8	24.8	133.6	39.8	112.0	*92.4*	19.6
September	245.0	163.6	177.8	90.6	359.8	*207.4*	152.4
May-Sept	*733.4*	*479.0*	*513.6*	*347.6*	*663.2*	*547.4*	115.8

Appendix 4: Average temperatures during the degree-day periods

Appendix 4: Average temperatures during the eight degree-day periods, for variety and location.

		Average temperature in degree-day period							
Location	**Variety**	**0-100**	**1-200**	**2-300**	**3-400**	**4-500**	**5-600**	**6-700**	**7-800**
Apelsvoll	**Edel**	18.94	13.86	15.57	13.97	14.14	16.67	13.06	13.60
Apelsvoll	**Heder**	22.36	14.85	14.87	14.60	14.53	14.27	16.29	11.98
Apelsvoll	**Helium**	13.31	15.69	13.97	14.19	16.07	13.45	14.80	
Apelsvoll	**Karmosé**	13.81	15.14	14.47	13.91	16.85	12.81	14.70	
Apelsvoll	**Magdalena**	13.81	15.14	14.47	13.91	16.85	12.81	14.70	
Apelsvoll	**Marigold**	16.52	14.21	14.74	14.73	14.16	16.70	12.56	16.50
Apelsvoll	**Olve**	18.94	13.86	15.57	13.97	14.14	16.67	13.06	
Apelsvoll	**Skaun**	22.26	13.36	15.35	14.54	14.04	15.49	14.93	12.23
Apelsvoll	**Tiril**	22.18	17.30	13.81	15.14	14.47	13.91	16.85	12.14
Jæren	**Edel**	19.62	17.40	15.67	16.26	15.85	15.88	18.55	
Jæren	**Heder**	20.00	19.12	15.50	16.87	16.00	15.48	17.22	
Jæren	**Helium**	18.72	15.47	16.55	15.93	15.66	18.68	16.67	
Jæren	**Karmosé**	17.40	15.67	16.40	15.79	15.88	18.98	15.12	
Jæren	**Magdalena**	17.40	15.67	16.40	15.79	15.88	18.98	15.65	
Jæren	**Marigold**	19.14	15.84	16.70	16.15	15.60	16.15	18.03	
Jæren	**Olve**	19.62	17.40	15.67	16.26	15.85	15.88	18.43	
Jæren	**Skaun**	20.80	19.14	15.97	16.38	16.27	15.60	16.15	19.25
Jæren	**Tiril**	20.80	19.14	15.97	16.38	16.27	15.60	16.15	21.00
Namdalen	**Edel**	15.23	14.63	17.10	19.22	13.35	14.20		
Namdalen	**Heder**	15.72	14.77	17.45	19.30	12.80	11.60		
Namdalen	**Helium**	14.77	17.45	19.30	12.67	14.09	15.63		
Namdalen	**Karmosé**	14.43	17.10	19.22	13.35	14.03	15.88		
Namdalen	**Magdalena**	14.43	17.10	19.22	13.35	14.03	14.03		
Namdalen	**Marigold**	14.51	15.72	18.67	15.35	13.35	14.36		
Namdalen	**Olve**	16.00	14.64	17.67	18.88	12.11	15.43		
Namdalen	**Skaun**	16.55	15.24	15.37	17.77	16.83	11.87		
Namdalen	**Tiril**	16.85	15.57	14.43	17.10	19.22	15.08		
Vågå	**Edel**	22.06	18.36	14.64	15.28	14.80	14.38	19.61	
Vågå	**Heder**	21.90	20.80	14.27	15.93	14.52	14.65	17.50	
Vågå	**Helium**	19.60	14.53	15.82	14.52	14.65	18.28	18.02	
Vågå	**Karmosé**	17.27	15.12	14.96	14.80	15.19	19.21	12.72	
Vågå	**Magdalena**	19.60	14.53	15.82	14.52	14.65	18.28	16.51	
Vågå	**Marigold**	22.06	14.40	15.56	14.74	14.63	16.36	18.80	
Vågå	**Olve**	22.22	15.72	15.28	14.73	15.28	14.97	20.85	
Vågå	**Skaun**	21.20	22.00	15.53	15.33	14.71	14.98	16.36	18.09
Vågå	**Tiril**	21.20	22.00	15.53	15.33	14.71	14.98	15.21	
Romerike	**Edel**	17.50	14.14	16.20	14.43	14.33	16.70	13.69	13.73
Romerike	**Heder**	19.94	13.37	16.26	14.53	14.31	16.10	13.79	
Romerike	**Helium**	14.14	16.20	14.43	14.33	16.70	13.69	15.08	
Romerike	**Karmosé**	14.37	15.85	14.77	14.26	16.67	12.93	15.02	
Romerike	**Magdalena**	14.37	15.85	14.77	14.26	16.67	12.93	15.83	
Romerike	**Marigold**	13.33	16.17	14.63	14.34	15.66	15.25	13.80	
Romerike	**Olve**	16.12	14.37	15.49	15.02	14.26	16.67	13.53	
Romerike	**Skaun**	21.42	13.33	15.97	15.03	14.34	15.18	15.71	13.18
Romerike	**Tiril**	21.42	13.33	15.97	15.03	14.34	15.18	15.71	11.50

Germany	**Edel**	13.87	15.72	19.37	24.75	20.13	14.99		
Germany	**Heder**	13.60	15.08	17.77	23.16	17.04	14.20		
Germany	**Helium**	14.90	18.90	20.74	23.16	17.04	19.94	19.06	
Germany	**Karmosé**	19.14	23.50	21.73	13.87	21.86	17.48		
Germany	**Magdalena**	19.42	22.22	22.16	15.83	21.80	17.58		
Germany	**Marigold**	14.90	18.90	20.74	23.16	21.66	17.04	19.94	23.50
Germany	**Olve**	14.09	15.54	19.14	23.50	14.99			
Germany	**Skaun**	13.87	15.72	19.37	24.75	20.13	14.99		
Germany	**Tiril**	13.87	14.90	18.90	20.74	16.64			
Sarpsborg	**Edel**	17.68	22.25	20.60	14.41	16.73	15.92	15.57	
Sarpsborg	**Heder**	14.46	21.62	22.13	15.56	15.67	16.48	15.83	
Sarpsborg	**Helium**	21.94	21.75	14.56	16.33	16.05	15.79	15.50	
Sarpsborg	**Karmosé**	21.94	21.75	14.56	16.33	16.05	15.79	17.96	
Sarpsborg	**Magdalena**	21.94	21.75	14.56	16.33	16.05	15.79	15.73	
Sarpsborg	**Marigold**	20.88	22.48	17.25	14.90	16.93	15.50	15.53	
Sarpsborg	**Olve**	18.38	22.12	19.14	14.56	16.95	15.70	15.90	
Sarpsborg	**Skaun**	13.44	20.88	22.40	15.92	15.03	16.93	15.64	15.18
Sarpsborg	**Tiril**	13.44	20.88	21.64	15.92	15.03	16.93	15.50	