baking quality in wheat: effect of delayed harvest, cultivars, growing conditions and nitrogen fertilization

neem lal pandey

Norwegian University of Life Sciences
Department of plant and environmental sciences (IPM)
Master Thesis 60 credits 2013

BAKING QUALTY IN WHEAT: EFFECT OF DELAYED HARVEST, CULTIVARS, GROWING CONDITIONS AND NITROGEN FERTILIZATION

M Sc. THESIS BY

NEEM LAL PANDEY

MASTERS OF SCIENCE IN PLANT SCIENCE

ECTS OF THESIS: 60

SUPERVISED BY

PROFESSOR ANNE KJERSTI UHLEN, PhD

A thesis submitted in partial fulfillment of the requirement for the degree of masters in Plant Science

Declaration

I, Neem Lal Pandey declare that this thesis is my original work and has never been submitted to any other university other than UMB for award of any type of academic degree. Sources of information other than my own have been acknowledged and a reference list has been appended.

Signature ……

Date …….

Acknowledgement

The report is my master thesis for the conclusion of my master program at Department of Plant and Environmental Science (IPM), Norwegian University of Life Sciences. I am grateful to the Government of Norway for providing opportunity for such a quality education free of cost even for International student like me.

I owe sincere thanks to my supervisor, Prof. Anne Kjersti Uhlen and appreciate her valuable guidance and support throughout the period of my project. Besides, I am very grateful to Eija Bakken for her immense help in field and laboratory work.

I am thankful to Mr. Raju Rimal, Subash Neupane, Nirmal Mani Dahal, Kedsirin and Ganesh Bahadur Rawat for their support and comments.

Finally, I express my heartily gratitude to my parents and family for their love, support and encouragement which was a valuable asset to pursue my studies.

Contents

List of tables

List of figures

Abbreviations:

ANOVA: analysis of variance

CP: Crude protein

FN: Falling number

HFN: Hagberg Falling Number

HMW-GS: high molecular weight glutenin subunits

LMW-GS: low molecular weight glutenin subunits

N: nitrogen

NIRS: near infrared reflectance spectroscopy

PHS: pre-harvest sprouting

PP: polymeric protein

SDS: Sodium dodecyl sulphate

SSDS: Specific Sodium dodecyl sulphate

Abstract:

FN, protein percent and gluten quality are amongst the most important quality parameters that determine the baking quality of wheat. Rainfall after physiological maturity causes PHS which reduces grain quality and value. To determine the effect of delayed harvest on PHS five spring wheat cultivars were used such that they were harvested one week after yellow ripeness, and then in 3 days interval for a period of six weeks. FN for each variety and harvest time was determined, as well as protein content and SDS sedimentation volume. Data from another set of wheat samples was used to study the effect of environment and nitrogen fertilizer on these quality parameters. This comprised data from the on-going research project, Future Wheat obtained from field trials of 12 varieties which were grown in Vollebekk and Apelsvoll in 2009, 2010 and 2011. FN, protein content and SDS sedimentation value was determined for each varieties and locations.

Results showed that FN, protein content and SDS sedimentation values were affected by delayed harvest. The FN showed stable and high values for a long time period (approximately one month), and then it decreased. Except Demonstrant all other 4 varieties maintained FN > 200 (threshold level set for bread wheat in Norway) until last harvest. These results are discussed against the actual weather data and the development of dormancy. Significant effects of environment, variety and N fertilization were found for FN, protein content, SDS sedimentation value and specific SDS value. Additional N fertilization at heading increased protein content and SDS sedimentation values in all varieties.

1 General Introduction:

Wheat (*Triticum* spp) is one of the most important and widely grown food crops with more than 25000 different cultivars (Sapone et al. 2012). Its cultivation was started with wild einkorn (diploid) and emmer (tetraploid) wheat around 10000 years ago during Neolithic Revolution, first series of agricultural revolutions. Due to its wide adaptability to diverse climatic conditions and multiple end-uses along with dynamic nature of genomes and polyploidy character, it has become a crop of financial and nutritional importance especially after the emergence of hexaploid wheat (Dubcovsky & Dvorak 2007) .

The trend of production and consumption of wheat is increasing world-wide. According to FAOSTAT (2013), the total average worldwide production of wheat in 2012 was 675 million tonnes with Norway contributing about 0.034% (235000 tonnes) of total world production. Large amount of the wheat produced is used for making bread, other baked goods, pasta and noodles or bulgar and couscous as in the Middle East and North Africa (Sapone et al. 2012). Nowadays people are more concerned about quality which is forcing processors to use wheat with specific quality attributes. Grain size, protein content and its composition as well as, starch content and its ability to gelatinize are important variables that determine wheat quality. And these characteristics depend on cultivar, growing conditions and other environmental factors and the interaction between cultivar and environment (Panozzo & Eagles 2000).

Quality for bread wheat is mainly determined based on falling number (FN), protein content and gluten quality. FN is a method aimed at determining the sprout damage and α-amylase activity in wheat grains which determines the flour quality for bread making (Wang et al. 2008). Timing, intensity and duration of rainfall affects pre-harvest sprouting (PHS) along with environmental conditions especially temperature (Biddulph et al. 2008). Large variation is found among varieties in their ability to resist PHS due to their inherited seed dormancy. Different studies revealed that FN can also be influenced by Nitrogen fertilization (Stewart & Dyke 1993). Similarly, the protein concentration and composition are found to affect the quality of baked products (Johansson 2002) which in addition are determined genetically and also affected due to environmental conditions (Johansson et al. 2001).

In many wheat producing regions there are challenges to meet criteria set for FN because of wet weather during harvesting time. This was particularly experienced in Norway in the year 2011 where most of the wheat produced had FN below 200 (threshold level set for food wheat in Norway). As sprout damage is highly dependent on rainfall, the predicted climate change can be more challenging for wheat producing areas that will have increased precipitation during the period of wheat maturation and harvest, as is predicted for south-east of Norway. So, breeding of more tolerant varieties as well as management practices to avoid sprout damage can be even more important in the future. Good knowledge on the phenomenon of PHS is an important basis for this.

1.1 Aims and Objectives:

This thesis focuses on: sprout damage as analyzed by FN in Norwegian varieties and their tolerance to PHS, the environmental conditions that cause PHS under Norwegian conditions and varieties ability to resist PHS upon delayed harvest. Two different materials were used,

- Field experiment with 5 varieties harvested at different dates to simulate delayed harvest and
- Field trials of commercial varieties grown at different locations and in different seasons that had different levels of sprout damage.

The aim of this study was to elucidate whether different harvesting times of wheat grain after yellow ripeness to about 45 days later will have effect on FN and also to analyze differences between the varieties in their ability to keep a high and stable FN. As protein content and gluten quality are important attributes for good quality wheat bread, analysis of these parameters are also included. The second aim of this study was to elucidate whether different varieties, their growing condition (nitrogen application), environment and their interactions have effect on quality parameters of wheat flour and thus, find the appropriate combination of fertilizer, environment and variety for best quality produce. In these regards, the work was carried out with following objectives:

- Examine the effect of different harvesting times after yellow ripeness of different wheat varieties in relation to environmental conditions, on FN.

- Examine the effect of genotypes, environment, nitrogen fertilization and their interactions on wheat flour quality parameters.

2 Literature Review:

Wheat is one of the most important cereal grain cultivated worldwide. Starchy endosperm storage tissue from wheat grain is used to produce bread, noodles, pasta and wide range of other food products (Tosi et al. 2011). For bread-making, grain crude protein concentration (CP%), gluten quality, Hagberg falling number (HFN) and specific weight are among the most important quality parameters (Gooding et al. 1997).

2.1 Starch:

Starch is the main component in wheat grain (54-72% of grain dry weight) and it affects the structure of baked products (Hoseney et al. 1978). It consists of 20-25% of the linear molecule, amylose and 75-80% of complex and highly branched molecule amylopectin (Stone et al. 2009). The starch is deposited in starch granules in the endosperm cells. Based on the size, mainly two types of starch granules are reported; the larger A-granules and the smaller B-granules. A types are larger than 10μm in diameter and appear 4 days after anthesis while B types are smaller than 10μm in diameter and are formed 12-14 days after anthesis (Dengate & Meredith 1984).In addition to this, there's also C type granules with size less than 5μm and are formed very late in grain filling (Bechtel et al. 1990). Although the number of A-granules is less (2-4%) than that of B-granules, A-granules represent the major mass (60-75%) of starch. A and B type granules consists of varying amount of amylose and amylopectin (Ao & Jane 2007).

2.1.1 Role in baking:

Breakdown of starch by amylases produce fermentable sugars for yeast fermentation. Besides, starch plays a vital role in producing optimal viscoelastic dough by diluting gluten and acting as a reservoir for water absorption (Burhans & Clapp 1942). Gelatinization takes place when starch is heated in excess of water, leading to large increase in viscosity which is the basis for dough to set in the baking process. The starch granules undergo several changes during gelatinization, where the partly break down of starch granules along with their swelling up to several times of its original size (Ahmed & Auras 2011) are the most important. Amylopectin is considered to be

responsible for water absorption, swelling and pasting of starch granules (Tester & Morrison 1990). However, amylose tends to leak out of the starch granules during gelatinization and contribute to the formation of starch gels, affecting the structure of final product (Hermansson & Svegmark 1996). Starch gelatinization sharply increase dough viscosity (Bloksma 1980) and creates cracks in cell membranes that prevents shrinkage of bread when cooled after baking(Kusunose et al. 1999).

The ratio of amylose to amylopectin and their molecular structure determines quality, texture and stability of end products as it affect solubility, gelatinization and retrogradation properties of starch (Blazek & Copeland 2008). Retrogradation is the recrystallization of amorphous starch paste when cooled after gelatinization. This process leads to gritty mouth feel. Normally the ratio between amylose and amylopectin is approximately 25:75 and this seems not to vary much between varieties. But starch mutants with 100% amylopectin have been found in wheat. Today, such varieties have been commercialized, and they are used for special products, but not for leavened bread and leavened baked products as they produce bread with poor crumb characteristics.

2.1.2 PHS and alpha-amylase activity:

Germination process occurring in grain with major physiological changes is termed as sprouting (Kruger 1994). If this phenomenon occurs in physiologically matured grains shortly before harvest, is termed as pre harvest sprouting (Fang & Chu 2008). During sprouting, among many physiological changes in grain, formation of generative enzymes and degradation of storage reserves are very important as these changes are responsible for reducing yield along with quality degradation of end-product (Kruger 1994).

Alpha amylase is one of the well known endo amylases that cleaves α, 1-4 glycosidic bond present in the inner part of amylose or amylopectin producing oligosaccharides as end-product (Pandey et al. 2000). Alpha-amylase activity in grain is triggered due to sprouting caused by rain during harvest period. This enzyme breaks down starch to glucose and maltose resulting in low viscosity of the flour-water slurry after gelatinization. This can be analyzed by HFN. HFN is time in seconds required for a stirrer to fall through a hot viscous medium of water and wheat flour solution in a tube incubated in a 100 ˚C water bath after 60 seconds of stirring (Humphreys

& Noll 2002; Lunn et al. 2001). Higher alpha-amylase activity in grain gives lower count of Falling Number resulting poor quality end-products (Edwards et al. 1989).

Some alpha amylases are needed for the yeasts to break starch into sugars by fermentation. This fermentation of starch produces carbon dioxide during leavening of the bread dough that helps to obtain high and optimal volumes of bread. However, too high alpha amylase activity will give too much breakdown of starch polymers resulting poor gelatinization properties, less binding of the water in the dough and sticky crumbs. Furthermore, dark crusts are formed because of the high sugar content that results in increasing Maillard reaction (caramelisation) (Gooding & Davies 1997).

2.1.3 Climatic conditions and PHS:

Rainfall prior to harvest creates favorable conditions for PHS in wheat grains. In white wheat producing regions like Australia, South Africa, Canada, Central Asia and Europe, PHS is a major problem (Biddulph et al. 2008) as rainfall occurs in most seasons during grain harvesting period . Therefore, dormancy and PHS become important considerations in these regions. Relatively cool weather and wet condition during harvest period result in high alpha-amylase activity in wheat grain, and thus lower FN (Smith & Gooding 1999). Climatic condition before 14 days of the physiological maturity of grains cause variation (non genetic) in seed dormancy between 10% and 65% (Strand 1989). Mares (1993) concluded that amount of rainfall during the 20-day period prior to harvest accounts for most seasonal variation in tolerance to sprouting.

Temperature during the grain development have effect on development of dormancy in seeds (Mares 1993). Nielsen et al. (1984) reported that lower temperature (minimum 8˚C and maximum 26˚C) is more favorable for the development of sprouting tolerant grain in comparison to higher temperature (minimum 17˚C and maximum 34˚C). Also, higher temperature of 2 weeks prior to maturity can lead to reduction in sprouting tolerance. This is also supported by the finding of Lunn et al. (2002) from their experiment on a sprouting tolerant variety Hornet. They found shorter dormancy period of grains in hot and dry years in comparison to cooler and wetter years. Besides temperature and rainfall, drought also has effect on seed dormancy. Mares (1993) found increase in sprouting tolerance of a wheat variety Kleiber due to extreme moisture stress as it showed higher FN after rain simulation in comparison to others that did not experience stress. Furthermore, moisture stress in combination to high temperature during grain fill can

create an induced dormancy in non-dormant seeds but not in dormant as found by Biddulph et al. (2007).

2.1.4 Tolerance to PHS:

Sprouting damage caused by rainfall is seen to be varied between and among wheat fields(Skerritt & Heywood 2000). Susceptibility to PHS varies among the varieties due to different rate of water infiltration into their kernel (Butcher & Stenvert 1973) particularly affected by awns and their associated structures. Process of wetting is slower in awnless spikes when compared to awned and thus, awnless cultivars are more resistance to PHS (King & Richards 1984). Moreover, King and von Wettstein-Knowles (2000) found that epicuticular waxes acts as water repellent in the mature ear and thus reduce sprouting.

Besides, physical factors some genetic factors also have control in seed dormancy. Red wheat is generally considered to be more tolerant to sprouting damage than white wheat. The red color (R) genes present in the homologous chromosome 3 of red wheat is considered to be responsible to impart dormancy in red wheat. Groos et al. (2002) mentioned that the dependency of dormancy to red color genes may be caused by pleotropic effect of these genes or due to linkage with other genes affecting PHS. The taVp1 loci was mapped by Bailey et al. (1999) on long arm chromosomes of 3A, 3B and 3D in wheat at 30 cM from R loci. taVp1 gene is orthologous to VP1 gene that is responsible for dormancy in maize (McCarty et al. 1991). Thus, if there is effect of grain color in dormancy it could be due to direct effect of R genes or their linkage to taVp1 gene.

However, flour from red wheat contains higher amount of bran specks than from white one which negatively influence appearance and acceptability of breads and noodles. This is forcing breeders to develop sprouting tolerant white wheat varieties (Kottearachchi et al. 2006). So, scientists are working to develop white wheat with significant level of seed dormancy and have developed several (Wu & Carver 1999). But level of dormancy is always much less in white-seeded wheat when compared to red one. Rasul et al. (2012), in his experiment on different varieties of red and white seeded spring wheat found genotypic variation as most influencing factor that affect FN, germination index and sprouting index (PHS traits) compared to genotype and environment interactions and suggested that PHS resistant cultivars could be developed by using genotypes regardless of environment.

2.2 Protein content

Carbohydrates, protein, amino acids, lipids and minerals are the major components in wheat grains that affect nutritional value and end-use quality. Among them protein is an important constituent that determines bread making quality (Pomeranz 1987; Shewry 2009). Protein content in fully matured wheat grain varies from 10-20% (Shewry et al. 1994) and normally from 10-15% in western Europe. If we assume 10 % protein content in wheat grain, it produced 66.3 x 10^9 kg total protein and 9.4 kg protein/capita/annum (assuming 7 billion world population) in the year 2012 (Balyan et al. 2013).

2.2.1 Role in baking:

Gluten forming storage proteins present in endosperm of wheat grains are responsible for rheological properties of dough that is required for the production of leavened bread and other diverse products (Gianibelli et al. 2001).Relation of protein content to loaf volume has been studied since long time. Finney and Barmore (1948) did a pioneering work in this area by finding that loaf volume of bread is affected by protein quantity, in their study on hard red winter and spring wheat cultivars. Further study in protein revealed that both quantity and quality of protein affect bread making properties like water absorption, oxidation requirements, loaf volume and crumb characteristics (Finney 1984). Water absorption in the flour is increased with increasing protein content resulting larger loaf volume and softer bread. It also shows effect on staling rates (bread with higher protein content can be stored longer) (Maleki et al. 1980).

2.2.2 Variation in protein content:

Grain yield and protein content are important parameters in wheat production (Groos et al. 2003). Protein content in wheat grain normally decreases with the increase in grain yield (Simmonds 1995). Negative correlation between these two traits is considered to be affected genetically (Groos et al. 2003) that is highly heritable . So, there are some chances to improve yield without affecting quality or vice-versa through breeding (Barraclough et al. 2010). Mobilization of nitrogen from various plant parts to head can increase grain protein content and reduction in dry weight of plant biomass (stem weight) can increase grain yield. So, understanding the genetic base for dry weight build up and nitrogen concentration of various plant parts can be useful for successful breeding of cultivars with high grain yield and high protein content (Malik et al. 2012).

Grain protein content and composition is affected by both genotype and the environment in which it is grown. Study on 212,600 lines of wheat in the World Wheat Collection showed that protein percent varied from 7 to 22% on a dry weight basis and genotype was considered to have effect on one third of variation (Vogel et al. 1976). Protein content in wheat grain can be increased by increasing level of nitrogen fertilizer application (Uhlen et al. 2004). But timing of nitrogen application could have different responses depending on the environment i.e. temperature in which wheat is grown. Split application of nitrogen during stem elongation or at heading can increase protein content in wheat grain. Such, application has become a common strategy in western Europe. In a study of Dupont et al. (2006) application of post anthesis nitrogen under moderate temperature (24˚C days and 17˚C nights) incresed rate of accumulation of protein as well as total protein content in wheat. But when grown in same condition under 37˚C days and 28˚C nights (higher temperature) post anthesis nitrogen did not have marked effect on rate of accumulation and total content of protein. However, protein percentage in grains grown at the higher temperature was higher than those grown at the lower teperature . Usually grain protein percent increase when environment conditions like drought and high temperature hinders grain yield to reach its potential (Fowler 2003). Bly and Woodard (2003) found postpollination application of nitrogen to be more effective for gaining wheat with higher protein content as well as higher yield when compared to application of nitrogen by boot stage.

2.3 Gluten protein:

Endosperm of wheat consists of 80% of total grain proteins and these proteins are not soluble in water at neutral pH or dilute salt solutions. Such insoluble storage proteins in endosperm are referred as gluten (Osborne 1907; Wieser 2007). Wheat gluten proteins can be classified into monomeric gliadins, lacking either disulphide bonds or containing only intra-chain bonds and the complex polymeric glutenins, with inter-chain disulphide bonds. Further gliadins are classified as α-, γ- and ω- types based on their mobility in acid PAGE gels and glutenins as low molecular weight glutenin subunits(LMW-GS) and high molecular weight glutenin subunits (HMW-GS) which link together by intermolecular disulphide bonds and form large insoluble polymers. HMW-GS is considered to have major impact on dough elasticity and thus has been studied in most detail (Shewry et al. 2003) although quantitatively they are less (10% of total storage protein) when compared to LMW-GS (40% of total storage protein) (Payne 1987).

The quality traits of wheat depends on its genetic constitution and these traits are the result of genes and their interaction with the environment (Gianibelli et al. 2001). Intra specific polymorphism present in gliadin and glutenin can be detected through gel electrophoresis. Multiple alleles present in different loci of homologous chromosomes 1 and 6 control the polymorphism of gliadin and glutenin subunits (Payne 1987). Glu-1 loci are located on the long arm chromosomes 1A, 1B and 1D of wheat and it consists of genes that encode HMW subunits of glutenin. On each locus there are 2 tightly linked genes – x-type and y-type. X-type encodes high molecular size subunits while y-type encodes low molecular size subunits. Due to differences in gene expression bread wheat possess 3 to 5 subunits (Payne 1987). All cultivars of wheat consists of the HMW-GS 1 Dx, Dy and 1Bx while only some consists of 1Ax and/or 1By (Gianibelli et al. 2001). Subunits 1Ax and 1Dx5+Dy10 are considered to have major role in determining bread-making quality where subunits 1Dx5+Dy10 is most important and responsible for production of larger size glutenin polymers (Gupta & MacRitchie 1994). So, in bread wheat, which are mainly characterized by subunits 1Dx2+1Dy12 and 1Dx5+1Dy10 in Glu-D1 locus, wheat with subunits 1Dx5+1Dy10 have superior bread quality (Rasheed et al. 2012).

2.3.1 Protein composition and gluten network:

Protein concentration and composition both affects quality. Even flours with equal protein content can produce bread with different loaf volumes. So, it is the quality of gluten protein that determines baking-quality at certain protein content. Although gluten protein is being studied since more than last 250 years (Shewry et al. 1995) the mechanism of gliadin and glutenin in dough formation is still a hot topic in literature (Li Vigni et al. 2013). However, most scientists agree on the fact that gluten is an important constituent that affects dough properties and thus viscoelastic behavior. And also glutenin fractions affect the strength and elastic properties of dough while gliadin fraction determines the dough viscosity and extensibility (Anjum et al. 2007).

Different baked products or even baking processes will require different viscoelastic properties. For a desirable viscoelastic properties of dough and quality of end-use product there should be balance between monomeric and polymeric protein fractions (Shewry & Tatham 1997; Wieser 2007). Such a balance between elasticity and extensibility is important in producing high-quality

bread. It helps to retain gas during fermentation to produce bread of larger volume. Extensibility is related to sheeting properties and low extensibility can reduce ovenspring (Khan & Shewry 2009). Bread volume from a cultivar depends on gluten strength of that cultivar which is determined by storage protein composition, amount of HMW-GS, Glu/Gli ratio and amount of SDS-soluble and SDS-insoluble polymeric proteins (Johansson et al. 2001).

2.3.2 Effect of management practices and environment:

Gluten protein strength depends on storage protein composition, amount of total HMW-GS, Glu/Gli ratio and amount of SDS-soluble and SDS-insoluble polymeric protein (PP), characters that are highly determined genetically. However environmental factors can modify gluten quality. Temperature along with nitrogen timing affects gluten strength (Johansson et al. 2005). Field studies show that variation in nitrogen application influences these parameters indicating that there is effect of nitrogen fertilizer in gluten strength and bread volume (Johansson et al. 2001). Godfrey et al. (2010) found an increase in proportion of gliadin proteins and dough extensibility with increase in nitrogen fertilization. There was an increasing trend in SDS sedimentation value with increasing temperature from 9 ˚C to 15 ˚C along with protein content (Uhlen et al. 1998). However, higher temperature (> 35˚C) during grain filling can enhance synthesis of gliadin reducing glutenin to gliadin ratio and thus result in weaker dough (Blumenthal et al. 1991). Johansson et al. (2001) in their experiment on 10 spring wheat found that different levels of nitrogen application affect glutenin and gliadin content which ultimately affects protein content and bread volume.

Quality characteristics like protein content, wet and dry gluten and rheological properties should be well defined by baking industries. So, many physical and chemical analysis should be performed before the flour is processed (Miralbés 2003). NIRS technique was used to measure protein content and SDS sedimentation test to measure gluten quality for this thesis research. Alpha amylase activity, an indication of PHS was determined by measuring Falling Number. Effect of late harvest of wheat quality has not been extensively studied. Information regarding probable changes in quality parameters after yellow ripening could be an important asset to plan for harvest time especially in regions like Norway where rainfall during harvest time is big problem.

3 Materials and Methods:

3.1 Material 1:

Five spring wheat cultivars, i.e. Bjarne, Zebra, Demonstrant, Sabin and Berserk were used in this study. Information about varieties is shown in table 1. Wheat seeds were sown on 2nd of may, 2012 in 10 plots in a randomized block design with two replications at Vollebekk research farm, Norwegian University of Life Sciences (UMB), Ås, Norway(59˚16'N and 11˚6'E) with seed rate of 20 kg/daa. NPK fertilization (22-3-10, Yara) of 55 kg/daa was given at the time of sowing. The field was treated by herbicide (Ariane) to control weeds. After yellow ripening (judged visually) samples corresponding to approximately 50g grains were cut from each plot using a scissor from 30th of August in every 3 days interval till 8th of October. Samples were dried in drying chambers at 25-30 ˚C until moisture content was below 14%, threshed, cleaned and then milled on Falling Number (FN) Laboratory mill 3100 (Perten Instruments AB, Huddinge, Sweden). The mill was carefully cleaned after each sample to avoid mixing.

All samples were analyzed for FN, protein content and SDS sedimentation volume as described below.

3.2 Material 2:

Material 2 consists of samples from field trials that were carried out in the on-going research project Future Wheat. The field trials performed at Vollebekk in 2009, 2010 and 2011, and at Apelsvoll in 2010 and 2011 were selected. The criterion for the selection was variation in FN. Each field trial contained 12 spring wheat varieties, and was laid out following a randomized block design with two replicates. The field trials included nine varieties of Norwegian/Nordic origin and three varieties of the HRS-type from Minnesota, US. Two N fertilisation strategies were included in the field trials of 2010 and 2011. These were N1) giving all N fertilisation at sowing using NPK fertiliser and N2) the same amount of NPK at sowing as N1 plus split application of 40 kg Nha^{-1} using calcium nitrate at heading (Zadox 49). The amount of fertiliser used at sowing was optimised for each location, and varied from 100 – 120 kg N/ha. Information about the varieties is shown in table 1, and details about the single field trials in table 2. Grain samples from each threshed plots were dried, cleaned, milled by Falling Number (FN)

Laboratory mill 3100 (Perten Instruments AB, Huddinge, Sweden), and analyzed by the methods described below. The results from the chemical analyses were made available for this master thesis.

Table 1: Overview of varieties used in material 1 and 2

The characteristics of the cultivars are according to Åssveen (2011) for varieties included in the Norwegian official variety testing

SN	Variety	Country	Characteristics
1	Avle	Sweden	Late, strong gluten
2	Bajass [1)]	Norway	
3	Bastian	Norway	Early, strong gluten
4	Berserk	Norway	Late, strong gluten
5	Bjarne	Norway	Late, strong gluten
6	Demonstrant	Norway	Late, strong gluten
7	Quarna [2)]	Switzerland	
8	RB07	USA	HRS
9	Sabin	USA	HRS
10	Tom	USA	HRS
11	Vinjett	Sweden	Very late, strong gluten
12	Zebra	Sweden	Late, strong gluten

[1)] New breeding line from Graminor, Norway, [2)] not tested in the Norwegian official variety testing.

Table 2: Overview of locations and management practices

S N	Location and year	Sowing date	Heading data (Zadox 49)	Anthesis (Zadox 65)	Yellow ripeness	Harvest date	Fertili-sation [1)] KgN/daa
1	Vollebekk 2009	29.4	27.6	30.6	7.8	19.8	13+0
2	Vollebekk 2010	3.5	3.7	8.7	16.8	1.9	13+4
3	Apelsvoll 2010	7.5	4.7	11.7	24.8	6.9	11+4
4	Vollebekk 2011	26.4	29.6	4.7	16.8	23.8	12+4
5	Apelsvoll 2011	28.4	1.7	7.7	22.8	6.9	11+4

[1)] Indicates amount of nitrogen given at sowing and additional amount given as split application.

3.3 Chemical analyses:

3.3.1 Falling number:

FN was analyzed using Falling Number 1800, Perten Instruments AB, Huddinge, Sweden) by following method no. 56-81 as described in AACC (2000). About 7 grams (according to moisture content in flour) of flour was weighted, and taken into two dry falling number tubes. Then 25 ml of distilled water was added in each tube and shook thoroughly to obtain homogenous dispersion. Without any delay viscometer stirrers were placed in both tubes and placed into the heating chamber of falling number apparatus and closed. The pick-up arm stop at top of tubes after stirring the solution for a minute and viscometer stirrer fell freely through gelatinized dispersion. After the process, the number of seconds required for the stirrer to fall through gelatinized dispersion, in addition to the 60 seconds of mixing was recorded as FN. Duplicate analyses for all samples (material 1 and material 2) were performed.

3.3.2 Protein content:

NIRS technique was used to measure protein percentage of whole-meal flour using Perten Inframatic 9200 according to the procedure described in AACC (2000) method No. 39-11. Calibration for whole-meal wheat flour was made by Perten Instrument AB, Huddinge, Sweden, with minor modification made by Department of Plant and Environmental Sciences, Ås, Norway. Samples of whole–meal were loaded in the calibrated instrument and readings were recorded, and are presented in dry weight basis.

3.3.3 SDS Sedimentation test:

SDS sedimentation value was determined according to method number 56-70 as described in AACC (2000). 6 g of whole wheat flour was weighted and transformed to a graduated cylinder in which 50 ml of water (colored by bromophenol blue dye) was added and the cylinder was shaken for 5 minutes. Then 50 ml of SDS solution was added in the cylinder and again left to shake for 5 minutes. The cylinder was allowed to stand to sediment the contents. After 15 minutes the volume of sediments was recorded as SDS sedimentation value.

3.4 Climatic data:

Climatic data from each location and year for material 1 and 2 was obtained from Bioforsk (2013). For material 1, weather data for the period from yellow ripeness to the last harvest date at Ås was collected. For material 2, weather data for the period from 14 days before yellow ripening to the harvest date of the field trials were collected. The weather data was processed and shown in the result part of the thesis.

3.5 Statistical analysis:

Micro-soft Excel 2007 version was used to make graphs and tables. Two way ANOVA and General Linear Model (GLM) was used to analyze the level of significance ($P<0.05$) of main treatments and their interactions by using Minitab software program (Minitab Ltd., Coventry, UK). The significance levels were set to 95% for all analysis.

Material 1 was analyzed by GLM using the model: Response = Harvest time + Variety + Harvest time*Variety + Replicate + Error. For material 2, the single field trials were analyzed by two way ANOVA (Vollebekk 2009), and with GLM for those having two nitrogen fertilizer treatments (model: Response = N fertilization + Variety + N fertilization*Variety + Replicate + Error). In addition combined analysis were performed using all experiments and N1, considering the year*location as different environments (model: Response = Environment + Variety + Environment*Variety + Replicate + Error). The environment was considered as fixed variable. Level of significance was shown as *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

4 Results:

4.1 Material 1:

Material 1 contains samples of five spring wheat varieties harvested from normal harvest time (1 week after yellow ripeness) and then in three days intervals for a period of six weeks simulating delayed harvest. The aim was to follow changes in FN during this period, but protein content and SDS sedimentation volume were also analyzed. The data were analyzed by two-way ANOVA using minitab. F-values and significance levels obtained are given in table 3.

Table 3: Variance ratio (F-values) for the main effects and interaction of cultivar and harvesting time on FN, SDS and Protein percent

	FN	Protein%	SDS
Cultivar	98 ***	340.47 ***	295.76 ***
Harvesting time	42.57 ***	9.51***	5.37 ***
Cultivar×Harvesting time	2.54 ***	0.88	0.44

4.1.1 Falling Number:

There was significant effect of cultivar, harvesting time and their interactions on FN (table-3). Fig. 1 shows the FN for all the varieties harvested from 30th of august to 8th of October that covers the period from normal harvesting time to until 6 weeks later. FN remained almost constant until ninth harvesting time (23rd September) and then started to decrease till thirteenth harvesting time (8th of October). Berserk and Bjarne had the highest FN in the first part of the period, compared to Sabin, Zebra and Demonstrant. Among the five varieties Demonstrant had a sharp decrease in FN after 9th harvesting and reached a final FN lower than the threshold level set for food wheat in Norway. FN for other varieties almost remained above 250 even at 6 weeks after yellow ripening, and showed a very good stability in FN upon delayed harvest in this experiment. Berserk showed a peak in FN at its third and fourth harvesting time. This was found in both replicates of the field trial.

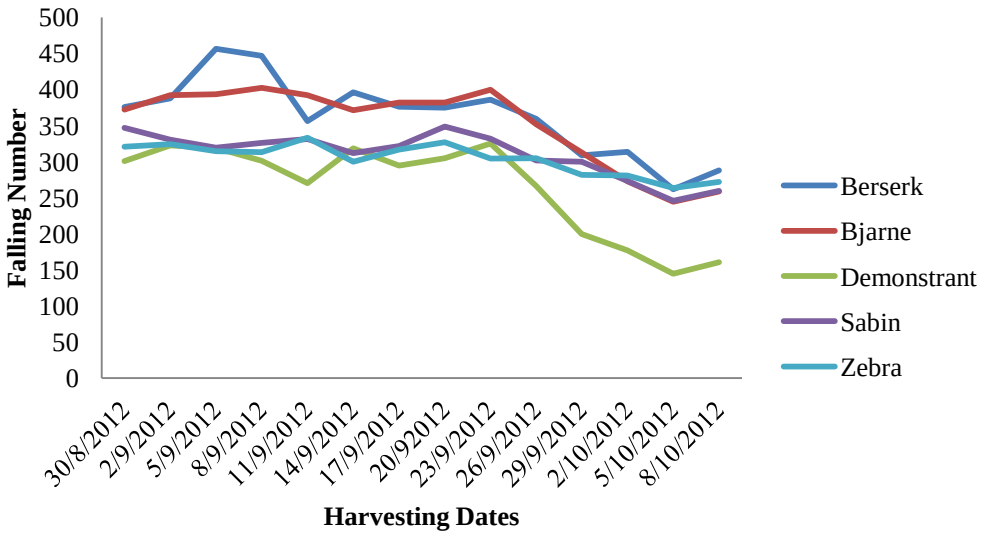

Figure 1: Average FN of two replications for five varieties harvested at three days interval, from 30th of August to 8th of October, 2012

4.1.2 Climatic data of Vollebekk:

Mean daily temperature and precipitation data recorded throughout the harvesting period at Vollebekk is shown in fig 2. Temperature was moderate (approximately 15 ˚C) for the first three harvests, then it started to drop till fourth harvest. There was rise in temperature again for a short period duration prior to fifth harvest. Then the temperature dropped and varied between 5 and 13 degree for rest of the period with even lower temperatures for the last two harvests.

In case of precipitation there was almost dry condition for first six harvests without heavy rainfall. Thereafter a wet period started with heavy rainfall for all harvests excluding 8^{th} and 9^{th}.

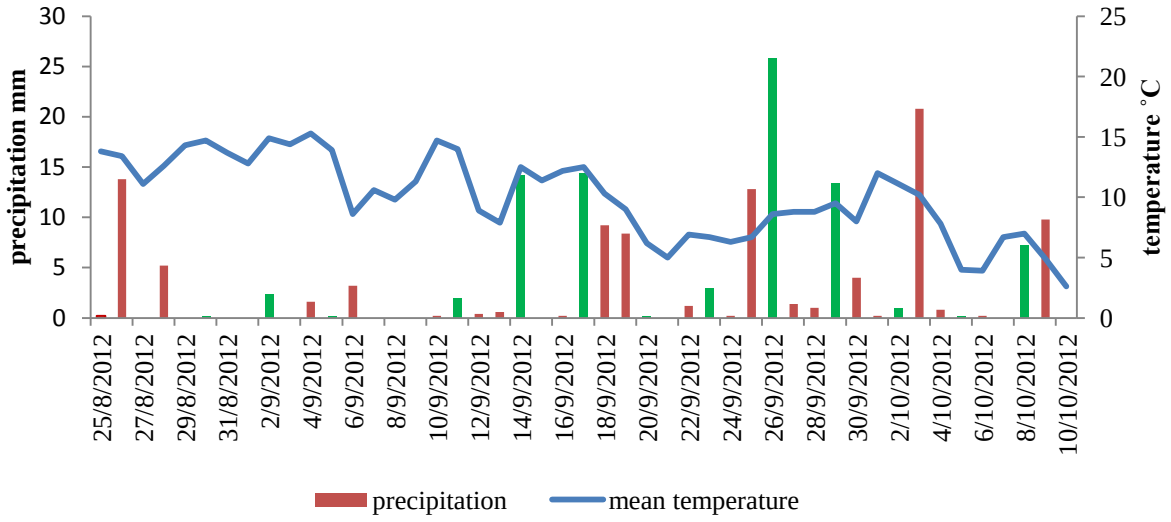

Figure 2: The daily mean temperature and precipitation at Vollebekk, from August 25 to October 10. Green colored bars for rainfall indicate the dates when wheat samples were harvested

4.1.3 Protein percent:

The average protein content, analyzed by NIR for all varieties and harvesting times are shown in figure 3. ANOVA result showed the significant effect of cultivar and harvesting time on protein percent (table 3). Protein content for all varieties remained almost similar till 7th to 9th harvesting times. Thereafter a moderate increase was obtained. Sabin had the highest protein percent while Zebra and Bjarne had the lowest. Among Norwegian varieties Berserk was observed to have the highest protein content.

The increase in protein content during the later harvest time was present for all varieties, and most pronounced for Demonstrant and Berserk. Protein content was analyzed by NIR, so the result could possibly have been interfered due to changes in light reflectance caused by PHS of the grains. Therefore, for first 3 and last 3 samples of the variety Demonstrant, nitrogen content was analyzed by Dumas method (Bremner & Mulvaney 1982) using Leco CHH 1000 at IPM lab of UMB, Ås, Norway. The result is shown in the table 4. This result also showed similar increasing trend for protein content during later harvesting times as found by NIR.

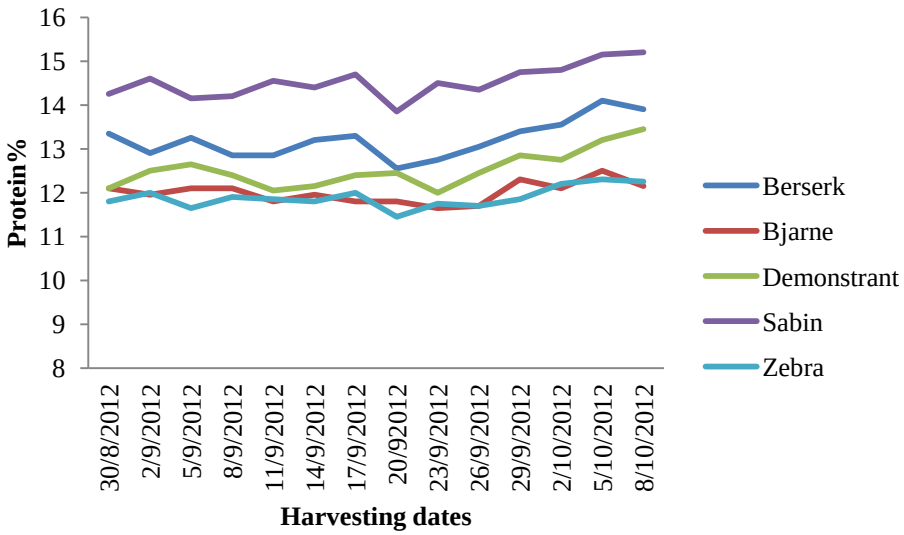

Figure 3: Average protein percent of two replications for five varieties harvested at three days interval, from 30th of August to 8th of October, 2012

Table 4: Nitrogen percent and protein content of demonstrant variety for first three and last three samples harvested

SN	Date	Nitrogen percent	Protein content
1	30-18-2012	1.96	11.21
2	2-09-2012	2.07	11.84
3	5-09-2012	2.07	11.81
4	2-10-2012	2.02	11.56
5	5-10-2012	2.08	11.85
6	8-10-2012	2.20	12.55

4.1.4 SDS value:

SDS sedimentation volume in accordance with AACC (2000) was analyzed for all the varieties and harvesting times, and averages for the two replications are shown in figure 4. ANOVA results showed significant effect of cultivar and harvesting time on SDS sedimentation value. Berserk had the highest SDS value, whereas Zebra and Sabin were having the lowest value. Within variety the SDS value remained similar for all harvesting times till 26th October, 2012, hence similar trend to the protein content. Thereafter there was moderate increase in SDS value for all varieties. Among five varieties, Berserk had the highest SDS value and Zebra the lowest.

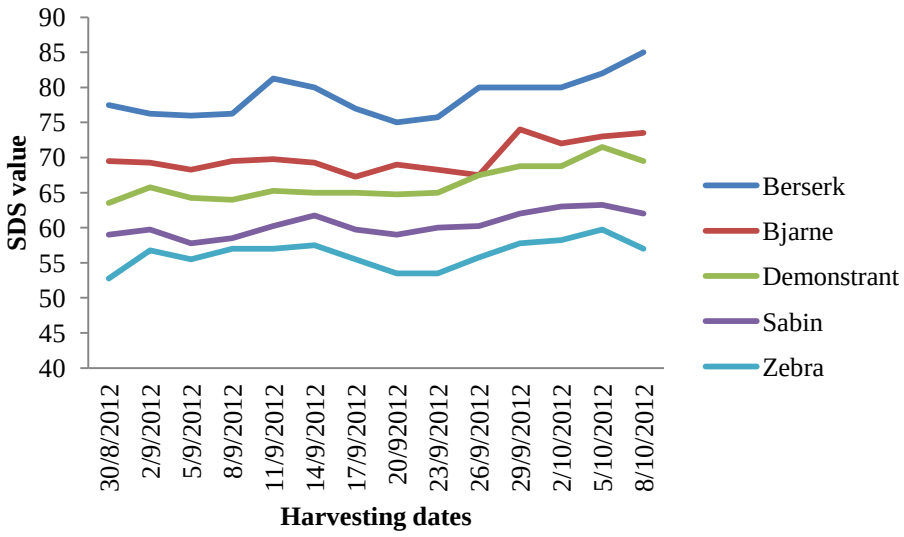

Figure 4: Average SDS value for two replications of five varieties harvested at three days interval, from 30th of August to 8th of October, 2012

4.2 Material 2:

Material 2 consists of samples from field trials at two different locations, grown in different years to investigate variation in PHS and gluten quality. Varieties were from Norway and Minnesota, including varieties used in material 1. Some of the field trials (2010 and 2011) also include effects of increased nitrogen fertilization, given as split application at heading.

Table 5 shows the averages of FN, protein percent, SDS and specific SDS for all varieties and replication with spring application (N1) of nitrogen from the field trials in the year from 2009-2011. Average FN and protein percent was highest for the field trial at Vollebekk in the year 2009 and the lowest FN was observed at Aplesvoll in the year 2011. Lowest protein percent was obtained at Vollebekk (2011), whereas average SDS value was highest for Apelsvoll (2011) and lowest at Vollebekk (2010). Similarly, Vollebekk (2011) field showed highest specific SDS value and Vollebekk (2010) the lowest.

Table 5:Average FN, protein percent, SDS and specific SDS of twelve varieties and two replications from Vollebekk and Apelsvoll harvested from the year 2009 to 2011 for normal N-application

SN	Field trials	Average of Falling No	Average of Protein%	Average of SDS	Average of Spes. SDS
1	Vollebekk 2009	315	13.7	76.8	57
2	Apelsvoll 2010	222	13	74.8	57.9
3	Vollebekk 2010	209	12.7	70.2	55.8
4	Apelsvoll 2011	165	13.3	84.2	63.4
5	Vollebekk 2011	264	11.8	77.6	66.3

*4.2.1 Variation between environments, varieties and variety*environment interactions:*

The data for FN, protein percent, SDS and specific SDS from all field trials and years with nitrogen application only at sowing was analyzed by ANOVA and the results are shown in table 6. There was significant effect of environment and variety for all the quality parameters. Interaction effect of environment and variety was significant for protein percent and specific SDS value.

Table 6: Variance ratio (F-value) for the main effects of location, variety and interactions of location and variety on FN, SDS, Protein percent and specific SDS

	Protein	FN	SDS	SSDS
Environment	30.18 ***	38.29 ***	27.55***	38.84 ***
Variety	40.52 ***	16.79 ***	35.29 ***	41.84 ***
E*V	3.09***	1.5	1.15	1.97**

Table 7 shows the average of FN, protein content, SDS and specific SDS for 12 varieties grown at Vollebekk and Apelsvoll in the year from 2009 to 2011 for N1. Large variation in FN was found between the varieties. The varieties Bajass, Berserk, Bjarne, Quarna, Sabin and Zebra had higher FN. Lower values, and below the threshold level set for food wheat in Norway was found in Avle, Vinjett and particularly in RB07. High protein content was found for the varieties from Minnesota (Sabin, Tom and RB07), having approximately 2% higher than those from Norway/Scandevia. Among the later groups Quarna, Bajass and Berserk had higher protein contents compared to Vinjett and Zebra, having lowest protein content. Although American varieties had higher protein content SDS value was higher for Bajass, Bastian, Berserk, Bjarne and Quarna. Vinjett showed the lowest SDS value. In case of specific SDS Bajass had the highest value and Sabin the lowest.

Table 7: Average FN, Protein percent, SDS sedimentation value and specific value for all varieties in both fields grown from 2009 to 2011 nitrogen application at the time of sowing only. abcdefg show the significant difference among the varieties. Values without common alphabets are significantly different.

Variety	Falling Number	Protein%	SDS	Specific SDS
Avle	174 c	12.5 cde	73.8 de	59.2 b
Bajass	290 a	12.7 cd	92.1 a	72.2 a
Bastian	239 abc	13.1 c	87.3 ab	66.7 a
Berserk	276 ab	12.7 c	84.5 ab	66.7 a
Bjarne	279 ab	12.1 def	81.7 bc	67.5 a
Demonstrant	247 ab	11.0 g	66 f	60.1 b
Quarna	278 ab	13.4 bc	81.8 bc	61.3 b
RB07	100 d	15 a	77.7 cd	52.9 cd
Sabin	265 ab	14.1 ab	68.2 ef	48.5 d
Tom	220 bc	14.9 a	74.2 de	50.1 d
Vinjett	175 c	11.4 fg	65.5 f	57.4 bc
Zebra	282 ab	11.7 efg	67.7 ef	58 bc

Figure 5 shows the interaction effects of environment and variety on protein content. RB07 had the highest protein content in 2009 Vollebekk field while Zebra had highest protein content in 2011 Appelsvol field and Tom in other remaining fields among the 12 varieties. Similar results can be seen in the figure below in which one variety showed better performance in one environment than another and vice-versa in another environment. For example when comparing Quarna and Bastian, these varieties had almost equal protein content in 2011 Vollebekk and Apelsvoll fields while in 2010 Vollebekk and Apelsvoll field Quarna showed higher protein content than Bastian and in 2009 Vollebekk field Bastian had little higher protein content than Quarna. Bastian, Berserk, Bajass, Avle, Bjarne Quarna showed good performance in Apelsvoll 2011 field in relation to protein content while Vinjett, Zebra and Sabin had highest protein content in Apelsvoll 2010 field and Demonstrant, Tom and RB07 in Vollebekk 2009 field. In average, the varieties from Minnesota are having higher protein contents, particularly RB07. Demonstrant had the lowest protein content in all the environments.

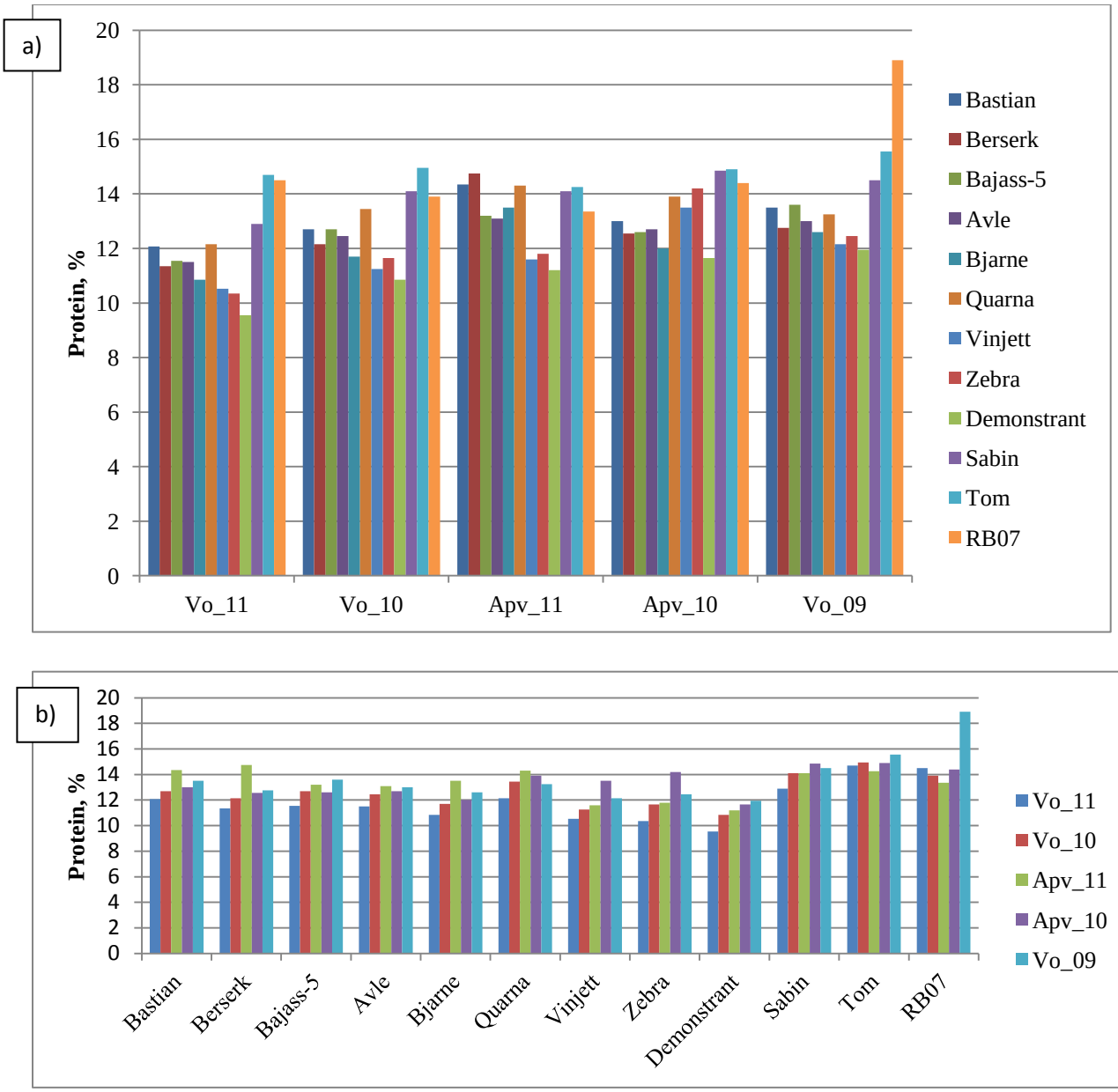

Figure 5: Interaction effect of environment and variety on protein content, as shown by bar plots for all varieties grown in the different environment where the environment are sorted after increasing protein content (a), and bar plot of all environments shown for different environments (b). Data are from all samples of wheat grown at Vollebekk and Apelsvoll in the year from 2009-2011 with nitrogen application at the time of sowing only

Specific SDS is suggested to more clearly show the differences between varieties in gluten quality. Figure 6 shows the interaction effect of environment and variety on specific SDS value. In Vollebekk field (2011) almost all varieties have higher values of specific SDS. Bajass had the highest specific SDS values in all environments. Interaction effect of variety and environment

could be seen clearly in most of the varieties. Bastian showed better performance than Berserk in Apelsvoll (2010 and 2011) while in others Berserk had higher specific SDS value. Similarly there was big difference in specific SDS value for RB07 among fields.

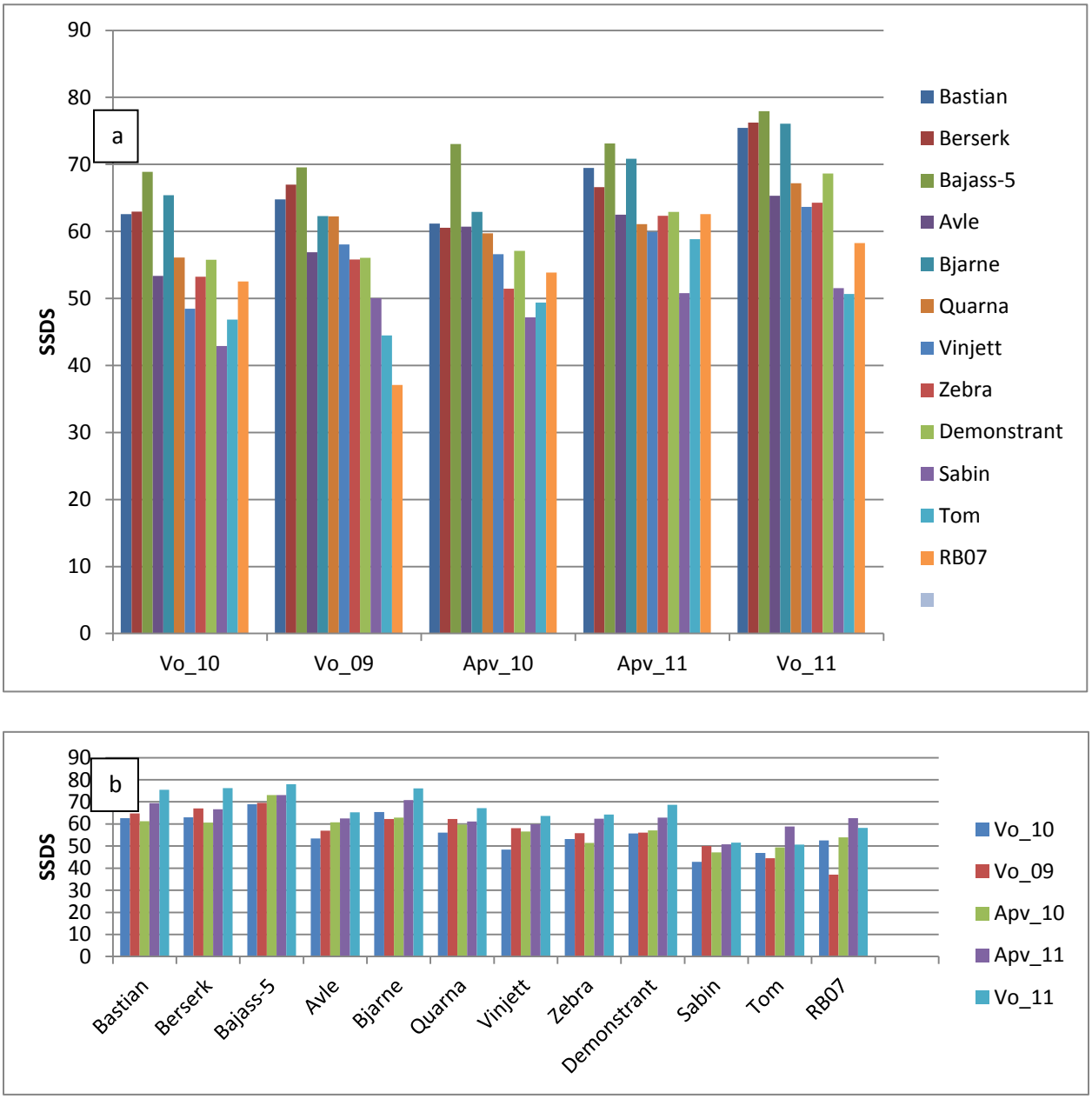

Figure 6: Interaction effect of variety and locations on specific SDS value, as shown by bar plots for all varieties grown in the different environment where the environment are sorted after increasing specific SDS value (a), and bar plot of all environments shown for the different environments (b). Data are from all samples of wheat grown at Vollebekk and Apelsvoll in the year from 2009-2011 with nitrogen application at the time of sowing only

4.2.2 Climatic data:

Mean daily temperature and precipitation for the period from 2 weeks before yellow ripening and to the harvest date of the field trails are shown in the figures below. Temperature in Vollebekk field did not vary much for the year 2009 and 2010. It remained nearly 15˚C for whole period. But in 2011 temperature was higher up to 20˚C, 2 weeks before harvest which decreased to 10˚C prior to yellow ripeness and again increased to 15˚C till harvesting. Before harvest there was wet period in Vollebekk for all years but comparatively 2010 field was wettest and 2011 driest. In Apelsvoll temperature was lower (10˚C) prior to harvest in 2010 while in 2011 it was higher (12 to 15˚C). Although there was some rain after yellow ripeness, a week before harvest was dry in Apelsvoll (2010). In 2011 there was comparatively more rain than in 2010 especially some days before harvest.

High FN at Vollebekk can be related to low temperature after yellow ripeness which helped grains to maintain dormancy even in wet environment. But Apelsvoll, 2011 field had very low FN. Higher temperature in combination to frequent rainfall before harvest might have caused that.

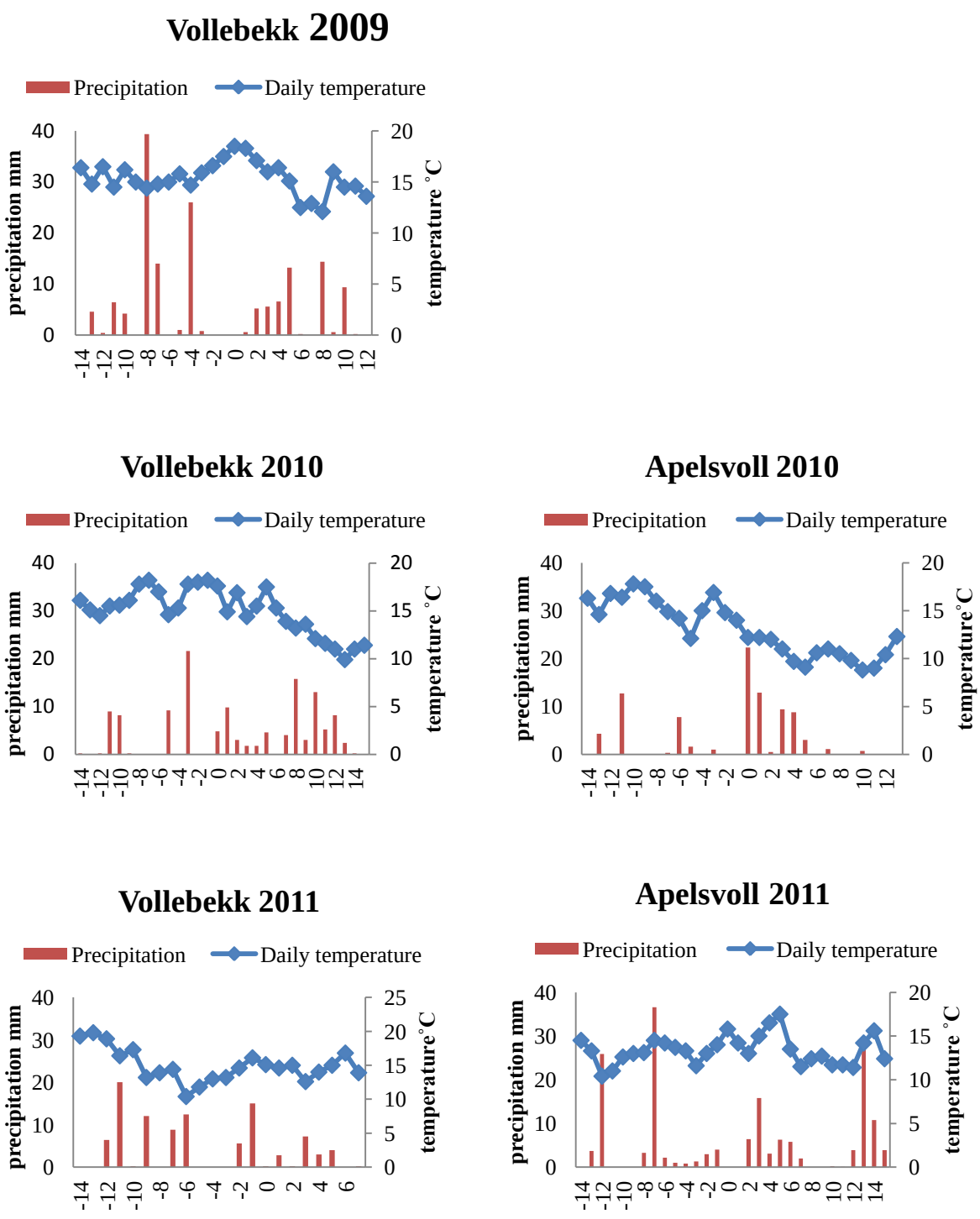

Figure 7: Mean daily temperature (degree celsius) and precipitation (mm) for the period from 14 days before yellow ripeness to harvest date for the Vollebekk and Apelsvoll in the year 2009, 2010 and 2011.Date for yellow ripeness is day 0.

Figure 8 shows the relation between the Protein content and SDS value in different varieties. There was weak positive correlation (R^2=0.251) between protein content and SDS values of 12 varieties.

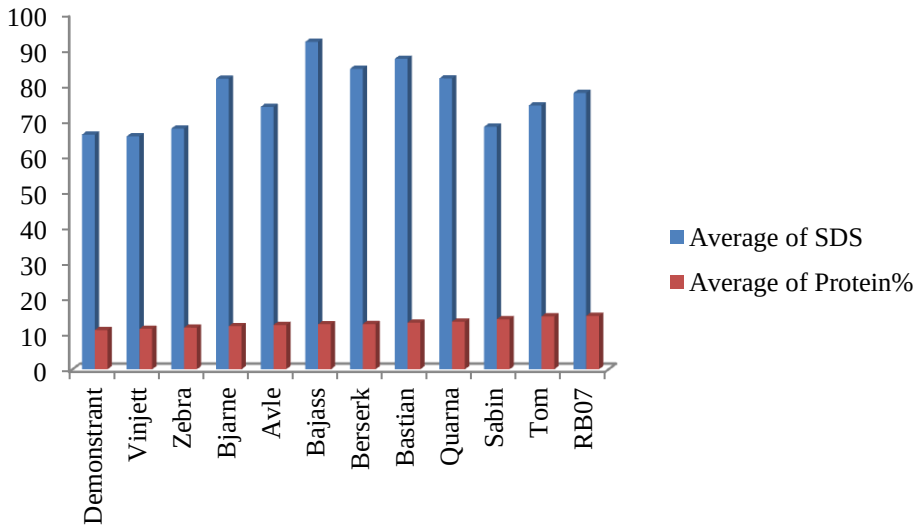

Figure 8: Average protein content and SDS value of 12 varieties from Vollebekk and Apelsvol field with N1 application from 2009 to 2011such that varieties are sorted as increasing protein content.

4.2.3 Effect of N fertilization along with variety and Environment:

The data from both fields for the year 2010 and 2011 with 12 varieties and 2 levels of nitrogen fertilizer treatments were analyzed by ANOVA using minitab and the result are shown in table 8 below. Result showed the significant effect of location and variety on all the quality parameters measured. Protein percent and SDS values were significantly affected by the two different nitrogen levels. Effect of nitrogen level was clearly observed on protein percent and SDS value and there was no effect on FN (table 8). No significant interaction between variety and N fertilization was found.

Table 8: Variance ratio(F-value) for the main effects of replication, variety and N-level and interactions of N-level and variety and location and replication on FN, SDS, Protein percent and specific SDS

	Protein %	FN	SDS	SSDS
Location	60.51 ***	47.39 ***	40.37 ***	74.59 ***
Nitrogen	228.14 ***	0.34	112.86 ***	1
Variety	58.94 ***	23.02 ***	45.65 ***	59.68 ***
N*V	0.65	0.92	1.22	1.11

The quality parameters for the two N fertilization regimes, averaged over all varieties and environment, are shown in table 9, and for the single varieties in table 10. A split application of additional 4kgN/daa, given at heading increased the protein content by 1.5% units in average for all the varieties and environments. Split fertilization also increased SDS by approximately 8ml, but no differences were found for specific SDS, indicating that the increase in SDS was to a general increase in concentration of the gluten proteins rather than changes in the gluten protein composition.

Table 9: Average FN, protein % and specific SDS for 12 varieties grown in the year 2010 and 2011 at 2 different nitrogen levels

Nitrogen level	Average FN	Average Protein%	Average SDS	Average Spes. SDS
N1	215	12.7	76.7	60.8
N2	219	14.2	84.5	60.3

Effects of 2 different N fertilizations on FN, protein content, SDS sedimentation value and specific SDS is shown in table 10. Split application of N at heading resulted in increase in protein content and SDS sedimentation value in all the varieties. But there was no marked effect in FN and specific SDS value.

Table 10: Average FN, protein content , SDS and specific SDS of the samples at Vollebekk and Apelsvoll in the year 2010 and 2011 at 2 different Nitrogen levels (1=Nitrogen allocation only at sowing, 2=1+ split application)

S N	Variety	Nitrogen Levels	Falling Number	Protein%	SDS	Specific SDS
1	Avle	1	138	12.3	73.8	59.8
		2	177	13.6	80.5	59.7
2	Bajass	1	263	12.5	91.5	73.2
		2	276	13.8	96.9	70.3
3	Bastian	1	228	13	87.2	67.2
		2	239	14.2	93.9	66.5
4	Berserk	1	258	12.7	84.3	66.6
		2	259	13.9	91.1	65.7
5	Bjarne	1	258	12	82.5	68.8
		2	274	13.5	91.8	68
6	Demonstrant	1	224	10.8	65.8	61.1
		2	254	12.3	77.2	63
7	Quarna	1	253	13.5	81.6	61
		2	252	15.2	91.5	60.5
8	RB07	1	100	14	79.6	56.8
		2	120	15.8	86.4	55
9	Sabin	1	243	14	67.1	48.1
		2	228	16	79.4	49.8
10	Tom	1	200	14.7	75.5	51.4
		2	164	16.5	77.2	46.8
11	Vinjett	1	138	11.2	64.2	57.3
		2	134	12.5	72.6	58.6
12	Zebra	1	278	11.5	67.2	58.5
		2	249	12.8	75.6	59.4

4.2.4 Effect of variety and N on Individual field:

ANOVA results for the single experiment from 2009 to 2011 in Vollebekk and Apelsvoll for twelve varieties, two replications and two nitrogen levels in the year 2010 and 2011 are shown in Table 11. There was significant effect of varieties for all quality parameters, for all locations and years. Nitrogen level had significant effect on protein percent and SDS for all fields in all years. Significant effect of nitrogen level on FN was also seen in both fields in the year 2010. Significant interactions between variety and N fertilization regime were found for specific SDS value at Vollebekk field (2010) and for FN at Apelsvoll field (2011).

Table 11: Variance ratio (F-value) for the main effects of replication, variety and N-level and interaction of N-level and variety on FN,SDS, Protein percent and Spes SDS

Year	Location	Quality Parameters	Variety	N-level	N-level×Variety
2009	Vollebekk	Protein	57.38 ***		
		FN	8.48 **		
		SDS	13.67 ***		
		Spes. SDS	28.58 ***		
2010	Vollebekk	Protein	51.23 ***	286.91 ***	1.501
		FN	62.06 ***	5.34 *	2.14
		SDS	28.96 ***	104.79 ***	0.86
		Spes. SDS	97.20 ***	0.46	2.35 *
	Apelsvoll	Protein	25.60 ***	193.66 ***	0.92
		FN	3.32 **	5.08 *	1.02
		SDS	20.42 ***	111.26 ***	0.551
		Spes. SDS	35.42 ***	0.92	1.60
2011	Vollebekk	Protein	58.05 ***	59.79 ***	1.33
		FN	41.57 ***	0.16	2.12
		SDS	33.88 ***	28.56 ***	0.92
		Spes. SDS	29.10 ***	0.71	0.30
	Apelsvoll	Protein	7.72 ***	23.22 ***	0.68
		FN	14.87 ***	3.78	3.47 **
		SDS	5.15 ***	6.18 *	0.97
		Spes. SDS	4.26 **	0.46	0.84

Figure 9 shows the interaction effect of variety and N fertilizer for Vollebekk 2010. Bastian, Berserk, Bajass, Quarna, Vinjett, Zebra, Demonstrant showed the positive effect on specific SDS value by additional application of 4 kg/daa N at heading. But there was decrease in specific SDS value due to additional application of N fertilizer in Avle, Bajrne, Tom and RB07.

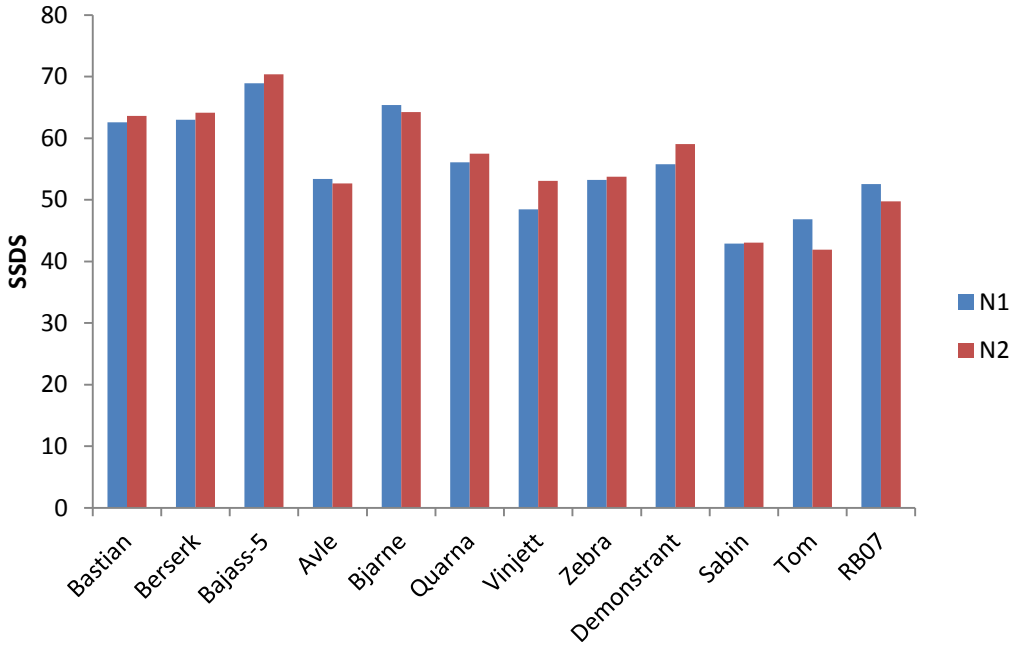

Figure 9: Plot showing the interaction effect of variety and nitrogen level on Spes SDS for year 2010, Vollebekk

Figure 10 shows the interaction effect of Nitrogen level and variety on FN for Apelsvoll 2011. Split application of Nitrogen decreased FN value in most of the varieties (Berserk, Bajass, Quarna, Zebra, Sabin, Tom and RB07). Sabin and Tom showed almost double value of FN when split application of Nitrogen was absent. While other varieties showed positive response of FN to split Nitrogen application at heading.

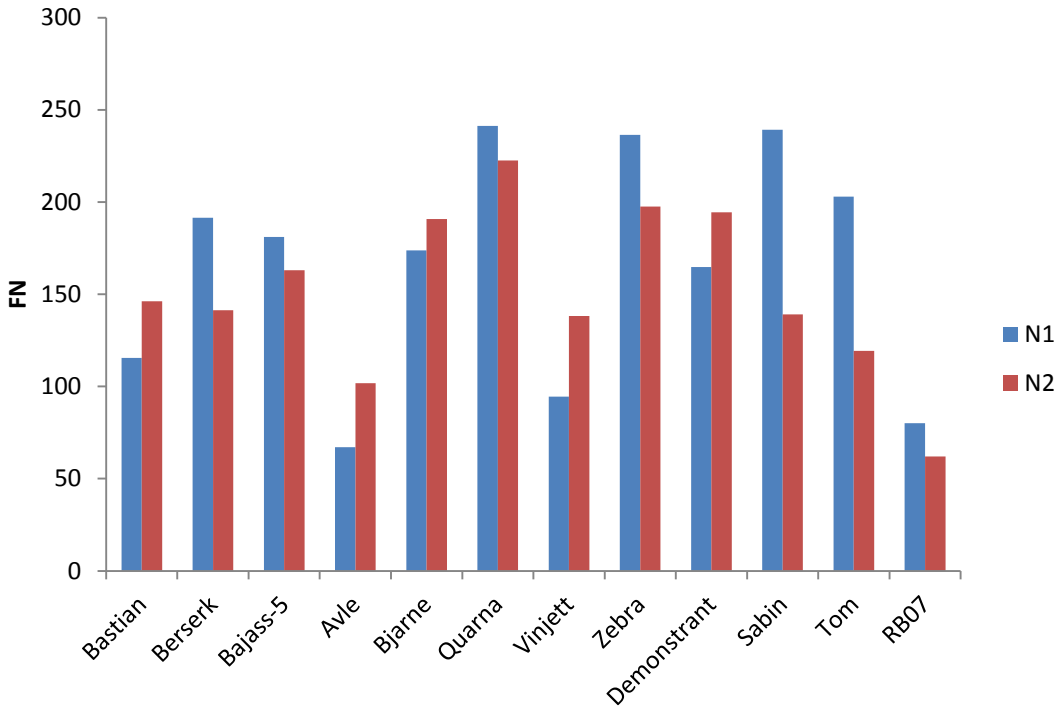

Figure 10: Plot showing the interaction effect of nitrogen level and variety on FN for year 2011 Apelsvoll

5 Discussion:

For wheat to be used for baking, the quality parameters FN, protein content and gluten quality are of overall importance to meet the requirements from the industry. The aim of this study was to explore deeper the variation in these quality parameters among spring wheat cultivars and how different weather conditions and harvest time may influence upon the variation.

The results showed that, both variety and environment affected all wheat quality parameters measured. However, effects of nitrogen fertilization were mainly seen on protein content and SDS sedimentation value. Different harvest times of wheat grain after yellow ripening also had effect on FN, protein content and SDS sedimentation value. The single experiments were exposed to different weather conditions that affected the measured quality parameters.

5.1 Material 1:

Material 1 was conducted to study effect of delayed harvest on these quality parameters, of which FN was considered as most important as PHS might commonly occur. In commercial wheat production, we see large variation in harvest time, and delayed harvest may be caused not only by difficult weather conditions but also by lack of harvest or drying capacity on the farm units. In this experiment, samples were harvested regularly from approximately one week after yellow ripening and until 42 days after that. The aim was to follow changes in FN during this time period, and to explore the ability of the different varieties to tolerate delayed harvest and maintain FN above 200, the threshold level to be accepted as food wheat in Norway. This type of study has not been done before with commercial wheat varieties that are recommended for Norwegian famers today.

Result from ANOVA implies that variation in cultivar and harvest time could significantly influence FN, and also the protein content and SDS sedimentation value. However, FN for each variety was high and stable for the first 9 harvests, which was unexpected. This can, however, be explained by the weather conditions during this period. There was relatively moderate temperature (15 ˚C) and lack of heavy rainfall for the first five harvests. This might be the reason that wheat plants withstand germination for such long time.

The ability to resist PHS after yellow ripening could be linked to the build-up of dormancy in the grains. Temperature and rainfall prior to harvest greatly influence dormancy of grain (Mares 1993; Nielsen et al. 1984), and lower temperature prior to yellow ripeness could generally cause deeper dormancy. Furthermore, cool and/or wet conditions after yellow ripeness could trigger development of secondary dormancy. So, favorable condition for dormancy development before yellow ripeness, as well as the following cool and dry condition after yellow ripeness could explain the ability of the crop to withstand sprouting for such long time.

However, in spite of wet condition from 6th harvest there was no marked effect in FN till 9th harvest. After that stage, FN decreased and the decrease was sharper for the variety Demonstrant which dropped below 200. Lower temperature (<15˚C) might have increased the duration of lag period for germination, so that lower FN was not detected until 9th harvest. Furthermore, dormancy of grains declines with time after yellow ripeness (Mares 1993) and the grains may germinate more easily at later stage of ripeness when compared to initial stage of ripeness and at wider range of temperature (Mares 1984). This might be the reason for germination of grain at 9th harvest and subsequently lower FN from that time. Sensitivity of ABA in wheat grains can affect level of germination of seeds and there are differences in it (Walker-Simmons 1987). This might be the reason that there was sharper decrease in FN in Demonstrant variety when compared to others.

The varieties used in this study, except for Sabin were tested for dormancy index at 150 degree days after yellow ripeness by UMB based on field trials located at the same field at Vollebekk in 2012. This result (given below) reveals a certain level of dormancy, which may partly explain the result from this study that FN of these varieties remain high for long period after yellow ripeness. However, the differences seen in Dormancy index between the varieties cannot explain the less tolerance for PHS seen in Demonstrant.

Variety name	Dormancy Index
Berserk	10.6
Bjarne	20.7
Demonstrant	20.88

Zebra	21.77

Initially, after yellow ripeness, FN for Berserk and Bjarne was higher in comparison to the other varieties but later after 9th harvest they were somehow similar to other varieties. Possibly there could be difference in alpha-amylase activity that explains the differences between varieties. Furthermore, as FN measures the viscosity of a gelatinized flour-water mixture, differences in water absorption linked to differences in fiber composition and non-starch polysaccharides or difference in polymerization of starch between these varieties could also be an explanation for such differences.

Significant differences in protein content and SDS sedimentation values among varieties reveal the genotypic variation in protein quantity and quality of these varieties. The variation seen in protein content and gluten quality is in agreement with the official variety testing results of Norway (Sundgren et al. 2013). The result is also supported by the finding that many genes and multiple QTLs have effect on grain protein concentration by Bogard et al. (2011) and on gluten strength by Peterson et al. (1992) and there is genetic variability among varieties. Some genes are known to effect gluten quality. The HMW gluten composition of these varieties is known and all varieties are having the Glu-D1 encoded 5+10, giving strong gluten quality. As the composition of HMW-GS in these varieties are fairly similar, it is possibly that allelic variation in LMW-GS or gliadins is responsible for the inherited variation in gluten quality among these varieties. Furthermore, variation in protein content that we see in field trials with similar nitrogen fertilization could also reflect variations in yield between the cultivars as high yielding cultivars normally will have lower protein content.

Protein content together with SDS value also showed effect of harvesting time. The values for both quality parameters tend to increase slightly from 9th harvesting time. It was surprising and beyond the expectation. One possible explanation for this could be that due to higher alpha amylase activity, starch breakdown to form some non gluten proteins in seeds. As there is some weak correlation between protein content and SDS value as seen in our result SDS value also might have shown increasing trend as protein during later harvests.

5.2 Material 2:

For materials 2 secondary data from the field trials of the on-going research project Future Wheat was used. These data were taken to simulate the effect of different environment and variety along with their interactions on FN, protein content and SDS sedimentation value. As N fertilizer is considered as an important factor that can influence these quality parameters, its effect was also seen through 2 different nitrogen applications in the field. i.e. N1- at sowing only and N2- same amount at sowing plus 40 kgN/ha at heading.

Field trials were selected for this study with no, medium and severe PHS and the selected experiments comprised of two different locations (Vollebekk and Apelsvoll), 3 years (2009-2011) in Vollebekk and 2 years (2010-2011) in Apelsvoll. Variation in temperature and precipitation can be observed between these two regions along with the years in which they were cultivated. In general, PHS occurs when dormancy in the grain is lost, and when the weather conditions prior to harvest are optimal for sprouting. Both criteria must be fulfilled to get severe PHS. Temperature and rainfall 14 days prior to harvest greatly influence development of dormancy, and lower temperature in this period gives deeper dormancy giving better PHS tolerance in grain and thus higher FN (Barnard & Smith 2009). Such effect of temperature and precipitation on FN can possibly be seen in the Apelsvoll field in 2010 where there was less rainfall and lower average temperature 2 weeks before harvest than in 2011. However, the severe PHS and low FN seen in the Apelsvol field in 2011could also be explained with frequent rain after yellow ripeness, giving delayed harvest, also combined with a relatively higher temperature.

It is well known that variation in sprouting damage can be seen between different fields, as well different sites within the same field (Skerritt & Heywood 2000). Along with environment, sprouting also depends on susceptibility of variety, under favorable condition, for sprouting. In addition to genetic variability in dormancy (Bewley 1997), morphological features as awns, waxes also affect sprouting (Butcher & Stenvert 1973). Waxes act as water repellent and awns affect water infiltration into the kernel and these morphological characteristics vary among the varieties. Such effects of variety and environment were also seen in our experiment where we found the significant effects of environment and variety on FN. Norwegian varieties are awnless,

but the variety Sabin, Tom and RB07 have awns. Thus, these varieties can be more susceptible to PHS, and this was seen at least for RB07. Differences in dormancy between the varieties also affect FN. Avle and Vinjett used in this experiment are less dormant and showed lower FN when compared to other varieties. However, less tolerance to PHS seen in Demonstrant from material 1 was not seen in material 2. The reason behind this is difficult to explain from our experiment and as Demonstrant is a promising spring wheat variety in Norway further experiments on this variety could be fruitful.

Protein content varied between environments and also between varieties. Pleiotropic QTLs present in the chromosomes 2A, 2D and 7D have been reported to have effect on protein concentration along with yield and leaf senescence of wheat(Bogard et al. 2011). Typically in this study, the varieties from Minnesota gave higher protein content. These varieties had longer straw and gave lower yields. Among the Norwegian varieties, the high yielding and newly recommended variety Demonstrant gave lower protein content. Thus, in this study variation in protein content due to variation in yielding potential may be present. Whether some of these varieties are having the QTLs for higher protein content or not, is not known.

Along with genotypes there are number of environmental factors that can cause variation in protein concentration as such nitrogen availability (McDonald 1992), temperature (Stone & Nicolas 1998), rainfall (Taylor & Gilmour 1971) , drought and water stress (Johansson et al. 2001) and disease and other biotic and abiotic stresses(Dimmock & Gooding 2002). Our study was limited to the effect of temperature, rainfall and variety on protein concentration and it showed the significant effect of these factors on protein content of different varieties.

Temperature at which wheat is grown may greatly influence crude protein in grains. Temperature from anthesis has an effect on duration of grain filling. Higher temperature usually results in faster growth rate but shorter grain filling period. Shorter grain filling period is expected to be more detrimental to carbohydrate accumulation than for protein. So, grain developed in higher temperature are usually smaller with less starch content which increases the protein percentage in grain when compared to grain developed at lower temperature. Besides this, rainfall prior to grain filling results in increase in N loss from field through leaching and other forms which dilutes the nitrogen reserves by vegetative parts of plant and finally the total protein content in grain (Taylor & Gilmour 1971). So, higher temperature and rainfall might be the main reasons

for low average protein content of 12 varieties in Vollebekk, 2011 field when compared to other fields.

Gluten strength can be affected by both genotype and environment, mainly temperature during maturation. In this study, large variation in SDS was seen between the environments, but this variation did not follow the protein content. Furthermore, large differences in specific SDS were also seen between environments. This shows the effect of different environments in gluten quality. Among environmental factors temperature during maturation is listed among the most important factor affecting gluten strength (Zhu & Khan 2001). Usually warm grain filling period produces stronger gluten in comparison to cool and wet grain filling period (Johansson et al. 1999). Generally, the seasons 2009-2011 can be characterized as relatively cool and wet seasons in Norway. A clear temperature difference between the selected environments that may affect gluten quality was difficult to find. Varietal difference in SDS is well known from the literature, and based on the HMW-GS 5+10 and 2+12, gluten can be classified as strong and weak gluten where the former type refers to strong gluten type (Payne et al. 1987). All varieties used in this study fall under the category of strong gluten type. However, other studies have found that HMW-GS 5+10 shows greater variability and are having less stability in different environments when compared to HMW-GS 2+12 (Johansson et al. 1999). The result also showed that highest SDS values were found in some of the varieties bred in Norway, as Berserk and Bajass. The varieties from Minnesota, included in the study as they are known to have excellent gluten quality, were not better than the best Norwegian varieties, indicating that the Norwegian varieties have good genetic potential to obtain strong gluten flours.

Wheat varieties show response to N fertilizer application. Increase in N application results in significant increase in gliadins and glutenins along with protein content and bread volume (Johansson et al. 2001). Timing of Nitrogen application can have effect on protein content and SDS sedimentation value where late (flowering) application gives higher value in comparison to early(booting) application (Luo et al. 2000). This is in accordance to our result where we find significant increase in protein content and SDS value when shifting from N application at sowing only to sowing plus heading.

6 Conclusions:

Result from Material 1 showed that different wheat varieties can maintain higher FN for a long period after yellow ripeness in field, depending on the weather conditions. This may be useful for farmers for making harvesting plan and save the expenses for drying of crops, but more knowledge is needed about the specific weather conditions causing PHS in this period, as well as the build-up of dormancy before yellow ripening. Result from Material 2 showed significant effects of environment, variety and N fertilization on FN, protein content, SDS sedimentation value and specific SDS value. Frequent rainfall after yellow ripeness results in delayed harvest and greater PHS. The result showed that protein content and gluten strength can be manipulated through selection of varieties along with split application of N fertilizer at heading.

7 References

AACC, C. (2000). Approved methods of the American association of cereal chemists. *Methods*, 54: 21.

Ahmed, J. & Auras, R. (2011). Effect of acid hydrolysis on rheological and thermal characteristics of lentil starch slurry. *LWT-Food Science and Technology*, 44 (4): 976-983.

Anjum, F. M., Khan, M. R., Din, A., Saeed, M., Pasha, I. & Arshad, M. U. (2007). Wheat gluten: high molecular weight glutenin subunits—structure, genetics, and relation to dough elasticity. *Journal of food science*, 72 (3): R56-R63.

Ao, Z. & Jane, J.-l. (2007). Characterization and modeling of the A-and B-granule starches of wheat, triticale, and barley. *Carbohydrate Polymers*, 67 (1): 46-55.

Bailey, P., McKibbin, R., Lenton, J., Holdsworth, M., Flintham, J. & Gale, M. (1999). Genetic map locations for orthologous Vp1 genes in wheat and rice. *Theoretical and Applied Genetics*, 98 (2): 281-284.

Balyan, H. S., Gupta, P. K., Kumar, S., Dhariwal, R., Jaiswal, V., Tyagi, S., Agarwal, P., Gahlaut, V. & Kumari, S. (2013). Genetic improvement of grain protein content and other health-related constituents of wheat grain. *Plant Breeding*.

Barnard, A. & Smith, M. F. (2009). The effect of rainfall and temperature on the preharvest sprouting tolerance of winter wheat in the dryland production areas of the Free State Province. *Field Crops Research*, 112 (2): 158-164.

Barraclough, P. B., Howarth, J. R., Jones, J., Lopez-Bellido, R., Parmar, S., Shepherd, C. E. & Hawkesford, M. J. (2010). Nitrogen efficiency of wheat: genotypic and environmental variation and prospects for improvement. *European Journal of Agronomy*, 33 (1): 1-11.

Bechtel, D. B., Zayas, I., Kaleikau, L. & Pomeranz, Y. (1990). Size-distribution of wheat starch granules during endosperm development. *Cereal Chemistry*, 67 (1): 59-63.

Bewley, J. D. (1997). Seed germination and dormancy. *The plant cell*, 9 (7): 1055.

Biddulph, T., Plummer, J., Setter, T. L. & Mares, D. J. (2008). Seasonal conditions influence dormancy and preharvest sprouting tolerance of wheat (< i> Triticum aestivum</i> L.) in the field. *Field Crops Research*, 107 (2): 116-128.

Biddulph, T. B., Plummer, J. A., Setter, T. L. & Mares, D. J. (2007). Influence of high temperature and terminal moisture stress on dormancy in wheat (< i> Triticum aestivum</i> L.). *Field crops research*, 103 (2): 139-153.

Bioforsk. (2013). http://lmt.bioforsk.no/.

Blazek, J. & Copeland, L. (2008). Pasting and swelling properties of wheat flour and starch in relation to amylose content. *Carbohydrate polymers*, 71 (3): 380-387.

Bloksma, A. (1980). Effect of heating rate on viscocity of wheat flour doughs 1. *Journal of Texture Studies*, 10 (3): 261-269.

Blumenthal, C., Batey, I., Bekes, F., Wrigley, C. & Barlow, E. (1991). Seasonal changes in wheat-grain quality associated with high temperatures during grain filling. *Crop and Pasture Science*, 42 (1): 21-30.

Bly, A. G. & Woodard, H. J. (2003). Foliar nitrogen application timing influence on grain yield and protein concentration of hard red winter and spring wheat. *Agronomy Journal*, 95 (2): 335-338.

Bogard, M., Jourdan, M., Allard, V., Martre, P., Perretant, M. R., Ravel, C., Heumez, E., Orford, S., Snape, J. & Griffiths, S. (2011). Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs. *Journal of experimental botany*, 62 (10): 3621-3636.

Bremner, J. & Mulvaney, C. (1982). Nitrogen—total. *Methods of soil analysis. Part 2. Chemical and microbiological properties* (methodsofsoilan2): 595-624.

Burhans, M. E. & Clapp, J. (1942). A microscopic study of bread and dough. *Cereal Chem*, 19: 196-216.

Butcher, J. & Stenvert, N. (1973). Conditioning studies on Australian wheat. III. The role of the rate of water penetration into the wheat grain. *Journal of the Science of Food and Agriculture*, 24 (9): 1077-1084.

Dengate, H. & Meredith, P. (1984). Variation in size distribution of starch granules from wheat grain. *Journal of Cereal Science*, 2 (2): 83-90.

Dimmock, J. & Gooding, M. (2002). The influence of foliar diseases, and their control by fungicides, on the protein concentration in wheat grain: a review. *The Journal of Agricultural Science*, 138 (4): 349-366.

Dubcovsky, J. & Dvorak, J. (2007). Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science*, 316 (5833): 1862-1866.

Dupont, F. M., Hurkman, W. J., Vensel, W. H., Tanaka, C., Kothari, K. M., Chung, O. K. & Altenbach, S. B. (2006). Protein accumulation and composition in wheat grains: effects of mineral nutrients and high temperature. *European Journal of Agronomy*, 25 (2): 96-107.

Edwards, R. A., Ross, A. S., Mares, D. J., Ellison, F. W. & Tomlinson, J. D. (1989). Enzymes from rain-damaged and laboratory-germinated wheat I. Effects on product quality. *Journal of Cereal Science*, 10 (2): 157-167.

Fang, J. & Chu, C. (2008). Abscisic acid and the pre-harvest sprouting in cereals. *Plant signaling & behavior*, 3 (12): 1046-1048.

FAOSTAT. (2013). http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor.

Finney, K. (1984). An optimized, straight-dough, bread-making method after 44 years [Wheat quality]. *Cereal Chemistry*, 61.

Finney, K., F & Barmore, M., A. (1948). Loaf volume and protein content of hard winter and spring wheats. *Cereal Chem*, 25 (29): 1-3.

Fowler, D. (2003). Crop nitrogen demand and grain protein concentration of spring and winter wheat. *Agronomy Journal*, 95 (2): 260-265.

Gianibelli, M., Larroque, O., MacRitchie, F. & Wrigley, C. (2001). Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. *Cereal Chemistry*, 78 (6): 635-646.

Godfrey, D., Hawkesford, M. J., Powers, S. J., Millar, S. & Shewry, P. R. (2010). Effects of crop nutrition on wheat grain composition and end use quality. *Journal of agricultural and food chemistry*, 58 (5): 3012-3021.

Gooding, M., Smith, G., Davies, W. & Kettlewell, P. (1997). The use of residual maximum likelihood to model grain quality characters of wheat with variety, climatic and nitrogen fertilizer effects. *The Journal of Agricultural Science*, 128 (2): 135-142.

Gooding, M. J. & Davies, W. P. (1997). *Wheat production and utilization: systems, quality and the environment*: Cab International.

Groos, C., Gay, G., Perretant, M.-R., Gervais, L., Bernard, M., Dedryver, F. & Charmet, G. (2002). Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a white× red grain bread-wheat cross. *Theoretical and Applied Genetics*, 104 (1): 39-47.

Groos, C., Robert, N., Bervas, E. & Charmet, G. (2003). Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. *Theoretical and Applied Genetics*, 106 (6): 1032-1040.

Gupta, R. & MacRitchie, F. (1994). Allelic Variation at Glutenin Subunit and Gliadin Loci,< i> Glu-1, Glu-3</i> and< i> Gli-1</i> of Common Wheats. II. Biochemical Basis of the Allelic Effects on Dough Properties. *Journal of Cereal Science*, 19 (1): 19-29.

Hermansson, A.-M. & Svegmark, K. (1996). Developments in the understanding of starch functionality. *Trends in Food Science & Technology*, 7 (11): 345-353.

Hoseney, R., Lineback, D. & Seib, P. (1978). Role of starch in baked foods. *Bakers Digest*, 52.

Humphreys, D. & Noll, J. (2002). Methods for characterization of preharvest sprouting resistance in a wheat breeding program. *Euphytica*, 126 (1): 61-65.

Johansson, Prieto-Linde, M. L. & Jönsson, J. Ö. (2001). Effects of wheat cultivar and nitrogen application on storage protein composition and breadmaking quality. *Cereal Chemistry*, 78 (1): 19-25.

Johansson, E., Svensson, G. & Tsegaye, S. (1999). Genotype and environment effects on bread-making quality of Swedish-grown wheat cultivars containing high-molecular-weight glutenin subunits 2+ 12 or 5+ 10. *Acta Agriculturae Scandinavica, Section B-Plant Soil Science*, 49 (4): 225-233.

Johansson, E. (2002). Effect of two wheat genotypes and Swedish environment on falling number, amylase activities, and protein concentration and composition. *Euphytica*, 126 (1): 143-149.

Johansson, E., Kuktaite, R., Andersson, A. & Prieto-Linde, M. L. (2005). Protein polymer build-up during wheat grain development: influences of temperature and nitrogen timing. *Journal of the Science of Food and Agriculture*, 85 (3): 473-479.

Khan, K. & Shewry, P. R. (2009). *Wheat: chemistry and technology*: American Association of Cereal Chemists, Inc (AACC).

King, R. & Richards, R. (1984). Water uptake in relation to pre-harvest sprouting damage in wheat: ear characteristics. *Crop and Pasture Science*, 35 (3): 327-336.

King, R. & von Wettstein-Knowles, P. (2000). Epicuticular waxes and regulation of ear wetting and pre-harvest sprouting in barley and wheat. *Euphytica*, 112 (2): 157-166.

Kottearachchi, N., Uchino, N., Kato, K. & Miura, H. (2006). Increased grain dormancy in white-grained wheat by introgression of preharvest sprouting tolerance QTLs. *Euphytica*, 152 (3): 421-428.

Kruger, J. E. (1994). Enzymes of sprouted wheat and their possible technological significance. In Bushuk, W. & Rasper, V. F. (eds) *Wheat*, pp. 143-153: Springer US.

Kusunose, C., Fujii, T. & Matsumoto, H. (1999). Role of starch granules in controlling expansion of dough during baking. *Cereal chemistry*, 76 (6): 920-924.

Li Vigni, M., Baschieri, C., Marchetti, A. & Cocchi, M. (2013). RP-HPLC and chemometrics for wheat flour protein characterisation in an industrial bread-making process monitoring context. *Food Chemistry*, 139 (1–4): 553-562.

Lunn, G., Kettlewell, P., Major, B. & Scott, R. (2001). Effects of pericarp alpha-amylase activity on wheat (Triticum aestivum) Hagberg falling number. *Annals of applied biology*, 138 (2): 207-214.

Lunn, G., Kettlewell, P., Major, B. & Scott, R. (2002). Variation in dormancy duration of the UK wheat cultivar Hornet due to environmental conditions during grain development. *Euphytica*, 126 (1): 89-97.

Luo, C., Branlard, G., Griffin, W. & McNeil, D. (2000). The effect of nitrogen and sulphur fertilisation and their interaction with genotype on wheat glutenins and quality parameters. *Journal of Cereal Science*, 31 (2): 185-194.

Maleki, M., Hoseney, R. & Mattern, P. (1980). Effects of loaf volume, moisture content, and protein quality on the softness and staling rate of bread. *Cereal Chem*, 57 (2): 138-140.

Malik, A. H., Andersson, A., Kuktaite, R., Mujahid, M. Y., Khan, B. & Johansson, E. (2012). Genotypic variation in dry weight and nitrogen concentration of wheat plant parts; relations to grain yield and grain protein concentration. *Journal of Agricultural Science*, 4 (11): p11.

Mares, D. (1984). Temperature dependence of germinability of wheat (Triticum aestivum L.) grain in relation to pre-harvest sprouting. *Crop and Pasture Science*, 35 (2): 115-128.

Mares, D. (1993). Pre-harvest sprouting in wheat. I. Influence of cultivar, rainfall and temperature during grain ripening. *Crop and Pasture Science*, 44 (6): 1259-1272.

McCarty, D. R., Hattori, T., Carson, C. B., Vasil, V., Lazar, M. & Vasil, I. K. (1991). The< i> Viviparous-1</i> developmental gene of maize encodes a novel transcriptional activator. *Cell*, 66 (5): 895-905.

McDonald, G. (1992). Effects of nitrogenous fertilizer on the growth, grain yield and grain protein concentration of wheat. *Crop and Pasture Science*, 43 (5): 949-967.

Miralbés, C. (2003). Prediction chemical composition and alveograph parameters on wheat by near-infrared transmittance spectroscopy. *Journal of agricultural and food chemistry*, 51 (21): 6335-6339.

Nielsen, M., McCrate, A., Heyne, E. & Paulsen, G. (1984). Effect of weather variables during maturation on preharvest sprouting of hard white wheat. *Crop science*, 24 (4): 779-782.

Osborne, T. B. (1907). *The proteins of the wheat kernel*: Carnegie institution of Washington.

Pandey, A., Nigam, P., Soccol, C., Soccol, V., Singh, D. & Mohan, R. (2000). Advances in microbial amylases. *Biotechnology and Applied Biochemistry*, 31: 135-152.

Panozzo, J. & Eagles, H. (2000). Cultivar and environmental effects on quality characters in wheat. II. Protein. *Crop and Pasture Science*, 51 (5): 629-636.

Payne, P. I. (1987). Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annual Review of Plant Physiology*, 38 (1): 141-153.

Payne, P. I., Nightingale, M. A., Krattiger, A. F. & Holt, L. M. (1987). The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *Journal of the Science of Food and Agriculture*, 40 (1): 51-65.

Peterson, C., Graybosch, R., Baenziger, P. & Grombacher, A. (1992). Genotype and environment effects on quality characteristics of hard red winter wheat. *Crop Science*, 32 (1): 98-103.

Pomeranz, Y. (1987). Bread around the world. *Modern Cereal Science and Technology. VCH Publishers Inc., New York, NY*: 258-333.

Rasheed, A., Mahmood, T., Kazi, A. G., Ghafoor, A. & Mujeeb-Kazi, A. (2012). Allelic variation and composition of HMW-GS in advanced lines derived from d-genome synthetic hexaploid/bread wheat (Triticum aestivum L.). *Journal of Crop Science and Biotechnology*, 15 (1): 1-7.

Rasul, G., Humphreys, G. D., Wu, J., Brûlé-Babel, A., Fofana, B. & Glover, K. D. (2012). Evaluation of preharvest sprouting traits in a collection of spring wheat germplasm using genotype and genotype× environment interaction model. *Plant Breeding*, 131 (2): 244-251.

Sapone, A., Bai, J., Ciacci, C., Dolinsek, J., Green, P., Hadjivassiliou, M., Kaukinen, K., Rostami, K., Sanders, D. & Schumann, M. (2012). Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC medicine*, 10 (1): 13.

Shewry, P. & Tatham, A. (1997). Disulphide bonds in wheat gluten proteins. *Journal of Cereal Science*, 25 (3): 207-227.

Shewry, P., Gilbert, S., Savage, A., Tatham, A., Wan, Y.-F., Belton, P., Wellner, N., D'ovidio, R., Bekes, F. & Halford, N. (2003). Sequence and properties of HMW subunit 1Bx20 from pasta wheat (Triticum durum) which is associated with poor end use properties. *Theoretical and Applied Genetics*, 106 (4): 744-750.

Shewry, P. R., Tatham, A. S., Halford, N. G., Barker, J. H., Hannappel, U., Gallois, P., Thomas, M. & Kreis, M. (1994). Opportunities for manipulating the seed protein composition of wheat and barley in order to improve quality. *Transgenic research*, 3 (1): 3-12.

Shewry, P. R., Tatham, A. S., Barro, F., Barcelo, P. & Lazzeri, P. (1995). Biotechnology of breadmaking: unraveling and manipulating the multi-protein gluten complex. *Bio/technology*, 13 (11): 1185-1190.

Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany*, 60 (6): 1537-1553.

Simmonds, N. W. (1995). The relation between yield and protein in cereal grain. *Journal of the Science of Food and Agriculture*, 67 (3): 309-315.

Skerritt, J. H. & Heywood, R. H. (2000). A five-minute field test for on-farm detection of pre-harvest sprouting in wheat. *Crop science*, 40 (3): 742-756.

Smith, G. & Gooding, M. (1999). Models of wheat grain quality considering climate, cultivar and nitrogen effects. *Agricultural and forest meteorology*, 94 (3): 159-170.

Stewart, B. & Dyke, G. (1993). Factors Affecting the Grain Yield, Milling and Breadmaking Quality of Wheat 1969-1972: II. Breadmaking quality in relation to variety and nitrogen fertiliser. *Plant Varieties and Seeds*, 6: 169-169.

Stone, B., Morell, M., Carbohydrates, K. K. & Shewry, P. (2009). *Wheat: Chemistry and Technology*: AACC, St Paul, MN, USA.

Stone, P. & Nicolas, M. (1998). The effect of duration of heat stress during grain filling on two wheat varieties differing in heat tolerance: grain growth and fractional protein accumulation. *Functional Plant Biology*, 25 (1): 13-20.

Strand, E. (1989). Studies on seed dormancy in small grain species. Part 2. Wheat. *Norwegian Journal of Agricultural Sciences*, 3.

Sundgren, T., Assveen, M. & Stabbetorp, H. (2013). Sorter og sortsprøving. *Jord- og plantekultur* 8.

Taylor, A. & Gilmour, A. (1971). Wheat protein prediction from climatic factors in southern NSW. *Animal Production Science*, 11 (52): 546-549.

Tester, R. F. & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal chem*, 67 (6): 551-557.

Tosi, P., Gritsch, C. S., He, J. & Shewry, P. R. (2011). Distribution of gluten proteins in bread wheat (Triticum aestivum) grain. *Annals of botany*, 108 (1): 23-35.

Uhlen, A., Hafskjold, R., Kalhovd, A.-H., Sahlström, S., Longva, Å. & Magnus, E. (1998). Effects of cultivar and temperature during grain filling on wheat protein content, composition, and dough mixing properties. *Cereal Chemistry*, 75 (4): 460-465.

Vogel, K., Johnson, V. & Mattern, P. (1976). Protein and lysine content of grain, endosperm, and bran of wheats from the USDA world wheat collection. *Crop science*, 16 (5): 655-660.

Walker-Simmons, M. (1987). ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. *Plant physiology*, 84 (1): 61-66.

Wang, J., Pawelzik, E., Weinert, J., Zhao, Q. & Wolf, G. A. (2008). Factors influencing falling number in winter wheat. *European Food Research and Technology*, 226 (6): 1365-1371.

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology*, 24 (2): 115-119.

Wu, J. & Carver, B. F. (1999). Sprout damage and preharvest sprout resistance in hard white winter wheat. *Crop Science*, 39 (2): 441-447.

Zhu, J. & Khan, K. (2001). Effects of genotype and environment on glutenin polymers and breadmaking quality 1. *Cereal Chemistry*, 78 (2): 125-130.