Relationships Between *Fusarium* Infestation, Mycotoxin Content and Baking Quality in Spring Wheat

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Lists Abbreviations

ALG	Albumins/globulins
LC-MS/MS	Liquid chromatography with coupled mass spectrometry
P F	Significance factor
RP-HPLC	Reversed-phase high-performance liquid chromatography
DON	Deoxynivalenol
FHB	*Fusarium* Head Blight
Ppm	Part per Million
BQ	Baking Quality
PC	Protein Content
PQ	Protein Quality
FN	Falling number
SDS	Sodium Dodecyl Sulfate
PHS	Preharvest Sprouting
TKW	1000 kernel weight
DAA	Days after anthesis

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Summary

Fusariums infestation in spring wheat (*Triticum aestivum*) has become more severe in Norway and it is often questioned if this also can affect the baking quality. In this study, two different materials were selected to investigate this hypothesis. One material consisted of wheat samples harvested at different developmental stages during grain filling, and both grain quality development and *Fusarium* infestation of different *Fusarium species* were followed. The second materials were consisted of wheat samples harvested at different years to investigate how different levels of mycotoxin content in grains influence baking quality.

This thesis performed and studied the modifications and quality changes during kernel development. Another distinctive aspect of this investigation was the detection of the *Fusarium species* infested at particular developmental stage in spring wheat and their infestation levels (%). In addition, mycotoxin content in grains and the its influence in baking quality were tested. A part of the plant material (material 1) been used in the investigation were collected in Vollebekk experimental farm in 2009, Ås, Norway. Materials were harvested from 10 days after anthesis (DAA). Harvestings were done at each 5 DAA until 40 DAA. The second part of the material was collected from Roverud, Norway in the years 2006, 2007 and 2008.

Both grain size and grain quality tend to develop normally. Infestation levels of different species revealed that F. avenaceum was extraordinarily highly dominant *F. species* obtained in this data. It was more than 50% at 20 DAA, and more than 70% at 40 DAA. The *Fusarium species* identified in this research presented the following order of prevalence; *F. avenaceum, F. culmorum, F. graminearum, F. equiseti.*

On the other hand, the potential relationship between mycotoxin content in the grains and baking quality parameters being tested in material 2 revealed that, there was no significant correlations been observed in general. However, slightly positive relation between protein content (%) compared to grain mycotoxin content couldn't expose any at least in this data.

Mycotoxins estimated means between the years 2006, 2007 and 2008 revealed insignificant in Roverud, even though the estimated means deviated as much as more than two folds greater mycotoxin in 2006 than in 2008.

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1.0 Literature

1.1 Mycotoxins and Social Impact

Mycotoxins are toxic secondary metabolites yielded by fungus on crops that caused mycotoxicoses to human and animals ingested a food and feed contaminated with such toxic substances. It affects the immune and metabolic systems of human and domestic animals (Agrios 2005) (Peraica et al. 1999).

For the last three decades, large number of papers, both national and international have been written on mycotoxins and DON in cereals. Most of which, if not all, have expressed a huge concern over potential negative impact of mycotoxins in contaminated foods.

Similar concerns were experienced locally, for which extensive number of scientific papers based on Norwegian studies (Langseth et al. 1997) (Langseth & Elen 1997; Liu et al. 1997) have been raised their concern in *Fusarium* head blight (FHB) and its consequences in the cereal feed and food chain.

In particular, spring wheat was highly associated with DON production than any other Norwegian cereals (Langseth & Elen 1997)

This problem has become ever growing worldwide concern since 1960, at which an outbreak of “young turkey birds or turkey X disease” had been reported in UK (Agrios 2005). Since then a potential danger for *Fusarium* head blight (FHB) was more frequently reported. Particular concern has been made on contaminated food and feed affected mainly by *Fusarium species* and *Microdochium nivale* (Wu et.al. 1997, Magan and Aldred 2007, (Placinta et al. 1999). (Reddy & Raghavender 2008) had also been connected the human poisoning outbreak at Kashmir in 1987 with consumption of bread made of flour infected by mould. Similarly, (Reddy & Raghavender 2008) had associated sporadic and unseasonal (disorders) of disease

outbreaks in human and animals caused by ingestion of contaminated food and feedstuff with mycotoxins, particularly DON.

Nevertheless, grains infected by FHB would produce various mycotoxins, particularly DON, in their pre-and postharvest (Placinta et al. 1999). However, the generation of mycotoxins might be a result of an extended or poor techniques of harvest, extended or poor storage (Placinta et al. 1999), but the main challenge for DON is the infection prior to the time of harvest.

Domestic animals ingested mycotoxins are often seen symptoms such as; weight reduction, hypoproteinemia, and loss of physical ability (Placinta et al. 1999). There are many other internal and physical symptoms caused by mycotoxins in domestic animals and humans. These problems are mainly exposed and hit to the lowest class of the social cross-section and their domestic animals. These lowest socio-stratum have little choice available for them to avoid eating (intended/unintended) of contaminated food ((Reddy & Raghavender 2008). Further unconfirmed report (McMullen, M. et al. 1997) is linking mycotoxins with potential danger affecting reproduction abnormality in domestic animals.

Accordingly, limits have been set by the Food and Drug Administration. They have established vomitoxin advisory levels. Vomitoxin is also known as deoxynivalenol (DON).

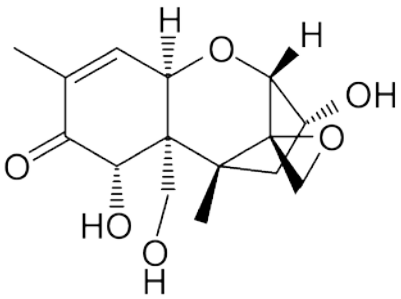


Figure 1: Deoxynevivalenol (DON) or vomitoxin

Its molecular formula is $C_{15}H_{20}O_6$ and belong to type B Trichothecenes group of mycotoxins (Beyer et. al. 2006;2009).

The specific limits are as follows;

* 1 part per million (1ppm) for finished grain products for human consumption.

* Cattle, over 4 months old: 10 ppm (providing grain at that level does not exceed 50 percent of diet).

* Poultry: 10 ppm (providing grain at that level does not exceed 50 percent of diet).

* Swine: 5 ppm (not to exceed 20 percent of ration).

* All other animals: 5 ppm (providing grains do not exceed 40 percent of diet)'

(McMullen, M. P. et al. 1997b).

Mycotoxins not only adversely affect human's and animal's health. But, there are also other aspects that mycotoxins might have detrimental effects such as alterations that *Fusarium* do to influence baking quality aspects in cereal grains and others food commodities. However, the ultimate interest and the goal of this study is to understand the nature of this detrimental effects and reflect applicable guidelines to achieve stable and wanted quality.

1.2 Fusarium Head Blight (FHB)

FHB is tan and brown discoloration that affects the base of the floret. Spikelets for which disease is progressed may turn to light tan and even become bleached in appearance. Disease symptoms may spread throughout the head, particularly, if the fungal attacks the rachis. Certain proportions of the rachis may develop dark brown color. Small orange clusters of spores or even black in color as reproductive structures called perithecia germinate on the spikelets. These structures appear when weather is favorable for pathogen reproduction. However, infected kernels become shriveled, white and chalky. Some other kernels can be pink or red in color.

There are various common diseases caused by *Fusarium species* in the growing crops, ornamental plants and naturally growing plants.

Some of the diseases and symptoms commonly found under *Fusarium* infected plants are; Brown Foot Rot, Early Blight, Dry Rot, Stem Rot, Dei-back, Storage Rot, Cob

and Stalk Rot. These diseases are not only expected to reduce the yield, but also affect the grain quality.

Norwegian Field surveys were conducted in order to investigate pathogenic strains associated with cereals hit by FHB, particularly barley, oats and wheat. The survey had demonstrated most frequently observed isolates from Norwegian cereals as *F. avenaceum, F. poae, F. culmorum* and *F. graminearum* ((Kosiak and Torp 1995; Sundheim et al., 1988; Langseth et al., 1997; Haave, 1985; Liu et al., 1997). Although, *Microdochium nivale* and others contribute similar role as *Fusarium* do in FHB formation in cereals. However, *F. avenaceum* and *F. culmorum* often cause FHB in many parts of Northern Europe (Van et al.1995; Parry et al. 1995; Brennan et. al. 2005). It might be is: due to both being soil-and plant residue borne fungi. Unlike others, *F. graminearum* is, generally, linked to most of FHB outbreaks in Europe, North and southern America, as well as some parts of far-east Asia (China and Japan).

Besides, several reports among them is McMullen et al. (2008) associated *F. culmorum* and *F. graminearum* with stalk rot of corn and root rot of small grains. The reports also linked DON contamination in wheat with FHB caused by other fungi generas such as; *Trichoteceum, Stachybotryts, Myrothecium*.

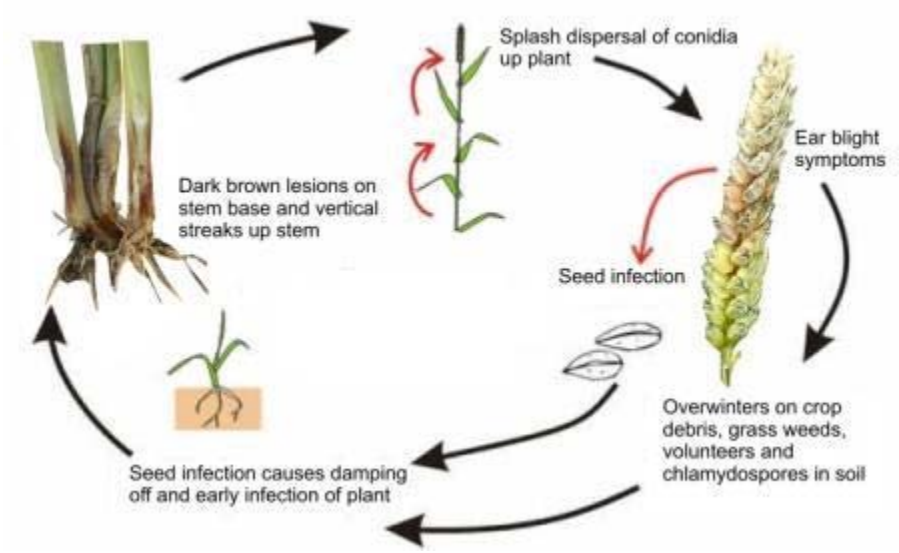
Worldwide detection of mycotoxins is mainly associated with some of the most frequently encountered *F. species* in wheat (Miedaner T., 1997; Jones & Mirocha, 1999; Doll et al., 2002). Although, FHB presence in spring wheat do not, necessarily, involve the presence of the only above mentioned *F. species.* Yet, it can certainly be a result of another *Fusarium* species or even with a combination of other factors as well.

In connection with their geographical distribution, *F. graminearum* is predominant in warmer climates, whereas *F. culmorum* is predominant under cooler (Wang et al. 2005) climates. Contrastingly, (Brennan et al. 2005) documented inversely effects on the temperature of *F. graminearum* and *F. culmorum* under greenhouse investigation. The study demonstrated that *F. culmorum* was propagated more effectively at 20 ° C, while *F. graminearum* propagated well with 16 ° C.

However, most often these reports were linking FHB, if not all, with mycotoxins development and grain quality reduction. Therefore, reviewing these articles might help and contribute to understand posible interaction between FHB and mycotoxin generation in gains, as well as alterations it does in baking quality.

1.2.1 *Fusarium* Life cycle

Fusarium spores overwinter in infected debris, grasses, grass weeds, volunteer plants and in the soil as chlamydospores, corn and grains (Agrios 2005). In wet conditions, spores are either blown or splashed by wind or rain on the plant, in ears. The source of the inoculums can be within the plant or outside inoculums that are from shorter or longer distance (McMullen, M. et al. 1997). However, its primary source in wheat crops is the seed source, though *Fusarium* survives in the soil and plant residues as saprophytic fungus. Hence, wheat crops are highly susceptible to FHB infection at flowering to hard dough stage. Infection, normally, begins at spore landing on anthers at pollination time (Gartner et al. 2008), which further grows into the kernel and bracts, as well as other segments of the head . Thus, infection doesn't cease until near to maturity. It further proceed colonizing to the vascular connection via xylem and phloem tubes (Kang & Buchenauer 1999). Primary infection mainly depends on air-bone inoculums. It might also initiate secondary infection, particularly to late tiller developing cultivars.



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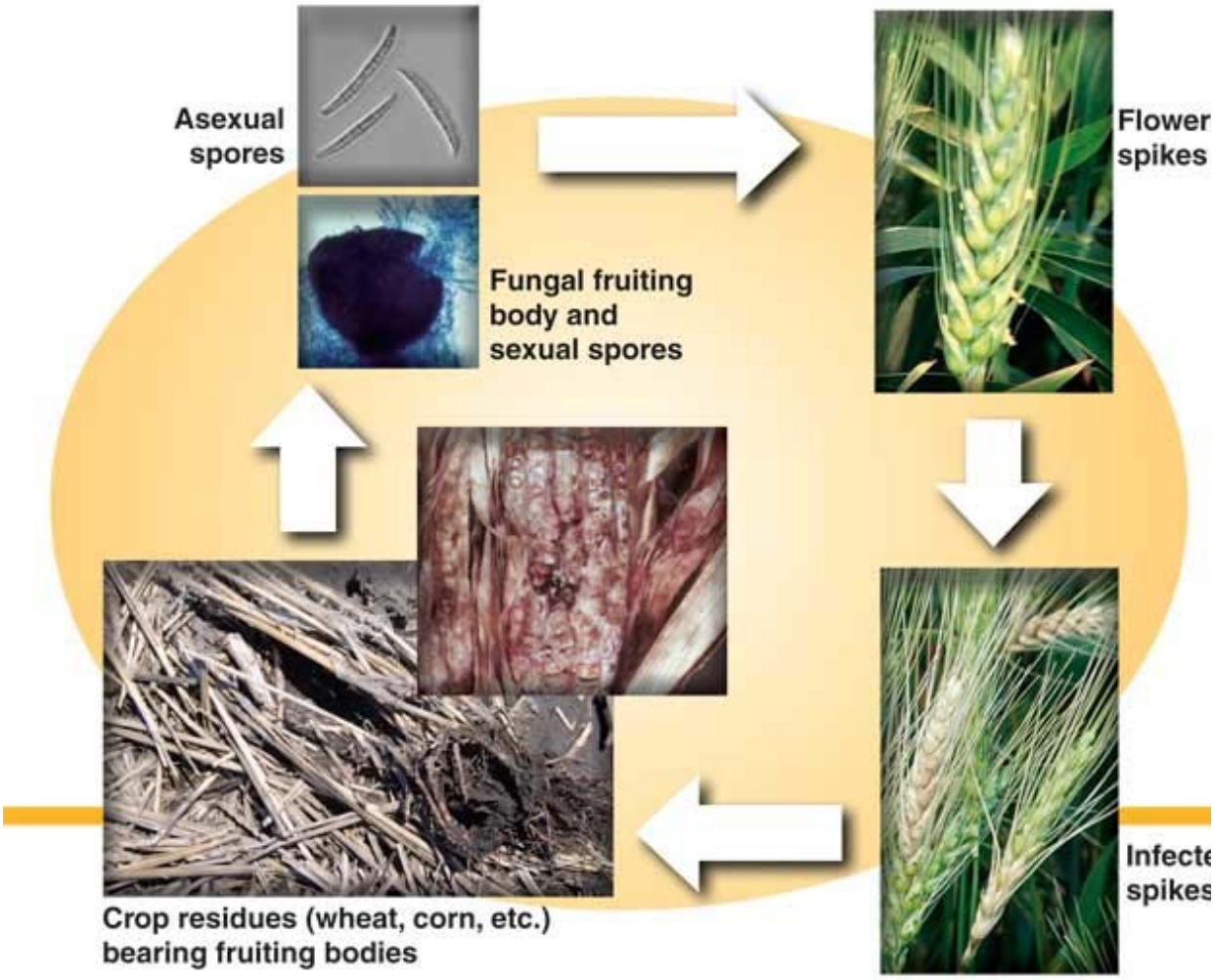

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(http://images.google.no/images?hl=no&q=Fusarium+life+cycle&um n. s)

Figure 2: *Fusarium* head blight disease cycle (McMullen, M. et al. 1997)

1.2.2 Favorable Weather Conditions for FHB

Its widely excepted that frequent rainfall, and high humidity (or heavy dews) at anthesis and also later during grain filling period may enhance infection development for FHB (Jones & Mirocha 1999) (McMullen, M. et al. 1997; McMullen, M. P. et al. 1997a).

Another research conducted by (McMullen et al. 2008) suggested the interaction of certain environmental factors; such as temperature and precipitation are closely associated with FHB severity. The study has underlined that susceptibility is likely to be high under prolonged and repeated periods of wetness, and high humidity at kernel development and grain filling stages for susceptible species. Particularly, wheat is susceptible early at the stage of anthesis.

Furthermore, (Paul et al. 2007) investigated the association between weather variables and inoculums of *Gebberella Zeae* (telomorph form of *F. graminearum*) on wheat canopies. This study showed wet and high humidity at anthesis or near flowering favors best for FHB inoculums disseminations.

Equally, (Brennan et al. 2005; Xu et al. 2007) have examined FHB incidence in wheat and small cereals. They documented that incidence of FHB are very likely to occur at anthesis due to temperature effect. Temperatures at 20 ° C shortens the duration of grain filling respectively, without necessarily increasing the rate of grain filling (Gooding et al. 2003). This implies that, a shorter duration of grain filling may result a lesser rate of grain filling. Therefore, a lack of proportionality between the rate of grain filling and the duration of grain filling may exist. This might lead to a reduced 1000 kernel weight (TKW) (Chowdhury et.al., 1978)

However, controlled environment study in Norway by (Brennan et al. 2005) confirmed that *F. graminearum*, and *F. culmorum* had both reduced 1000 kernel weight (TKW) at 20 °C. The study demonstrated also that *F.culmorum* caused more visual disease at 16 ° C than 20 ° C, while *F. graminearum* caused more visual disease at 20 °C than 16

º C. There are others reporting F. graminearum as dominant in both temperate and warmer climates. But obviously, both *F. graminearum* and *F. culmorum* can be distributed well under both warmer and cooler climates.

Furthermore, a chance of FHB epidemics is high in warm and humid periods at flowering (Buerstmayr et al. 2003) Dill-Macky and Jones 2000;(Buerstmayr et al. 2002) under circumstances where inoculums are abundant.

On the other hand, 258 mm yearly average rainfall had been reported in South Africa. In this condition, sporadic and severely FHB outbreaks confirmed on cereals (Wilma M. KRIEL – Zacharias A. Pretorius 2008). In this regard, a combination of lower temperature and higher relative humidity than average during flowering was associated with the incidence.

There are, nevertheless, other studies that confirmed relationships between inoculums abundance, FHB development and DON accumulation (Abbas and Mirocha 1988, Alves-Santos et al., 2002).

Despite wind and water conidia dispersal, *F.graminearum* has abundant sexual stage by forming perithecia (Trail 2009). Thus, the formation of perithecia leads to multiple infection periods due to release of fungus spores into air and water splashes on different growing seasons. This may further result more sporadic outbreaks of FHB in small cereal fields than any other inoculums sources.

However, the interaction of two or more of the basic factors; temperature, rainfall, humidity and the presence of abundant inoculums determines the seasonal outbreak of FHB in small cereal fields.

Therefore, reduction and abundance of inoculums might be involved agronomic and management practices as; tilling and crop rotation been explained elsewhere in the management strategy (1.2.3).

Favorable weather for FHB is, generally, regarded when the temperature falls over 20 coincides with wet and Higher RH at anthesis.

In relation to grain quality: tendency to severity of Fusarium infestations and mycotoxin development are both critical at anthesis. Both of them are generally perceived to affect baking quality and grain quality as whole.

1.2.3 Management Strategy

Tillage and crop rotation play an important role to minimize chance for inoculums survival (Paul et al. 2007). Preferable crop rotation involves sequencing wheat with non host crops or non cereal species (Dill-Macky & Jones 2000). Based on this fact, wheat sequenced with soybeans reduced FHB infestations and FHB severity in respect to wheat-wheat or any cereal- wheat sequences; for which tillage has been disregarded. The most irrespective cropping system and the least recommended that might lead severe FHB outbreak, and the worst case scenario can be succession of wheat with maize as prior crop (Gartner et al. 2008).

In Norway, where soybean is not common crop, oil-seeds may be better option and potential candidate to break the sequential pattern of cereal-wheat cropping system.

With and without an appropriate crop sequences, tilling is an important practice to reduce chance for residue-borne seasonal inoculums survival (Bai & Shaner 1994; Dill-Macky & Jones 2000). Regarding this, research was conducted by (Khonga & Sutton 1988) to study *Gebberella zeae* seasonal inoculums production and inoculums survival in wheat and maize residues. The researchers suggested that perithecia and macroconidia production were not observed at buried residues. It further documented higher perithecia density in wheat grains and maize kernels than other plant parts. Another studies in the USA (McMullen et al. 2008; Christensen et al. 1929) confirmed that a permanent variation in weather enhances FHB epidemics out breaks. Both studies had explicitly recommended the use of tilling is a better choice to enhance decomposition of infected tissue and minimize inoculums accumulation.

In Norway study on tillage systems on cereals was conducted by Henriksen et al. (1999). For this paper significant increase was found under reduced tillage systems

(harrowing, spring tillage, and no-till systems) for inoculums build-up and mycotoxins grains content. Hence, mean *Fusarium* incidence appears to be consistent with reduced tillage, precipitation and soil type. Under some circumstances, where soil is typical silt with high precipitation rate, high *Fusarium* infection has been observed.

Therefore, in respect with this review and other studies such as Henriksen et al. (1999), mentioned that a plowing can be used to eliminate, or even minimize inoculums abundance in Norwegian cereal fields.

Various studies, however, emphasized the use and application of resistance varieties and cultivars for FHB. Relevant study is made by (Buerstmayr et al. 2003), for which it strongly recommended the use of resistant cultivars to reduce loss of yield and quality by FHD. (McMullen et al. 2008; Gartner et al. 2008) equally recommended the use of resistant varieties. He particularly highlighted the positive impact of resistant spikelet against initial infection, as well as kernel resistance to fungal penetration. These physiological characteristics can be the basic criteria for the selection of the resistant cultivars. Such characters can contribute reduction for DON accumulation in kernels prior to FHB infection.

On the other hand, sowing date and the selected cultivar needs to adjust earlier kernel filling time before weather becomes favorable for FHB infection (McMullen, M. et al. 1997)

On different approach, bio-control research conducted by Allen Xue (2007**)** in Canada has found significant effect on FHB control. These researchers found a strain of plant pathogenic fungi *Clonostachys rosea (*ACM94); where this project aimed at FHB control without a fungicide application. The biocontrol method can be used with (or without) minimum fungicide (Tebuconazole) application in order to perform pesticide and herbicide reduction policy.

Triazole fungicide applied at anthesis played significant role for controlling FHB. It also plays a similar role on DON contamination in durum wheat, when FHB pressure is severe (Blandino et al. 2009).

Recalling basic experimental hypotheses, controlling FHB and even minimizing *Fusarium* infestation occurrence, does not only improve baking, but may also insure higher production rates per unit area.

1.2.4 *Fusarium* Identification

Identification of sporodochia and a conidial formed from CZID were commonly used to identify *Fusarium spp*. isolated in cultures. Although, *Fusarium species* do greater complexity to identify them, yet certain characteristics function as a checklist. The checklist used for *Fusarium* cultures based mainly to macroconidia; size, shape, apical and foot cell shapes. Microconida size, shape and phialides also provide an important contribution for *F. species* identification. Furthermore, clamydospores, colony size and colors are also used as tool for identifying *Fusarium* isolates. A certain *Fusarium species* may present rapidly, sparsely or even densely colonies. Such characteristics often help to suggest only a few and exclude larger number of *Fusarium spp*. Species of *Fusarium* often vary a season to another due to the weather variation. However, *Fusarium graminearum, Fusarium culmorum , Fusarium avenaceum , Fusarium equiseti* identification checklist are incorporated and described here:

Fusarium graminearum Schwabe *(F. graminearum)* is common name similar to *Gibberella Zeae* Schwein (Petch). *Gibberella Zeae is* known in its sexual stage. In addition, its common synonym name is *Fusarium graminearum* group 2.

It, however, has a very high host and geographical distribution, but most basic hosts are maize, wheat, and barley as well as other annuals and perennials. It often presents sparsely and pale orange sporodochia and usually fast growing. Macroconidia is relatively slender, sickle-shaped to almost straight. It, usually, has tapered apical cell and distinctly foot-shaped basal cell. Besides, 5-6 septated thicker cell-wall is often seen. There is also a moderately curved to straight with ventral surface straight and the dorsal side smoothly arched (Leslei, J. F. & Summerell, B. 2006). Micro conidia are known to be absent. *F. garmirearum* can be confused with other *F. species*.

But, the morphology of macroconidia and the absence of microconida can differentiate it from many confusable *F. sp.*

Unlike the one above, *Fusarium culmorum* (W. G. Smith) Saccardo, (*F.culmorum*) has no known sexual stage. It is linked to pathogenecity in cereal crowns, and plant residues in soils in temperate areas. It produces relatively rapid growing sporodochia. Sporodochia is pale orange in color but becomes brown to dark brown with age.

The macroconidia is short and stout. It has rounded apical cell and poorly developed foot cell. It, usually, is 3-4 septated. Micro conidia aren't known, so far. The dorsal side is near to be curved, but the ventral side is almost straight.

In contrast to the both above, *Fusarium avenaceum* (Fries) Saccardo (*F. avenaceum)* is an identical to *Gibberella avenacea Cook* . *Gibberella avenacea Cook* is known at its sexual stage. *Fusarium avenaceum* (*F. avenaceum*) is also called as common synonyms name. It occurs as saprophyte in temperate areas. It is also pathogenic to many plant species including cereals such as wheat and barley (John F. Leslei & Brett A. Summerell 2006). Slightly curved to straight needle-like macroconidia are formed on CZID and water agar (WA). Micro conidia are produced sparsely on CZID for some isolates. It moreover, presents variable septation (1-3) and shapes. Sporodochium is a pale-orange in color also in the CZID agar. *F. avenaceum* can grow relative fast or relatively low. Generally, it is long, slender and thin walled. It also has long tapering to a bent apical cell. Basal cell is usually notched, but might be foot-shaped. Chlamydospores are not known to exist in this organism. *F. avenaceum* was not linked to human and toxicoses, but ground cultures reported to be toxic to chicken, mice and rabbit (John F. Leslei & Brett A. Summerell 2006). Yet, *Fusarium equiseti (corda) saccardo* (*F.equiseti*) vary from those above in many aspects. Macroconidia length ranges $25-120\ \mu m$ with a strong dorsiventral curvature. It is 5-7 septated at most often. In addition, distinctly foot shaped basal cell and whip-like or filamentous tapering apical cell is abundant (John F. Leslei & Brett A. Summerell 2006). It belongs to Gibbosum group and has no known microconidia occurrence.

There are a number of plants that *F. equiseti* is reported to be pathogenic, it also causes some human disorders.

1.3 Baking Quality

Baking quality has been investigated to understand the factors that are closely linked to quality modifications in the spring wheat grains. Investigation was also conducted to comprehend the role Deoxynivalenol (DON) content plays on quality alterations in cereal grains. The grain quality is determined by its end-use product. To achieve a standard quality for bread-wheat, analytical methods are developed by AACC among others, such as; Falling Number (FN), 1000 Kernel weight, Protein content in %, and rheological tests. Limits are been set to mycotoxins in most countries. Therefore, grains with higher mycotoxin levels are not, generally, accepted as good quality grains and may even become unfit to human and animal use (Placinta et al. 1999, NorFors-UR ,12. 03. 2010).

F. graminearum was highly associated with high DON accumulation in spring wheat (Xu et al. 2008; NorFors-UR, 12.03.2010). These studies have associated DON to affect negatively to the product end-use flexibility. They further, highlighted the inhibition of protein synthesis due to the presence of these toxic substances in the grain.

Therefore, *Fusarium* infested grains with mycotoxins above a certain threshold level should be avoided, because of quality and health concerns. Although, there are several recent reports been mentioned that *Fusarium* infection and DON can affect negatively on baking quality, yet the biological mechanisms, severity of infestations and DON concentrations which are enough to give an effect is not known. This is the reason why, the area of DON and its correlation with baking quality is still deserves further attention in order to investigate it.

1.3.1 Important Parameters in Wheat Baking Quality

Protein Content

Protein content (%) is the sum of individual protein fractions distributed as; glubolins/albumins and gliadins/glutenins fractions. Protein content required to form a standard bread baking quality should be 10-13% and of hard wheat. This improves dough development time, as well as dough volume. Soft wheat may contain less than 8 % protein for pastry baking. Other general purposes require only 8-10 % protein content. Protein content can be analyzed by near infrared reflectance, NIR Inframatic 9200, Perten Instruments. NIR is spectroscopy method using the near-infrared region of electromagnetic spectrum (800nm-2500)**.** The NIR was used since 1970 by the United States Department of Agriculture and Canadian Grain Commission for protein and moisture analysis for wheat and barley. Since then, worldwide acceptance for NIR was gained during the years by using it to numerous applications in cereal analysis. At present, the NIR analysis is used almost all cereal grain types.

Protein Quality (PQ)

In respect to bread –making quality, higher SDS-sedimentation volume was associated with stronger gluten and sound bread making quality. Gluten is, basically, divided in gliadins and glutenin components. In particular, SDS-sedimentation volume is a result of swelling of glutenin strands (Eckert et al. 1993). In other words, SDS-sedimentation volume is based on the amount of large and insoluble proteins (insoluble in SDS solution). It is also confirmed that SDS-sedimentation volume test is a small-scale test, easy to perform and highly reproducible.

Falling Number (FN)

Falling Number (FN) is based on the ability of α- amylase to liquefy a starch gel. The FN is indirectly measured the enzyme activity and defined it as a time in seconds required to allow a stirrer to fall a measured distance through a hot aqueous flour or meal gel undergoing liquefication AACC (2002).

In wheat, Pre-harvest sprouting (PHS) refers to the germination of grain in the ear. This occurs after physiological maturity of seeds and before harvest.
In addition, different grains within a given ear may present variation in PHS due to 'basal response thresholds to water availability and hormonal signals' (*Seed dormancy* s.n.). In other words, grains in the same ear may absorb different amounts of waters, due to difference in their nodding angels. Therefore, the amount of water imbibed in the grain will, hopefully, reflect as a level of PHS damage.
However, pre- and post harvest sprouting is an overwhelmingly destructive factor in producing low grade quality of wheat (Walker-Simmons & Ried 1993; Warner et al. 2000). Water loss at post harvest is prerequisite demand in wheat handling and management (*Seed dormancy* s.n.). High alpha-α-amylase activity affects decrease in FN values, thereby producing relatively low grade in wheat grain quality, and sometimes unfit to human and animal consumption AACC (2002).

High α-amylase activity is associated with kernel sprouting; both meal and flour are inversely correlated with FN (Gartner et. al. 2008). Higher amylase activity forms sticky bread crumb, lower bread volume and Lower FN value. When the activity is intermediate, firm and soft bread crumb, higher bread volume and reasonable FN value (250-300) is formed. Under conditions when amylase activity is very low, a dry bread crumb with a diminished bread volume and higher FN (ca. over 400) is expected to form.

The α-amylase activity involves both endogenous and exogenous factories like to induce kernel sprouting. Whereby, grains having FN values below 200 are classed as lower class grains according to Norwegian system, hence further graded to fodder class. Other countries have developed similar grading systems. Lower FN grains produce sticky bread undesirable for slicing. It is sticky because of reduced gelatinization of starch.

Retention of gasses influences positively in flour quality. They promote higher volume and softer texture which is a desirable bread quality. It is affected by the gluten quality.

In contrast, grains with high amylase activities are, generally, recognized as having breads with lower volumes, wet and sticky bread crumb due weak gluten quality.

Therefore, FN is the scale used to determine amylase activity in grains. Grains having lower FN values have higher α-amylase activity and poor bread-making quality. Pre- and postharvest wet conditions can cause lower FN in grains (Gartner et. al. 2008). This is the intention underlying FN to be expressed under the list of major parameters in wheat baking quality.

SDS- Sedimentation Volume

In considering baking quality, protein quality is trustworthy parameter to investigate.

Protein quality/gluten quality is based on the glutenins and gliadins protein composition (Bushuk 1998b; Shewry et al. 1999; Weegels et al., 1997; Kuktaite et.al., 2004).

The balance of these two major groups determines the wheat flour suitability for different products. Unique balance of gliadins and glutenins provides an optimal quality in the grain end-use product. Protein composition in wheat is derived from the relative proportions of; protein content, the ratio of polymeric to monomeric protein content, the ratio of HMW to LMW glutenin subunits and the proportion of x- & y-type HMW glutenin subunits (Hoseney, 1986; Lawrence et al. 1988; Uthayakumaran et al., 1999, 2000). Glutenin consists of approximately 20 HMW-GS (high molecular weight gluten subunits) and 80% LMW- GS (lower-molecular-weight gluten subunits). It is responsible for dough firmness in bread baking. Both gliadins and glutinins form gluten together. Wheat grains are categorized in to various classes in the market. Classes are distinguished by kernel hardness, bran color, grains morphology and growth habit. Soft white grains are commonly used for cookies, pastries, cakes and flat breads. Such grains are often accurately predicted to have high flour extraction rates, weak glutens and lower protein concentrations. Variation in bread-making quality of wheat depends on gluten characteristics. Therefore, sodium dodcylsulfate (SDS)-sedimentation volume is a universal accepted method to determine gluten. It can affect dramatic variation in wheat protein % (Orth and Bushuk 1972; Marchylo et al. 1989; Gupta et al. 1993a; MacRitchie and Gupta 1993). Unlike to soft white wheat, hard

wheat presents higher protein concentration, and stronger gluten, among other things. Therefore, SDS sedimentation volume can be a good tool to predict weakness and strength of the gluten, as well as baking quality.

Quality Variations between Varieties

An extremely large number of studies have so far investigated the variations in baking quality which is linked with varietal variations. Most of these had sufficiently substantiated the variations in bread-making quality is partly related with the variations in wheat varieties (Schofield 1994; Dreere et.al, 1988; Lawrence et al. 1988; Uthayakumaran et al. 2001; Butow et al. 2003a, 2003b; Juhasz et al. 2003). For instance, Schofield 1994 had tested two winter wheat cultivars, Hereward and Riband, and to their varietal gluten variations. The study demonstrated that a Hereward cultivar has showed better rheological properties than Riband. It confirmed inter-cultivar differences in viscoelasticity of the glutenins. The study of Dreere et al., 1988 among those mentioned above who have tested varietal gluten variations had showed variations in bread-making quality based on variety differences. However, all above studies have investigated and revealed the difference in wheat protein composition in order to account for dough strength and extensibility. Dough properties mainly vary due to variation in protein content, the ratio of polymeric to monomeric proteins, the ratio of HMW to LMW glutenin subunits and the relative proportion of x- and y-type HMW glutenin subunits.

These variations may significantly be contributed to partly by varietal based gluten variation in determining their gluten properties and the ability of gluten to perform the viscoelastic network. Higher protein content and good balance between these different components is necessary for the bread-making quality in order to satisfy quality related criteria.

The quality reduction affected by Temperature and Water Stress

Proteins composition can, significantly, be modified by certain environmental factors. These factors make-up the composition of gluten properties of wheat and its nature in dough formation. For instance, short period of higher temperature (over 35 ° c) during filling time alters gluten composition and reduces molecular weight distribution by interfering disulfide inter-chain formations, as to weakening it (Lafiander et al., 1999) which are extremely important in determining rheology properties. This process favors the gliadins fraction and produce weaker dough properties. Interestingly, higher temperature can yet promote bread-quality. Extended and gradual increase pattern in temperature prolongs the rate of filling. This prolonged rate of filling is favorable for better quality. Mature kernel needs high temperature to enhance good and stronger dough quality (Wrigley and Bekes 1999). Adequate temperature is necessary, particularly, at storage time. However, very high temperature alters the composition and gives weaker dough properties. Nevertheless, temperature range between 20 -35 might be the relevant temperature range in grain filling period as stated by (Wrigley and Bekes 1999). Moreover, a combination of temperature, drought stress and Nitrogen application (primary and secondary) might cause significant variation in protein fraction in the grain at post-anthesis. The study of Wrigley and Bekes (1999) revealed decrease in the fraction of albumins-globulins coefficients and increase in the fraction of storage proteins (gliadins and glutens). Gooding et. al. (2003) examined the effects of timing and severity of the drought and increased temperature on grain development in controlled environment in winter wheat (Hereward). They tested the protein content, FN, SDS, grain specific weight among other things and their potential influence by temperature and drought stress. Results revealed significant FN increase by both stress and temperature except wet and raining conditions. The temperature (over 35 °C) and drought stress increase applied before the end of grain filling period showed negative effect on the ratio of glutenin to total grain protein. However, SDS-sedimentation volume was not affected by temperature, but affected by drought stress.

On the other hand, a research in winter wheat quality conducted by (Graybosch et al. 1995, Blumenthal et al. 1993) demonstrated negative correlation between SDS sedimentation volume and stress conditions, specially increased temperature (> 32° C) in more than 90 hours and reduced RH (40%) in kernel filling period. The correlation was explained because of gliadins proteins fraction increased at the loss of glutenin proteins proportions, at an extended time spans. Furthermore, SDS-sedimentation volume was positively associated with dough strength according to mixogragh resistance and good volume loafs.

However, the increase of temperature up to certain degree has been associated to reduction in wheat quality. Although this is well documented elsewhere, but not seen in Norway and Scandinavia as a whole.

Quality changes due to Fertilizer Type and Timing

Shewry et al. 1997, 1999, Zhao et al., 1999a among other numerous studies associated quality variation in wheat grains with certain management practices. Among them was recently greenhouse pot experiment in Norway (Flaete et al. 2005) on wheat baking quality. This study had clearly documented that optimization of management strategies can improve wheat grain quality. Although, not comparable to field experiment, it revealed deficiencies in certain fertilizer inputs that had prevalent influence on bread-quality. Most notably Nitrogen and Sulfur (S) proportions and their timing play an important role in bread-making quality. Depending on the type and differences in glutenin subunits (GS) in (HMWGS) and glutenin subunits (LMWGS), meal flours can produce viscoelastic dough with sound texture loaves and large volume. (Flaete et al. 2005) confirmed that primary fertilization enhances mainly yield by producing high number of grains/ears. The study recommended that, further, split fertilization in spring wheat to insure quality and proper filling of kernels at the time of maturity. In respect to that, protein fractions in wheat depends mainly on the N-quantity per grain accumulated during grain-filling (Triboi et al. 2003).

On the other hand, S-and N status do shift gluten fractions. This may be due to several reasons; one of which might be wheat crop intensification. This intensification may increase the use of N application without increasing S-fertilizer. Although N is well known to increases protein content (%). This increase doesn't necessarily insure proper balance between gliadins/glutens (Zhao et al. 1999). Due to consequences of the fertilizer type, depletion of S rich-proteins is more often intervened, where off high rate of N alters protein composition towards higher proportions of gliadins in respect to glutenins (Castle & Randall 1987; Zhao et al. 1999).

However, insoluble amount of glutenins are considered to be responsible for the alteration of loaf-volume (Sapirstein & Fu 1998).

Despite its positive influence, interaction of high N may affect severe *Fusarium* infestations in cereals and easily be exposed to lodging.

1.3.2 Baking Quality (BQ) and Rheology Alteration Due to *Fusarium* Infection

Alterations in baking quality caused by *Fusarium* damaged kernels (FDK) had often been a major concern in grain quality. Profound discussions considered mainly; shriveled kernel and their modification of protein quality, DON content and rheological alterations in response to *Fusarium* infection.

Shriveled Grains

Shriveled grains are smaller head scabbed infected grains that are leading to both serious loss of yield and degradation of grain quality (McMullen et al., 2008). They produce what is known "shrunken kernel". These deformed kernels (misshapen kernels) are also often known "tombstone kernels". They are indicative as poorly filled kernels due to fungal infection and classed them as lower grade grains (Gartner et al.

2008; Jones & Mirocha 1999), causing lower flour extraction. These kernels induce alteration in several components in the kernel; e. g. starch, cellulose, hemicelluloses, protein quality and test weight of grains (Boyacioglu & Hettiarachchy 1995; Gartner et al. 2008)

However, fungal protease degrades starch as an α-amylase is activated in FHB infected kernels (Gartner et al. 2008). High level of α-amylase activity in grains leads to lower FN value; thereby resulting grains discriminated as lower quality grains. Although, they are poor in their rheological properties, but are not necessarily producing lower protein %. In some cases, slightly increase in protein by shrunken kernels had been observed by (Dexter et al. 1996; Gartner et al. 2008). Boyacioglu & Hettiarachchy (1995) obtained similar conclusion as above one, in which an increase was observed in protein % (content).

Deleterious effects on Gluten Quality Caused by DON and Proteases from *Fusarium species*

Deleterious effects of *Fusarium species* on gluten quality has been addressed in this chapter. How gluten quality can be modified under severe *Fusarium* infection will be of particular concern and in bread making quality as a whole.

It is widely held view that DON and proteases from *Fusarium species* do cause changes in gluten proteins. It will, therefore, be reviewed in this topic how these factors interact with rheological characteristics and baking quality in general.

Biotic factors attacking grains produce proteases (Sivri et al. 1999). These proteases play an important role for the modification of gluten composition by interfering polymerization degree of gluten formation.

Moreover, prevalent reduction had been observed on protein quality and bread making properties (Wang et al. 2005b) in winter wheat (*Triticum aestivum* L). However, protease activity found in the flour was negatively correlated with SDS-sedimentation volume. Furthermore, proteases activity was positively correlated with both free amino

acids and the degree of the infection. Distinct reduction had been observed on both high molecular weight glutenin subunits and total glutenin. The infection diminished the dough quality leading to a deformed loaf shape.

Similarly, significant change has been confirmed in glutenins fraction when (Eggert et al. 2010) investigated in wheat (*Triticum aestivum L*) and emmer (*Triticum dicoccum)* inoculated with *F. culmorum* and *F. graminearum.* Eggert et al. (2010) stated that 'The trichothecene mycotoxins are potential inhibitors for protein biosynthesis owing to a strong immunosuppressive effect'. Highly mycotoxin concentrated food and feeds reduce uptake, increase vomiting and immunosuppresion in animal and humans.

It demonstrated further that the amount of total gluten protein didn't changed, whereas gliadins/ glutenins ratio shifted remarkably in favoring to gliadins. These changes affected severely in wheat gluten quality due to alterations occur in balance of the gliadins and glutenins. The changes in the glutenins synthesis at a later grain development stages could be claimed due to reduction in protein synthesis as result of *Fusarium* infection. It, in addition, explained that high N-supply showed significant effect in shifting gliadins/glutenins ratio by enhancing expression of certain genes and depression of others. This reduction is recognized as detrimental effects in wheat grains and physical dough properties , as well as bread-making characteristics creating weaker dough properties (Nightingale et al., 1999; Dexter et al. 1996). However, in many aspects, severe *Fusarium* infection does effect deleteriously on grain quality. It, obviously, does cause poor bread making quality and limited end-use product. Some of the basic reasons for changes can be of alterations in glutenin/gliadin balance, there by resulting weaker dough, reduced bread volume, etc (Kieffer et. al. 1998; Belitz et. al. 1986; Abang Zaidel et. al. 2007)

2.1 Experimental Hypotheses

Fusarium infestations in spring wheat (*Triticum aestivum*) have become more severe in Norway. Grains heavily infected by *Fusarium* infestations may develop higher mycotoxin content and DON in particular.

First part of the claim of the experimental research alternative hypotheses is; do grains severely infected by *Fusarium species* affect reduction in baking quality?

Second part of the claim of the experimental hypotheses is that; are grains with higher DON content can be associated to poor baking quality?

However, establishing null Hypothesis $H_{0,}$ as an opposite one, can be stated as this; grains with higher levels of DON content can't be associated to poor baking quality.

There are, additional, relevant questions being conducted as an approach for testing the hypothesis such as;

What are (is) the common *Fusarium* species involved in infestations in the field trial under investigation?

Which *Fusarium* species dominated at what stages in wheat development?

Does the quality changes with the stage of grain development after anthesis?

However, validity of either two of the initial questions need to be substantiated with data obtained from the research.

2.2 Experimental Aim

This part of the work aims to investigate *Fusarium* species that affects Norwegian spring wheat at different developmental stages and their influence in grain quality alterations. It is expected that grains having higher *Fusarium* infestation rates might cause quality alteration. On the other hand, mycotoxin content in grains and its potential association with baking quality will be examined in the study.

Therefore, our goal of this experiment is to verify and produce adequate evidence as a form of data to validate or invalidate a test of the hypothesis.

3. 0 Wheat Materials (1 and 2) and Methods

3.1.0 Wheat Material 1:

The wheat materials used in this experiment were collected from a field trial laid out as randomized block design with two replicates. Plot size was 1.5 m *6 m. 7 (Seven) different treatments harvested at different developmental stages after anthesis, were randomized within each replicate. The trial was conducted at Vollebekk Experimental Farm, at the Department of Plant and Environmental Sciences, Norwegian University of Life sciences at Ås, Norway in 2009. The cultivar used was “Bjarne” NK 97520, which was released by Graminor AS in 2002. The first harvesting time was 10 days after anthesis (DAA), and thereafter in 5 intervals until yellow ripeness. Thus, harvesting times were 10 DAA, 15 DAA, 20 DAA, 25 DAA, 30 DAA, 35 DAA and 40 DAA. The block design and the harvesting times are shown in table1.

Table 1: Block design for the different treatments of wheat material 1

Replicate I	DAA	Replicate II	DAA
101	25	202	35
103	30	203	20
104	20	204	40
105	35	205	30
106	40	206	15
107	10	207	25
108	15	208	10

3.1.1 Field Information

The materials were sown on 29, April, 2009. Furthermore, heads emerged on the 27th of June for all plots. In addition, flowering date was 30th of June.

Although all plots were sown at the same time, but they were harvested on the following dates; 09.07.09, 14.07.09, 19.07.09, 24.07. 09, 29.07.09, 03.08.10, and finally 08.08. 09.

3.1.2 Harvesting, Drying, Threshing, and Cleaning

The material 2 were harvested by hand (the ears) using a sickle. After the harvest, the ears were immediately dried at 25 C, threshed and cleaned. And then the grain samples were sent to Bioforsk Plantehelse. At this point, grain (seed) samples were packed in carton-paper bags and stored at room temperature (25 °C) in greenhouse facility for Bioforsk Plantehelse at Kjirkejorda for storage.

3. 2.0 Wheat Material 2

The seed samples were collected by Bioforsk Plantehelse, during the years 2006, 2007 and 2008 from farmers' fields at different locations in Hedmark County. The cultivar used here is Bjarne as referred under material 1(3.1). For the first selection, 44 seed samples grown at various locations across Southern to the middle of Norway with varying DON content were selected and accessed from Bioforsk plantehelse. The selection based criteria to choose from among numerous seed lots were their level of DON content as a lower, medium and high DON content samples. This approach appeared not to be an appropriate fit, owing a great variability in the sample population due to vast extended geographical locations throughout South and the middle of Norway. But then, the second selection criteria were to choose a set of data, presumably drown from different seed populations grown in different years at one location. Among the locations, Roverud was selected due to having the largest sample size. Therefore, seed lots (N=21) used in this investigation were selected to see if DON content in spring wheat can affect baking quality. These seed lots were collected

from various locations nearby Roverud village. Wheat materials are assumed to be equal around the station, though they differ due to micro-habitats. Similar treatments were applied to the selected wheat materials every year except possible variations related to yearly weather changes at a site-like Roverud (any site). Finally, 3th selection was done for further study of gluten quality by Kieffier test. Samples used in the Kieffier test were wheat samples from Roverud-2006. Roverud locates, Solør-Odal County, on the longitude and latitude (60.25254, 12.088158), Kongsvinger municipality, Hedmark County, Norway.

Table 2: Samples Analyzed from wheat material 2, for DON correlated with baking quality. The materials are grown (2006, 2007 and 2008) throughout south and the middle of Norway (N=44).

years	samples	DON	Locations
2006	6	90	Moelv
2006	14	410	Ilseng
2006	15	330	Ilseng
2006	16	200	Ilseng
2006	17	0	Kise
2006	18	90	Kise
2006	23	7400	Heradsbygd
2006	30	3700	Roverud
2006	33	4500	Roverud
2006	41	90	Roverud
2006	48	370	Rygge

2006	112	2400	Øsaker
2007	733	450	Ramnes
2007	737	390	Ilseng
2006	738	90	Ilseng
2007	740	90	Ilseng
2007	744	90	Moelv
2007	745	90	Moelv
2007	794	1800	Roverud
2007	795	8700	Roverud
2007	797	2500	Roverud
2007	802	320	Roverud
2007	803	16000	Åsnes
2007	804	2000	Roverud
2007	805	1600	Roverud
2007	806	2000	Åsnes
2007	851	220	Rakkestad
2007	1012	90	Kise
2008	1205	0	Tjølling
2008	1295	100	Gjennestad
2008	1296	1200	Tjølling
2008	1206	1206	Tjølling
2008	1233	460	Heradsbygd
2008	1236	1200	Roverud
2008	1238	950	Roverud
2008	1241	380	Roverud
2008	1248	100	Roverud

2008	1249	1800	Roverud
2008	1250	610	Roverud
2008	1293	430	Svelvik
2008	1297	750	Ramnes
2008	1299	120	Ramnes
2008	1300	560	Roverud
2008	1313	200	Tjølling

Table 3: Overview of wheat material 2 grown at Roverud (3.2.0) for studying relationship between DON and baking quality.

Sample Identity	Year of Production	Sowing Date	Harvesting Date	
25	2006	08.mai	25.aug	
31	2006	08.mai	25.aug	
33	2006	08.mai	25.aug	
34	2006	07.mai	24.aug	
41	2006	08.mai	24.aug	
122	2006	09.mai	05.sep	
794	2007	27.apr	22.aug	
797	2007	24.apr	25.aug	

802	2007	21.apr	26.aug
804	2007	18.apr	23.aug
805	2007	29.apr	05.sep
807	2007	02.mai	13.sep
924	2007	02.mai	13.sep
1236	2008	05.mai	03.sep
1238	2008	06.mai	05.sep
1240	2008	08.mai	02.sep
1241	2008	03.mai	30.sep
1248	2008	05.mai	28.aug
1249	2008	07.mai	12.sep
1300	2008	13.mai	28.sep
1301	2008	13.mai	28.sep

3.2.1 Harvesting, Threshing, Drying and Cleaning

The material 2 are harvested by plot harvester. After harvest, materials were packed in carton-paper bags (sacs) and dried at room temperature (25 ° C) in drying chamber until moisture content reached below 15%. In addition, they were threshed and cleaned by hand.

Seed lots were further packed with carton-paper bags and stored in room temperature (25 ^{0} C) at Kjirkejorda, Ås.

3.3 Methods of Baking Quality Analysis

Baking quality parameters were determined in Vollebekk Experimental Farm Laboratory, UMB, ÅS, Norway. Rheological tests were also done by Nofima, Ås, Norway. Grain milling to whole meal flour, 1000 kernel weight (TKW), protein content in %, SDS sedimentation volume, falling number and rheological test were determined according to methods approved and adopted in Norway.

Grain Weight

NumiGral solid stat 1800 was used as a seed counter apparatus to count the number of grains in 1000 kernels to find out their weight in g, so-called 1000 kernel weight in grams. This apparatus had counted approximately 200 kernels that were weighed. The weight of kernels were multiplied by 1000/ the number of counted kernels. Through this simple calculation TKW is determined.

Grain Milling

Laboratory Milling Falling Number 3100

No table of figures entries found., AB; Sweden, using a 0,5 mm sieve is used for milling .

Protein Content

Milled flour was used to analyze the protein content in percent (%) for both materials 1 and 2. The protein was analyzed using NIR by Inframatic 9200 (Perten Instrument, AB, Sweden). The protein content is expressed on dry weight bases. Whole-meal flour analyses are using 25 ml cuvette and calibration made by Perten Instruments.

Falling Number

Falling Number was analyzed by ICC standard method 107/1(56-70, AACC 1995), by using Falling Number 1700 (Perten Instruments, AB, Huddinge, Sweden).

Two parallels of 7 g whole meal flour were weighed in two different 100 ml glass tubes. However, weight of whole –meal flour is adjusted according to the moisture in the flour.

25 ml of distilled water was added to the flour in each cylinder. The content (25 ml dist and 7 gr. Flour) was suspended together by hand before loaded it to falling number apparatus. Then the tube is placed on boiling water. In 60 seconds, the viscometer-stirrer does fall a prescribed distance through the gelatinized dispersion, including stirring time.

FN value depends on amylase activity in the suspension and calculated it by transforming it to Perten Liquefaction Number (PLN) (PLN = 6000/(FN – 50). PLN is nearly linear with the amylase activity. The method was adapted by AACC (1995).

SDS- Sedimentation volume

The AACC method (AACC, 2002) was used to determine SDS- sedimentation volume. 6 g of whole meal flour was weighed into 100 ml SDS-sedimentation test cylinder and added with 50 ml water containing bromophenol-blue (4 mg l^{-1}). The materials in the cylinder were shaken in some seconds by hand up and down to insure materials are fully suspended before mounted them to the shaker. The content was shaken with laboratory shaker in 5 minutes, Chemie-Glass-Technik, H.W. Fisher and K.G. Bielefeld, Laboratoriumsgeräte. Then, 50 ml SDS solution containing lactic acid was added to the suspension in the cylinder and placed on the shaker for 5 more minutes. Finally, the whole suspension was set to a stable bench for sedimentation for 15 minutes. Sedimentation volume was thereby recorded.

Kieffer Method

Kieffer resistance and extensibility were analyzed by Nofima, Ås, Norway**.** Extensograph test was performed with the Stable Micro Systems (SMS)/Kieffer Dough and Gluten Extensibility. Rig (Kieffer et al. 1998) for the TAXTplus Texture Analyzer (Stable Micro Systems, Godalming, UK). Gluten dough were prepared in a

Glutamate 2100 (Perten, Sweden) from whole meal flour. A 2% NaCl solution was used for mixing the dough and washing out the salt-soluble components.

3.4.0 Methods of Analysis for Fusarium Infestations and Fusarium Species Identification of Wheat Materials 1(3.1.0), and Analysis of Mycotoxin Content for Wheat Material 2 (3.2.0)

3.4.1 Determinations of Fusarium infestation in wheat grains (3.1.0)

Wheat seeds from the 14 lots described under material 1 (3.1) were placed evenly on Czapeks Iprodione Dicloran (CZID) in 9cm Petri dishes, using 10 plates per seed lot, with 10 seeds on each plate on the 29.01.2010.

The plates were randomized like; 1, 2, 3…140 in order to conceal their identity.

The plates were then packed with plastic bags (8 plates per bag) and moved to a room with controlled environment for incubation. The room temperature was set at 22 ^{0}C, but may vary +/- 1 °C due to light and dark effect. Light conditions were 12 hours white light plus near ultraviolet light (UVA), and 12 hours dark. The plates were shifted each day between the upper and lower bench. The seeds were incubated for 10 days. Additional grains from seed lots 13 and 14 both harvested at 40 DAA were surface sterilized before the incubation. The seeds were immersed in 70 % ethanol for 1 minute, rinsed twice with sterile water and dried between two layers of sterile filter paper. Sterilized seeds incubated under the same conditions as non-sterilized ones.

At the end of the 10 days 0f incubation, *Fusarium* infestations were determined using a Leica “Aristoplan” compound microscope at 100X 250X magnification.

Table 4: The Czapeks Iprodione Dicloran medium **(**CZID) (Thrane 1996)

Chemical	content
KH2PO4	1 g
	0.5 g
	0.5 g
	20 g
	0.2 ml
	To 1 L

3.4.2 Mycotoxin Analysis

The mycotoxin kernel concentrations were analyzed by Evira, Finland.

Evira, uses liquid chromatography-mass spectrometery (LC-MS/MS) for the determination of several mycotoxin groups in the food and feed cereals. The method is newly used multimethod.

Deoxynivalenol (DON) limits of detection (LOD) and limits of quantification (LOQ) is considered to be LOD $= 45\ \mu\frac{g}{kg}$ and LOQ = 90 μ g/ kg, Evira (2008).

3.5.0 Weather Conditions During Grain Filling Period for material 1 and 2

3.5 .1 Temperature, Precipitation and Relative Humidity under Wheat Material 1(3.1.0)

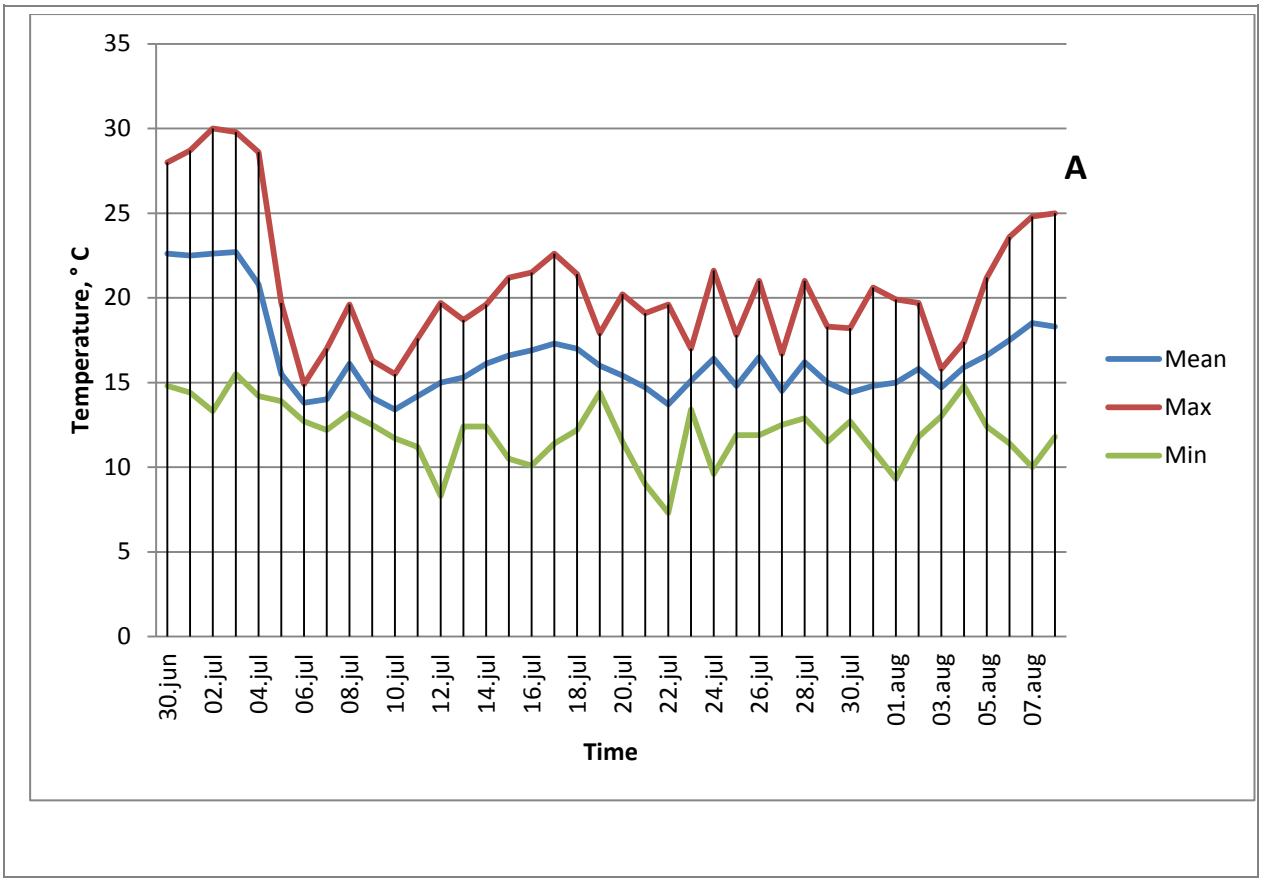
The weather data has been obtained from Bioforsk Meteorology Service.

www.lmt.bioforsk.no/lmt/index.php?weatherstation=5&loginterval=1&tid=12777836

The temperature data for Ås (average, maximum, and minimum) during grain filling for material 1 is shown in figure 3A. Accumulated day-decrees at different harvesting times are also shown in figure 3B.

The temperature data on figure (3) indicated the time window by which grain filling had been occurred. Mean temperature was 23 ºC around 30.06.-03.07, decreasing later to18.5 º C and followed around that mean until physiological maturity was near to ceases or even ceased. However, the temperature fluctuated between18 to 16 º C from 7th days after anthesis until kernel maturity.

Relative Humidity % (RH) was shown on figure 3A. It was around 45% at flowering and increased to 60% at 5th days after anthesis (5 DAA). Further increase of the RH up to 90% continued on the following days (10 DAA).



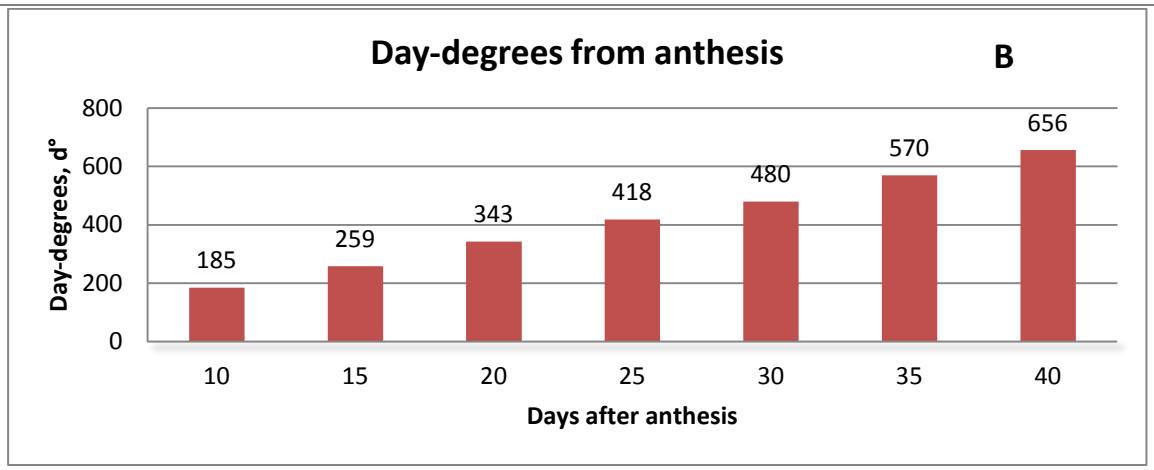


Figure 3: Temperature during the grain filling period (A) top, accumulated day-degrees from flowering to physiological maturity (B), below.

Anthesis initiated around at around 30.06 .10. At that day it was happen to be a dry and lower RH. Since then the RH start to raise and become stagnated at 80% at maturity (40 DAA).

Precipitation pattern during the grain filling periods was also shown on figure 3B.

This figure showed frequent rain from early July through grain period (40 DAA). Some events with heavy rain in the later period of grain filling were also shown.

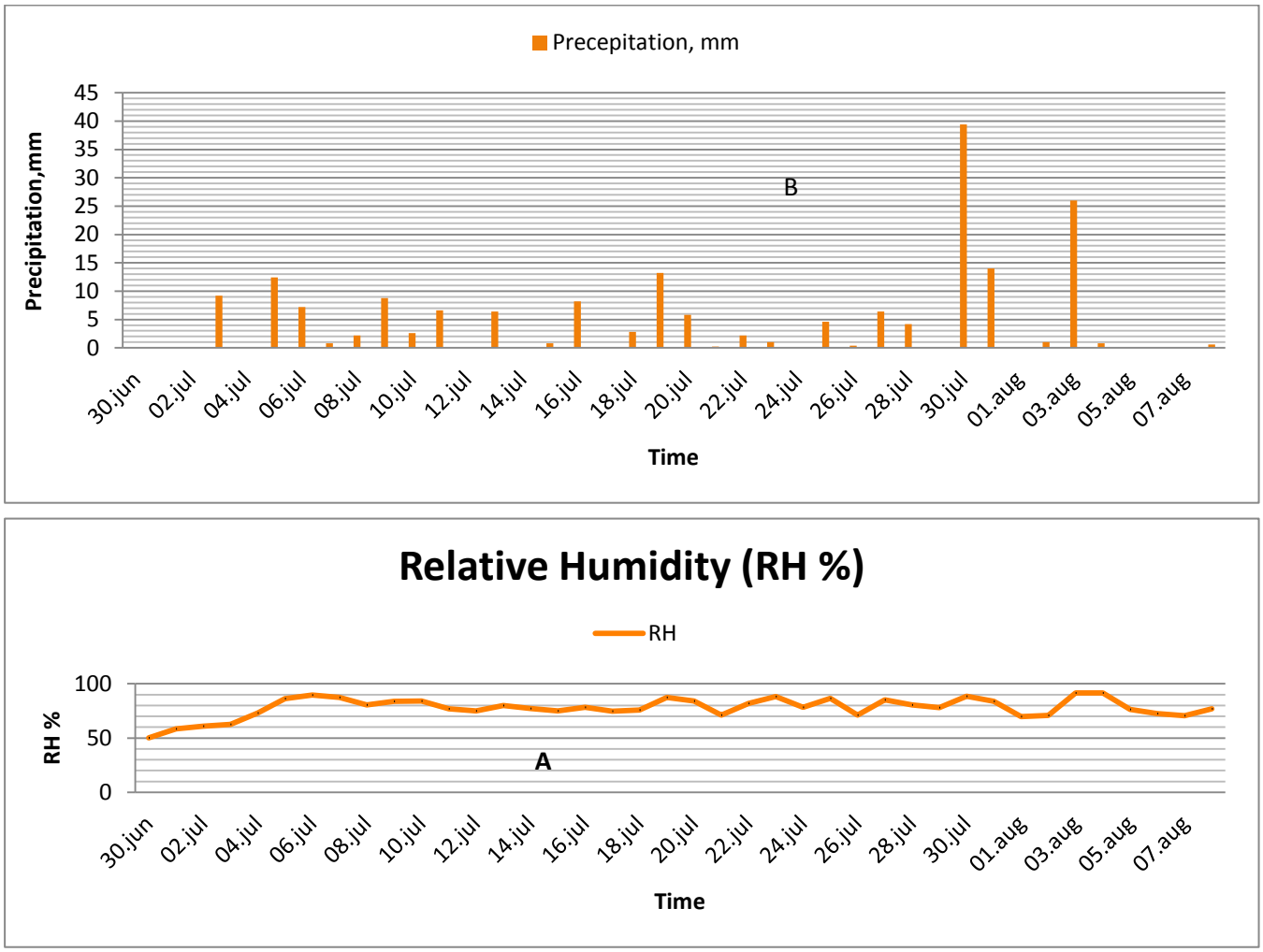


Figure 4: Relative Humidity, % (A) left, and Precipitation (B) right, during kernel filling period (from flowering to maturity).

3.5.2 Temperature, Precipitation and Relative Humidity under Wheat Material 2 (3.2.0)

The weather data for Roverud in 2006, 2007 and 2008 (temperature, rainfall and RH) during grain filling for material 2 has been illustrated on figures 5, 6 and 7.

The data was accessed from Bioforsk Meteorology Service through personal Communications. It illustrated the weekly mean values during the grain development.

Figure 5 is an illustration of the weekly mean temperature during grain filling period in 2006, 2007, 2008.

The figure showed slight increase in temperature from the first flowering week by 16 and 17 °C to 20 °C on 3rd week in 2006. Then, it turned to decrease to 17 °C on the 3rd week of anthesis. The temperature data in 2007 appeared to be somewhat stable at15-17 °C during the grain filling period. However, the temperature data in 2008 didn`t

Seemed stable as 2007, but showed relative increase on 2nd week from below 15 to 20°c 0n 3rd week and turned to decrease to below 13 °c.

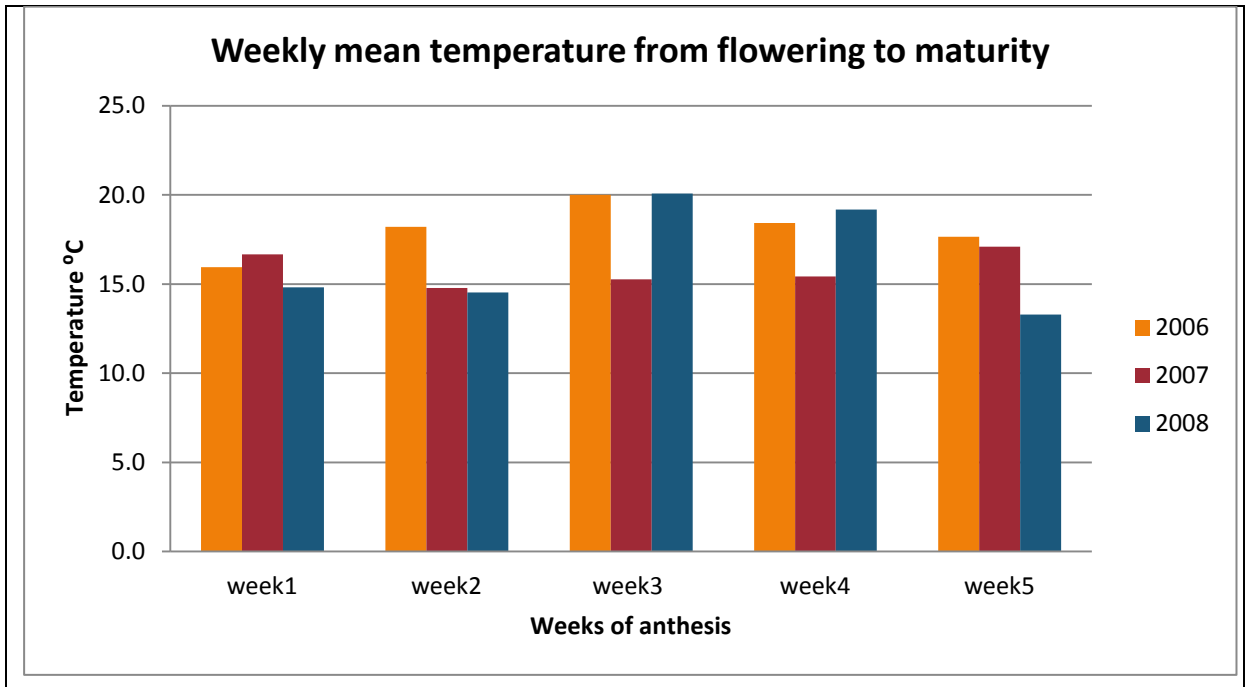


Figure 5: Weekly mean temperature during anthesis to physiological maturity coded as week1 to week5 for material 2 (3.2.0)

Similarly, relative humidity (RH %) had been shown in figure 6. RH % in 2006 tended to be all time lower during grain filling compared to the other years and was consistent to 69%. The RH % was also stable and high in 2007 at all 5 weeks of grain physiological maturity and was close to 80%. Unlike other years, 2008 had markedly been associated with RH % irregularity as it rises and falls from 70 to 91%.

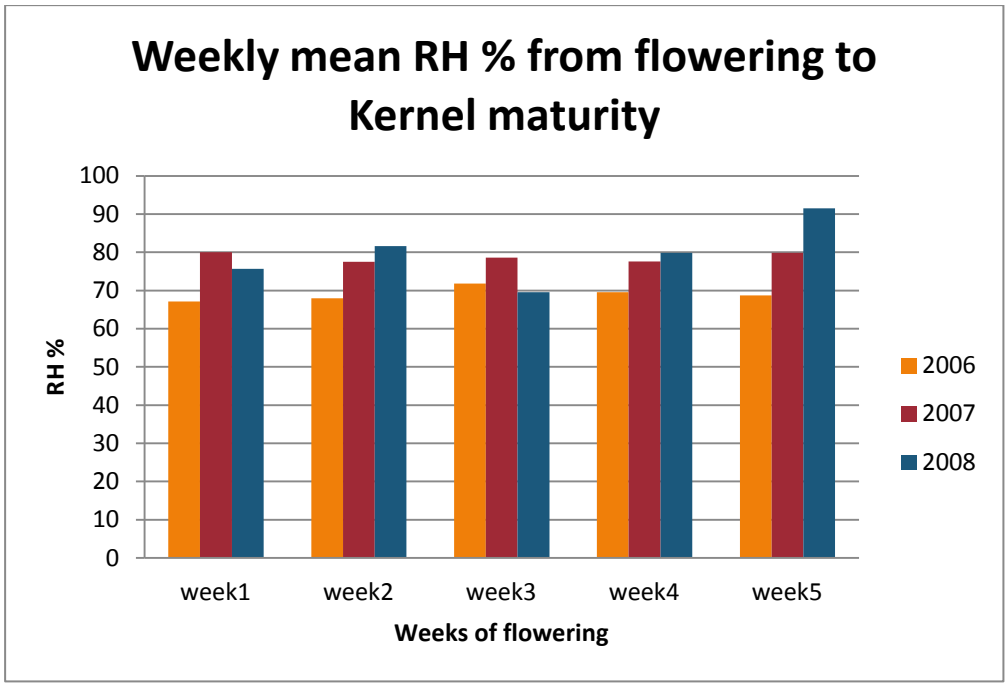


Figure 6: Weekly mean relative humidity (RH %) from anthesis to kernel maturity denoted as week1 to week5 for the material 2 (3.2.0)

The precipitation (figure7) revealed decreases from week1 of anthesis for all years. Unlike week1, week2 after anthesis displayed variably increase in precipitation values recorded in 2007and 2008, but not 2006. 2006 was, however, gradually and constantly decreasing to a lowest precipitation level during grain filling compared to other years. Initially, 2008 had the lowest (22 mm) precipitation level for all years on week1 but it presented increasing pattern up until 4th week (anthesis) when it peaked at highest (35 mm). Precipitation had fallen to below 20 mm at harvest in the 5th week of anthesis.

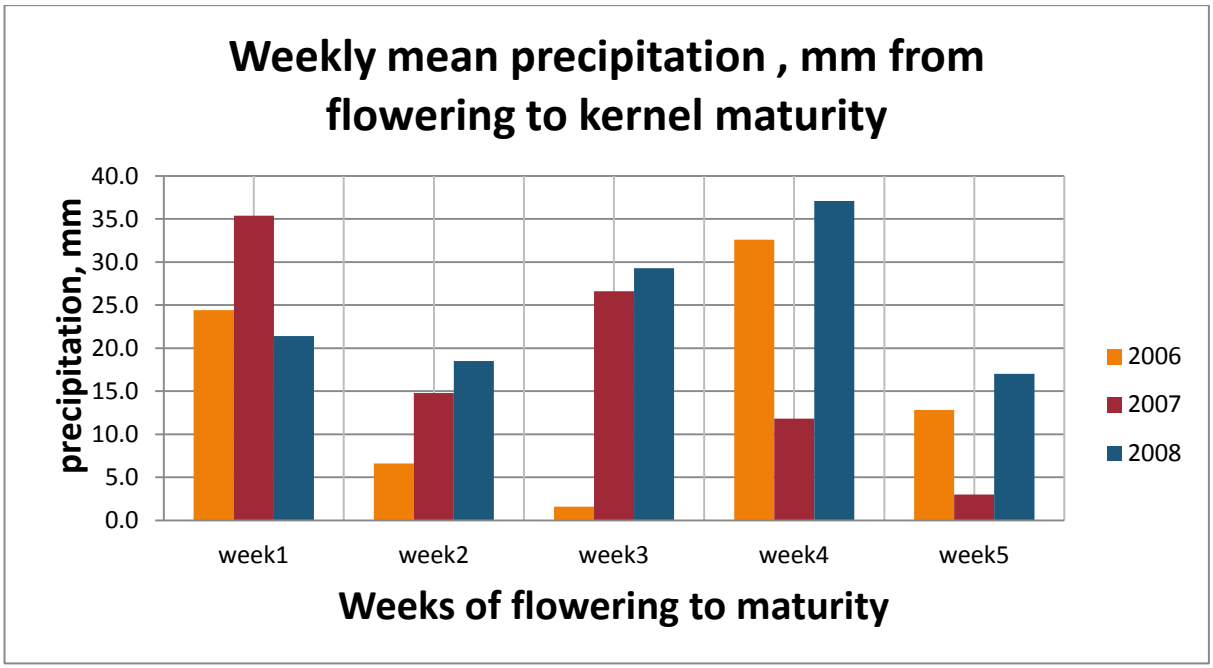


Figure 7: Weekly mean precipitation, mm, from flowering to kernel maturity at week5 denoted as week1 to week5 (3.2.0).

3. 6.0 Methods of Grain Mycotoxin Content and the Correlated with Baking Quality in Wheat Materials 2 (3.2.0)

In order to determine correlation between DON content in grains and baking quality, these parameters were determined; 1000 kernel weight (TKW), protein % (Pc), SDS-test , falling number (FN) and Kieffer.

The equipments and methods used in this section are described earlier under chapters (3.3). Furthermore, mycotoxins concentrations in grains using liquid chromatography-mass spectrometery (LC-MS/MS) was also performed both as indicated below (3.6.1).

3.7.0 Analysis of Data

The analysis of the data in the *Fusarium* infested grains and baking quality analysis obtained from material nr.1 were done with using Microsoft Office Excel 2007. In addition, ANOVA was performed by Minitab 15. 95 % significance was tested in sections (3.4.1 and 3.3). However, analysis of data drawn from wheat material 2

(3.2.0) and the correlation analysis of grains mycotoxin concentrations with baking quality were used with statistical software Minitab 15. Particularly, analysis of variances (ANOVA) and regression analysis were run. R-sq in %, and p-value were tested.

4.0 RESULTS

4.1 Results of Material 1(3.1.0)

There are numerous factors affecting grain quality alterations in spring wheat (*Triticum aestivum*). As a result of the alterations or modifications baking quality alterations are among those remarkably affected by changes. Could alterations be a partly associated to certain stages (DAA) of grain development? Does grain quality develop parallel as development progresses? These are the common questions of interest. Therefore, following tests were performed here, as of the initial hypotheses of this study and partly because it might cover up questions concerning baking quality formations in spring wheat as whole.

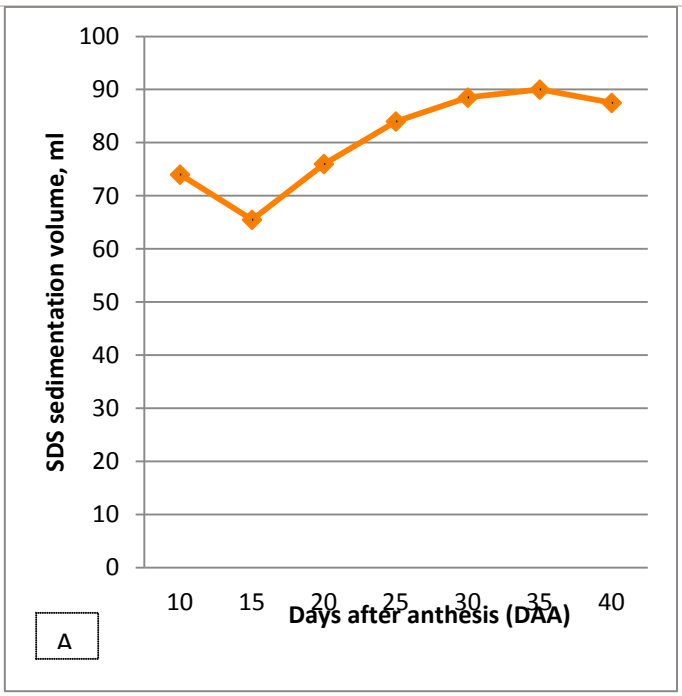
4.1.1 SDS-Sedimentation volume and Protein Content (%)

Figure 8 showed the result of the protein content (%) (right) and SDS-sedimentation volume (left). The result is obtained from grains harvested at different days after anthesis (DAA). It showed initial decrease for SDS (8A) followed by systematic and linear increase with time until it ceased at 25 DAA for Bjarne cultivar until physiological maturity ceased. We assume increase in linear fashion. The correlation coefficient expresses the linear relationship between SDS-sedimentation volume and DAA (SDS = 62, 57 + 0, 7286 DAA)

Minitab 15 output revealed positively significant (P=0,013) increase in SDS-volume correlated with DAA and the R-Sq= 74, 0 % indicating good model regarding the data set.

Protein content (%) was high at early developmental stages (8B). But, systematic significant (P=0,023) linear reduction has been observed with the time in days after

anthesis (DAA) until kernel maturity has been ceased in protein content at 95% to certain point at 25 DAA. The correlation coefficient gives a measure of the strength of the linear relationship between protein reduction and time in days after anthesis (protein = 19,07 - 0,2054 DAA) showing a perfect model (R-Sq= 67,89%).



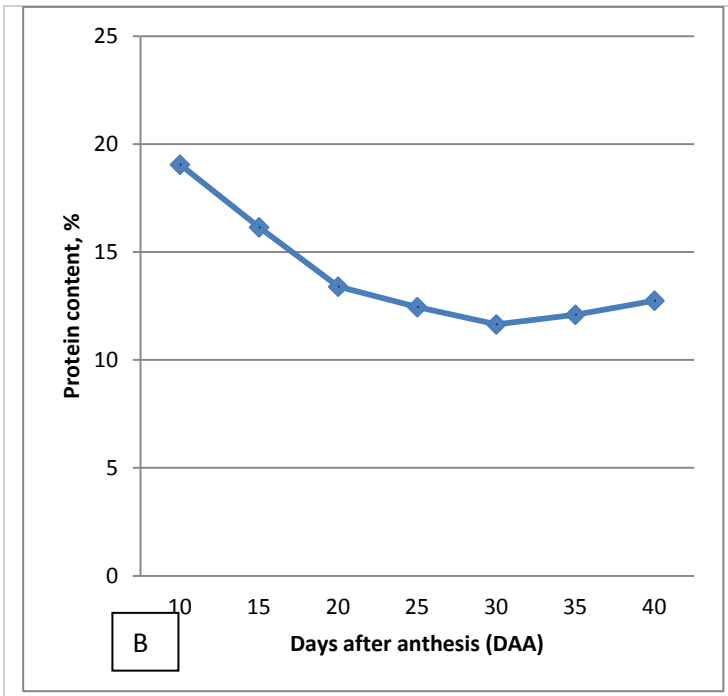


Figure 8: SDS-sedimention volume for different developmental stages (DAA) (A), protein content**(B)** for various developmental stages. Grains were harvested each 5 days after anthesis (DAA), starting from 10 DAA to 40 DAA. The regression equation is SDS = 62,57 + 0,7286 DAA and the regression equation is
protein = 19,07 - 0,2054 DAA

4.1.2 1000 Kernel Weight

Figure 6 is a result obtained from 1000 kernel weight (TKW) test during various developmental stages. TKW is a result in gram (g) produced by grains harvested each

5 days after anthesis (DAA). The result showed positively significant (p-value=0,000) linear relationship with DAA until grain development ceased. It revealed also a relevant fashion for a developing grain in response as filling progresses during development. The regression analysis with R-Sq = 97, 4% predicts perfect model (1000KW = 3,892 + 0, 9679 DAA) to predict how the TKW increase linear with DAA.

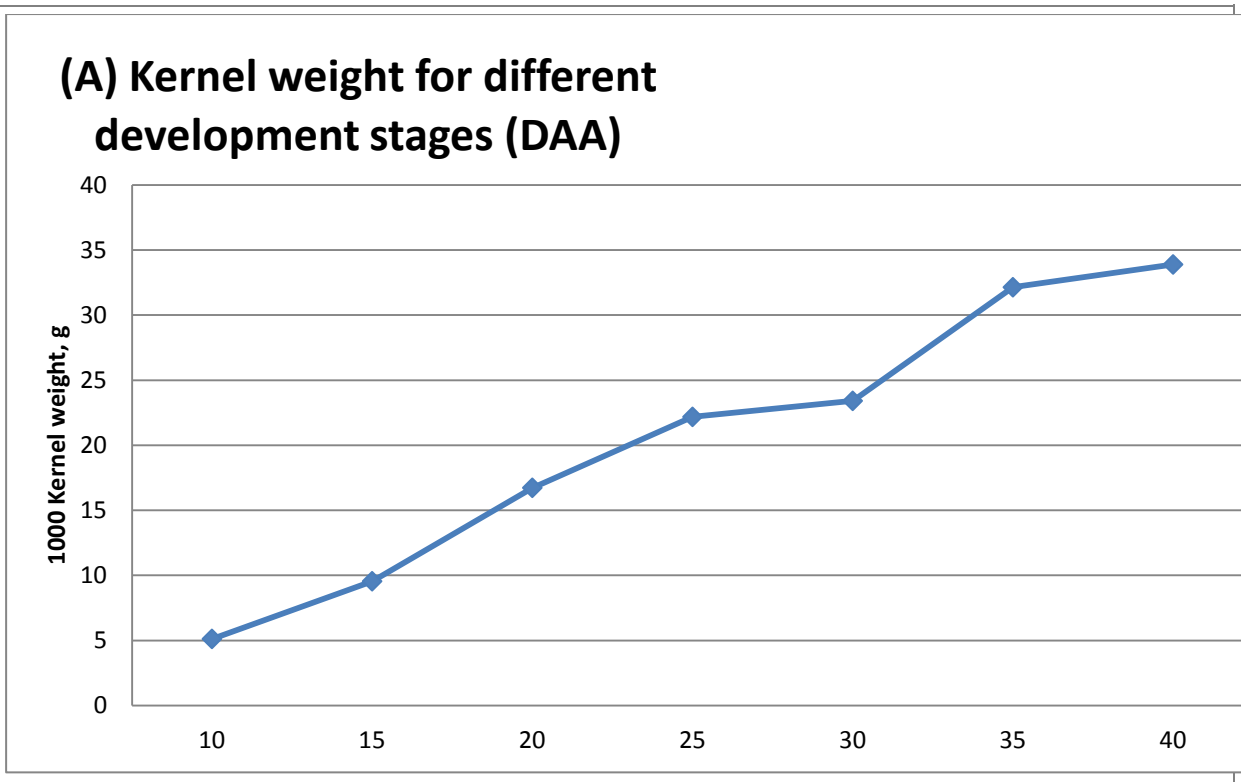


Figure 9: 1000 Kernel weight harvested at different days after anthesis (DAA). 1000 kernels were weighed from 10 days after anthesis (10 DAA) and harvested each 5 DAA until last havest at 40 DAA was performed

The regression equation is 1000KW = 3,892 + 0,9679 DAA

4.1.3 Kieffer Resistance and Extensibility Test (material 2)

Kieffer resistance and extensibility test was shown on table 8. There is correlation been observed in resistance due to mycotoxin levels in the grain? Similarly, correlation

has not been seen between extensibility and DON at least this data and this experiment.

Table 5: results of Kieffier analysis (N=6), resistance (force 1) and extensibility as distance 1 and 2 and predetermined DON content in gains were analyzed from the wheat materials 2 grown in 2006. Samples used in the Kieffier test were wheat samples from material 2 grown at Roverud-2006.

Years	Total average	Force 1	Distance 1	Distance 2	
	Sample	N	mm	mm	DON
2006	25	0,4262	89,23417	98,30183	1800
2006	31	0,538806	110,0757	116,2527	1500
2006	33	0,61262	113,7198	119,787	4500
2006	34	0,525363	90,74667	95,68067	1500
2006	41	0,272151	128,64	141,77	90
2006	122	0,788254	90,75233	97,892	840

There is very large variation in the data, but is not correlated to DON.

4.1.4 Identification for *Fusarium* Species Infested in Grains (mat 1)

Fusarium infection in spring wheat (*Triticum aestivum*) is becoming a major concern in Norway. Therefore many, if not all growers of spring wheat, believed that severely infected grains by *Fusarium species* may develop higher quantities of mycotoxin contents in particularly DON. These DON levels are often associated with potential degradation in grain quality in general and baking quality. In connection to such assumptions, some hypothetical questions are often asked; do grains severely infected by *Fusarium species* affect reduction in baking quality? What are (is) the common *Fusarium species* involved in infestations? And which stages in wheat development (DAA) are what *F. species* occur more over others? Some of the above questions are answered hereunder and the test results were illustrated as figures and descriptive text.

Figure 10 is a result of microscopic representation of macrocodia morphology of different *Fusarium* species produced in (CZID) after a period of 3 weeks. It showed

F. culmorum, F. avenaceum, F. graminearum and *F. equiseti* as the common and most frequent *F. species* detected in the experiment and coded them as A, B, C and D.

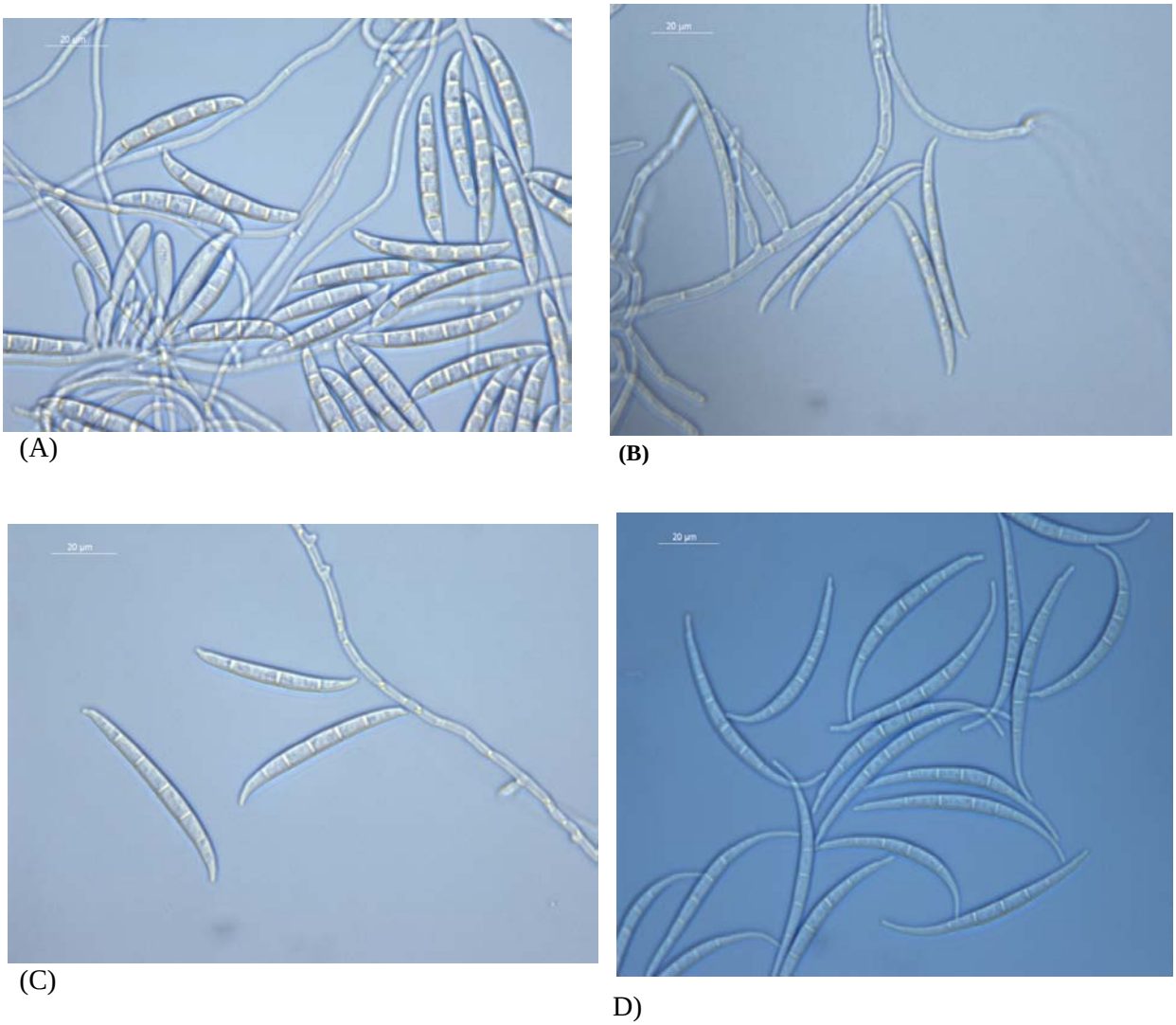
The result showed *Fusarium species* changes between different developmental stages (20 DAA compared with 40 DAA) of *F.* populations regarding to their infestation and abundance levels. Most frequently detected F. specie were; *F. culmorum* (A), *F.avenaceum* (B*), Fusarium graminearum* (C*),* and *F. equiseti* (C). Macrocondia morphology have been illustrated in chronological order of A, B, C and D.

There was also a *F. poae* for which was the 5th dominant *F.spp.* but not illustrated here.

F. poae among other *F. species* were indicated as others.

Figure 10A is *F. culmorum* macroconidia that ranges, more often, 30-60 µm and 4-7 µm in size with thick wall. It septated from 3-5. It has also distinct foot cells and pointed apical cells and slightly curved. There is no perithecia of *Gibberella Zeae* (the perfect stage of *Gibberella Zeae*) been reported to exist.

F.avenaceum macrconidia has been demonstrated in Figure 6B. It ranges usually between 50-80 µm, and sized 2.5- 4 µm. They are slender and long. They also present a standard curve for all most everywhere. Occurrence of prethecia is not common. Figure 10C showed *F. graminearum* macroconidia which is usually ranges 25-50 µm. It most frequently is from 3-7 septated. It is usually straight or slightly curved. Tapered apical cell and distinctly foot-shaped basal cell. Under warmer and prolonged wet conditions on a diseased straw and spikes can usually result super prethecia to develop.



(A) **(B)** (C) D)

Figure 10: *Fusarium* macrocodia produced in water agar after ca. 3 weeks. *F. culmorum* macroconidia (A), *F. avenaceum* (B). F. graminearum macroconidia (C), *F. equiseti* (D). The scale bar= 20 µm.

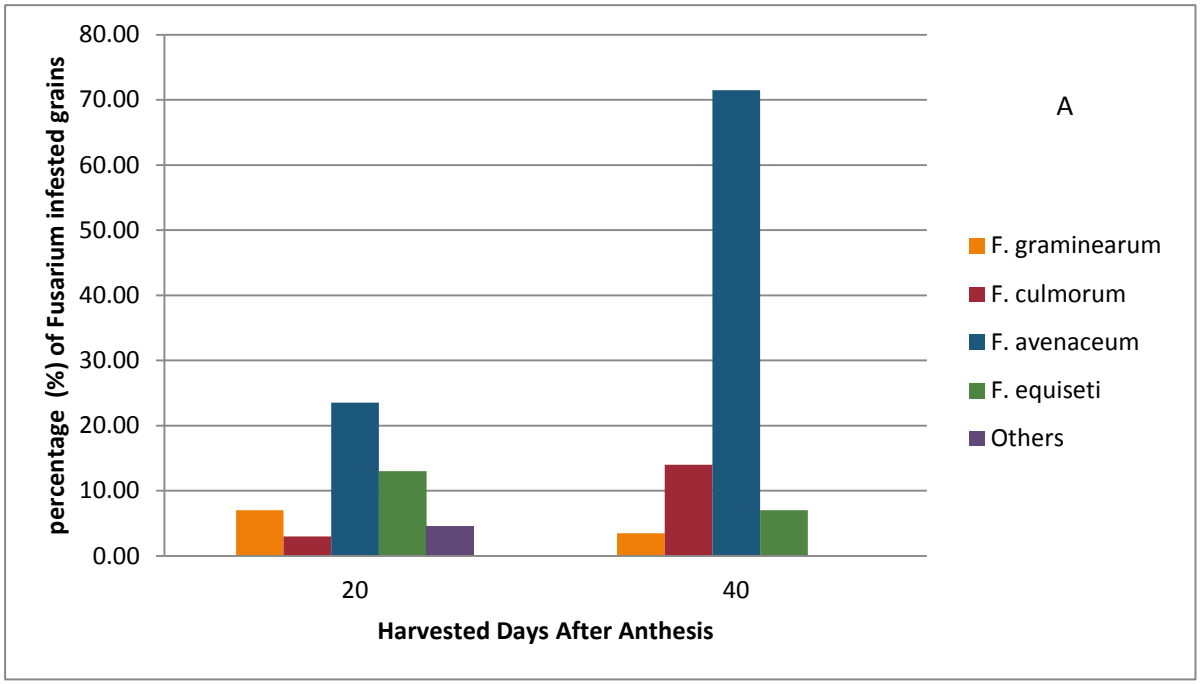
Figure (10D) represents *F. equiseti* macroconidia length ranges 25-120, and sized 3.5-6 µm. It often is 5-7 distinctly septated. It has prominent foot shape seem to become elongating. Tapered and elongated or even whip-like basal cell is also common.

4.1.5 Incubation tests: Direct incubation and Surface Sterilization (mat 1)

Figure (11) is a result achieved from isolates of *Fusarium* infested spring wheat (3.2.1) grains harvested at different days after anthesis (DAA). Figure 11A is the result obtained from grains directly incubated. They are not been washed with 70% ethanol. Comparison is shown between 20 DAA and 40 DAA levels of *Fusarium* infestations. Figure 11B showed the result obtained from grains treated with surface sterilization. The grains are washed with 70% ethanol before incubation. Two stages were also compared, 20 DAA and 40 DAA.

During identification performance, various species were harvested, but the only most frequently observed species are shown below as *F. avenaceum* was the most predominant on both stages of development and both procedures of incubations.

Although it was not significant, but Minitab One-way ANOVA was run revealing P-value= 0,147.



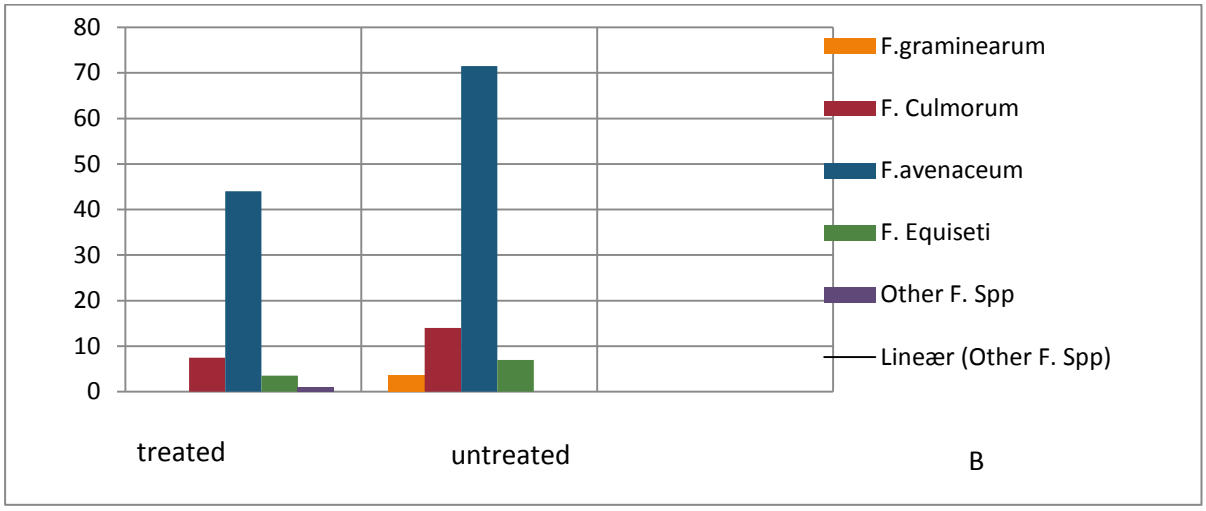


Figure 11: *Fusarium* infested grains harvested at different days after anthesis (20 DAA and 40 DAA) and incubated directly with ZCID media (A), *Fusarium* infested grains harvested at diffefent days after anthesis (20 DAA & 40 DAA) , treated with 70 % ethanol (surface sterilization) and untreated grains incubated with ZCID media (B)

4.2.0 Results of Material 2(3.2.0)

Deoxynivalenol (DON) content in the grain might negatively affect baking quality in spring wheat. This is showed a result of baking quality correlated with DON content in the spring wheat grain (3.2.0). Parameters been investigated under this chapter are; 1000 kernel weight, protein content %, falling number (FN) and SDS-sedimentation volume. The correlation between DON content in the grains and these parameters are of a particular concern in this experiment.

Table 6: Illustrated results of baking analyses of spring wheat material 2 and DON contents of 44 samples grown from various locations in south and the middle of Norway.

samples	kernel count	1000 kernel wt.	protein %	moisture %	Falling Number	SDS	DON	Station nr.	location
6	206	33,7	15,8	11,6	352	74	90	71	Moelv
14	254	46,7	13,6	11	426	54	410	26	Ilseng
15	250	38,8	14,4	11,4	464	59	330	26	Ilseng
16	241	41,1	14,8	11,6	456	58	200	26	Ilseng
17	317	41,3	14	11,1	510	59	0	27	Kise
18	197	45,8	13,9	11,6	499	55	90	27	Kise
23	639	33,0	13,4	11,2	505	59	7400	97	Heradsbygd
30	568	30,7	13,2	12,3	251	62	3700	40	Roverud
33	380	32,4	14,2	11,7	271,5	63	4500	40	Roverud
41	262	39,6	14	11,4	440	66	90	40	Roverud
48	609	28,5	12,7	11,7	430	49	370	41	Rygge
112	398	42,9	12	11,8	403	46	2400	118	Øsaker
733	302	33,1	14,5	11,2	512	75	450	38	Ramnes
737	350	34,2	12,3	11,2	368	53	390	26	Ilseng
738	425	30,9	13,2	11,1	416	71	90	26	Ilseng
740	337	43,0	12,8	11,2	206	66	90	26	Ilseng
744	314	43,8	13,3	10,8	486	70	90	71	Moelv
745	276	32,1	14,1	11,1	518	78	90	71	Moelv
794	349	35,4	14,4	11,4	308	72	1800	40	Roverud
795	354	33,1	13	11,4	516	62	8700	40	Roverud
797	256	43,5	13,1	11,4	377	67	2500	40	Roverud

802	269	36,7	11,5	11,3	436	57	320	40	Roverud
803	310	34,1	12,7	11,2	523	56	16000	72	Åsnes
804	330	36,8	13,7	11,4	410	74	2000	40	Roverud
805	424	37,8	12,6	11,2	443	67	1600	40	Roverud
806	208	39,6	14,1	11,5	423	76	2000	72	Åsnes
851	214	38,8	12,5	11,4	323	64	220	37	Rakkestad
1012	223	36,0	12,7	11,2	317	68	90	27	Kise
1205	360	30,5	15,7	12	311	90	0	50	Tjølling
1295	198	34,1	13,7	11,5	275	63	100	88	Gjennestad
1296	267	36,8	14,7	12,1	157	64	1200	50	Tjølling
1206	208	41,1	14,5	11,9	314	68	1206	50	Tjølling
1233	168	33,4	11,1	10,9	346	61	460	97	Heradsbygd
1236	135	35,6	13,5	11,3	293	77	1200	40	Roverud
1238	292	30,7	11,3	10,8	181	62	950	40	Roverud
1241	243	36,4	12,6	11,2	208	72	380	40	Roverud
1248	213	33,6	10,2	11	229	50	100	40	Roverud
1249	212	31,0	13,9	10,9	270	74	1800	40	Roverud
1250	309	30,0	12,8	11,2	259	79	610	40	Roverud
1293	392	37,0	13	11,3	315	60	430	47	Svelvik
1297	235	42,2	11,5	11,1	303	70	750	38	Ramnes
1299	312	32,8	12,4	11,2	223	69	120	38	Ramnes
1300	254	32,5	13,7		225	80	560	40	Roverud
1313	280	37,2	13,3	10,9	296	69	200	50	Tjølling

This table(6) showed the results of the analytical data obtained from 44 seed samples of spring wheat form material 2 as the first selection of Bjarne cultivar grown from various locations throughout southern and the middle of Norway.

In addition, the following table (7) showed the results of the analytical data achieved from the second selection of 21 seed samples of spring wheat from material 2 grown at Roverud village, Kongsvinger municipality, Norway in years 2006, 2007 and 2008.

Table 7: Illustrated results of baking analyses of spring wheat material 2 correlated with DON contents (N= 21) grown at Roverud village, Kongsvinger municipality, Norway

Location	Years	1000 kernel wt.	protein %	Falling Number(FN)	SDS	DON

Roverud	2006	33. 2	14	248	63	1800
Roverud	2006	31.5	14,2	219	63	1500
Roverud	2006	32.4	14,2	271,5	63	4500
Roverud	2006	33.8	12,9	303,5	60	1500
Roverud	2006	39.6	14	440	66	90
Roverud	2006	33.1	13,1	208	74	840
Roverud	2007	35.4	14,4	308	72	1800
Roverud	2007	43.5	13,1	377	67	2500
Roverud	2007	36.7	11,5	436	57	320
Roverud	2007	36.8	13,7	410	74	2000
Roverud	2007	37.8	12,6	443	67	1600
Roverud	2007	30.3	14,2	346,5	73	500
Roverud	2007	34.1	13,09	489	82	630
Roverud	2008	35.6	13,5	293	77	1200
Roverud	2008	30.7	11,3	181	62	950
Roverud	2008	33.8	13,8	276	84	330
Roverud	2008	36.4	12,6	208	72	380
Roverud	2008	33.6	10,2	229	50	100
Roverud	2008	31.0	13,9	270	74	1800
Roverud	2008	30.8	13,9	271	83	560
Roverud	2008	33.8	11,8	268	72	360

Linear relationship between protein content and DON values were investigated. This was investigated as baking quality and SDS are both dependent on protein content. Regression analysis showed insignificant positive relationship between grain protein content and DON. R-Sq (16.9 %) showed fair increase model in protein content as a function of DON, though insignificant as of p-value (p-value = 0,064). It fails to exhibit neither negative correlation between the two parameters, nor it gives good fit to this data. There is a little to predict that high DON could produce lower protein content.

There are other correlations been investigated in material 2, selection 1. Especially SDS sedimentation volume, 1000 kernel weight, FN and protein % were among the parameters correlated with DON. Significant variations were not observed from these investigations.

Table (7) is a result obtained in comparing DON levels between the years 2006, 2007 and 2008 from spring wheat material 2 (3.2.0) grown at Roverud municipality, Norway. The table showed the results received from the 2nd wheat material selection (N=21).

It illustrated a maximum, mean and minimum of DON values correlated with certain parameters; e. g. Falling Number (FN), Protein %, TKW and SDS-sedimentation volume. Whether these data indicates an evidence for association between high DON mean and lower baking quality based on the above parameters in a given year were examined. The result couldn't substantiate an association between DON mean and the other parameters, though tendency appears to exist between high DON mean in 2006 and reduced SDS-sedimentation volume. Lowest DON mean value and highest SDS-sedimentation volume mean found in 2008. The table showed comparisons of DON means between different years 2006, 2007 and 2008 at Roverud, Norway. DON Comparisons were used with Minitab 15 output. P -value was 0,193 (p-value = 0.193) at 5% level of significance.

Though, statistically not significant, but variation in DON is much high in 2006.

Table 7: showed the maximum, mean and minimum for DON in years2006, 2007 and 2008 of spring wheat (3.2.0). It also showed means of FN, Protein%, 1000 KW and SDS-sedimentation volume obtained in corresponding with specified DON means at specified years at Roverud, Norway

years	No of samples	Maximum DON means µ g/ kg	DON µ g/ kg Means	Minimum DON µ g/ kg Means	Mean Falling Number (FN)	Mean Protein %	Mean TKW	Mean SDS
2006	6	4500	1705	90	299	13,7	33,8	64
2007	7	2500	1335,7 µ g/ kg	320	401.4	13,23	36,30	70
2008	8	1800	710 µ g/ kg	100	249, 5	12.63	33,11	71

In addition, regression analysis was run to see possible correlation between FN and DON content in the grain. The result showed no correlation regarding the p-value (p-value = 0,909) and R-Sq= 0, 1 and. DON can't be as input variable to predict FN as response variable based on current data (N=21).
Similarly, DON levels do not show correlation with SDS in this data (N=21). Regression analysis revealed P-value = 0,551 and R-Sq = 2.0%

5.0 Discussion

This experiment was performed to investigate quality alterations that occur in developing grains from anthesis to physiological maturity. In this experiment, the nature in which wheat grain quality develops after flowering had been investigated.

In addition, potential grain quality modifications that are caused by *Fusarium species*, as well as *Fusarium* species involved in infestations at various developmental stages in grain were to examine. In order to assess and predict the negative consequences of *Fusarium* infested grains, might this work contribute and play a vital role to minimize and control seasonal *Fusarium* outbreaks that Norwegian wheat grower are facing from year to year. Therefore, the following parameters were determined; 1000 kernel weight (TKW), protein content (%), Falling Number (FN) and SDS sedimentation volume and rheological tests. Result of the above analyses are discussed her.

In this respect, 1000 kernel weight (TKW) showed a positive linear correlation with time of development from initial phase to physiological maturity (10 DAA to 40 DAA). Initially, TKW was 5 g at 10 DAA, while 34.0 g were weighed at 40 DAA. This showed usual increase in weight and reflects the growing pattern which can be seen from ordinary developing wheat kernels until physiological maturity was ceased.

Furthermore, developing wheat kernel is governed by cell division and expansion for which are stimulated by water uptake in the grain as the cell expansion continues. Nevertheless, the maturation of grain is predetermined by when the cell expansion in the grain ceases and grain growth ends.

Result is somewhat closely to several other studies (Egli, 1998; Ellis, 1992; Chowdhury 1978; Gooding 2002). Although these studies were aiming and approaching differently, but they were all lacking spontaneity in the area of filling and maturity in grains. It equally, however, appeared to show a close association between the moisture content, desiccation, persistence in time, the longevity of grain filling and the ultimate grain size which are the combining factors that are characterizing TKW.

In addition, SDS – sedimentation volume results showed linear and gradual increase, except at (10-15 DAA) as it showed decline and increased again (from15 DAA). This fall appears to be analytically arisen error, though it didn't appear in the repeated analysis of SDS-sedimentation. However, eventually, slight increase has been detected until physiological maturity has been reached and grain development ceased. Gradual increase in SDS-sedimentation volume is often seen in developing grain at linear phase. The SDS volume was positively increasing and was consistent with time in days after anthesis. Controlled environment study by Gooding (2002) had showed consistency in SDS-volume with water stress during linear phase of the grain growth.

However SDS-sedimentation volume of this result can't be explained by water stress during grain filling and maturity as figures 3 and 4 have indicated the precipitation and temperature pattern during filling. It does not show any particular water stress during filling. Linearly positive increase in SDS-sedimentation volume might be explained to desiccation level that can be enhanced by high temperature (20 C) at flowering.

Unlike, SDS-sedimentation volume, protein Content has been elevated on the initial phase at 10 DAA (18%) of immature developing grain. By post-anthesis decrease in protein level has been observed. This trend looks like normal one, due to higher rate of initial starch accumulation compared to protein accumulation. Initial protein content at 10 DAA was ca. 19% and then reduced gradually until 30 DAA ca. 12%.

Based on the findings of many authors (Bushuk, 1998b; Shewry, 1999; Weegels et al. 1997; Hosney, et al.1986; Uthayakumara, 1999) SDS-sedimentation volume is high correlated with balance of balance of gliadins and glutenins and their composition. Therefore high SDS-sedimentation values promote good baking quality in grains.

Incubation Result

Macrocodia formed in CZID and harvested after 3 weeks of incubation from various *Fusarium species* were identified. The comparisons made between 20 DAA and 40 DAA in respect to their *Fusarium* infestation levels were discussed here. They showed a harvest made from 20 days after anthesis and 40 days after anthesis. There were

larger number of different stages being identified, but neither the data nor discussion is shown here. The most dominant *F. species* identified in this experiment were in this order; *F. avenaceum*, *F. culmorum*, *F. equiseti*, and *F. graminearum.* This result agreed well with many other Norwegian and Nordic wheat researchers (Haave, 1985; Elen et al., 1997; Van et al.1995; parry et al. 1995; Stack 2000). However, the presence and absence, their occurrences, as well as the dominance of these *Fusarium* species might be of more seasonal due to the prevailing environmental factors in given locality. It implies that, although certain species may be expected, but occurrence of other species is not anticipated and varies between localities and seasons. Likewise, it partially corresponds with the finding s of (Brennan et al. 1995; Paul et al. 2007; Bai & Shaner 1994; Buerstmaret al. 2003; 2002) and many others all found similar (or even expressed close association). Besides, they equally associated the occurrence of these populations due to a combination of higher temperature with/and higher RH may have greater influence in infestation levels and disease development.

Nevertheless, *F. avenaceum* displayed highly dominant *F. species* in this experiment. It comprised 50 % of the overall total infested grains at 20 DAA (24%). At maturity (40 DAA), *F. avenaceam* has also been 71%. The greater variability between the *Fusarium species* can be explained as being byproduct of the prevailing weather condition at the growing season. The mean temperature of the growing season had been low; such as 15 ° C, and the mean precipitation was somewhat below 10 mm for most of the growing periods. This weather pattern appears to become more competitive to *Fusarium avenaceum* than others. Yet, partly, because Fusarium avaneceum is soil-borne and also having saprophytic behavior. It also reflects that spectacular suppression done by *F. aveneceum* over the other species. In addition, the result showed generally low *Fusarium* infestation at 20 DAA compared with 40 DAA.

Moreover, figure 8B showed the results of grains washed with 70% ethanol in order to determine relative effect of endogenous strains and possibly eliminate or even minimize exogenous fungal strains which are secondary or saprophytic pathogens. The surface sterilization washes out most of the exogenously applied counterparts. There is

clear variation in *Fusarium* infested grains between the treated and untreated gains. Treated grains demonstrate relatively low *Fusarium* infestations when compared with untreated regime. *F.ave*naceum is far more dominant for all *F. species* in both situations. 44% of the total infested grains of *F. avenaceum* had been observed under the treated regime and 71.5% infested grains under non-surface sterilized grains. The second dominant *Fusarium specie* is *F. culmorum* in both treatments (7.5% and 14%). *F. equiseti* made a slight increase from 3.5% in treated seeds to 7% under untreated grains.

The result in general appeared to be relevant outcome to experimental expectations. Thus, comparing treated and untreated revealed variation, where higher infestations associated with untreated. Because most of the exogenous F. strains were disinfected and removed from the treated regime.

Comparisons of DON Levels between the Years 2006, 2007 and 2008

Growth conditions and agronomic pattern of these materials are assumed to be equal. Relatively minor and major variations on prevailing climatic factories might exist from one growing season to another. These factors can cause significant changes in the cereal grain quality in general, and wheat quality in particular, as well as mycotoxin development in the wheat grain. The mycotoxin is highly correlated with loss of grain quality by many scientists. Due to the effect of the unstable weather at grain filling periods, physical and chemical properties of the wheat grains may become unstable and unwanted. Among the most prevalent factories that might effect a significant variation in the physical and chemical properties include; precipitation, relative (RH) and temperature. This instability may cause unwanted secondary metabolites in wheat grains such as DON, and by varying levels from year to year.

For this consideration, the result showed no significant variations ($p= 0,192$) observed in DON between the years 2006, 2007, and 2008, though, DON means for different years revealed large variations. There is a more than 3-folds higher DON level in 2006

than in 2008. The reason for invariability been observed, might be that the variations of the data in the same year may be large compared to variations in DON between the years (F=1.81).

Highest DON mean was 1705 found in 2006, where lowest DON mean was 710 observed in 2007. The maximum DON value was 4500 recorded in 2006, while lowest DON value was 90 recorded in the same year (2006).

It is wide held view to consider negative trend between high DON level and specific parameters in the baking quality such as TKW, protein content and falling Number (FN). In this regard, Minitab 15 was run to see if lower FN can be correlated to high DON content in the grain or not? The result revealed no correlation between FN and DON content in the grain based in P-value (p =0,909).

This result disagreed with other publication (Gartner et al. 2008; Jones & Mirocha 1999) where high α-amylase activity in grains leads to lower FN values. Similarly, protein analysis observed revealed insignificant (0,064) correlation between protein % and DON, but revealed slight positive correlation based R- Sq (16,9%).
This result coincided with some other papers (Dexter et al. 1996; Gartner et al. 2008; Boyacioglu & Hettiarachchy1995), where slightly increase in protein content was documented. The reason for that is, actually, unclear, but might be of one of two reasons. One reason might be the loss of water that occur in grains affected by fungal that effect increase in protein %. And the other reason is that fungal protein might, also, be accounted or detected as wheat protein, or even it can be the combination of both reasons.
In addition, Minitab 15 data had showed no correlation observed in SDS-sedimentation value plotted against DON as a function of DON been the input variable according to P-value (p-value =0,551) and R-Sq is (R-Sq 2%)

However, (Wang et al. 2005b) documented a contrasting result to this one. The study stated that SDS-sedimentation volume was negatively correlated with proteases and accumulation of DON in the grain.

In addition, in the experiment Kieffer method was also used to analyze bread making quality correlated with DON content in the grain. Unfortunately, correlation was not found when resistance and extensibility were correlated. However, large variations were being observed in the resistanc data(N= 0,272151, N= 0,788254 in this data (N=6), but not correlated to DON content in the grain. Several papers have reported varying decree of correlation between resistance and extensibility (Kieffer R. 2001; Kieffer and Weiser, 2006; Belitz et. al. 1986; Belitz et. al. 1986).

Resistance is a test of sound wheat functionality, where 0.5 (N) and higher resistance can be classed to a sound wheat value, where below this could not be accepted as sound wheat due to lower resistance. The resistance and extensibility are the basic properties governing the dough functionality.
On the other hand, relative proportions between gliadins and glutens determines its functionally. Modification can also occur when alteration caused shift between HMW-LMW, where stronger dough could be shifted to extensible due to protein content and quality.

Conclusion

In the baking quality been investigated during various development stages of spring wheat grains, both grain size and grain quality tend to develop normally as time moves towards physiological maturity.

Infestation levels of different *Fusarium* species revealed *F. avenaceum* is highly abundant in both grain developmental stages 20 DAA, and 40 DAA. F. avenaceum was also most frequently encountered species in both treatments; surface sterilized and non-sterilized regimes. However, *F. species* detected showed up the following prevalence order; *F. avenaceum, F. culmorum, F. equiseti, and F.graminearum.*

DON content in grains correlated with different parameters in baking quality revealed no correlation, but slightly correlation between protein content (%) and DON content in grains was documented. All parameters observed to correlate with DON revealed insignificant in general.

DON means variations between years 2006, 2007 and 2008 also showed not significant, but the interesting fact is that the samples have huge variation in DON means between years.

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