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Protein Content and Gluten Quality of Norwegian Grown Wheat Influenced by Fertilization

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List of Abbreviations

ANOVA: analysis of variance
PC%: protein content
FN: Falling Number
N: nitrogen
P: phosphorus
K: potassium
SDS: Sodium dodecyl sulfate
NIR: near infrared reflectance
Rmax : maximum resistance to extension
Ext: extensibility
TGW: thousand grains weight
TW: test weight

Abstract

Protein content and protein quality are important factors for bread making performance of the flour. The protein content widely varies between the types and classes of wheat, in amounts from 8-20%. It is strongly dependent on growing conditions, soil fertility and temperature during the season, fertilizer inputs, and nitrogen in particular. Higher protein content can be achieved by giving higher N fertilization and particularly by using split application methods. The protein quality tends to vary according to variations of the gluten proteins. Because there are large numbers of different combinations of storage protein components, their evaluation is complex and hard. Baking and different chemical tests, as well as the rheological tests on flour, dough and gluten are often regarded as good parameters to determine wheat quality.

During recent years the Norwegian food wheat production has been decreasing, especially since 2008. This is caused mainly by decrease in production area as well as by high precipitation during maturation and harvest in autumn, giving pre-harvest sprouting with extremely difficult harvest in 2011. However, autumn for the season 2013/14 was dry, showing slight increase in use of Norwegian wheat for flour and a specially good season followed in 2014, giving good gluten quality and spring wheat with high protein content compared to the later seasons. A high proportion of Norwegian wheat of about 70% was again used in 2014/2015 seasons. This study investigated the effect of fertilizers on the main quality traits, protein content and gluten quality and suggested possible strategies for management in agricultural practice on how to reach higher productivity.

Three experiments examined effects of nutrient availability on quality parameters with special focus on viscoelastic properties of gluten. The results from material showed strong effects of N fertilization regimes, giving variations in both, protein content and viscoelastic properties. The results showed increased Ext and decreased Rmax, which combine the same trend in accordance with earlier seasons in Norway. In the results where the effect of P and K fertilization on gluten viscoelastic properties was studied, the same effect was found. Ext increased and Rmax decreased with increased P level. Also, interesting trend of increased Rmax at the higher K level was found. Considering, there are significant interactions between P and K levels on viscoelastic properties of gluten, it is of interest to explore how some other factors influence the variability in protein content and not only nitrogen fertilization.

1 GENERAL INTRODUCTION

1.1 Wheat

For over 10,000 years wheat species cultivated as food crops represented the major factor of society development, culture and population growth. The common wheat or bread wheat (*Triticum aestivum* var. *aestivum*) accounts today more than 90% of world wheat production (Gooding, et al 1997). It is the main human food crop, beside the rice, with more than 70% of a total worldwide production (711 million tons) being used for food (FAO, 2014) and a per capita yearly consumption of 65kg in 2011. According to the latest FAO's forecast for global wheat production published in March 2015, production of wheat is anticipated to reach 722 millions of tons in the current year.

The popularity of wheat as a human food crop lies in ability of wheat grain to be milled and used to make flour for leavened, flat or steamed products as different kind of breads, biscuits, porridge, crackers, pies, pancakes, muffins, rolls, doughnuts, cookies, cakes, breakfast cereals, muesli, pasta, noodles, couscous, etc. (Gooding, 2009). Besides, the wheat grains contain 8-20% storage protein making it the most important plant source of human protein.

Wheat, just like the other cereals, in a human diet ensures energy as concentrated source of carbohydrates (mainly starch) with useful amounts of protein, fat, minerals, vitamins and fiber. The whole grain wheat flour contains all of the grain; bran, germ and endosperm, in contrast to refined white flours which contain only endosperm in the process of making flour. The whole grain flour is used for making breads and other baked goods and it is typically mixed with other bleached and unbleached lighter flours. It is more nutritious than refined white flour, although white flour can be fortified by adding some micronutrients lost in a processing. Still it does not contain the macronutrients of the wheat's bran and germ like the whole grain flour does, significantly lacking in fibers.

The nutrition value of 100g of whole grain wheat flour in a raw form typically contains 72,6% of carbohydrates on a 14% moisture basis, 12,7% of protein, 1,9% lipids, 12,2% dietary fiber, B vitamins, minerals Ca, I, Mg and Se and other components, like water, ash; (Percent Daily Values, (%DV); USDA SR-21); (Table 1).

Table 1. The nutrition value of 100g of whole grain wheat flour; Percent Daily Values; %DV Source: USDA SR-21

Carbohydrates			Vitamins		
Amounts Per Selected Serving		DV	Amounts Per Selected Serving		DV
Total Carbohydrate	72,6g	24 %	Vitamin A	9IU	0 %
Dietary Fiber	12,2g	49 %	Vitamin C	0mg	0 %
Sugars	0,4g		Vitamin E (Alpha Tocopherol)	0,8mg	4 %
			Vitamin K	1,9mcg	2 %
Fats & Fatty Acids			Thiamin	0,4mg	30 %
Amounts Per Selected Serving		DV	Riboflavin	0,2mg	13 %
Total Fat	1,9g	3 %	Niacin	6,4mg	32 %
Saturated Fat	0,3g	2 %	Vitamin B6	0,3mg	17 %
Monounsaturated Fat	0,2g		Folate	44mcg	11 %
Polyunsaturated Fat	0,8g		Vitamin B12	0mcg	0 %
Total Omega-3 fatty acids	38mg		Pantothenic Acid	1mg	10 %
Total Omega-6 fatty acids	738mg		Choline	31,2mg	
			Betaine	72,8mg	
Protein & Amino Acids					
Amounts Per Selected Serving		DV	Minerals		
Protein	13,7g	27 %	Amounts Per Selected Serving		%DV
			Calcium	34mg	3 %
Other			Iron	3,9mg	22 %
Amounts Per Selected Serving		DV	Magnesium	138mg	34 %
Alcohol	0g		Phosphorus	346mg	35 %
Water	10,3g		Potassium	405mg	12 %
Ash	1,6g		Sodium	5mg	0 %
Caffeine	0mg		Zinc	2,9mg	20 %
Theobromine	0mg		Copper	0,4mg	9 %
			Manganese	3,8mg	90 %
			Selenium	70,7mcg	101 %

Among other edible cereals, wheat has unique properties. Flour has the ability to develop protein complex called gluten, which is required for the production of leavened bread. Starch is deposited in starch granules in the endosperm cells (Bushuk, 1985). As the main carbohydrates fraction of wheat flour, it is also important for the bread quality, as the functional gelatinization properties of starch are essential during baking. Gelatinization occurs when starch is heated in excess of water, leading to increase in dough viscosity. This affects the structure of the final baked products (Bushuk, 1985). Furthermore, starch represents an important commercial product as an excellent source of energy, but it is second in economic value compared to gluten.

According to the bread baking quality, wheat is divided in two quality classes: hard and soft wheat. The classification is based on the hardness of the endosperm, due to the binding between starch and protein (Aamodt, et al., 2004). During milling, the kernel with hard endosperm gives coarser flour, easy to sift and to use for leavened products. Soft endosperm makes flour finer, easily aggregating at sifting and it is used for a biscuits and cookies.

By mixing flour and water, the water-insoluble proteins hydrate and form gluten, a viscoelastic network in which starch granules and other dough components are embedded. The skeleton of the dough is build by gluten. Its viscoelastic properties have ability to retain carbon dioxide formed by yeast. This allows baking of leavened bread possible (Uhlen, et al., 1989).

Wheat with higher protein has higher water absorbing capacity as well as greater loaf volume potential. The protein content widely varies between the types and classes of wheat, in amounts from 8-20%. It is strongly dependent on growing conditions, soil fertility and temperature during the season and fertilizer inputs, particularly nitrogen (Carson, et al., 2009). However, further nitrogen application results in increased vegetative growth, which is opposite to the expected higher protein in wheat. Heavy rains during maturation could result in leaching nitrogen from the soil, and subsequently to lower protein content. This may increase agronomic yield, but resulting in reduced protein content. (Carson, et al., 2009).

The protein content of the grain is more influenced by the environment and fertilizer practice, then by genotype. Therefore, increasing the protein content without affecting the grain yield is a challenging breeding task. In addition, there is generally a negative relationship between grain yield and grain protein content (Uhlen, et al, 1989).

Higher protein content can be achieved by giving higher N fertilization and particularly by using split application methods. The challenge for agricultural practice will be to determine the correct amount of N to be used as a split application in different seasons with different weather conditions.

Furthermore, another approach for a better bread making properties is to improve the protein quality. It tends to vary according to variations of the gluten proteins. There are large numbers of different combinations of the storage protein components and their evaluation is a complex and hard task (Uhlen, et al., 1989). Baking and different chemical tests, as well as the rheological tests on flour, dough and gluten are often regarded as good parameters to determine wheat quality (Tronsmo, et al., 2002).

Both, protein content and protein quality (composition and the properties of gluten proteins) are important factors for bread making performance of the flour (Tronsmo, et al., 2002).

1.2 Wheat proteins

Italian scientist Beccari started first with studying the wheat endosperm proteins and in 1748 reported the preparation of water-insoluble fraction from wheat. Many scientists continued his work developing a classification for cereal seed proteins. Based on their extraction and different solubility, wheat seed proteins were classified into four different groups by Osborne procedure (Osborn, 1907): **albumins** (soluble in water and dilute buffers), **globulins** (soluble in salt solutions), **prolamins** (soluble in ethanol) and **glutelins** (soluble in dilute acid or alkali). In 1970, Chen and Bushuk divided further the glutelin fraction according to solubility in dilute acetic acid, where prolamins are named **gliadins** and glutelins are named **glutenins** (Aamodt, et al., 2004). Glutenins and gliadins are storage proteins while albumins and globulins are mostly enzymes. There are other classifications developed but those are not discussed in this thesis. Today the classification by Osborne is still used, basically for 2 major reasons. The groups of proteins could be easily prepared, and gliadins are mainly responsible for the viscosity and glutenins for the elasticity of the gluten (Shewry et al., 2007).

1.3 Gluten proteins

The gluten proteins of wheat are responsible for the unique ability of wheat flour to form viscoelastic dough. When mixed with water, the proteins develop a viscoelastic network which allows the entrapment of carbon dioxide liberated from fermentation. This allows leavened bread to be baked (Gooding et al., 1997). As mentioned, the method where gluten is isolated from dough by washing out starch and water solubles was described first by Beccari. The remaining cohesive viscoelastic mass, gluten, consists mostly of gluten proteins (70%) glutenins and gliadins, water and of insignificant amounts of small starch granules, non-starch polysaccharides and flour lipids (Shewry et al., 2007). The variation in flour quality with respect to bread making properties is influenced by protein fraction, primarily the variation in the gluten fraction. Gluten and dough are viscoelastic materials and possess both elastic and viscous characteristics (Aamodt, et al., 2004). The elastic properties of gluten and dough can be attributed to the glutenins, while the gliadins provide viscous properties.

1.4 Norwegian wheat production

The wheat production in Norway increased rapidly from the mid-1970s until 2000. According to the data from Statistic of Norway, the proportion of Norwegian wheat in total quantity of its consumption of bread wheat varied between 20-70% for the period 1990-2013. Very stable productivity was achieved from 2004/05 seasons to 2008/9. The most favorable wheat seasons in Norwegian history were seasons after harvest in 2005, 2006 and 2007 (Lillemo, et al., 2011). During recent years the Norwegian food wheat production has been decreasing, especially since 2008. It is caused mainly by decrease in production area as well as by high precipitation during maturation and harvest in autumn giving pre-harvest sprouting with extremely difficult harvest in 2011. However, the autumn during the season 2013/14 was dry, showing slight increase in use of Norwegian wheat for flour and an exceptionally good season followed in 2014, giving good gluten quality and spring wheat with high protein content compared to the later seasons. A high proportion of Norwegian wheat of about 70% was again used in 2014/2015 seasons (Uhlen, Wheat Quality Project 2014, nofima.no).

Growing conditions as well as high and stable yields are very variable in Norway. The current spring wheat area spreads at about 50-55,000 ha, representing almost one half of the total Norwegian wheat production (Lillemo, et al 2011). Even though the humid climate favors production of high yielding wheat crops, there is always a great need for certain amounts of imported wheat with very strong gluten.

A great effort is made in plant breeding programs to assure high quality of domestic wheat for bread-making and high protein content. Pre-harvest sprouting, presence of *Fusarium* spp and other microorganisms influenced by different weather conditions in different seasons, represent even bigger challenge for Norwegian industry, already exposed to wheats of variable quality (Mosleth, 1989; Tronsmo, et al., 2002).

1.4.1 Wheat classification in Norway

In Norway, the wheat varieties are divided in 5 classes according to the gluten quality. The spring wheat is grouped into the first three classes. Sorted according to the gluten strength, class one being the strongest; four of five classes are categorized as strong wheat. The remaining 5th class consists of wheat with quite weak gluten quality (Table 2).

According to this classification, the national agricultural policy wanted to enhance the utilization of Norwegian wheat leading to self-sufficiency in productivity and needs for food consumption (Lillemo, et al., 2011). A small premium is paid to those who reach increased protein content and is included for the wheat quality classes 1 to 4. Therefore almost all of produced wheat that satisfied quality standards is used for food consumption. This doesn't give the flexibility to the baking industry to choose and select the certain varieties for their needs. Besides, some varieties tend to be popular due to agronomic reasons. According to this, import of some quantities of wheat with very strong gluten will always be necessary to balance the variations among wheat from different classes and assure stable bread making quality.

*Table 2. Classification of Norwegian bread wheat varieties according to the baking quality. Spring wheat varieties are written in bold. The varieties in brackets are old varieties, not any longer on the current seed market. New varieties which entered the market after 2007 are signed with**

Class **1** strong	Class **2** strong	Class **3** strong	Class **4** strong	Class **5** weak
(Bastian)	Bjarne	Zebra	Magnifik	Finans
Mirakel*	Rabagast*	Demonstrant	Olivin	(Mjølner)
	(Berserk, Avle, Tjalve; Portal)	Krabat*	Kuban, Ellvis	
			(Lars, Bjørke,Vinjett)	

1.4.2 Fertilization practices for spring wheat

Wheat crop requires balanced fertilization with more than 17 elements. The most frequently applied are macronutrients nitrogen (N), phosphorus (P), potassium (K) and sulfur (S)[1]. (Havlin, et al., 2005). Other secondary- (Ca, Mg) and micro- (B, Cl, Mn, Fe, Zn, Cu, Mb, Se) elements are applied in a case of deficiency of one or several nutrients to satisfy crop demand. These situations happen more likely on specific soil types under certain conditions (Gooding, 2009).

High-yielding varieties as well as intensive growing systems have increased demands for nutrients during growing period. As a result of season and variations within a season, there are higher rates of removal of essential nutrients from the soil. Recommended amount of fertilizers for spring wheat with regard to yield, quality and environmental impact is 150-180kg/ha of N, 30kg/ha of P and 60kg/ha of K per season.

Knowing the principals of fertilization strategies for wheat is obligatory to plan fertilization for each field according to soil analyses, previous cropping, tillage systems, expected yield as

[1] S sulfur is a secondary plant nutrient

well as nutrient demand of the crop. Furthermore, it is also important to know the nutrient content of the plant itself. The normal content in grain for N is 2,0%; for P is 0,34% and for K is 0,48% (Fertility book).

Typically for the spring wheat P and K are applied at planting (Lillemo, et al., 2011). They are both less prone to leaching and timing of application is therefore not so critical (Gooding, 2009).

Start fertilization is beneficial to secure P closer to the seedlings at the earlier stages of plant development. To improve P uptake, it is recommend to be applied early in the season. Potassium availability improves plant's tolerance to frost and drought, provides straw strength and resistance to many diseases (Taiz et al., 2010). It encourages healthy growth and improves the quality of the product. Deficiency occurs more on light, sandy soil than on heavier clay soil types (Yara Approach, 2014).

Phosphorus is important for energy storage and transfers in cells and is essential for the photosynthesis (Taiz, et al., 2010). It stimulates root development and helps plants to be early established in the season. It encourages maturity as well. Deficiency occurs more in high alkaline soil types (chalk) or in acidic like sand and peat soil types (Yara Approach, 2014).

Nitrogen is the most in focus. It gives greatest responses both in crop yield and in quality. It is the main component of proteins and therefore of enzymes and nucleic acids. Through effect on chlorophyll it influences the protein production (Taiz, et al., 2010).

Increasing N availability provides many benefits for the plants: greater biomass, larger plants and leaves, greenness of leaves, stimulates the tillering, delays the senescence (Taiz, et al., 2010). N is the main determinant of the canopy size as well as the grain yield. Final yield and the protein content are both dependent on the N availability.

In contrast to P and K, nitrogen can easily be leaked. To avoid nitrate leakage in environment and to improve efficiency of nitrogen application, it is recommended to split the nitrogen fertilizer (Carson). Rate given in spring application is reduced, about one half of the whole planned amount; one portion at the later growth stage, approximately at the start of stem elongation (Z31) and the rest at heading (Z49) to achieve high protein content (Lillemo, et al., 2011; Gooding, 2009; Zadox, et al., 1974). Time of application in later stages is flexible, between Z30 and Z49, adjusted to the precipitation and soil moisture conditions, crop fertility and expected yield (Riley, et al., 2012).

According to effect on crop quality, particularly on grain protein concentration, N alters the composition of gluten proteins. A higher application on N changes the composition of gluten proteins, increasing the proportion of gliadins and therefore the ratio of gliadin/glutenin, which results in increased dough extensibility (Koga, 2015; DuPont, et al., 2006).

Sulfur deficiencies have been more prevalent last decades. Deficiency results in yield reduction as well as gluten quality. It is an essential component of several plant amino acids, as in building blocks of protein. S uptake is closely linked to the N uptake (Carson, et al., 2009). Sulfur deficiency in grain occurs when the N/S ratio is higher than 17:1 and the grain concentration of S, less than 0,12% (Wrigley et al 1984). According to the impact on baking performance, S affects viscoelastic properties of dough where S amino acids ensure the inter-chain S-S bonds to maintain the network of storage proteins (Gooding, 2009). A positive correlation was found between S concentration and loaf volume; grain S was a better indicator of pan bread quality than grain N concentration (Zhao et al 1997).

2 THESIS

2.1 Introduction and literature

The principal goal for the agriculture sector is 20% increase in food production within 2030. To achieve this goal, it will be of major importance to improve management practices, which will lead to increased productivity and secure good quality of food wheat in Norway. The percent of usage of Norwegian grown wheat in flour used for food has been decreasing recent decades due to reduced production and insufficient quality (Uhlen, et al., 1989; Tronsmo, et al., 2002). The Norwegian milling and baking industry wants to achieve optimal protein content, considering current is too low and is reporting a decrease in protein level of Norwegian grown varieties since 2008/9 (Norwegian Agricultural Authority; Statistics of Norway). Furthermore, the gluten quality varies between seasons, as between regions and fields within the season, due to great variations among cultivars grown, weather condition challenges, use of home grown wheat and import from different sources (Tronsmo, et al., 2002).

To reach the national goal and increase the Norwegian grain production of wheat with optimal quality for food, measurements and knowledge need to be improved. This will be a great challenge for Norwegian industry exposed to wheat of variable quality. In dealing with this challenge, it will be essential to establish good dialogue and competence for each member of value chain - from plant breeders, producers of seeds, grain producers, handlers and buyers to millers, bakers and at the end to the consumers. Improving measurements for quality traits in breeding programs and management practices in fertilization techniques will reduce variation in protein content of the food wheat and secure expected higher quality.

2.2 The objectives of the thesis

The baking industry has set many quality traits to food wheat related to bread making. The most important among which are: optimal protein content and optimal gluten quality.

The primary objective of this thesis is to study the impacts of fertilization and nutrient availability on Norwegian grown wheat quality with focus on protein content and gluten quality. The thesis is divided in three parts, based on three different field trials performed in the season 2014 at NMBU, Ås: 1) Fertilization trial with P and K; 2) Fertilization trial with N given at different plant development stages as a split application; 3) Fertilization trial with four varieties and N given as a split application at different development stages.

The questions to be studied are:

- How does different P and K availability impact yield and wheat quality parameters?
- How can different N management strategies affect yield, protein content and the gluten quality?
- How will increasing protein content, as a result of late N fertilization or P and K availability, affect the viscoelastic properties of gluten?

2.3 The significance of the thesis

The aim of this thesis, as a part of projects Quality Wheat and Agropro (for PK experiment), is to ensure a good knowledge base and secure a sufficient quality and better utilization of Norwegian grown wheat, in challenging weather conditions, year-by-year variation and reaching the national goal for food production. It will provide important information for the Norwegian milling and baking industry and give possible solutions how to increase quality of flour from Norwegian grown wheat. It will investigate the effect of fertilizers on the main quality traits, protein content and gluten quality and recommend possible strategies for management in agricultural practice on how to reach higher productivity.

3 MATERIAL AND METHODS

3.1 The Wheat material

The wheat material used for the thesis has been chosen according to the genetic variation in protein quality and the variability in protein content. All are spring cultivars of Norwegian grown or bred wheat performed under different fertilizing regimes. All are strong gluten quality classes (1-3) according to the baking quality classification (Table 2). There were 16 grains and 16 straw samples, 2 repetitions from PK experiment; 36 grain and 36 straw samples, 2 repetitions for NAPE experiment and 32 grains samples, 2 repetitions for AKU experiment (Table 3).

Table 3. Wheat material used on three different field trials at Vollebekk and Låven performed in the season 2014, NMBU, Ås, Norway

Experiments	Cultivar	Class	Growing location	No. Of Replications	No. Of trials
PK	Krabat	3	Låven	2	16
NAPE	Bjarne	2	Vollebekk	2	36
AKU	Bastian Avle Bjarne Zebra	1 2 2 3	Vollebekk	2	32

3.1.1 Material 1

One spring wheat cultivar, Krabat (strong gluten, class 3), was used for this trial, called PK experiment. The field plot experiment was carried out in the period April-August 2014 in a long- term PK fertilization field trial established in 1966, at Låven (59°39'N; 10°45'E), Ås, Norway. The field trial has an experimental design with four phosphorus (P) levels and two potassium (K) levels on 16 plots in a randomized block design with two repetitions for each P and K level. For this experiment K levels were chosen as **III** (10kg K/daa) and **IV** (15kg K/daa) and for P levels namely as: **a** (0 kg P/daa); **b** (1,6kg P/daa); **c** (3,2kg P/daa) and **d** (4,8kg P/daa). The plot size for each P and K level combination was 7.5x3.6m; in total 4x2x2=16 plots and two repetitions. The field trial covered area of 28,8x30,0m^2. There was no irrigation during season. Weed control followed common practice.

The N, P, K fertilizers were applied before sowing in the following amounts: 11,6kg N/daa (OPTI-KAS^{tm}, Yara, Norge AS); 1,6; 3,2; 4,8 kg P/daa (OPTI-P 0-20-0, Yara Norge AS) and 10; 15 kg K/daa (60erKali (KCl), Yara Norge AS). There was no split application of N during the season.

Considering that moisture content was in range from 32-34%, the wheat material was harvested by scissors and divided on spikes and straw. Harvested area (middle of the plot) for all plots was 1x1,5 m^2 only for the plot number 12 was 0,7x1m^2. The harvest lasted from 29-31.07.2014.

The grain samples, from the threshed grain harvest were dried, cleaned and milled to the whole-meal flour for quality analysis of the grains. The grain yield was recorded and the physical grain analysis and grain flour quality analysis were done.

3.1.2 Material 2

The Norwegian spring wheat cultivar Bjarne (strong gluten, class 2) was grown on a field trial at Vollebekk, NMBU, Ås (59°16'N; 11°6'E) in the second experiment named NAPE. The field trial was carried in the period April-August 2014 as a part of the on-going research project "Quality Wheat". This fertilization trial was based on N given as a split application at different plant development stages. The experiment was performed in a randomized block design with three repetitions and 18 plots each. All analysis in thesis has been done for 2 repetitions (Table 4). The amount of 51 kg/daa of NPK (Yara Mila 20-4-11, Yara, Norge AS) was used at sowing. Two N fertilizers, OPTI-NS (27-0-0, Yara, Norge AS) and calcium nitrate (Yara Liva CALCINIT, Yara, NorgeAS) were given as a split application, in amounts of 4, 6, 8kg in 3 different development stages: Z30-31 (stem elongation), Z37-39 (flag leaf) and Z49-51 (heading), (Zadox et al., 1974).

Plot size was 2x8m^2 on a total field area of 40x24m^2. Irrigation and weed control follow common practice. The middle of the plot was harvested with combiner. After threshing, samples of whole plants were taken (0,5m^2) of the remaining plot to record yield of straw and analyze chemical content of the straw. The grain samples from the threshed grains harvest were taken, dried, cleaned and milled to whole-meal flour and quality analyses of the grains was done.

3.1.3 Material 3

Four Norwegian spring wheat cultivars Bastian, Avle, Bjarne and Zebra were grown on a trial field at Vollebekk, NMBU, Ås (59°16'N; 11°6'E) in the third experiment named AKU. Bastian cultivar has the strongest gluten (Class 1), Avle and Bjarne have strong gluten (Class 2) and Zebra has less strong gluten compared to the first three cultivars (Class 3). The field trial was carried in the period April-August 2014 within the on-going research project "Quality Wheat". The sowing date was 24th of April 2014. Nitrogen was given as a spring, basic fertilization in amounts of approximately 10kg/daa N (N1 fertilization regime). The fertilization trial was based on N given as a split application at following plant development stages:

N2) fertilization = spring + 4kg of N at fully flag leaf stage (Z37);
N3) fertilization = spring + N2 + 4kg at early heading stage (Z49); and
N4) fertilization = spring + N2 + N3 +4kg at anthesis (Z65).

Increasing protein content in four cultivars was expected to affect the viscoelastic properties of gluten, as a result of late N fertilization. Plot size was 1,5x5m^2. Irrigation and weed control follow common practice. N2) fertilization was given 1st of June; N3) on 11th of June and N4) on 16th of June 2014. The middle of the plot was harvested with combiner. The grain samples from the threshed grains harvest were taken, dried, cleaned and milled to whole-meal flour and quality analysis of the grains was performed.

3.2 Physical grain analysis and milling

Grain analysis and milling were performed for all three experiments with a same schedule.

Thousands grain weight (TGW) expressed in grams and test weight (TW) expressed in kg/hl, was determined in samples from each plot and repetition. Only for NAPE experiment, TW wasn't performed because of a lack of the grains. TW is the weight of a measured volume of grain and usually depends on grain size, shape and density. Kernel weight is a measurement of the kernel size, generally expressed as the weight of thousand kernels in gram. TW can vary from 60kh/hl to 84kg/hl, and wheat above 76 kg/hl is considered acceptable on the market. If the TW value is less than that, the wheat is considered to have poor bread making quality (Protic et al., 2007). The recommended value that gives neither premium nor reduction in price in Norway for TW is 79 kg/hl (Felleskjopet 2013/14). Studies have shown there is a generally significant correlation between test weight and flour yield; higher TW increases flour yield capacity (Marshall et al., 1986).

The harvested grains were milled as a whole meal on Falling Number Laboratory Mill 3100 (Perten Instruments AB, Huddinge, Sweden) using a 0,8mm screen. Samples of approximately 100g of grain were milled for each sample from all experiments.

3.3 Flour analysis

3.3.1 Total N content (%)

The total N content of the whole-meal flour (grains and straw) was determined by the Dumas method according to Bremner and Mulvaney tests performed only for the PK experiment. The protein content was calculated by multiplying the N content by 5,7 and expressed as g/kg. For NAPE and AKU experiments protein percentage was measured by near infrared reflectance (NIR) technique for whole-meal flour (grains) by using Perten Inframatic 9200 (Perten Instruments AB, Huddinge, Sweden).

Content of Mg, P, S, K, Ca, Fe and Cu of whole-meal flour was determined by an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) 5300 DV (Perkin Elmer, Waltham, MA, USA). Samples were decomposed in an Ultraclave with HNO3 10% ultrapure at 260C and 5MPa, before the analysis by ICP-OES. Certified reference material Wheat Flour 1567a (National Institute of Standards and Technology, Gaithersburg, MD, USA) was used and analyzed with all samples from straw and grains from all three experiments.

3.3.2 Protein content (NIR)

The protein content was calculated by multiplying the N content by 5,7 and expressed as g/kg. For NAPE and AKU experiments protein percentage was measured by near infrared reflectance (NIR) technique for whole-meal flour (grains) using Perten Inframatic 9200 (Perten Instruments AB, Huddinge, Sweden) while following the procedure described in AACC (2000) method No. 39-11. Samples of the whole-meal flour were loaded in the instrument calibrated for the wheat and the results were recorded and presented on dry weight basis. The protein content is given in % on dry weight basis, too.

3.3.3 Falling number (FN)

Falling Number (FN) was determined for all flour (grain) samples from each experiment using falling Number 1800 (Perten Instruments, AB Huddinge, Sweden) by following the method 56-81, described in AACC (2000). Considering the moisture content of the flours, approximately 7 grams of flours were weighed; more accurately 6,61g for PK experiment; 6,79g for NAPE experiment and 6,72g for AKU experiment and taken into dry falling number tubes, adding 25 ml of distilled water in each. There were 16 flour samples for PK; 36 flour samples for NAPE and 32 flour samples for AKU. The tubes were shaken to obtain homogenous dispersion, immediately placed with stirrers into the heating chamber of the FN apparatus at the same time and closed. After 60 seconds of stirring the solution, the pick up arm stop at the top position and the stirrers sink into the gelatinized dispersion. After the process, FN is recorded as the number of seconds required for the stirrer to fall down through a hot gel, in addition to 60 seconds of mixing. FN analysis were performed for all samples from all experiments and recorded as FN averages of the two tubes of each sample.

3.3.4 SDS Sedimentation test

Sodium dodecyl sulfate sedimentation volume (SDS) was determined according to the AACC method 56-70 (2000). The 6 grams of the whole-meal flour was measured; put into the 100ml tube and mixed with 50ml of distilled water (colored by bromophenol blue dye) for 5 minutes. Then the same cylinder was filled with 50ml of SDS solution and shaken again for 5 minutes. The cylinders were removed and placed on the table for 15 minutes to sediment the content. The volume of sedimentation was recorded as SDS sedimentation values for all flour samples from all experiments.

3.3.5 SMS/Kieffer Dough and Gluten Extensibility Rig

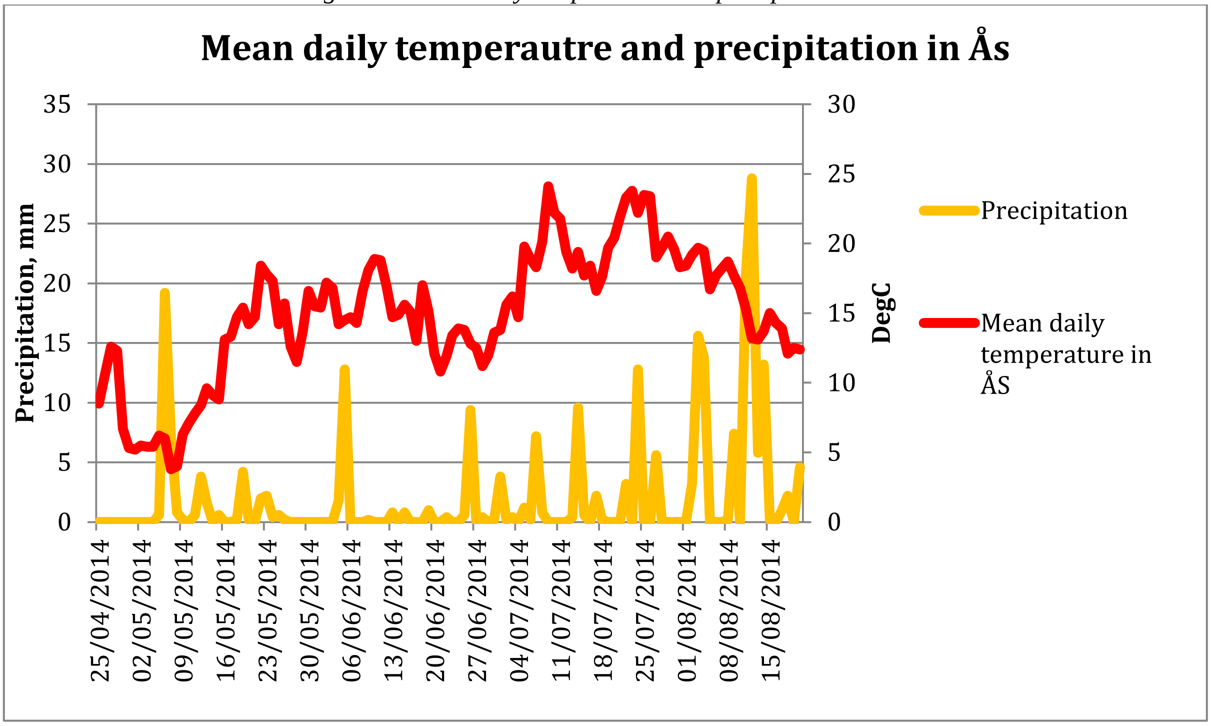
Viscoelastic properties of gluten were determined with the SMS/Kieffer Dough and Gluten Exstensibility Rig for all flour grain samples of PK experiment. For AKU, Kieffer was performed only for the Bjarne and Zebra cultivars.

For NAPE, Kieffer was performed only on representative samples. Samples were chosen statistically with three levels of N and two Zadox stages, Z30-31 and Z49-51. Gluten was prepared from 10g whole-meal flour in a Glutomatic 2100 (Perten Instruments AB, Hægersten, Sweden). Prepared gluten was centrifuged, incubated in a mold for 45 minutes on 30°C before analysis. Three pieces of gluten were stretched with Kieffer-rig extensograph. The R_{max} (maximum resistance to extension) and Ext (extensibility) were analyzed by using software TA (Texture Analyzer). Average results from three pieces were used in statistical analysis.

3.4 Temperature data

Climatic data from both location, Låven and Vollebekk, Ås were obtained from Bioforsk meteorological database. The recorded data were the mean daily temperatures during the growing season from April to August 2014. Data were recorded in order to show the general weather conditions for the season 2014 (Figure 1).

Figure 1. Mean daily temperature and precipitation in Ås



3.5 Statistical analysis

All samples were analyzed using analysis of variance (ANOVA) with two models, balanced ANOVA and general linear model (GLM). The main treatments and their interactions were analyzed using Minitab software, version 17 (Minitab, Inc. State College, PA, USA). For graphs and tables Microsoft Excel was used (version 2010). Level of significance was shown as: *p<0,05; **p< 0,01; ***p< 0,001.

4 RESULTS

4.1 Material 1 – P and K fertilization regimes applied on Krabat

One spring wheat cultivar, Krabat (strong gluten, class 3) was used for this field trial experiment. PK field trial had an experimental design with four phosphorus (P) levels and two potassium (K) levels on 16 plots with two repetitions for each P and K level (Table 4). The main aim was to follow how four P levels influence the changes in yield and wheat quality parameters. Furthermore, the two high K levels were selected in investigation. Balanced ANOVA was performed on the results of grain, flour and gluten analyses to provide an overview of the relationships between fertilizers and wheat quality parameters. Protein content, TW, TGW, FN, SDS, Rmax, Ext. and grain yield are given in table 4, averaged over the two replicates. The results from ANOVA (table 5) shows there was significant effect of P amounts on yield, protein content, test weight and Rmax.

Table 4. Grain yield, TW, TGW, FN; SDS, Protein %, Tot. N, R_{max} and Ext. of Norwegian spring wheat variety Krabat. Different amounts of P (a,b,c,d) and K (III, IV) were given on a field trial named PK experiment

K kg/daa	P kg/daa	Grain Yield, kg/daa 1)	TW, kg/hl	TGW, g	FN, s	SDS, ml	Protein, %	Tot. N, %	Rmax, N	Ext, mm
10	0	249,41	84,9	41,91	388	65	13,78	2,42	0,50	106,91
10	0	234,18	85,14	39,17	416	67	14,40	2,53	0,50	122,54
10	1,6	493,16	85,16	38,75	425	70	12,42	2,18	0,70	107,25
10	1,6	553,16	84,83	40,28	406,5	70	12,71	2,23	0,62	117,90
10	3,2	458,91	84,49	58	374,5	69	12,72	2,23	0,62	112,96
10	3,2	610,64	85,2	38,94	395,5	69	12,23	2,15	0,66	108,48
10	4,8	702,57	84,6	40,29	414	78	13,57	2,38	0,67	125,18
10	4,8	493,59	84,22	39,09	378,5	65	10,88	1,91	0,63	80,37
15	0	332,07	84,67	40,17	422,5	64	13,52	2,37	0,52	115,19
15	0	309,50	85,15	41,6	387	69	13,58	2,38	0,48	107,59
15	1,6	397,58	85	39,8	421,5	73	12,07	2,12	0,89	112,85
15	1,6	481,74	84,85	42,98	408,5	78	14,11	2,48	0,64	117,05
15	3,2	547,98	85,93	39,7	410,5	71	12,34	2,17	0,73	115,77
15	3,2	550,46	85,22	44,2	391	70	12,11	2,12	0,65	93,26
15	4,8	539,77	84,1	45,78	413	68	11,71	2,05	0,88	106,75
15	4,8	543,93	84,32	43,05	395	70	11,06	1,94	0,97	96,52

1) 15 % moisture

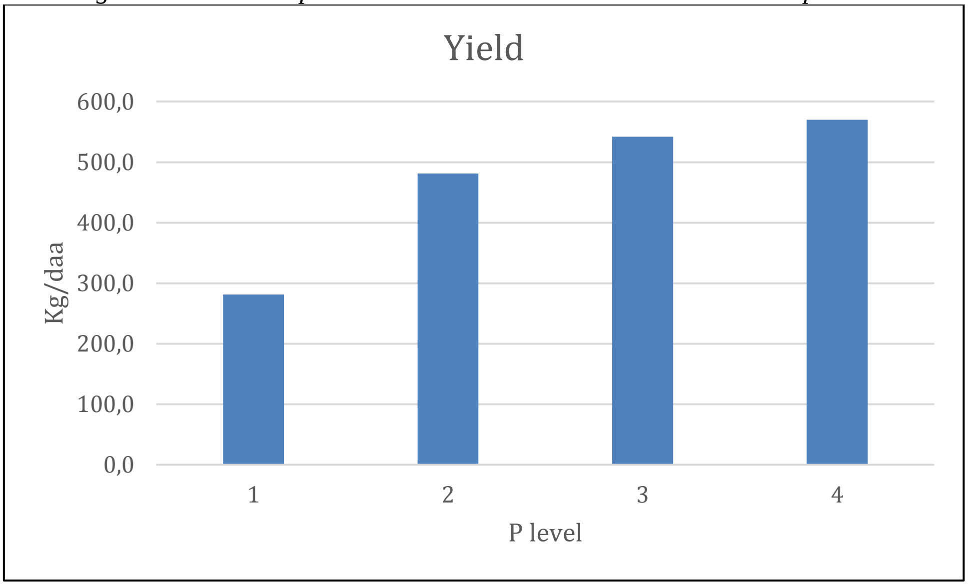
Table 5. Mean squares and significance level calculated with Balanced ANOVA on grain yield, TW, TGW, FN, SDS, Protein% and Total N

	Grain Yield	TW	TGW	FN	SDS	Protein%	Tot. N	Rmax	Ext
K	0,11	0,3	0	0,52	0,44	0,39	0,39	$7,70^{*}$	0,09
P	$13,9^{**}$	$5,87^{*}$	0,74	1,14	2,01	$3,71^{*}$	$3,71^{*}$	$10,03^{**}$	0,6
P*K	1,10	1,84	0,88	0,17	0,77	0,44	0,44	2,47	0,07

*Mean squares of F at 95-99% significance level

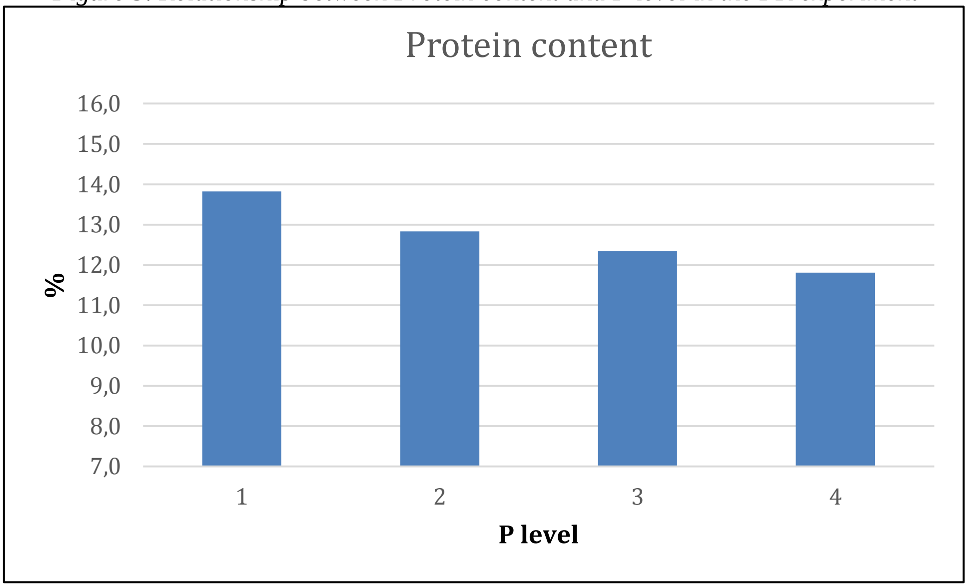
**Mean squares of F at 99-99,9% significance level

Figure 2. Relationship between Grain Yield and P level in the PK experiment



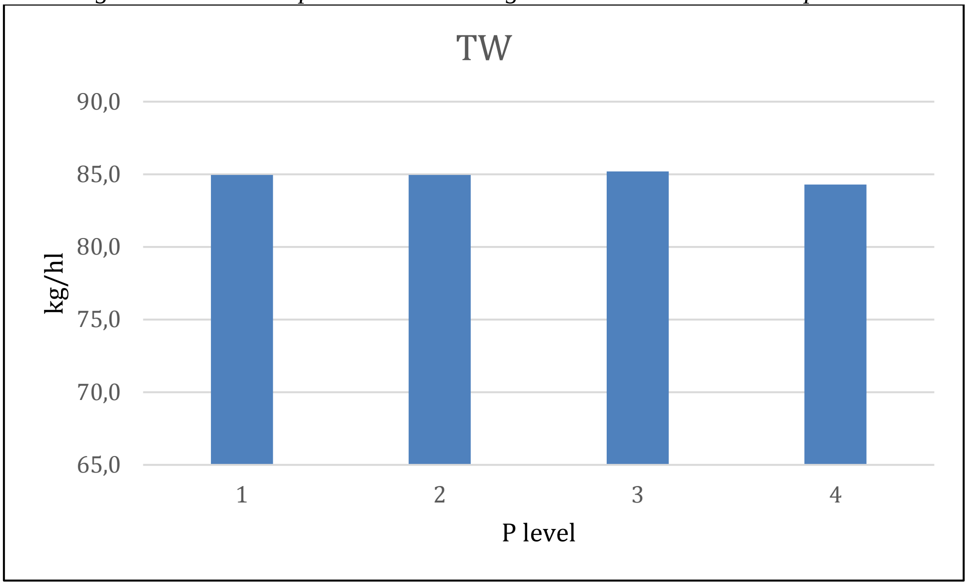
There were strong differences in yield between plot combinations with 0kg P/daa and 10kg K/daa. For both replicates the yield was 234,18kg/daa and 249,41kg/daa, given at 15% of moisture. The 4,8kg P/daa and 10kg K/daa combination gave the highest yield compared to all other combinations, 702,57kg/daa. The results showed that yield was increasing with increasing in P amounts (Figure 2). The plots with 1,6kg P/daa and 10kg K/daa amounts of P and K, as well as 3,2kg P/daa and 10kg K/daa had an increase in yield between 458 and 610 kg/daa.

Figure 3. Relationship between Protein content and P level in the PK experiment



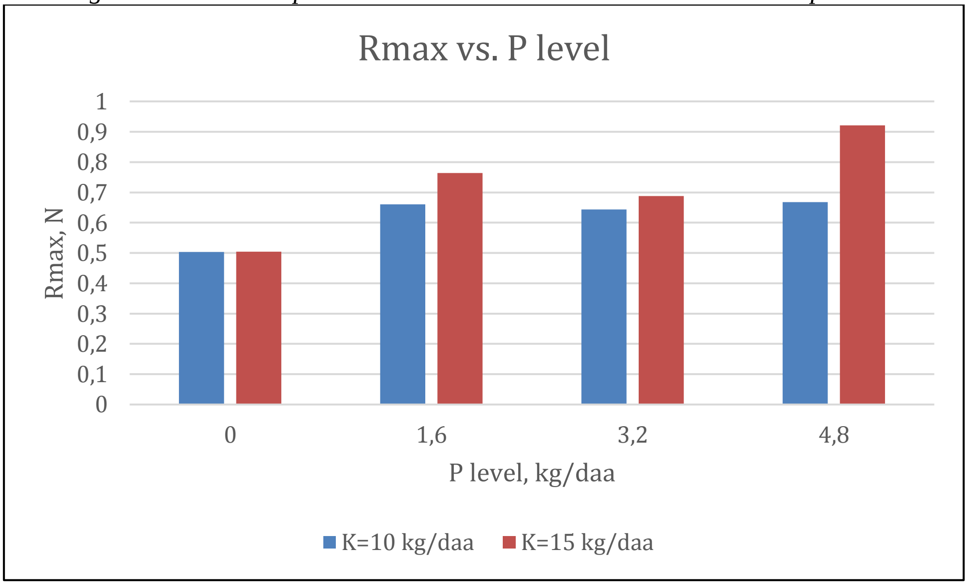
ANOVA result showed the significant effect of P on protein content and total N. The current minimum requirement for wheat for bread making in Norway is 11,5 for PC%. According to this results showed that protein content increased as P amounts decreased in the trials. The smallest values of PC% was 10,87% and 11,06% for plots fertilized with the highest dosage of P (4,8kg P/daa). The highest PC% was 14,39% in unfertilized plot with P and 14, 111 for 1,6 kg P/daa (Figure 3). The whole protein content values ranged from 10,87-14,39%.

Figure 4. Relationship between Test Weight and P level in the PK experiment



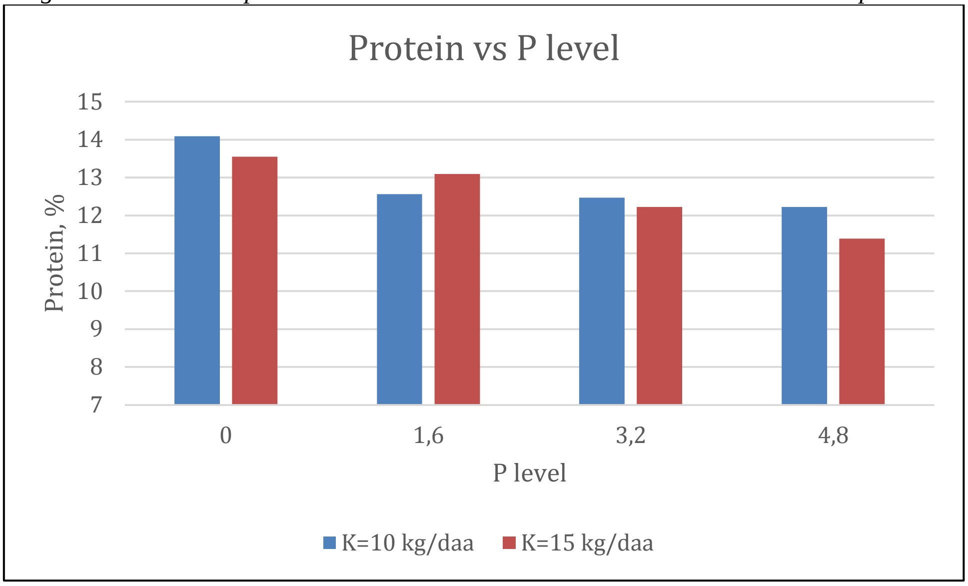
The significant effect of P on TW was noted. The highest P level in the experiment showed lower TW (84,1kg/hl); while the other P levels are similar. However TW values are very high due to the fact that grains were very well developed and well filled (Figure 4).

Figure 5. Relationship between R max and P and K level in the PK experiment



The Kieffer Dough and Gluten Extensibility rig were used to measure the viscoelastic properties of gluten. There was significant difference between P and K relative to Rmax. The Rmax values varied between 0,52 and 0,969 (Table 4). Higher R max was observed at the highest P level and this is the main explanation for higher Rmax at K level. Looking at the highest level of P, 4,8 kg P/daa, the protein content decreases the most between K level of 10kg K/daa and K level of 15kg K/daa, and this could be explanation for Kieffer results for K (Figure 6). This effect can be seen in Figure 6, where Kieffer Rmax of gluten decreased with protein content.

Figure 6. Relationship between Protein content and P and K level in the PK experiment



4.2 Material 2 – N split fertilization regimes applied on Bjarne

The Norwegian spring wheat cultivar Bjarne (strong gluten, class 2) was grown on a field trial at Vollebekk. Two N fertilizers, OPTI-NS and calcium nitrate, were given as a split application, in amount of 4, 6, 8kg in 3 different developmental stages Z30-31 (stem elongation), Z37-39 (flag leaf) and Z49-51 (heading); (Table 6). The aim was to follow how different N management strategies affect yield, protein content and the gluten quality. Balanced ANOVA was performed on the results of grain, flour and gluten analyses to provide an overview of the relationships between fertilizers and yield and wheat quality parameters.

Table 6. Grain yield, TW, TGW, FN; SDS, Protein %, Tot. N, R_{max} and Ext. of Bjarne. Three amounts of N given at different developmental stages on a field trial named NAPE experiment

Nitrogen KG	Stadium	Fertilizer	Grain Yield, kg/daa 1)	Protein, %	TGW, g	FN, s	SDS, ml	Tot. N, %	Rmax, N	Ext, mm
4	30-31	opti-kas	543	13,43	38,3	381	100	2,36		
4	30-31	calcium nitrate	529	12,33	31,4	376	90	2,16	0,81	83,81
4	30-31	opti-kas	571	11,84	35,46	314	89	2,08		
4	30-31	calcium nitrate	566	13,09	33,9	338,5	94	2,30	1,13	104,07
4	37-39	calcium nitrate	518	12,56	34,69	396	95	2,20		
4	37-39	opti-kas	533	13,24	32,71	379,5	96	2,32		
4	37-39	calcium nitrate	571	12,67	39,08	358	90	2,22		
4	37-39	opti-kas	543	11,90	34,38	351,5	99	2,09		
4	49-51	opti-kas	530	12,83	37,69	319	100	2,25		
4	49-51	calcium nitrate	516	13,91	35,47	330,5	99	2,44	0,86	103,82
4	49-51	calcium nitrate	541	13,08	36,55	359,5	98	2,30	0,95	96,98
4	49-51	opti-kas	551	12,10	36,3	372,5	90	2,12		
6	30-31	calcium nitrate	562	12,60	34,6	346,5	100	2,21	1,04	78,45
6	30-31	opti-kas	555	13,25	33,57	364	99	2,33		
6	30-31	calcium nitrate	565	13,73	32,85	390,5	99	2,41	0,95	95,95
6	30-31	opti-kas	540	13,35	40,41	363	99	2,34		
6	37-39	calcium nitrate	554	13,49	35,81	345,5	100	2,37		
6	37-39	opti-kas	548	13,92	34,65	388,5	100	2,44		
6	37-39	opti-kas	542	13,12	34,67	346	95	2,30		
6	37-39	calcium nitrate	550	14,01	35,47	366,5	99	2,46		
6	49-51	calcium nitrate	569	13,40	37,87	330	91	2,35	0,93	105,36
6	49-51	opti-kas	535	13,80	37,54	378	98	2,42		
6	49-51	calcium nitrate	483	14,40	37,04	373	99	2,53	0,97	112,48
6	49-51	opti-kas	499	13,62	36,49	367	99	2,39		
8	30-31	opti-kas	555	13,12	34,37	383	100	2,30		
8	30-31	calcium nitrate	548	13,78	32,86	349	100	2,42	0,83	92,20
8	30-31	calcium nitrate	563	13,61	33,47	346,5	95	2,39	1,12	94,10
8	30-31	opti-kas	525	13,71	33,05	348,5	100	2,41		
8	37-39	opti-kas	550	14,63	35,88	308,5	100	2,57		
8	37-39	calcium nitrate	541	14,49	34,81	354	100	2,54		
8	37-39	calcium nitrate	588	14,91	34,5	324	100	2,62		
8	37-39	opti-kas	562	14,07	36,57	365	98	2,47		
8	49-51	calcium nitrate	579	14,89	32,69	384,5	100	2,61	0,82	110,70
8	49-51	opti-kas	549	14,95	36,73	356	98	2,62		
8	49-51	opti-kas	556	14,84	38,49	355	98	2,60		
8	49-51	calcium nitrate	544	14,50	33,9	339,5	99	2,54	0,72	108,01

Table 7. Mean squares and significance level calculated with Balanced ANOVA on Grain Yield, Protein content, TGW, FN, and SDS

	Grain Yield, kg/daa 1)	Protein, %	TGW, g	FN, s	SDS, ml	Rmax, N	Ext, mm
N amount	1,19	24,71***	1,45	0,63	4,88*	0,59	0,27
Zadox stadium	1,28	5,25*	3,92*	0,04	0,09	1,84	9,55*
Typ fertilizer	0,51	1,34	4,18	0,04	0,25	0,45	0,87
N amount*Zadox stadium	0,96	1,20	0,58	0,89	0,85		
N amount*Typ fertilizer	0,45	0,25	0,85	0,28	0,13		
Zadox stadium*Typ fertilizer	0,06	0,19	3,75*	0,04	0,28		
N amount*Zadox stadium*Typ fertilizer	0,10	0,67	2,43	0,26	1,32		

*Mean squares of F at 95-99% significance level
**Mean squares of F at 99-99,9% significance level
***Mean squares of F at 99,9% significance level

Figure 7. Relationship between Protein Content and N amounts

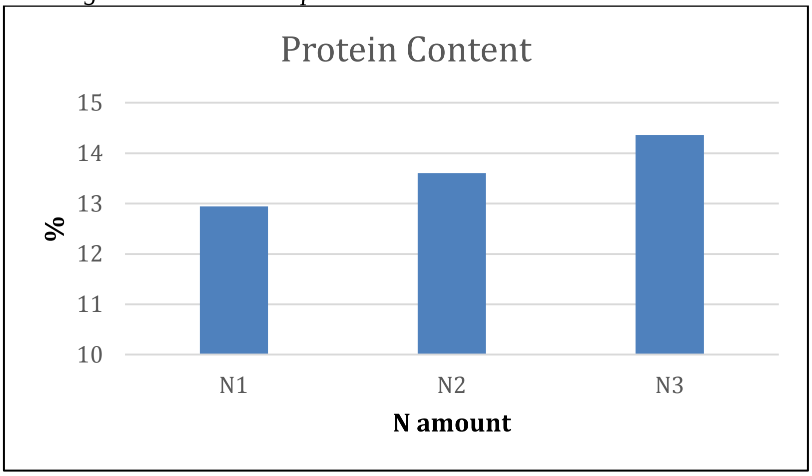
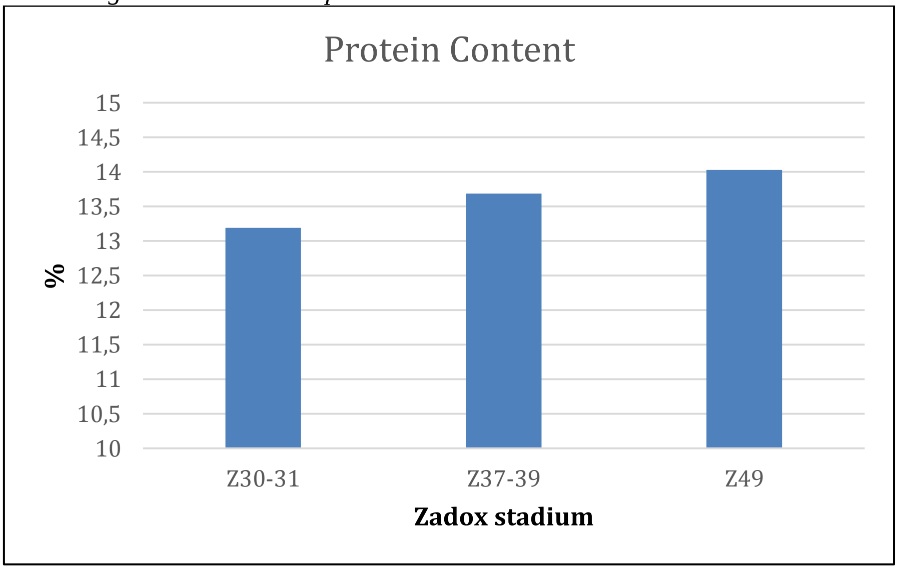


Figure 8. Relationship between Protein content and Zadox stadium



The ANOVA results show there was significant effect of N amounts and Zadox stadium on protein content. Split application during stem elongation, early flag leaf and heading increased protein content (Figures 7 and 8).

Figure 9. Relationship between Rmax and   N amount

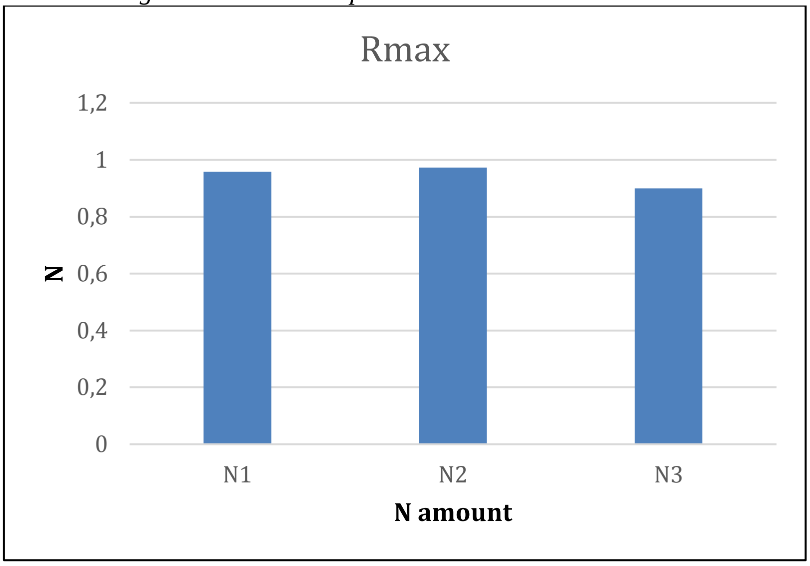
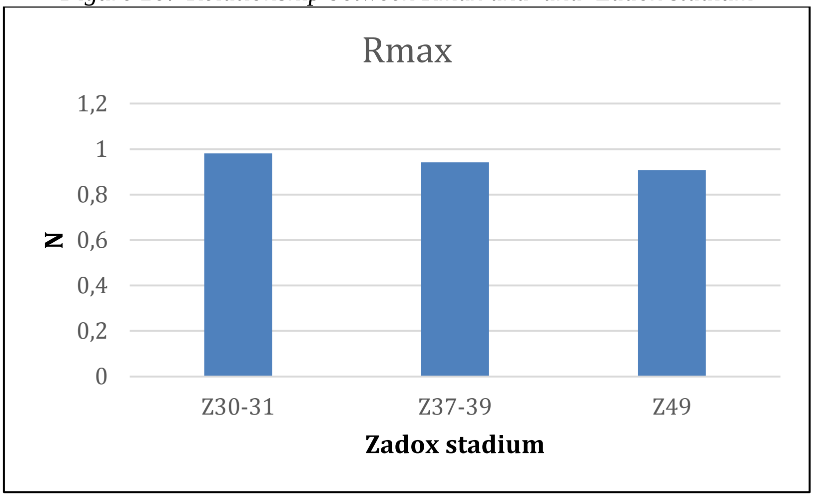


Figure 10. Relationship between Rmax and and Zadox stadium



The results from ANOVA statistics show there were significant effects of N amounts and Zadox stadium on Rmax. R max values were the most affected in N2 amount level (6kg), whereas the different developmental stages caused decrease in Rmax (Figure 9 and 10).

Figure 11. Relationship between Extensibility and N amounts

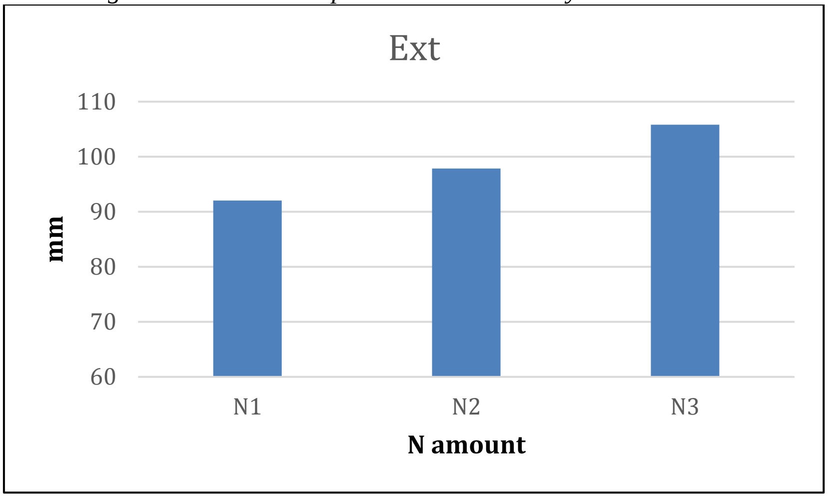
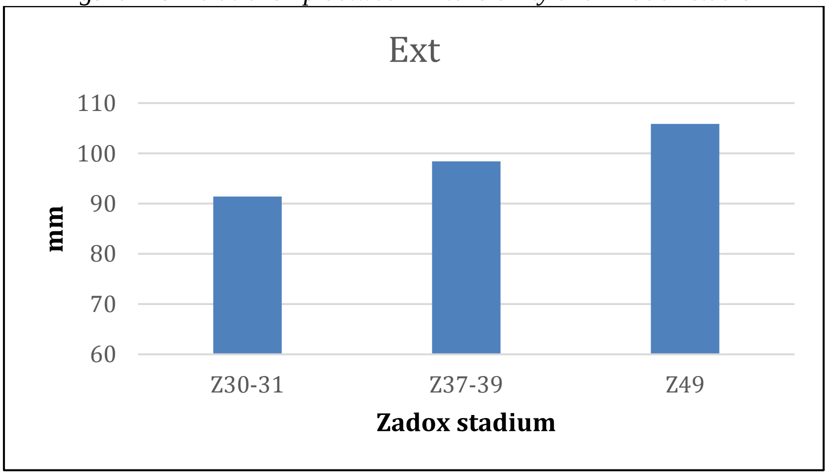


Figure 12. Relationship between Extensibility and Zadox stadium



The ANOVA results show that extensibility was affected by Zadox stadium and higher N amounts. This confirms the fact that increased N amounts influence gluten strength. Extensibility values varied between 78,45 to 112,48mm. It is clearly seen in Figures 11 and 12 how Ext level raises with increased N amounts and in different developmental stages. The N fertilizer application given as a split application in steam elongation, flag leaf an heading could improve gluten quality.

4.3 Material 3 – N split fertilization regimes applied to four varieties Bastian, Bjarne, Zebra, Avle

Four Norwegian spring wheat cultivars Bastian, Avle, Bjarne and Zebra were grown on a trial field at Vollebekk. This fertilization trial was based on N given as a split application, giving increase in protein content at different plant developmental stages and with four cultivars differing in gluten strength. Bastian is the cultivar with the strongest gluten (Class 1), Avle and Bjarne with strong gluten (Class 2) and Zebra with less strong gluten compared to the first three cultivars (Class 3). The aim was to follow how N given as spring and split application and choice of cultivar influence changes in wheat quality parameters, particularly the viscoelastic properties. The fertilization regimes in this experiment was chosen to give a wide variation in protein content to investigate this, and thus some of the regimes are not relevant for use in commercial production. Increasing protein content in four cultivars was expected to affect the viscoelastic properties of gluten, as a result of late N fertilization.

Balanced ANOVA was performed on the results of flour and gluten analyses to provide an overview of the relationships between fertilizers, cultivars and wheat quality parameters (Table 8). Nitrogen as N1) was given as a spring, basic fertilization; N2) fertilization as spring +4kg of N at fully flag leaf stage (Z37); N3) fertilization as spring + N2 + 4kg at early heading stage (Z49); and N4) fertilization as spring + N2 + N3 +4kg at anthesis (Z65). The results from ANOVA shows there was significant effect of N amounts and cultivars on protein content and quality parameters (Table 9).

Table 8. Grain yield, TW, TGW, FN; SDS, Protein %, Tot. N, R_{max} and Ext. of 4 Norwegian spring wheat varieties. Different amounts of N (1,2,3,4) given at different developmental stages were given on a field trial named AKU experiment

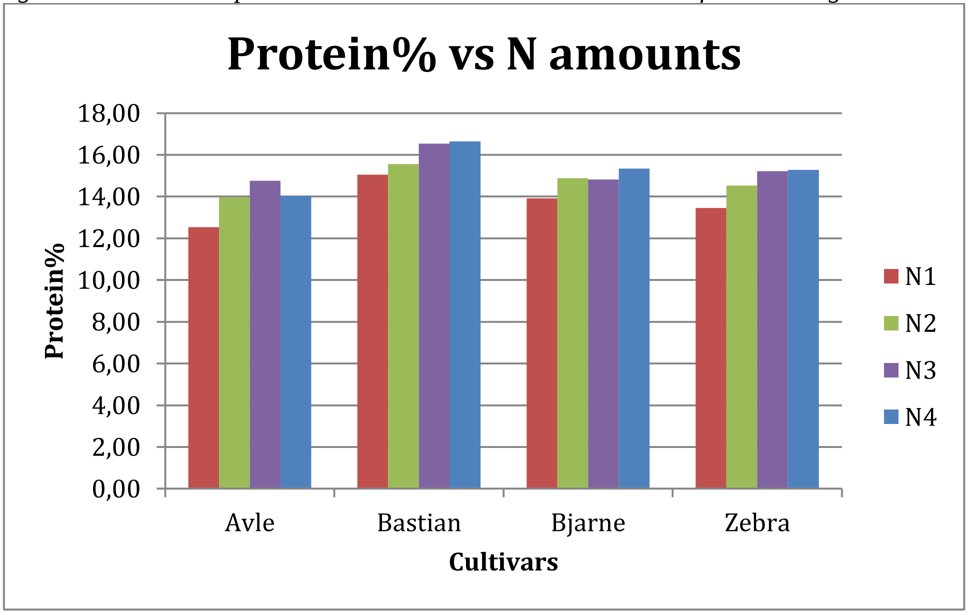
Rep.	N level	Cultivar	TW, kg/hl	TGW, g	Protein, %	FN, s	SDS, ml	SDS/P ml/%	Grain Yield, kg/daa 1)	Rmax, N	Ext, mm
1	N1	Avle	82,14	39,04	12,54	368	72	5,74	542,76		
1	N1	Bastian	79	32,17	15,05	429,5	99	6,58	491,00		
1	N1	Bjarne	77,5	31,85	13,91	399	96	6,90	459,94	0,95	97,18
1	N1	Zebra	81,95	43,32	13,45	354,5	73	5,43	491,00	0,86	109,71
1	N2	Avle	83,3	38,32	13,97	355	80	5,73	542,76		
1	N2	Bastian	79,34	32,03	15,56	374	99	6,36	491,00		
1	N2	Bjarne	80,12	34,36	14,88	393,5	98	6,59	501,35	0,96	99,72
1	N2	Zebra	83,6	41,5	14,54	349	74	5,09	501,35	0,80	129,07
1	N3	Avle	83,93	39,05	14,76	370	86	5,83	470,29		
1	N3	Bastian	80,46	34,63	16,53	438,5	96	5,81	501,35		
1	N3	Bjarne	78,42	31,93	14,82	405	98	6,61	480,65	0,92	110,96
1	N3	Zebra	81,93	42,77	15,22	362	81	5,32	480,65	0,83	132,83
1	N4	Avle	83,36	38,41	14,02	381,5	82	5,85	511,70		
1	N4	Bastian	79,9	32,39	16,64	398	96	5,77	470,29		
1	N4	Bjarne	79,1	33,58	15,33	389	98	6,39	522,06	0,97	126,67
1	N4	Zebra	82,46	42,67	15,28	362	73	4,78	501,35	0,82	129,77
2	N1	Avle	83,43	38,85	12,48	374	75	6,01	501,35		
2	N1	Bastian	81	33,8	14,25	397	95	6,67	501,35		
2	N1	Bjarne	77,3	32,53	13,22	390,5	89	6,73	449,59	0,92	101,17
2	N1	Zebra	83,2	42,64	12,83	347,5	72	5,61	522,06	0,85	111,52
2	N2	Avle	83,2	39,57	14,36	364	82	5,71	501,35		
2	N2	Bastian	79,84	32,26	16,25	429	96	5,91	470,29		
2	N2	Bjarne	77,9	31,84	14,76	405,5	96	6,50	511,70	0,95	110,86
2	N2	Zebra	82,77	45,68	14,25	356	74	5,19	522,06	0,80	128,77
2	N3	Avle	83,6	43,86	14,14	390	82	5,80	532,41		
2	N3	Bastian	79,9	33,14	16,47	402,5	99	6,01	480,65		
2	N3	Bjarne	77,9	35,55	14,71	368	97	6,59	491,00	0,87	114,98
2	N3	Zebra	81,8	42,04	14,76	355	78	5,28	501,35	0,78	133,85
2	N4	Avle	83,8	40,26	14,93	372,5	88	5,89	532,41		
2	N4	Bastian	80,1	38,05	16,30	406,5	96	5,89	501,35		
2	N4	Bjarne	78,68	33,32	15,22	400,5	96	6,31	449,59	0,75	116,31
2	N4	Zebra	81,9	42,19	15,50	340	79	5,10	511,70	0,87	134,76

Table 9. Mean squares and significance level calculated with Balanced ANOVA on TW, TGW, Protein content, FN, SDS and Grain Yield

	TW, kg/hl	TGW, g	Protein, %	FN, s	SDS, ml	Grain Yield, kg/daa 1)	Rmax, N	Ext, mm
N amounts	1,09	0,68	50,05***	0,35	7,81**	0,49	0,46	20,25*
Cultivar number	91,69***	55,82***	46,41***	18,49***	147,25***	3,49*	7,73*	59,91***
N amounts *Cultivar number	1,28	0,59	0,65	0,41	1,84	0,69	0,89	2,26

* Mean squares of F at 95-99% significance level
** Mean squares of F at 99-99,9% significance level
*** Mean squares of F at 99,9% significance level

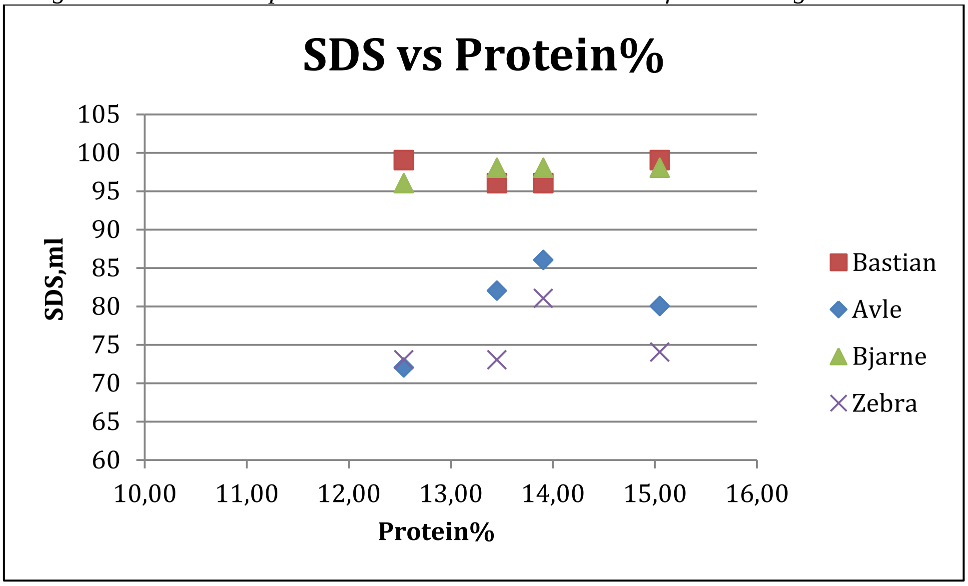
Figure 13. Relationship between Protein content % vs N amounts for 4 Norwegian cultivars



There was significant effect of different N amounts on protein content as well as on SDS for all cultivars. The PC% varied from 12,48 to 16,64%. A split application of additional 4kgN/daa given at Z49 increased the protein content the most for all cultivars (Figure 13). This confirmed that higher protein content could be achieved by giving higher N fertilization and particularly by using split application methods.

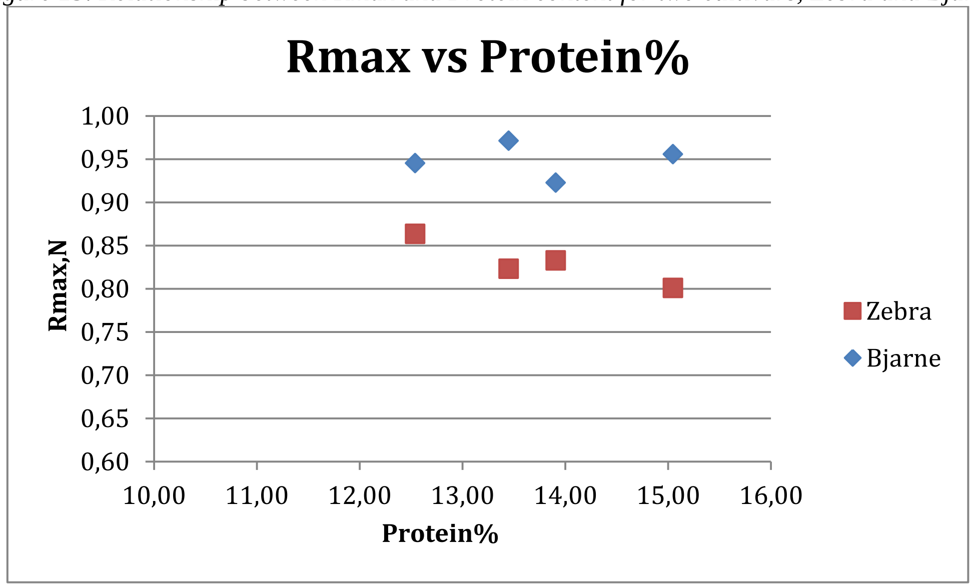
There were significant effects on all quality traits by different cultivar (Table 8). Wheat cultivars were significantly affected by N fertilizer application. Bastian cultivar had the highest protein content in all N application regimes. This is the cultivar with the strongest gluten (Table 2). Bjarne and Zebra showed very similar response followed by Avle. The same effect was seen on increased protein influenced by increased N fertilization (Figure 13).

Figure 14. Relationship between SDS vs Protein content % for 4 Norwegian cultivars



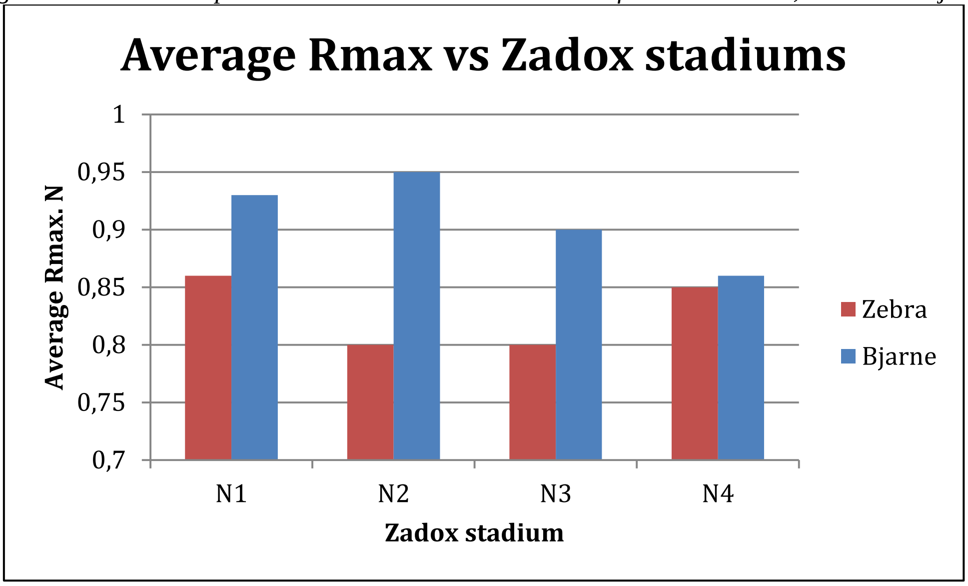
The results from ANOVA show there was significant effect of N amounts on SDS. Bastian and Bjarne showed the highest SDS values from 96 to 99 ml and these variations did not follow the protein content (Figure 14). SDS values for Avle and Zebra showed greater variability from 72 to 88ml while SDS increased with increased PC%. Avle had better results, which confirmed stronger gluten according to Zebra (Table 2). Zebra had the lowest SDS value of 72ml (Figure 14). SDS results shows that gluten strength can be affected by different genotypes according to different results for different cultivars.

Figure 15. Relationship between Rmax and Protein content for two cultivars, Zebra and Bjarne



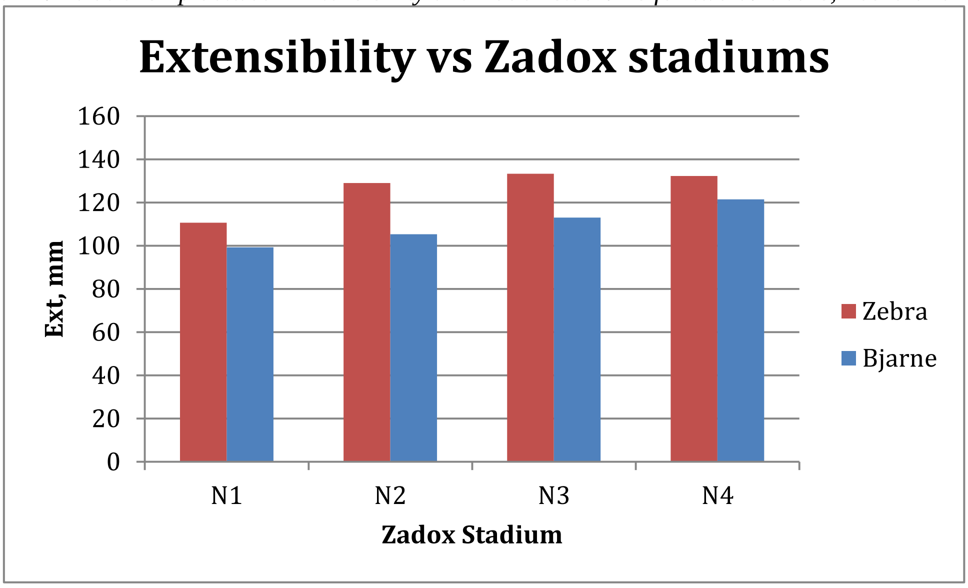
The ANOVA results show there was significant effect of N amounts and cultivar on Rmax. It was analyzed and compared between two cultivars, Bjarne and Zebra. Bjarne had higher Rmax than Zebra at all N regimes. Furthermore, Bjarne had relatively similar Rmax values from N1 to N4, whereas for Zebra the Rmax decreased from N1 to N4, as the protein increased (Figure 15). These results support earlier findings in which nitrogen application is important for gluten structure, which determines bread-making quality.

Figure 16. Relationship between Rmax and Zadox stadiums for two cultivars, Zebra and Bjarne



Different cultivars alter differently the viscoelastic properties of gluten proteins and this is clearly seen in figure 16. The results from ANOVA show there was a significant effect of Zadox stadium on Rmax for Bjarne and Zebra. Spring application and split application at fully flag leaf seems to be the best choice for timing N application according to gluten quality. Bjarne had better results, with the average Rmax 0,91 comparing to Zebra where it was 0,82.

Figure 17. Relationship between Extensibility and Zadox stadiums for two cultivars, Zebra and Bjarne



Both cultivars Bjarne and Zebra showed the increased Ext values with increased N amounts given in all split applications (Figure 17).

5 DISCUSSION

The baking industry has set many quality traits to food wheat related to bread making. The most important among which are: optimal protein content and optimal gluten quality.

The primary objective of this thesis was to study the impacts of fertilization and nutrient availability on Norwegian grown wheat quality with the focus on protein content and gluten quality. The thesis is divided in three parts, based on three different field trials performed in the season 2014 at NMBU, Ås: 1) Fertilization trial with P and K; 2) Fertilization trial with N given at different plant development stages as a split application; 3) Fertilization trial with four varieties and N given as a split application at different development stages.

5.1 Material 1

Material 1 was conducted to study effect of P and K fertilizers applied on spring cultivar Krabat. There was no split application of N. The significant effect of P amounts on yield, protein content, test weight and Rmax was found.

The analysis showed there is a negative correlation between yield and protein content. Protein content in wheat grains normally decreases with the increase in grain yield (Simmonds, et al., 1995). PC% is more influenced by environment and fertilizer practice, then by genotype. Therefore, increasing the protein content without affecting the grain yield and vice versa is a great breeding task.

Yield increased with higher P levels. It could be argued that low P availability decreased the yield, with less ability to take up N, leading that more N is available for heading and during grain filling to give a higher protein. At 0 level of P a severe decrease in yield was found. This was observed also on the field during the early plant development.

Significant positive correlations were reported between test weight and grain yield (Troccoli et al., 2000). This was not the case in this trial. There was no significant correlation between yield and TW. Both, TW and yield were influenced by P amounts, and there was a large variation in yield with increasing P levels, but rather small differences in TW. This indicates that yield differences are caused by P limitations early in the plant development giving weaker canopies and less tillers for the lower P levels (and lower numbers of grains to be filled), whereas the conditions during grain filling were sufficient to develop large and well-filled grains for all P levels. Lower TW indicates lower quality. According to the recommended value of 79 kg/hl for TW in Norway, all samples showed higher rate of TW.

ANOVA result showed the significant effect of P on protein content and total N. The whole protein content ranged from 10,87-14,39%, showing that Krabat nearly satisfied the minimum requirement set for food wheat for all the treatments, but mixed with strong flour gluten for expected quality bread baking properties. The current minimum requirement for wheat for bread making in Norway is 11,5 for PC%.

There were no significant effects of different P or K level neither on SDS, FN and TGW nor on their interactions. Quality traits set in Norway for bread wheat required FN values to be above 200. According to that, Krabat samples had very high FN and it could be argued that there was no pre-harvest sprouting. The SDS sedimentation volume is a predictive test for bread making, positively correlated to PC%. Here was no significant correlation between SDS and PC%. High values of PC% and FN, but low SDS calculated from quality analysis, showed that high PC% does not necessary mean high gluten content and strong quality (Wang et al., 2008). Considering low SDS it was expected that viscoelastic properties of gluten from Krabat flour show the same effect. This could be connected with classification of Norwegian cultivars in which Krabat is classified in the 3rd class according to baking quality. Increased protein content due to increased fertilization has resulted in reduced Rmax and increased extensibility. Krabat had higher Ext but lower Rmax K level. Higher R max was observed at the highest P level and this is the mainly explanation for the higher Rmax at K level. Considered the protein content decreased more from both K level at highest P level, which could also be a part of explanation. Furthermore, there is a positive effect of higher K level on Rmax at the highest P level. This was an interesting result, but unfortunately not documented until now in a literature. Further tests need to be done in order to elaborate this.

There is a lack of studies of possible influence P and K amounts on quality traits. But, there are many dealing with P and K influence on yield (Simmonds, et al., 1995; Groos et al., 2003). This experiment showed that there might be some future steps in this direction and in a wider context of fertilizing management, even though it is a fact that N fertilized trials are mostly used to study effect of protein content and viscoelastic properties of gluten.

5.2 Material 2

Material 2 was conducted to study effect of N split fertilization applied on spring cultivar Bjarne at different development stages**.** ANOVA results showed there was significant effect of N amounts and Zadox stadium on protein content. By increasing level of nitrogen fertilizer application an increasing in protein content is expected (Aamodt, et al., 2004). N given as a split application during stem elongation or at heading, increased protein content in wheat. Increased protein content, especially accompanied by higher temperature during grain filling, affects significantly gluten quality properties (Koga, 2015).

Quality traits set in Norway for bread wheat required FN values to be above 200. For the SDS sedimentation volume values are expected to be 70 and above. The SDS sedimentation volume is the best predictive test for bread making, positively correlated to PC%. In this experiment were calculated high values of PC%, FN and SDS from quality analysis. It showed that high PC% increased SDS, which was associated with general increase in concentration of gluten proteins rather than changes in the gluten protein composition. This result coincides with results of many other studies proving that SDS is correlated with protein content (Zhang et al., 2008).

Timing of N level significantly affected PC% as well as FN. Results from ANOVA implied that N given as split application at stem elongation and heading stages could influence FN. Falling number is considered as the most important indicator of pre harvest sprouting. Very high values observed at this trial showed that cultivar Bjarne can be accepted as food wheat in Norway.

Increased protein content affected significantly gluten quality properties. There was significant effect of Zadox stadium on extensibility as well as the effect of N amounts on the same trait.

More extensible gluten was influenced by higher N amounts. This result supports studies where increased effect of N influenced gluten properties. An increase of dough extensibility was found with increase in nitrogen fertilization (Godfrey et al., 2010).

In this experiment, result shows there was no significant effect on yield and Rmax by N amount, Zadox stadium or type of fertilizer as well as their interactions. Types of fertilizer did not affect any of observed parameters.

5.3 Material 3

Material 3 was conducted to study effect of N split fertilization applied on four spring cultivars at different development stages. Cultivars also differ in gluten quality; all are strong gluten quality classified in three classes according to the baking quality set for Norway.

The protein content of the grain is more influenced by environment and fertilizer practice, than by genotype. Higher protein content can be achieved by giving higher N fertilization and particularly by using split application methods. The challenge for agricultural practice is to determine the correct amount of N given as a split application in different seasons with different weather conditions.

Wheat cultivars were significantly affected by N fertilizer application. Different cultivars utilize available N differently (Bushuk, 1985). Increased N application results in significant increase of gluten proteins, mainly gliadins and glutenins, of protein content and bread volume (Johansson et al., 2001). Considering that protein quality is determined genetically it was expected that different cultivars would show different results. They have different genes coding for different polypeptides (Aamodt et al., 2004). Bastian had the highest protein content in all N application regimes. This is the cultivar with the strongest gluten (class 1). Bjarne and Zebra showed a very similar response followed by Avle. The same effect was seen on increased protein influenced by increased N fertilization. According to the effect on crop quality, particularly on grain protein concentration, N alters the composition of gluten proteins.

Split application also increased SDS, which could be explained by general increase in concentration of gluten proteins rather than changes in the gluten protein composition. Bastian and Bjarne showed the highest SDS values. The SDS results shos that gluten strength can be affected by different genotypes according to different results for different cultivars. Furthermore, variations in SDS might be a result of temperature effect, mainly during maturation. It was found that warm grain filling period produces stronger gluten (Johansson et al., 2001).

There were significant effects on all quality traits by different cultivars. This was analyzed and compared between two cultivars, Bjarne and Zebra. The Rmax varied between 0,78 to 0,86 in Zebra and between 0,87 to 0,96 for Bjarne. It was found that the resistance of the dough, Rmax is increasing with protein content (Aamodt 2004). Rmax decreased in Zebra with increased protein and increased amounts of N application, and did not significantly change for Bjarne. A significant effect on the viscoelastic properties of gluten was expected, as a result of late N fertilization (Johansson et al., 2001).

An increase of dough extensibility with increase in nitrogen fertilization was also noted (Godfrey et al., 2010). Both cultivars Bjarne and Zebra showed increased Ext values with increased N amounts given in all split applications. These results support the earlier findings that nitrogen application is important for gluten structure, which than determines bread-making quality. (Uhlen, et al.,1998 ; Moldestad et al.,2011).

6 CONCLUSION

Protein content and protein quality are important factors for bread making performance of the flour. And both are complex parameters to examine. The protein content widely varies between the types and classes of wheat, in amounts from 8-20%. It is strongly dependent on growing conditions, soil fertility and temperature during the season and fertilizer inputs, nitrogen in particular. Higher protein content can be achieved by giving higher nitrogen fertilization and particularly by using split application methods.

A different approach in order to achieve better bread making properties is to improve the protein quality. It tends to vary according to variations of the gluten proteins. Because there are large numbers of different combinations of storage protein components their evaluation is usually a complex and hard task. Baking and different chemical tests, as well as the rheological tests on flour, dough and gluten are often regarded as good parameters to determine wheat quality.

To study variation in protein content, wheat can be grown on nitrogen-fertilized trials whereas nitrogen is given with different amounts. In this thesis, for the first time the possible effects of higher phosphorus and higher potassium levels on protein content and gluten quality were experimented.

Three experiments examined the effects of nutrient availability on quality parameters with special focus on viscoelastic properties of gluten. The results from material 2 and 3 showed strong effects of N fertilization regimes, giving variations in protein content and viscoelastic properties. Increasing Ext and decreasing Rmax as shown in the results, are in accordance with reports from earlier seasons in Norway. The results from material 1, where the effect of higher P and higher K fertilization on gluten viscoelastic properties were studied, showed same effect. Ext increased and Rmax decreased with increased P level. That seems to be related to the differences in protein content and linked to the differences in yield. Also, interesting trend of increased Rmax at the higher K level was observed. Considering, there are significant interactions between P and K levels on viscoelastic properties of gluten, it is of interest to explore how the protein content varies depending on other factors rather than only nitrogen fertilization.

On five different cultivars the effect of split application of nitrogen given at the different developmental stages was shown, as well as the effect of higher P and higher K fertilization on the same parameters. As a result, all of them varied in protein content and viscoelastic properties of gluten. The results showed that protein content and gluten quality can be manipulated through choice of cultivars and possible management strategies with higher nitrogen, but also with higher phosphorus and higher potassium levels.

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