



# **Baking quality of winter wheat**

## – the effect of variety, cultivation site and nitrogen supply

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*Höstvetets bakkvalitet – inverkan av sort, odlingsplats och kvävetillförsel*

Linnea Gustafsson

Master thesis in Food science • 30 hp  
Swedish University of Agricultural Sciences, SLU  
Department of Molecular Sciences  
Agricultural Programme – Food Science  
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Linnea Gustafsson

**Supervisor:** Roger Andersson, Swedish University of Agricultural Sciences, Department of Molecular Sciences  
**Assistant supervisor:** Louise Selga, Swedish University of Agricultural Sciences, Department of Molecular Sciences  
**Examiner:** Annica Andersson, Swedish University of Agricultural Sciences, Department of Molecular Sciences

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Faculty of Natural Resources and Agricultural Sciences  
Department of Molecular Sciences

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## Abstract

Wheat is the most important cereal crop for breadmaking. Different wheat varieties tend to yield varying bread quality, which is affected both by genotype and environmental factors. The present study aims to investigate the genetic and environmental effect on bread quality. This was done to determine whether different breadmaking properties are most influenced by wheat variety, cultivation site or nitrogen supply. Seven winter wheat varieties were cultivated at two sites in Sweden, with varying amount of nitrogen fertilisation (one low and one high), resulting in four different environments. These samples were analysed to evaluate flour, dough and bread quality properties. The parameters were evaluated using principal component analysis (PCA) and those contributing most to the overall variance in data were further analysed using two-way analysis of variance (ANOVA).

In the PCA, genetic and environmental differences formed an orthogonal pattern, influencing the bread quality parameters differently. Breadmaking properties, such as peak viscosity of the flour, bread volume and dough tenacity were linked to genotype, whereas protein content of the flour was linked to environment. For those samples that were cultivated with low nitrogen supply, cultivation site had no effect on the baking quality. For the samples cultivated in Svalöv, nitrogen supply did not have any effect on the baking properties. In conclusion, the genetic effect seems to be greater than the environmental effect on the breadmaking properties and bread quality. However, future research is needed to elucidate whether the observed patterns and obtained results can be confirmed and further explained in universal studies.

*Keywords:* bread, baking quality, winter wheat, variety, cultivation site, nitrogen supply, genotype, environment

## Sammanfattning

Vete utgör den viktigaste spannmålsgrödan för brödbakning. Olika vetesorter genererar olika brödkvalitet, vilket påverkas både av genotyp och miljöfaktorer. Denna studie ämnar undersöka effekten av genetik och miljö på brödkvalitet. Detta gjordes för att fastställa huruvida olika brödbakningsegenskaper påverkas mest av vetesort, odlingsplats eller kvävetillförsel. Sju stycken höstvetesorter odlades på två platser i Sverige med olika kvävegödslingshalter (en låg och en hög), vilket resulterade i fyra olika miljöer. Dessa analyserades för att utvärdera mjöl-, deg- och brödkvalitetsegenskaper. Parametrarna utvärderades med principalkomponentanalys (principal component analysis, PCA) och de som bidrog mest till den totala datavariansen analyserades vidare med tvåvägs variansanalys (analysis of variance, ANOVA).

Ett ortogonalt mönster bildades i PCA:n mellan de genetiska och miljömässiga skillnaderna, vilket påverkade parametrarna för brödkvalitet på olika sätt. Brödbakningsegenskaper, såsom mjölets toppviskositet, brödvolum och seghet hos degen var kopplade till genotyp, medan mjölets proteininnehåll var kopplat till miljön. Odlingsplatsen hade ingen effekt på bakkvaliteten för de prover som odlades med låg kvävegiva. Kvävegödsling hade ingen effekt på bakegenskaperna för proverna som odlades i Svalöv. Sammanfattningsvis verkar effekten av genotyp vara större än miljön vad gäller brödbakningsegenskaper och brödkvalitet. Framtida forskning behövs dock för att klarlägga om de observerade mönstren och de erhållna resultaten kan bekräftas och vidareförklaras i allmängiltiga studier.

*Nyckelord:* bröd, bakkvalitet, höstvete, sort, odlingsplats, kvävetillförsel, genotyp, miljö

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# Abbreviations

ANOVA	Analysis of variance
BU	Brabender unit
CO <sub>2</sub>	Carbon dioxide
cP	Centipoise
FU	Farinograph unit
GI	Gluten Index
HFN	Hagberg falling number
HMW-GS	High molecular weight glutenin subunits
LMW-GS	Low molecular weight glutenin subunits
N	Nitrogen
NIT	Near Infrared Transmittance
PCA	Principal component analysis
RVA	Rapid Visco Analyser
SS bonds	Disulphide bonds

# 1. Introduction

In Sweden, wheat (*Triticum aestivum*) is an important food crop, especially for breadmaking. In addition, wheat is extensively grown due to its production and monetary yield. Alongside, bread is one of the major foodstuffs being consumed (Heenan et al. 2008). During the period 1980-2018, Swedes almost doubled their bread and pastry consumption to 74 kg per person and year (Swedish Board of Agriculture 2019). However, of all bread being produced, over eighty thousand tons of bread is wasted yearly in Sweden (Brancoli et al. 2019). This contributes to the overall worldwide issue with food waste, as it is estimated that 33-40 percent of the food being produced never will be consumed (FAO 2013; WWF-UK 2021). Meanwhile, one-tenth of the world population is malnourished (FAO et al. 2021) and the world population continues to grow steadily, estimating that the number of inhabitants will increase to 9.7 billions in year 2050 (United Nations 2019). This implicates a worldwide issue. Hence, an efficacious wheat cultivation and subsequently bread production is vital. To meet a growing world population and thus a greater demand of food, wheat production has to increase due to its importance of being a source of plant-based protein (Young & Pellett 1985), which is in line with a sustainable diet. This applies pressure on research and breeders to find wheat varieties that are suitable for food production and in turn for high-quality breadmaking.

Bread quality can be defined differently depending on interest. However, bread volume is the most commonly used bread quality indicator. Other breadmaking parameters are also important for the bread quality, such as dough development time, extensibility of the dough, water absorption capacity of the flour and dough tenacity. Breadmaking potential is mainly affected by gluten proteins, which in turn are influenced both by the wheat genotype as well as the environmental conditions (Malik et al. 2013). To obtain a desired viscoelastic dough, content and composition of gluten proteins are of importance. Wheat varieties vary in their gluten composition, which influences the formation of a large and complex gluten protein network and thus its breadmaking potential (Johansson et al. 2013). In contrast, the concentration of protein is dependent on both genotype and environmental factors. Nitrogen fertilisation aid to increase the wheat protein content, which is one basis for payment for farmers. However, depending on genotype, different varieties tend to prioritise either increased protein content or yield upon fertilisation with

nitrogen. Moreover, environmental factors such as temperature, precipitation and soil properties affect the growth ability and in turn, the bread quality.

## 1.1. Aim and objectives

The aim of this study was to investigate the genetic and environmental effect on bread quality, such as bread volume, dough development time, dough tenacity and water absorption capacity of the flour. Thus, the purpose was to provide insight how wheat variety, cultivation site and nitrogen supply influenced baking quality in white bread baked with winter wheat. To gain insight in the abovementioned aspects, the objectives of this study were the following:

1. Study the effect of variety on different bread quality parameters
2. Evaluate whether different environments, with a focus on cultivation site and nitrogen supply, influence the baking quality of bread.

## 1.2. Delimitations

This study was limited to only scope seven different winter wheat varieties. These were chosen by Lantmännen, based on their interest. Additionally, the samples cultivated at the two different sites were only from one cultivation year. Thus, it could be challenging to draw conclusions about baking quality based on cultivation site due to annual variations in weather. Nevertheless, some differences may be valid. Furthermore, the samples were fertilised with different amounts of nitrogen. For each cultivation site, three and four different levels of nitrogen input were respectively cleaned and analysed by NIT per variety (see section 3.1.1 and 3.1.2). Only two of them were chosen respectively for further analysis due to lack of time. However, some differences between the nitrogen inputs might be seen. Based on these delimitations, generalisation of the results beyond this study may be limited.

## 2. Background

To get an overview of the basic knowledge within this field of area, an underlying background will be presented below. This section will cover wheat as a cultivated crop, its history and composition. The varieties used in the present study will also be presented. Furthermore, the processing of wheat to flour and bread will be described and the principle behind the methods used for determination of flour, dough and bread characteristics will be given.

### 2.1. Wheat

Worldwide, wheat is the single largest cereal crop being cultivated and harvested (FAO 2019). Totally, the hectares of harvested wheat accounts for 30 percent of the total harvested area of cereals. Wheat is an important cereal crop within both food and feed industry (Delcour & Hosney 2010a). Globally, around two-thirds of the total wheat production is intended for food (Grote et al. 2021).

#### 2.1.1. Wheat history and cultivars

Since first being domesticated over twelve thousand years ago, wheat has developed into thousands of different cultivars (Kiszonas & Morris 2018). Bread wheat originates from the hybridisation between a progenitor of emmer wheat and a wild grass (IWGSC 2014). Emmer wheat was in turn the result of a hybridisation between einkorn and a wild grass. The genus *Triticum* consists of both bread wheat (*T. aestivum*) and durum wheat (*T. durum*) (Budak et al. 2013). Moreover, there are other wheat species such as einkorn, emmer and spelt wheat. These were cultivated on Gotland already before the birth of Christ, but are not very well cultivated in Sweden nowadays (Hjelmqvist 2009).

#### 2.1.2. Cultivation of wheat in Sweden

In year 2020, the Swedish agricultural land comprised 3 013 000 hectare, out of which 85 percent consisted of arable land (Jordbruksverket 2021). In total, 1 006 700 hectares was designated to grain cultivation. Wheat is the single largest cereal crop being cultivated in Sweden, composing 45 percent of the cultivation

area of cereal grains. This corresponds to around 15 percent of the total Swedish agricultural land. Winter wheat is the commonest wheat type to cultivate in Sweden, accounting for 89 percent of the total area of cultivated wheat. Wheat is also the cereal crop produced in largest quantity, accounting for 59 percent of all cereals being produced, giving the highest yield (FAO 2019).

### 2.1.3. Winter vs. spring wheat

Wheat can be subdivided into spring wheat and winter wheat, depending on the sowing time. Winter wheat varieties require vernalisation, which is a longer period where the crop is exposed to low temperatures, to induce flowering of the plant (Yan et al. 2006). In Sweden, winter wheat constitute 41 percent of the total cereal yield, whereas spring wheat account for only 7 percent (Lantmännen n.d.). In contrast, spring wheat is dominating internationally (WWF 2020). Winter wheat has a higher yield potential, which is one argument for choosing winter wheat over spring wheat (Jordbruksverket 2011). In addition, the use of winter wheat is more widespread as around half of the yield goes to animal feed, one fourth is exported and the last fourth is used for food. In contrast, almost all spring wheat is used for food. In comparison with winter wheat, spring wheat has a higher protein content and is therefore useful for breadmaking (Lantmännen n.d.). However, spring wheat is costlier due to its higher protein content, a basis for payment for farmers. Therefore, spring wheat is usually blended with winter wheat to increase the protein content. As winter wheat is most commonly produced in Sweden, winter wheat varieties were chosen in this study in order to compare their breadmaking potential.

### 2.1.4. Varieties used in the present study

In the present study, seven winter wheat varieties were used: Julius, Festival, Brons, Hallfreda, Kask, Norin and Bright. All of the following information regarding the varietal characteristics in section 2.1.4, has been provided by Tina Henriksson, a senior plant breeder at Lantmännen in Svalöv. According to Henriksson (2021), there are several factors to consider in wheat breeding, some that are basis for payment and some that are not. Straw strength and disease resistance are two important quality parameters. Straw strength is dependent on the nitrogen (N) supply, as greater amount decreases the straw strength. Upon fertilisation, the plant can either use the N to increase the protein content or the yield by producing more kernels. In contrast, baking parameters are not as important upon breeding as the abovementioned ones. Other important factors for the farmer when selling a wheat variety as bread wheat are the Hagberg falling number (HFN), hectolitre weight and protein content. HFN is a quality indicator as an HFN above 250 seconds generates a payment bonus for the farmer. A variety having an unstable HFN is risky as this parameter is very weather dependent. If subjected to precipitation during harvest,

the HFN decreases rapidly, leading to sprout damage as the germination process is initiated in the grains. An HFN below 250 seconds leads to a price deduction for the farmer as the wheat is no longer suitable for breadmaking. Hectolitre weight is an important parameter for the farmer as it is also a basis for payment. Moreover, the farmer gets paid on a protein content basis since protein is an important quality indicator in bread wheat.

### *Julius*

In Sweden, Julius is the major milled wheat variety and has been for a long period of time. It is a medium early variety having a relatively stable HFN and yield. However, yield is not as high in comparison with other varieties, as Julius prioritise to increase the protein content over yield upon N fertilisation. Julius function as a control wheat, as it is very appreciated at Swedish mills and the grinders are set accordingly. It is therefore desired that other varieties have similar properties and quality as Julius.

### *Festival*

Festival is a variety suitable for organic cultivation, primarily developed to resist common bunt (*Tilletia caries*). It has a lower yield and straw strength, which however is not as important as in conventional cultivation due to the absence or limited application of nitrogen in organic farming. It is a variety yielding high bread volume, even with a lower protein concentration.

### *Brons*

Brons is an all-round bread wheat with a stable (but not very high) HFN and good straw strength. It is late in development and as with Festival, Brons yields high bread volume at lower protein content. In contrast to Julius, Brons prioritise yield over protein concentration upon N fertilisation. Thus, it is more difficult to increase the protein content in Brons.

### *Hallfreda*

Hallfreda is a new variety mainly developed to withstand common bunt (*Tilletia caries*) and dwarf bunt (*Tilletia controversa*). It is used both in conventional and organic cultivation. Moreover, it is medium early in development, has a high and stable HFN but poor straw strength, certainly when fertilised with nitrogen. In conventional farming, Hallfreda is therefore treated with growth regulators to improve the straw strength. It is suitable for breadmaking, showing good baking properties, but not as good as Festival.

### *Kask*

Kask is a new variety that is believed to be a good bread wheat as it has shown good potential in small-scale baking, yielding high bread volume. It is medium early in development, has a stable HFN, a high and good quality protein content and good straw strength. Moreover, it has better disease resistance properties in comparison with Julius and is therefore hoped to replace Julius to some extent in the future.

### *Norin*

In comparison with the other varieties, Norin is earliest in development. Thus, it is disfavoured in trials as it is not optimally sown and fertilised. Norin is a stronger gluten quality wheat that has shown good baking quality. However, it is not very similar to Julius, which is the most common and desired wheat variety in Sweden.

### *Bright*

Bright is a Danish variety prioritising yield over protein content. Even though it has a very high protein content, the baking results have been inconsistent with large differences in baking quality. Sometimes it is baking good and sometimes not. However, the reason behind this is not yet known.

## 2.1.5. Wheat flour composition

Wheat flour primarily consists of 70-75 % starch, around 14 % water and 10-12 % protein (Goesaert et al. 2005). Furthermore, non-starch polysaccharides and lipids are minor constituents in wheat flour, accounting for around two percent each. Two of the major components, starch and protein, will be described more in detail below.

### *Starch*

The major component in wheat grains is starch, acting as an energy reserve in the starchy endosperm (Goesaert et al. 2005). Starch is a glucose polymer arranged in granules of varying sizes and shapes, and is mainly composed of amylose and amylopectin in an approximate 1:3 ratio (Shewry et al. 2013). Amylose and amylopectin differ in their molecular size and structure (Goesaert et al. 2005). Amylose is smaller and linear, whereas amylopectin is larger and branched.

The starch granules are important for water uptake during dough mixing, being able to absorb almost half of their own weight in water. Upon baking, gelatinisation and swelling occur within the starch granules as a result of high temperature. The granules become disrupted, thus some of the soluble starch (mainly amylose) leaches out. When the bread cools down, retrogradation of the starch occur. A network of soluble amylose is formed and amylopectin regain its lost crystallinity.

As a result of milling, some of the starch granules becomes damaged (Jukić et al. 2019). The amount of damaged starch is dependent on milling settings but also

by the kernel hardness. Damaged starch has both positive and negative effects on bread quality. In breadmaking, damaged starch is important for its greater water absorption capacity in comparison with native starch. Furthermore, enzymatic degradation is more common in damaged starch, providing the yeast with maltose monomers to ferment. However, too much damaged starch (>10 %) causes an overhydrated and sticky dough (Calvin 2016). Thus, 4.5-8 percent damaged starch is thought to be optimal for baking (Arya et al. 2015).

### *Protein*

Gluten proteins are important in breadmaking and accounts for 80-85 % of the total protein content in wheat (Goesaert et al. 2005). The gluten proteins can be divided into monomeric gliadin and polymeric glutenin, found in an approximate 1:1 ratio in wheat. Glutenin, accounting for 40-50 % of the total gluten proteins, consists of 80 % low molecular weight glutenin subunits (LMW-GS) and 20 % high molecular weight glutenin subunits (HMW-GS) (Johansson et al. 2013). These builds up the gluten protein polymers through disulphide (SS) bonds. HMW-GS constitutes the backbone of the gluten network while LMW-GS extends it. During breadmaking, a network of gluten proteins is formed, yielding a viscoelastic dough (Goesaert et al. 2005). This network entraps carbon dioxide (CO<sub>2</sub>), which increases bread volume. Hence, bread quality is determined by the quantity and quality of gluten proteins. Glutenin gives the dough strength (resistance to extension) as well as elasticity. Gliadin on the other hand, provides the dough with viscosity and extensibility (Wieser 2007). To achieve a desired dough viscoelasticity, an optimal ratio between these proteins is required, which in turn will affect the final bread quality. Thus, both protein content and protein quality are affecting the wheat flour quality (Goesaert et al. 2005). These attributes are influenced by the genetic and environmental effect (Tran et al. 2020), which will be further explained below.

## **2.2. Environmental and genetic effect on baking quality**

As mentioned, wheat flour quality and thus breadmaking potential are influenced by both protein content and quality. In turn, the concentration of protein is determined both by genotype as well as different environmental factors (Malik et al. 2011), while protein quality is influenced by genotype only (Brennan 2012). Thus, both environmental and genetic factors have an effect on the breadmaking potential of wheat (Xue et al. 2016), especially on the “size and complexity of the gluten protein polymer” (Johansson et al. 2013).

### 2.2.1. Genotype

Genetic variation in gluten composition results in different breadmaking potential between wheat varieties (Brennan 2012). Wheat varieties differ in their number and combination of HMW-GS and LMW-GS (Johansson et al. 2013). Each genotype has between three and six different HMW-GS, as well as between seven and sixteen LMW-GS. These are differently distributed, resulting in a large genetic variation. Moreover, the number of cysteines differ between the different glutenin subunits, influencing the ability to form SS bonds. A larger and more complex gluten network provides greater gluten strength and baking quality, as more CO<sub>2</sub> can be retained, yielding a bigger volume. Based on this, different varieties will genetically have varying ability to build a strong gluten network. Furthermore, a correlation between the time of plant development and the polymer structure is also based on genetics (Malik et al. 2013). A complex gluten network seems to be related to a long plant development time (Malik et al. 2011)

### 2.2.2. Environment

Among the environmental factors, N fertilisation is a result of human impact and is thus easiest to amend. Other conditions, including temperature, precipitation and soil are environmental factors also need to be managed. Temperature, the amount of N fertilisation being applied, when during the season nitrogen is applied and whether or not the nitrogen is available to the plant after fertilisation are affecting the structure of the gluten protein polymer to the largest extent (Johansson et al. 2013). Especially the ratio between gliadin and glutenin is affected by environmental factors. Thus, the amount of glutenin is primarily dependent on the environment. In the plant development stage, a higher glutenin to gliadin ratio is favoured by more N supply and lower temperature. In addition, a stronger gluten network will be formed if the wheat grains, having more glutenin, are subjected to high temperature, more N fertilisation or precipitation.

#### *Nitrogen fertilisation*

In the production of wheat, nitrogen is a limited and thus an essential nutrient (Zhang et al. 2021). Therefore, nitrogen has an important role in wheat cultivation. A low N availability in the soil will result in lowered yield and protein content. The reason behind this statement is the fact that nitrogen is a fundamental component of amino acids. In turn, amino acids constitute the protein building blocks. Fertilising with nitrogen is an effective strategy to increase protein content as well as the grain yield to a certain degree before the uptake is satiated.

## 2.3. Bread

Bread has been eaten by humans for thousands of years (Goesaert et al. 2005). Only a few ingredients are needed to make bread, namely flour, water, yeast and salt. In traditional breadmaking, wheat flour is mostly used. However, rye is commonly used in some parts of the world, particularly in the Nordic countries. In addition to the essential ingredients, bread may also contain sugar, shortening, enzymes, surfactants and oxidants. These ingredients are added to improve flavour, texture, functionality or shelf life. In the present study, wheat flour, water, yeast, salt, table sugar, rapeseed oil, barley malt flour and ascorbic acid were the ingredients used for breadmaking. Flour together with water constitute the bread structure as they form a viscoelastic dough (Delcour & Hoskeney 2010b). Yeast is involved in the fermentation process, where it converts carbohydrates into CO<sub>2</sub> and ethanol. Salt makes the dough stronger and gives the bread a good taste. Table sugar is added to enhance the fermentation process. Rapeseed oil acts as a plasticiser and increases bread volume. Barley malt flour increases alpha-amylase activity in flour. Ascorbic acid is an oxidant accelerating maturation of the flour, yielding a stronger gluten.

### 2.3.1. Milling

Wheat kernels can be milled into flour in order to expand the product portfolio of cereal foods (Delcour & Hoskeney 2010c). During milling, the kernels pass through break rolls, which separates the starchy endosperm from the bran. Wheat flour is the starchy endosperm and is further milled into finely flour particles. Bran, which is a milling fraction and regarded as a by-product (Onipe et al. 2015), consists of both the aleurone layer, pericarp, seed coat and nucellar epidermis (Delcour & Hoskeney 2010c). A laboratory mill is useful when having smaller wheat samples, as in the present study, to further evaluate different flour properties. Bühler mill is one of the most common laboratory mills (Wheat Marketing Center 2008a). By using the obtained information, industrial mills can adjust the settings to optimise the extraction yield.

### 2.3.2. Breadmaking

Bread is most commonly made from wheat flour. No wonder considering its great dough forming and gas retention potential, yielding a leavened product (Delcour & Hoskeney 2010b). According to the authors, the straight-dough system, which has been used in the present study, is a widely used breadmaking procedure. In this system, a dough is formed by mixing all ingredients together into optimal development. The dough is then fermented, punched, divided into loaf pieces, moulded into loaf shapes and most often (but not always, as in the present study) proofed in baking pans prior to baking. Physiochemical changes occur at all steps in the breadmaking procedure, as outlined below (Delcour & Hoskeney 2010b).

### *Mixing*

During dough mixing, water molecules moisten the flour particle surface (Delcour & Hoskeney 2010b). This increases the resistance to extension, resulting in an optimally mixed viscoelastic dough when the flour particles are fully hydrated. Moreover, the hydrated starch granules begin to interact with the continuous and hydrated gluten proteins during the dough preparation, forming a network. However, overmixing should be avoided as the SS bonds within the protein matrix will be broken down, leading to a weakened dough. Air is also incorporated in the dough upon mixing. This lays the foundation for the number and size of the gas cells where CO<sub>2</sub> diffuses during fermentation.

### *Fermentation*

During dough fermentation, the yeast consumes oxygen and sucrose, if being included to boost the yeast activity (Delcour & Hoskeney 2010b). Based on wheat flour weight, around two percent added sugar can be fermented in a straight-dough system. This is in line with the present study, where 1.8 % of sugar was used. The resulting anaerobic process produces ethanol and CO<sub>2</sub>, lowering the pH. Due to saturation of the aqueous phase, CO<sub>2</sub> diffuses and expands the gas cells. Moreover, the expansion is kept due to retained CO<sub>2</sub> in the hydrated gluten protein network. Further fermentation is enabled through subsequent punching. By doing this, the dough ingredients are redistributed and a larger number of gas cells are formed.

### *Moulding and proofing*

Moulding means that the dough is flattened with pressure and then rolled into a loaf shape, resulting in alignment of the gluten fibrils (Delcour & Hoskeney 2010b). This is followed by proofing, where the loaves are fermented in a temperature, humidity and time regulated environment. Meanwhile, the loaf increases in volume.

### *Baking*

Upon baking, the dough expands immediately, which is called oven spring (Delcour & Hoskeney 2010b). This is caused by further yeast fermentation, CO<sub>2</sub> formation and gas cell expansion. Furthermore, the high temperature causes vaporisation of the dough surface, forming a crust as it becomes dry. Steam can be added to the oven in order to generate a greater crust as it slows down the vaporisation. Following vaporisation, browning occurs on the crust due to the Maillard reaction between reducing sugars and free N groups. Moreover, starch gelatinisation is initiated during baking at 65 °C, at which temperature the dough also is transformed into a bread. Upon cooling, the bread is not collapsing due to the existing gas-continuous system.

## 2.4. Analytical methods

In order to evaluate different flour characteristics, several analytical methods were used in this study. Their function will be presented below.

### 2.4.1. Rapid Visco Analyser (RVA)

Starch is one of the major components in wheat grains (Delcour & Hosney 2010e). As most cereal-based food undergoes some sort of heating and cooling before consumption, understanding the changes of starch is of great importance. Upon exposure of surplus water, the starch granules swell due to water absorption. Increasing the temperature makes the starch gelatinise and the viscosity increases. Rapid Visco Analyser (RVA) is an instrument being used to analyse such viscous properties of cereal starch (Balet et al. 2019). A rotational viscometer records the viscosity under controlled heating, a holding period at a constant temperature and cooling, at known speed and time, resulting in a pasting profile (Figure 1).

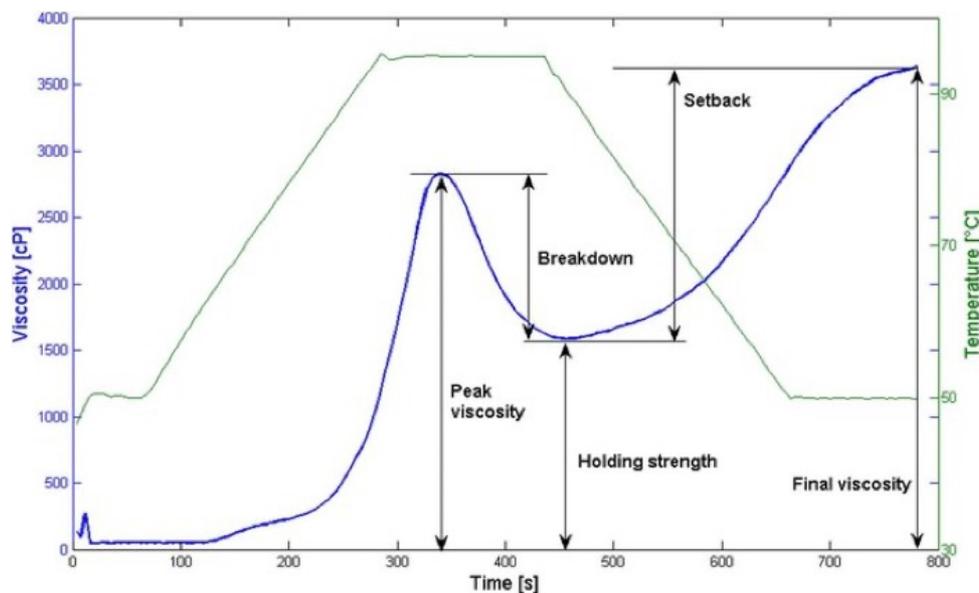


Figure 1. Typical Rapid Visco Analysis (RVA) profile of heat treated flour in water (Keppler et al. 2018:363) (CC BY 4.0). Viscosity is the resistance of a fluid to flow and is a function of temperature and shear (Delcour & Hosney 2010e).

## 2.4.2. Alveograph (Alveolab)

Alveograph, the so-called alveolab, is an instrument used to measure rheological properties correlating to baking quality of the flour. These properties include tenacity (resistance to deformation), elasticity, extensibility and baking strength of a dough. The principle is based on the inflation of air into a dough patty, forming a bubble (Delcour & Hoskeney 2010d). The pressure is measured in a biaxial mode and registered in an alveogram (Figure 2). This process is thought to imitate the expansion of gas cells during dough fermentation.

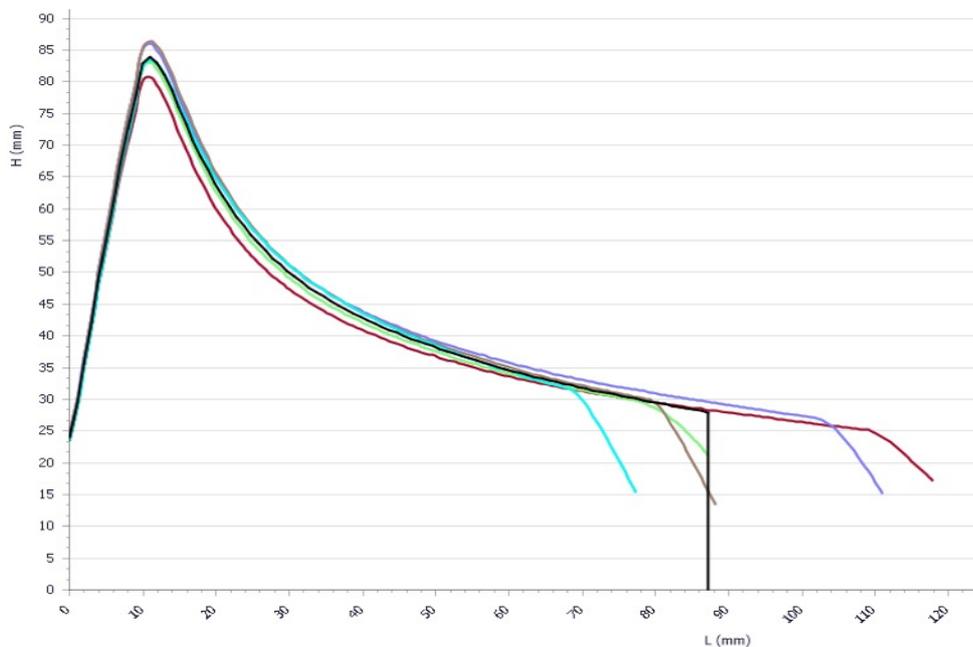


Figure 2. A typical alveogram. On the x-axis, the length ( $L$ ) is designated, whereas height ( $H$ ) is on the y-axis. The five patties are shown with different colours. The mean is marked with the black line.

## 2.4.3. Farinograph

A farinograph is used to measure rheological properties and behaviour of a dough (Mondal & Datta 2008; Diósi et al. 2015). By adding water to wheat flour and yielding a pre-determined consistence of the dough, resistance to mixing is recorded in a farinogram (Figure 3) (Rosell et al. 2001; Diósi et al. 2015). Along with dough properties, the instrument estimate parameters such as water absorption, required mixing to develop a dough and tolerance to overmixing (Wheat Marketing Center 2008b). Upon initial mixing, the resistance is rising until a maximum level where optimum dough development is reached (MacRitchie 2016). At this dough development maximum, all starch and protein particles are hydrated, meaning that they cannot absorb more water (Delcour & Hoskeney 2010b). Further mixing leads to decreased resistance (degree of softening), which is caused by gluten proteins being broken down (MacRitchie 2016; Delcour & Hoskeney 2010b).

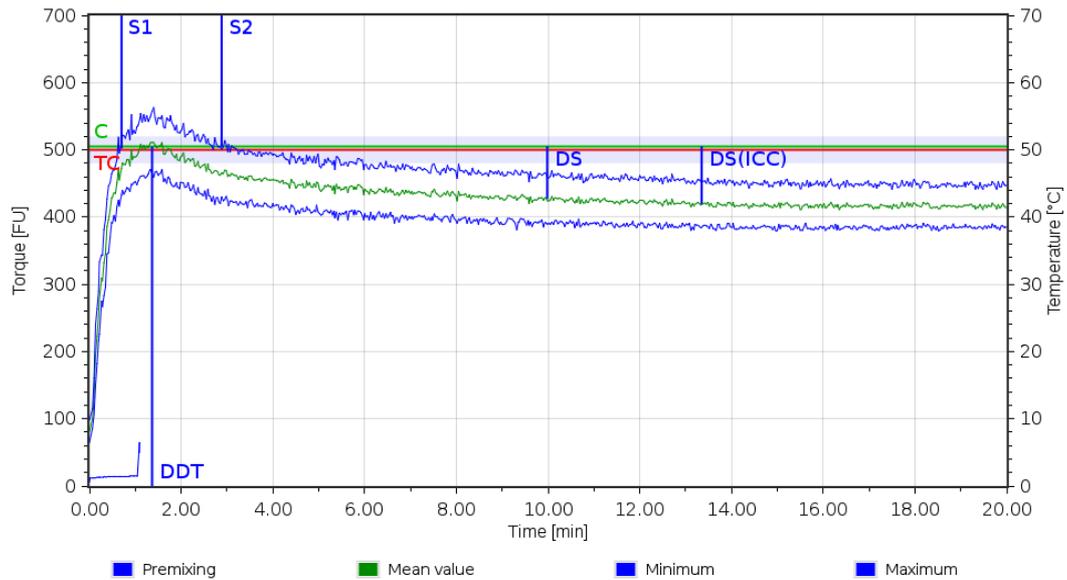


Figure 3. An example of a farinogram where DDT (dough development time) is aimed to reach 500 FU (entitled TC). The actual FU is entitled C (consistency). The difference between S2 and S1 is designated as S (stability). DS is the degree of softening. Farinograph unit, FU, is an arbitrary unit used to designate dough resistance (Delcour & Hosenev 2010f).

#### 2.4.4. Extensograph

What kind of behaviour the dough possess with regard to viscoelasticity can be determined by an extensograph (Rosell et al. 2001). The extensograph measures viscoelastic properties such as extensibility of the dough as well as its resistance to extension, which is recorded in an extensogram (Figure 4). Resistance to extension is the force needed to stretch a dough until it breaks, which can be correlated to dough strength (Delcour & Hosenev 2010d). Extensibility is the dough elasticity and its ability to stretch without breaking, which is recorded as the length when it breaks. These parameters assess flour characteristics, correlating to gluten strength and baking quality of a dough (Boros et al. 2009).

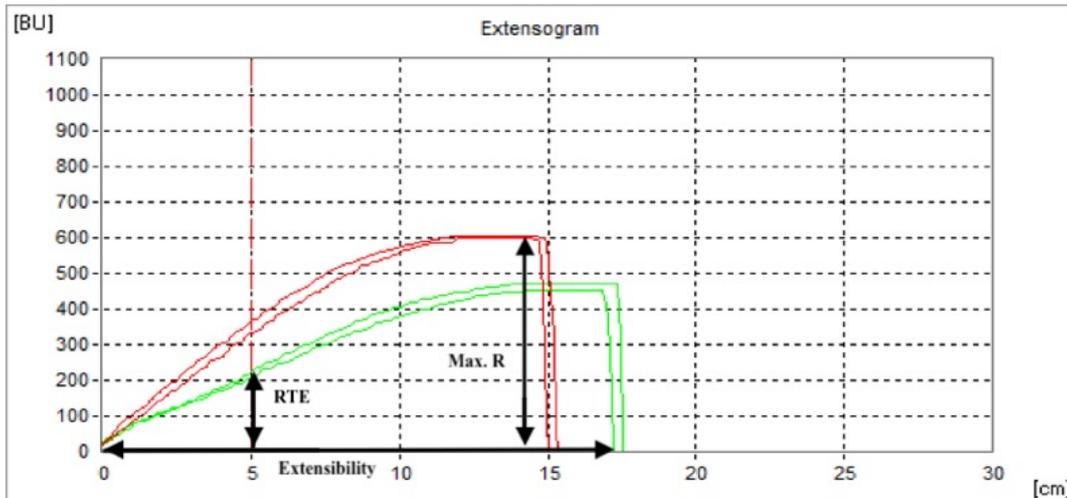


Figure 4. A typical extensogram with extension (cm) on the x-axis and resistance (BU) on the y-axis. Resistance to extension (RTE) at 5 cm, maximum resistance (Max. R) and extensibility are marked in the figure. Brabender unit, BU, is an arbitrary unit used to designate dough resistance (Delcour & Hosney 2010d).

#### 2.4.5. Hagberg falling number

To determine sprout damage, enzymatic activity and thus grain quality, HFN can be measured (Wang et al. 2008). HFN is the recorded time it takes for a stirring rod to reach the bottom of a test tube, which is correlated to the viscosity of a heated mixture of wheat flour and water (Delcour & Hosney 2010e). An HFN between 250 and 280 seconds is ideal, yielding a bread with high volume and a texture which is firm and soft. A lower HFN is due to preharvest sprouting, which is associated to high alpha-amylase activity (Ji & Baik 2015). The enzyme hydrolyses starch into sugars and alpha-dextrin upon heating, lowering the viscosity which results in a low HFN. In turn, the resulting bread volume will be low and the crumb sticky (PerkinElmer n.d.). On the contrary, a higher HFN is due to low enzyme activity, which also diminish the bread volume and makes the crumb dry. By knowing the HFN of the flour, different flours can be blended in order to reach a desired alpha-amylase activity, yielding an optimal bread volume.

#### 2.4.6. Wet gluten & Gluten Index

Wet gluten is defined as the residual viscoelastic mass remaining after washing the dough with a sodium chloride solution (Oikonomou et al. 2015). After subjecting the wet gluten to centrifugation, forcing the gluten through a sieve, the gluten index (GI) might be obtained. Defining GI is a way to measure the gluten strength, indicating whether the gluten is weak (GI < 30 %), normal (GI = 30-80 %) or strong (GI > 80 %).

## 3. Experimental Procedure

All wheat samples being used in the present study were provided by Lantmännen. Seven winter wheat varieties were used: Julius, Festival, Brons, Hallfreda, Kask, Norin and Bright. These were cultivated at two locations in Sweden, namely Svalöv and Bjertorp. At these cultivation sites, the varieties were fertilised with three and four different nitrogen inputs respectively, yielding 49 samples with varying protein content. In Svalöv, the N supply was either 120, 180 or 240 kg ha<sup>-1</sup>. In Bjertorp, the applied N was either 120, 180, 240 or 280 kg ha<sup>-1</sup>.

### 3.1. Selection of material

In advance of milling, the wheat samples were cleaned and analysed in a Near Infrared Transmittance (NIT), as further described below. This was done to select which samples that were going to be milled and further analysed.

#### 3.1.1. Cleaning

The 49 different samples of wheat grains were cleaned in a sieving machine. Sieves with 2.50 mm and 4.50 mm width were used to remove kernels that were too large and too small. Moreover, straw, husk and other impurities that accompanied with the threshing were removed during the cleaning procedure. From each sample, 10 kg was taken out for milling.

#### 3.1.2. Near Infrared Transmittance (NIT)

NIT was used to analyse the wheat kernel protein content. Infratec<sup>TM</sup> NOVA from FOSS was the instrument being used. The sample was poured into the instrument and then analysed using *wheat* as method. Afterwards, the obtained protein content was compared between the wheat samples. Samples were selected based on their protein content, as it was desired to be as close to Julius as possible for the low nitrogen supply and the high nitrogen supply respectively. In total, 28 samples were chosen for milling and further analyses.

## 3.2. Study design

The design used in the present study includes seven winter wheat varieties: Julius, Festival, Brons, Hallfreda, Kask, Norin and Bright. These were cultivated at two locations in Sweden, with two different nitrogen supplies: one low and one high. In turn, this resulted in four different environments.

## 3.3. Milling

One day prior to milling, the wheat kernels were conditioned in a rotating chamber for one hour per five kilograms of sample. Depending on the moisture content derived from the NIT, the amount of added water was adjusted to reach a final water content corresponding to 16 %.

A Bühler laboratory mill was used to grind the wheat samples into wheat flour. All samples were milled in accordance with the pre-made settings done by Lantmännen. When all wheat kernels had been milled, the sieve levers were pulled out in order to let the final flour particles out from the mill. After milling, the resulting eight fractions (six flour and two bran) were weighed respectively in order to calculate the extraction yield. Afterwards, the flour fractions were blended and homogenised in a dough mixer for approximately 20 minutes. Wheat flour was then stored refrigerated in plastic containers until further usage. The bran fractions were not used further in this study.

## 3.4. Wheat flour analyses

After milling the wheat kernels into flour, several methods were used to measure properties of flour, dough and bread, as further described below. Prior to analysis, the flour moisture content was determined using NIT, with *wheat flour* as method.

### 3.4.1. Ash

To analyse the content of minerals, organic matter was burned, retaining the residue being ash. Approximately 2.5-3.5 g of wheat flour was added to a crucible, which was put into a muffle furnace ash oven at 900 °C for 90 minutes. Afterwards, the crucible was left to cool in a desiccator and then weighed. All recordings were registered in software Labmaster, calculating the ash content automatically.

### 3.4.2. Wet gluten & Gluten Index

In order to get information about the amount and quality of gluten, wheat flour was analysed using a Perten Glutomatic instrument together with Gluten Index. First,

10 g of flour was weighed into a Glutomatic wash chamber with an 88-micrometre polyester sieve. Then, 5 ml of 2 % salt solution was dispensed to the flour. Wet gluten was the remainder after washing the flour with brine, which was transferred to a sieve cassette and centrifuged. The amount of gluten that passed through the sieve and the remainder were weighed separately. Together, they constituted the total wet gluten content. The procedure was done in duplicates and a mean value was taken. The gluten index could then be calculated using the following formula:

$$\frac{\text{Total wet gluten} - \text{amount of gluten that passed through}}{\text{Total wet gluten}} \times 100$$

### 3.4.3. Rapid Visco Analyser (RVA)

An RVA Tecmaster from Newport Scientific was used to analyse viscosity in a suspension of wheat flour and water. Around 25 g distilled water and 3 g of wheat flour, corrected for moisture content in the sample, were put into an aluminium canister. Suspension was initiated and clumps were dissolved by vigorously stirring a plastic paddle in the canister. This was also done to prevent adhesion of flour particles to the paddle. The canister with the paddle were attached to the RVA and inserted into the heating block. The instrument was run in a combination of varying shear and temperature applied over time, creating a viscosity curve of the material in the Thermocline for Windows™ (TCW) software program. The viscosity curves were then overlaid in Microsoft Excel in order to simplify comparisons (Figure 10 and 11).

### 3.4.4. Hagberg falling number

A Perten Falling Number instrument was used to measure HFN. Approximately 7.00 g flour, corrected for moisture content, was put into a test tube. Further on, 25 ml of distilled water was poured into the test tube, which was vigorously shaken to obtain a homogenous mixture. A stirring rod was then placed into the test tube, which was further placed into a water bath. The device enabled stirring of the sample during 60 seconds. Subsequently, the stirring rod was dropped from the top of the test tube, recording the time until it reached the bottom. The procedure was done in duplicates, yielding an average HFN.

### 3.4.5. Alveograph (Alveolab)

An Alveolab instrument from Chopin Technologies was used to analyse the dough viscoelastic properties at constant hydration. First, 250 g of wheat flour was poured into a chamber in the Alveolab instrument. Then, 2.5 % brine was added to the flour in adjusted amount depending on the flour moisture content, in order to achieve flour hydration to a predetermined value. A dough was formed through mixing and kneading during eight minutes. The dough was then extruded into five patties. Each

patty was sheeted twelve times to obtain a fixed thickness and was then formed into equal discs using a die. After resting in an isothermal box for about 15-20 minutes, the patties were individually placed in the instrument and inflated by injecting air at constant pressure and flow rate until the bubble ruptured. The resulting pressure, length and time were recorded in alveograms (Appendix 1).

### 3.4.6. Farinograph

A Farinograph-TS from C.W. Brabender Instruments was used to measure dough resistance and water absorption, among other things. Based on an estimated water absorption (given by performing a NIT analysis), around 300 g of wheat flour (adjusted for moisture content to equal 14 %) was poured into a farinograph bowl. Distilled water was titrated automatically through an Aqua-Inject equipment to the flour upon stirring, forming a dough. A consistency corresponding to  $500 \pm 20$  FU was desired to be obtained. After 20 minutes, a corrected water absorption was given by the farinograph, which was used for the extensograph and for baking. A resulting farinogram was given at each run (Appendix 2).

### 3.4.7. Extensograph

The extensograph was run based on the ICC 114/1 method. A dough was first prepared in a large farinograph bowl using a Brabender Farinograph<sup>®</sup>-AT. Around 300 g wheat flour, adjusted for moisture content to equal 14 %, was put into the farinograph bowl together with various amount of water, in which 6 g of sodium chloride had been dissolved. The amount of water was based on the corrected water absorption given from the previous farinogram. However, to compensate for the effect of salt, 3 % less water was used. After five minutes of mixing, the dough was divided into two pieces of  $150 \pm 0.1$  g respectively. Each dough was subjected to 20 revolutions in a rounder and then rolled into a cylinder in a shaping unit. Each cylindrical dough piece was clamped in dough holders, followed by resting for 45 minutes in a humidified chamber. Afterwards, the cradle was placed on a balance arm and a hook was stretching the dough until broken. The procedure with shaping, resting and stretching was repeated a second time. Hence, the force-time chart was registered in extensograms after 45 and 90 minutes (Appendix 3).

## 3.5. Breadmaking

French loaf bread was prepared by using three kg of flour based on a 14 % moisture content. The recipe consisted of 3000 g wheat flour, 150 g baker's yeast, 54 g salt, 54 g sugar and 54 g rapeseed oil. Moreover, based on the water absorption obtained from the farinogram, various amount of 12 °C water was added to the flour prior to mixing. Despite this, 25 ppm of a 0.2 % ascorbic acid solution (corresponding 75 g

for 3 kg of flour) was added to the water as a flour improver. For a wheat flour with 56 % water absorption, the amount of water corresponded to 1650 g in total (75 g ascorbic acid solution + 1575 g tap water). Due to high HFN, a certain amount of barley malt flour was added to all flour samples except one. The amount was depending on the obtained HFN as well as the desired HFN of 280 seconds and the malt quality. To prepare the dough, all ingredients were mixed into a dough at low speed for five minutes, followed by mixing at higher speed until the dough was optimally set. The dough was laid for 30 minutes in a plastic covered bowl. Then, the dough was divided into six pieces of 420 g each. Each piece was folded from the corners into the centre twice, followed by resting for another 10 minutes. The dough was then moulded in a Tregor moulder from Merand and proofed for 60 minutes at 37 °C and 85 % relative humidity. Afterwards, the loaves were baked for 25 minutes at 220 °C in an oven containing 10 % steam and allowed to cool in room temperature.

### 3.5.1. Loaf volume, height and width

After being cooled, the loaf volume was measured by using a TexVol from Perten Instruments. Based on the volume, three loaves were chosen. The largest loaf and the two loaves having the smallest volume were removed. This was done to reduce the effect of human on the final result. The remaining three loaves were measured for height and width. Finally, a mean was respectively taken for volume, height and width of the three loaves.

### 3.5.2. Loaf porosity

By using the Dallmans scale with 0.5 gradings (between 1-8), the loaf porosity was determined for the three chosen loaves. This was done by comparing the bread crumb with the scale. Afterwards, a mean was taken for each sample.

## 3.6. Statistical analysis

Obtained data was compiled and analysed using two statistical techniques. The multivariate dataset was first submitted to a principal component analysis (PCA) using SIMCA 17 (Sartorius Stedim Data Analytics AB), using two principal components. The resulting two-dimensional plots were used to find correlations between the variables. Moreover, the data was analysed in Minitab® 19. Two-way analysis of variance (ANOVA) with Tukey pairwise comparison was used to obtain mean values and significant differences between different varieties and environments. A 95 % confidence interval was used throughout the whole statistical procedure.

## 4. Results

To get an overview of the parameters that were used in the following figures and tables, Table 1 and 2 are showing the abbreviations in alphabetical order and what analysis they originate from. Moreover, an explanation what the parameters mean as well as their unit (if having one) are also given.

*Table 1. Parameters that were analysed in the different analytical methods, the abbreviations used, units and a description of what they mean*

<b>Parameter</b>	<b>Description</b>	<b>Unit</b>	<b>Analysis</b>
Ash	Ash content	%	Ash
Breakdown		cP	RVA
C	Consistency	FU	Farinograph
DDT	Dough development time	min	Farinograph
DS	Degree of softening (10 min after start)	FU	Farinograph
DS (ICC)	Degree of softening (ICC / 12 min after max.)	FU	Farinograph
Energy	Area under the curve	cm <sup>2</sup>	Extensograph <sup>1</sup>
Ext.	Extensibility	mm	Extensograph <sup>1</sup>
Final Visc.	Final Viscosity	cP	RVA
FN	Falling number	s	Falling number
FQN	Farinograph quality number	mm	Farinograph
G	Swelling Index		Alveograph
GI	Gluten Index	GI	Wet gluten
H <sub>2</sub> O	Moisture content	%	Alveograph
Height		mm	Bread
HS	Holding strength	cP	RVA
Ie	Elasticity Index	%	Alveograph
L	Length (Extensibility)	mm	Alveograph
Max.	Maximum	BU	Extensograph <sup>1</sup>

<sup>1</sup> Tested after 45 and 90 minutes resting.

Table 2. Parameters that were analysed in the different analytical methods, the abbreviations used, units and a description of what they mean. Continued from Table 1

Parameter	Description	Unit	Analysis
P	Height (Tenacity)	mmH <sub>2</sub> O	Alveograph
P/L ratio			Alveograph
Pasting T	Pasting temperature	°C	RVA
Peak time		min	RVA
Peak visc.	Peak viscosity	cP	RVA
Porosity			Bread
Protein F	Flour protein content	%	NIT
Protein G	Grain protein content	%	NIT
RN	Ratio Number		Extensograph <sup>1</sup>
RN Max.	Ratio Number (Max.)		Extensograph <sup>1</sup>
RTE	Resistance to extension	BU	Extensograph <sup>1</sup>
S	Stability	min	Farinograph
Setback		cP	RVA
Volume		ml	Bread
W	Area under the curve (Deformation energy = flour strength)	10 <sup>-4</sup> J	Alveograph
WAC	Water absorption corrected for default consistency	%	Farinograph
WAM	Water absorption corrected for default moisture content	%	Farinograph
Wet gluten	Wet gluten content	%	Wet gluten
Width		mm	Bread

<sup>1</sup> Tested after 45 and 90 minutes resting.

The plots originating from the PCA illustrates how the analysed parameters and the different varieties correlate respectively. This is depicted in Figure 5, 6 and 7. A total of 50.4 % of the variance was included in the two first principal components, of which 32.3 % was explained by the principal component 1 and 18.1 % by the principal component 2.

Overall, parameters originating from the extensograph, farinograph, alveograph and wet gluten analysis seems to contribute more to the variance than those coming from the RVA, falling number and ash analysis (Figure 5). The closer the variables are to the plot centre, the lesser impact they have on the variance in the data. Information about relationships between parameters are given in the loading plot. Parameters that are grouped closely together far away from the plot centre are

positively correlated, whereas parameters that are positioned on the opposite side of the centre are negatively correlated.

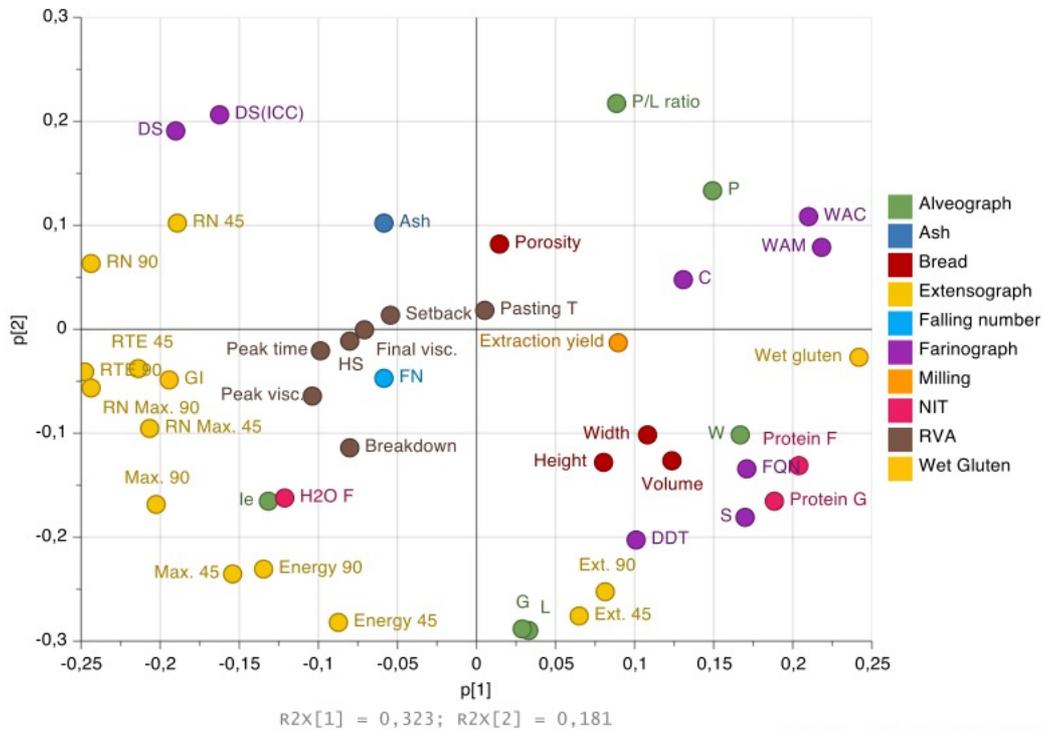


Figure 5. Loading plot, coloured according to analysis. In total, 50.4 % of the variance was covered in these two principal components. For abbreviations, see Table 1 and 2.

Along principal component 1, the variation is mainly caused by differences in parameters such as wet gluten, GI, protein content, water absorption, RTE, RN and DS between the samples. In principal component 2, the parameters extensibility, energy, G, L, DS and P/L ratio seems to contribute to the sample variation. Tenacity (P) seems to be positively correlated to water absorption capacity (WAM), but negatively correlated to water content in the flour. Moreover, G and L are positively correlated with extensibility.

#### 4.1. Genetic effect

By looking at the score plot in Figure 6, Julius seems to be the variety contributing most to the variance in the multivariate dataset. Also, the different varieties are to some extent grouped. Samples that are closely positioned in the score plot are positively correlated and have similar properties. Moreover, it is notable that the varieties are grouped in a similar pattern along the diagonal from the upper right corner to the bottom left corner. However, some samples depart from this pattern.

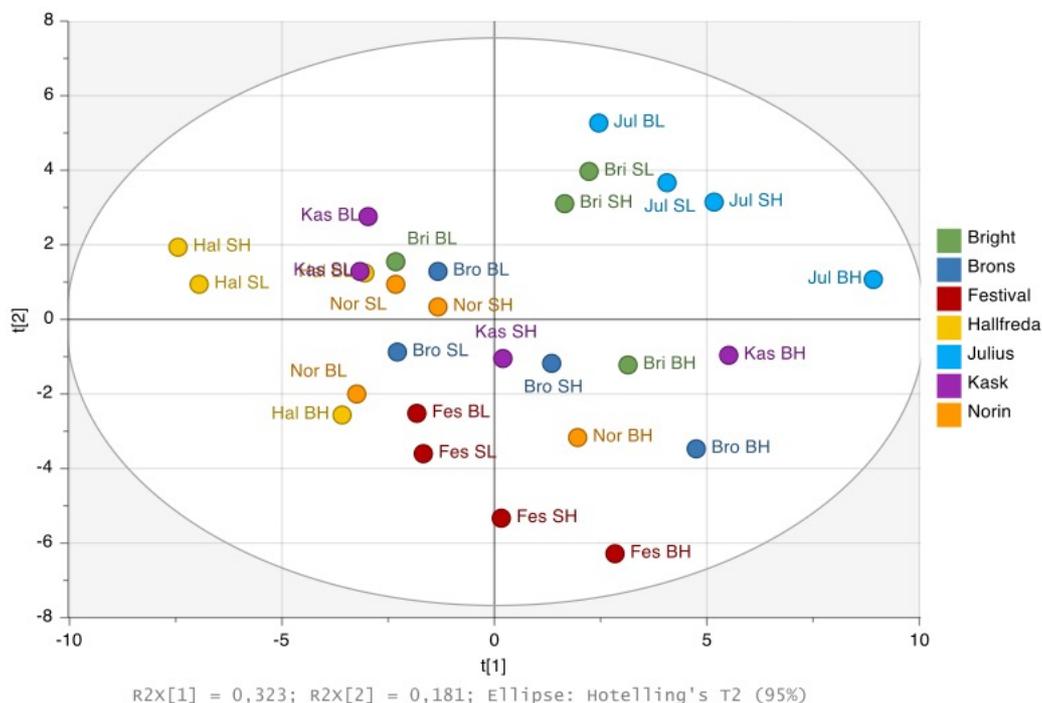


Figure 6. Score plot, coloured according to variety. The colour shows which samples that are from the same variety but cultivated in different environments (different sites with different amount of N fertilisation). Kas SL and Hal BL are overlapping. For abbreviations, see Table 3.

By comparing the score plot (Figure 6) and the loading plot (Figure 5), Julius seems to be related to parameters such as P/L ratio, P and water absorption. Brons BH and Norin BH are related to bread volume, whereas Festival BH correlates with extensibility. All abbreviations used (B, H, L, S, 1-4) for the different environments are explained in Table 3.

Table 3. A summary of the abbreviations and numbers used for the different environments and a description of what they mean

Abbreviation	Description
B	Cultivated in Bjertorp
H	High nitrogen supply
L	Low nitrogen supply
S	Cultivated in Svalöv
Environment 1 = SL	Cultivated in Svalöv with low nitrogen supply
Environment 2 = SH	Cultivated in Svalöv with high nitrogen supply
Environment 3 = BL	Cultivated in Bjertorp with low nitrogen supply
Environment 4 = BH	Cultivated in Bjertorp with high nitrogen supply

## 4.2. Environmental effect

The same score plot was also coloured based on the four different environments in the study design (Figure 7). The score plot visualises that the varieties within the environments to large extent are related. However, for environment 2 and 4, Hallfreda seems not to be as intercorrelated as the other varieties. It is notable that the different environments contributed to the variation in analysed parameters. Environment 4 (corresponding to high nitrogen supply in Bjertorp) seems to be related to parameters like wet gluten, protein content, deformation energy, bread volume, stability, dough development time and extensibility. Parameters that are all depending on the protein content and gluten quality. This is not surprising as environment 4 had the highest protein content, as seen below in Table 4.

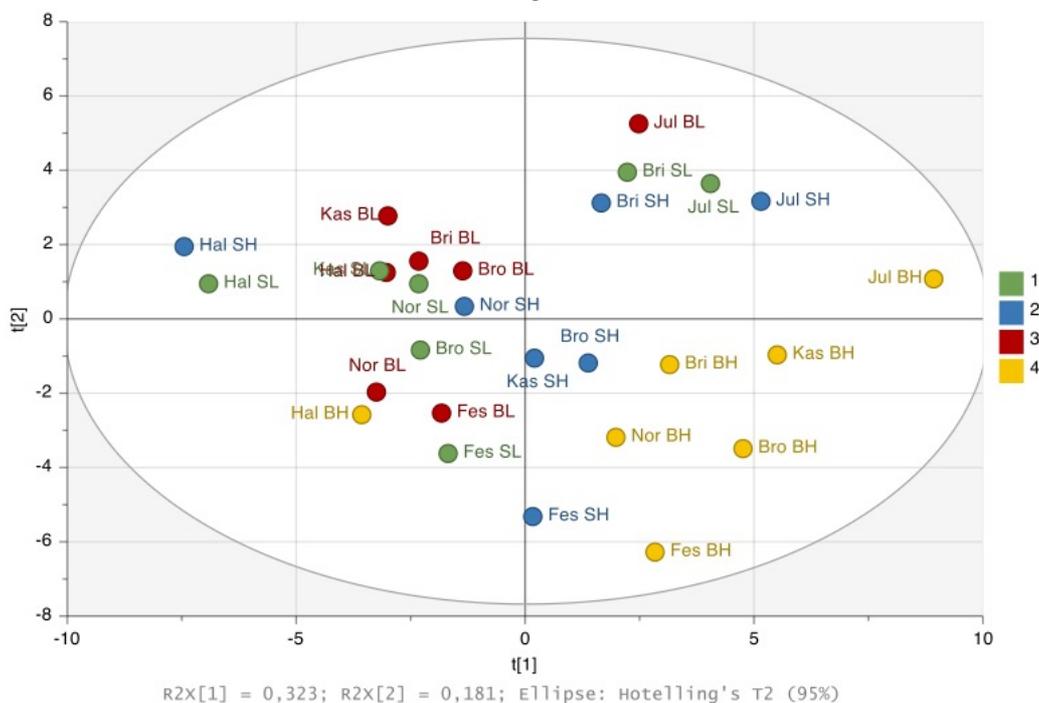


Figure 7. Score plot, coloured according to environment. The colour shows which samples that are cultivated in the same environment (the same cultivation site and the same nitrogen supply). Kas SL and Hal BL are overlapping. For abbreviations, see Table 3.

In contrast to the score plot coloured according to variety, the grouping based on environment is different. As mentioned, the varieties are grouped from the upper right corner to the lower left corner of the score plot. The environments are on the other hand grouped from the upper left corner to the lower right corner.

### 4.3. ANOVA

In Table 4, some of the analysed parameters from the ANOVA are depicted. The parameters were chosen based on their contribution level to the PCA variance. This was done in order to determine whether there was any significant difference between the varieties and between the different environments.

As previously mentioned, the varieties and the environments were aligned in different directions in the score plots, forming an orthogonal pattern. This can also be visualised in Table 4 by looking at the p-values. Tenacity (P) is positioned in the upper right corner of the loading plot (Figure 5). In the score plot (Figure 6), Julius is positioned at the same spot. Julius is related to P, which indicates that this parameter is positioned in the variety direction. This is confirmed in the ANOVA, as the p-value is zero, whereas it is not significant for the environmental factor. On the contrary, the protein content is rather dependent on the environment and not the variety. Where the protein parameters are located on the loading plot, environment 4 is also located on the score plot (Figure 7). This is also visualised in Table 4, where the p-value is zero for environment but not statistically significant for the genetic factor.

Hallfreda has a significantly lower wet gluten content and a significantly higher RTE 90 and DS in comparison with the other varieties (Table 4). Julius has the highest water absorption capacity and is significantly different from the other varieties. P, which is correlated with the water absorption, was also highest in Julius. Environment 4 has the significantly highest P and water absorption capacity.

For the majority of parameters, environment 4 is significantly different from the other environments ( $p \leq 0.05$ ). For this environment, both the cultivation site and the amount of nitrogen fertilisation influences as they differ from environment 3 (same cultivation site but lower nitrogen input) and environment 2 (another cultivation site but similar nitrogen input). DS (degree of softening) is the only parameter being significantly different between environment 2, 3 and 4. For the same parameter, there is a large variation between the varieties as well, where Festival and Hallfreda are significantly different the other varieties.

Table 4. Mean values for the analysed parameters of the different varieties and environments. The letters in the data labels designate whether the samples are significantly different or not. Letters in the same row that are dissimilar imply that the mean values are significantly different.<sup>1</sup> Nitrogen (N) supply (kg ha<sup>-1</sup>) is also included

Parameter	R-sq(adj) <sup>2</sup>	p-value	Variety							Environment				
			Julius	Festival	Brons	Hallfreda	Kask	Norin	Bright	p-value	1	2	3	4
<b>DS (FU)</b>	81.41	0.000	22.5 <sup>cd</sup>	17.0 <sup>d</sup>	21.3 <sup>cd</sup>	68.8 <sup>a</sup>	41.8 <sup>bc</sup>	40.0 <sup>bcd</sup>	47.0 <sup>ab</sup>	0.000	45.5 <sup>ab</sup>	33.7 <sup>b</sup>	52.0 <sup>a</sup>	16.3 <sup>c</sup>
<b>Ext. 45 (mm)</b>	72.85	0.001	164 <sup>b</sup>	183 <sup>a</sup>	174 <sup>ab</sup>	170 <sup>ab</sup>	159 <sup>b</sup>	167 <sup>b</sup>	161 <sup>b</sup>	0.000	161 <sup>b</sup>	163 <sup>b</sup>	167 <sup>b</sup>	182 <sup>a</sup>
<b>Ext. 90 (mm)</b>	57.27	0.008	153 <sup>ab</sup>	173 <sup>a</sup>	159 <sup>ab</sup>	156 <sup>ab</sup>	145 <sup>b</sup>	154 <sup>ab</sup>	149 <sup>b</sup>	0.003	149 <sup>b</sup>	154 <sup>b</sup>	151 <sup>b</sup>	167 <sup>a</sup>
<b>G</b>	74.09	0.000	17.0 <sup>c</sup>	22.7 <sup>a</sup>	19.4 <sup>bc</sup>	18.3 <sup>bc</sup>	20.5 <sup>ab</sup>	20.6 <sup>ab</sup>	19.3 <sup>bc</sup>	0.001	19.4 <sup>b</sup>	19.6 <sup>b</sup>	18.5 <sup>b</sup>	21.3 <sup>a</sup>
<b>L (mm)</b>	73.74	0.000	59.5 <sup>c</sup>	105 <sup>a</sup>	76.5 <sup>bc</sup>	69.0 <sup>bc</sup>	85.8 <sup>ab</sup>	86.3 <sup>ab</sup>	75.5 <sup>bc</sup>	0.002	77.0 <sup>b</sup>	79.1 <sup>ab</sup>	69.7 <sup>b</sup>	92.4 <sup>a</sup>
<b>N supply</b>	90.68	0.015	150 <sup>b</sup>	165 <sup>b</sup>	180 <sup>ab</sup>	220 <sup>a</sup>	175 <sup>ab</sup>	190 <sup>ab</sup>	175 <sup>ab</sup>	0.000	128.6 <sup>c</sup>	205.7 <sup>b</sup>	128.6 <sup>c</sup>	254.3 <sup>a</sup>
<b>P (mmH<sub>2</sub>O)</b>	85.14	0.000	130 <sup>a</sup>	66.5 <sup>d</sup>	96.5 <sup>b</sup>	79.3 <sup>bcd</sup>	70.3 <sup>cd</sup>	86.3 <sup>bc</sup>	76.5 <sup>cd</sup>	0.110	81.1 <sup>a</sup>	85.3 <sup>a</sup>	86.4 <sup>a</sup>	92.9 <sup>a</sup>
<b>Protein F (%)</b>	73.68	0.079	11.0 <sup>a</sup>	10.8 <sup>a</sup>	10.5 <sup>a</sup>	10.1 <sup>a</sup>	10.6 <sup>a</sup>	10.8 <sup>a</sup>	10.9 <sup>a</sup>	0.000	10.2 <sup>b</sup>	10.6 <sup>b</sup>	10.2 <sup>b</sup>	11.7 <sup>a</sup>
<b>Protein G (%)</b>	77.25	0.151	12.2 <sup>a</sup>	12.3 <sup>a</sup>	12.3 <sup>a</sup>	11.7 <sup>a</sup>	12.3 <sup>a</sup>	12.1 <sup>a</sup>	12.4 <sup>a</sup>	0.000	11.7 <sup>c</sup>	12.3 <sup>b</sup>	11.5 <sup>c</sup>	13.1 <sup>a</sup>
<b>RTE 45 (BU)</b>	44.50	0.021	193 <sup>b</sup>	230 <sup>ab</sup>	236 <sup>ab</sup>	248 <sup>a</sup>	235 <sup>ab</sup>	218 <sup>ab</sup>	207 <sup>ab</sup>	0.037	234 <sup>a</sup>	231 <sup>ab</sup>	227 <sup>ab</sup>	203 <sup>b</sup>
<b>RTE 90 (BU)</b>	81.40	0.000	238 <sup>d</sup>	301 <sup>bc</sup>	309 <sup>bc</sup>	384 <sup>a</sup>	292 <sup>bc</sup>	330 <sup>b</sup>	274 <sup>cd</sup>	0.002	312 <sup>a</sup>	302 <sup>ab</sup>	328 <sup>a</sup>	274 <sup>b</sup>
<b>Volume (ml)</b>	68.92	0.000	1972 <sup>a</sup>	2060 <sup>a</sup>	1956 <sup>a</sup>	1671 <sup>b</sup>	2126 <sup>a</sup>	2061 <sup>a</sup>	1969 <sup>a</sup>	0.112	1930 <sup>a</sup>	2001 <sup>a</sup>	1930 <sup>a</sup>	2034 <sup>a</sup>
<b>W (10<sup>-4</sup>J)</b>	78.54	0.000	281 <sup>a</sup>	229 <sup>bc</sup>	256 <sup>ab</sup>	211 <sup>bc</sup>	202 <sup>c</sup>	254 <sup>ab</sup>	197 <sup>c</sup>	0.000	209 <sup>b</sup>	222 <sup>b</sup>	215 <sup>b</sup>	284 <sup>a</sup>
<b>WAM (%)</b>	75.23	0.000	58.5 <sup>a</sup>	53.8 <sup>b</sup>	55.3 <sup>b</sup>	53.5 <sup>b</sup>	53.6 <sup>b</sup>	55.4 <sup>b</sup>	55.4 <sup>b</sup>	0.002	54.1 <sup>b</sup>	54.8 <sup>b</sup>	54.7 <sup>b</sup>	56.6 <sup>a</sup>
<b>Wet gluten (%)</b>	71.19	0.001	28.9 <sup>a</sup>	25.7 <sup>a</sup>	25.0 <sup>ab</sup>	20.1 <sup>b</sup>	25.8 <sup>a</sup>	25.1 <sup>ab</sup>	28.5 <sup>a</sup>	0.000	24.1 <sup>b</sup>	26.1 <sup>ab</sup>	22.9 <sup>b</sup>	29.2 <sup>a</sup>

<sup>1</sup> Please note that the letters should be compared between varieties only and environment only (clearly distinguished with an edged line).

<sup>2</sup> R-sq describes what percentage of the variance that can be explained by the model. Adjusted R-sq minimises the effect of the number of terms in the model.

## 4.4. Bread volume

As seen in Table 4, Hallfreda has a significantly lower bread volume compared to the other varieties. There was no significant difference in volume between the different environments, even though environment 4 has a significantly higher protein content in the flour. The high protein content might be explained by a significantly higher nitrogen input. Therefore, the genetic effect seems to be greater on bread volume than the environmental factor. Additionally, by comparing the protein content in the wheat flour and the bread volume, the correlation is not very strong (Figure 9). Nevertheless, some of the varieties are aligned in a similar pattern, even though some samples deviate. This is an interesting observation as Hallfreda is the only variety being significantly different from the others. However, the pattern seen within the different varieties cannot be seen if looking at the different environments. Instead, they are arranged in clusters. This is confirmed in Table 4, as flour protein content is significantly different for environment but not for variety. In contrast, volume is significantly different for variety but not environment.

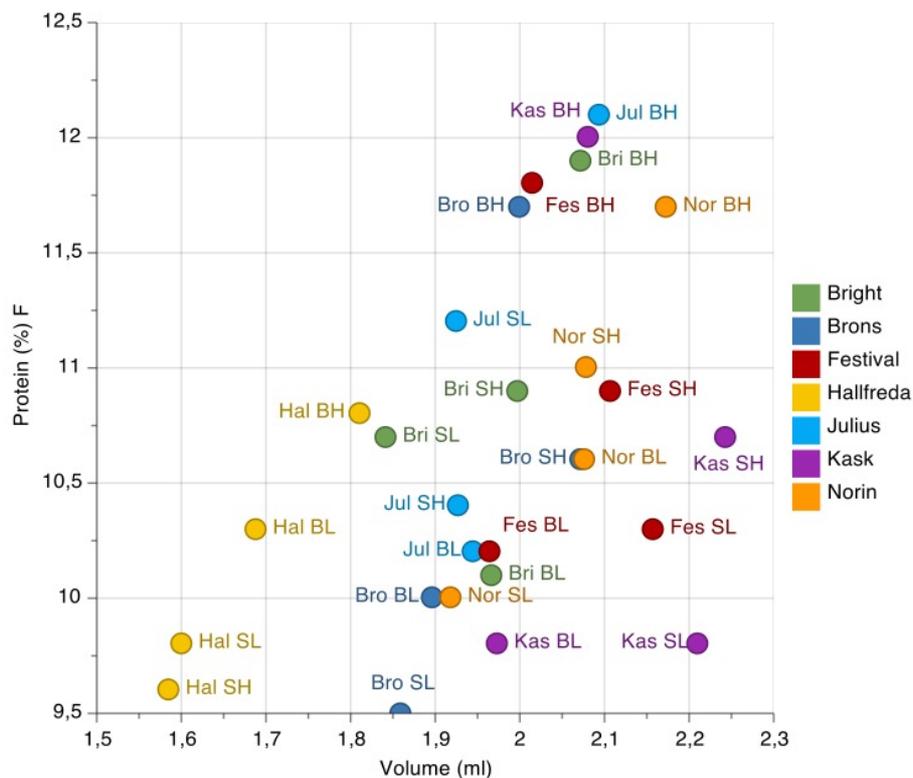


Figure 8. Scatter plot between protein content in the flour and bred volume. The plot is coloured according to variety. For abbreviations, see Table 3.



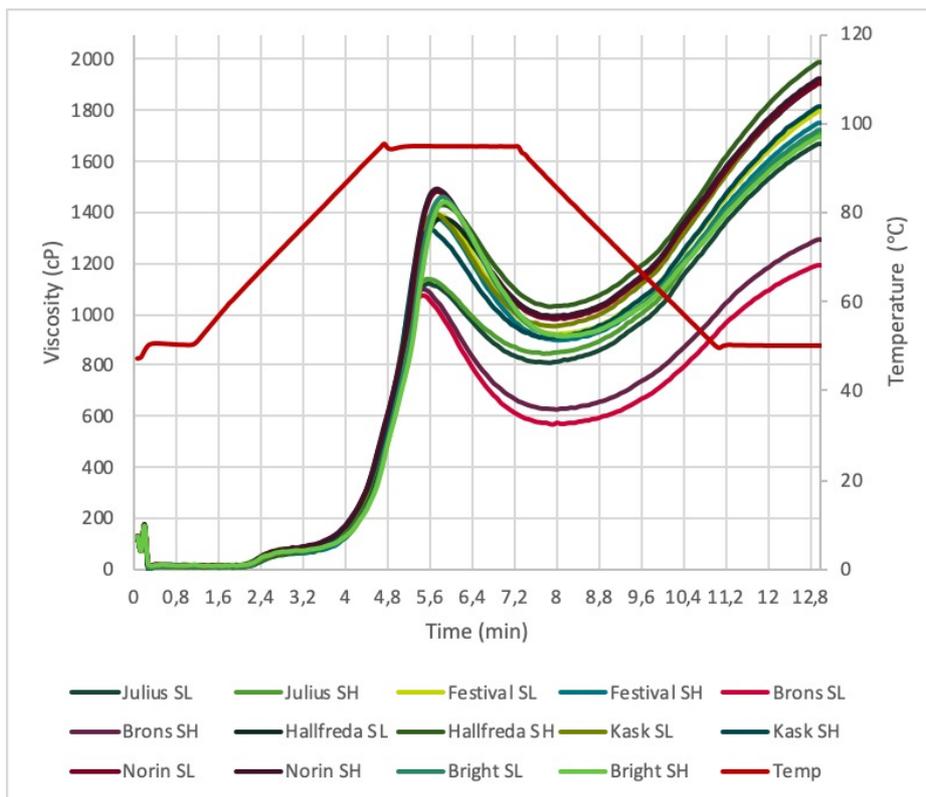


Figure 10. Pasting properties of the different samples cultivated in Svalöv.

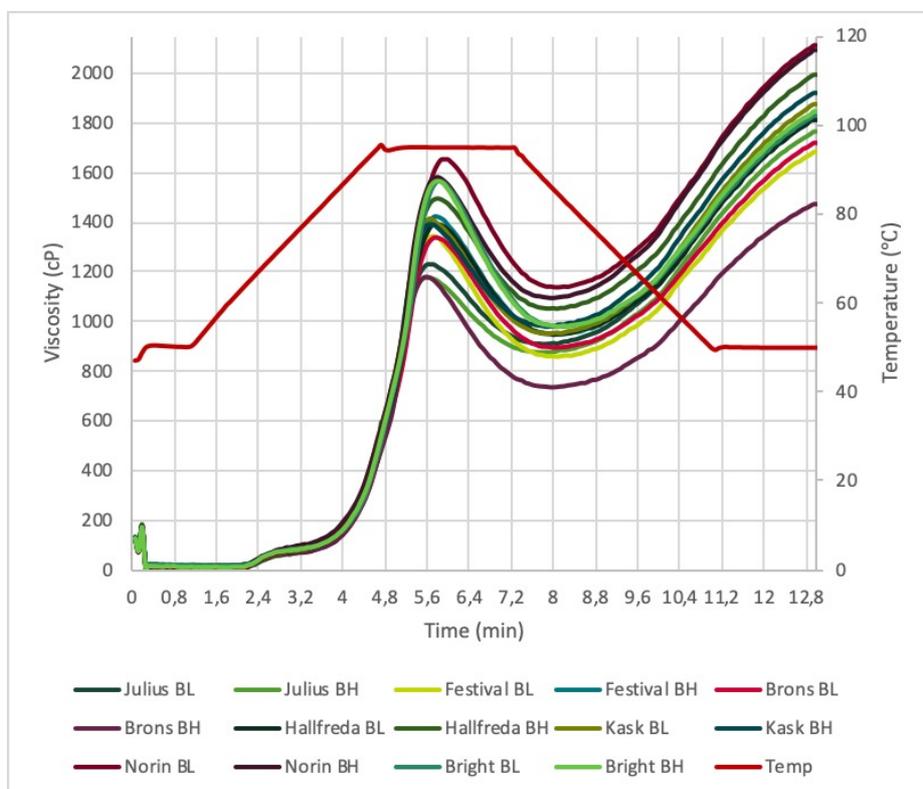


Figure 11. Pasting properties of the different samples cultivated in Bjertorp.

By comparing the different varieties and the different environments in Figure 12, the varieties differ more than the environments. Hence, it seems that the genetic factor affects the outcome more than the cultivation site and the nitrogen supply. The wheat varieties cultivated in Bjertorp have in general higher viscosity than those cultivated in Svalöv, but only environment 3 (low nitrogen input in Bjertorp) is significantly different from the others, not environment 4 (high nitrogen input in Bjertorp).

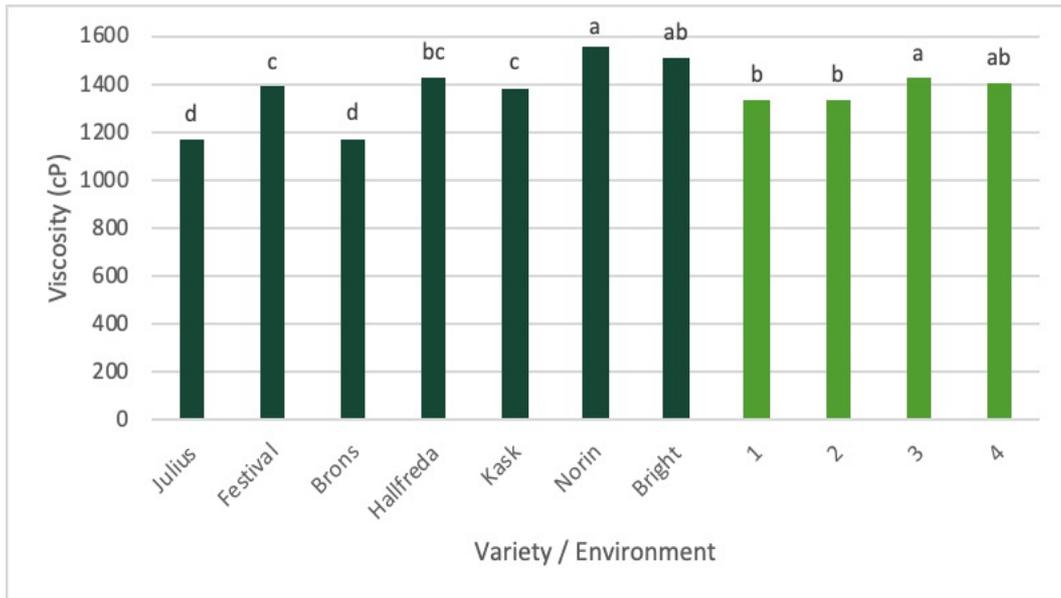


Figure 12. Mean peak viscosity (cP) for the seven different varieties (mean values for all four environments) and the four different environments (mean values for all seven varieties). The letters in the data labels designate whether the samples are significantly different or not. Letters that are dissimilar imply that the mean values are significantly different. Please note that the letters should be compared between varieties only and environment only (clearly distinguished with different colours).

## 5. Discussion

The underlying reason behind the observed orthogonal pattern between the genetic and environmental differences, being aligned in different directions in the score plots, is not described in the literature. Nevertheless, the pattern is an interesting observation that may be affirmed in the future.

In the loading plot, P was positively correlated to WAM but negatively correlated to water content, which agrees with previous knowledge (Jødal & Larsen 2021). When the concentration of water increases, tenacity is lowered. Meanwhile, tenacity decreases with decreased water absorption capacity. Furthermore, G and L are both a measure of extensibility (*ibid.*), explaining the positive correlation with the extensibility parameter.

### 5.1. Peak viscosity

Genotype was believed to cause the varietal difference in peak viscosity. Assuming that the genetic factor is greater than the environmental factor, there must be something within the flour particles influencing the peak viscosity. According to one study, peak viscosity was believed to be correlated with protein content and composition between different wheat varieties (Singh et al. 2016). Higher protein content was assumed to be correlated with lower peak viscosity. However, no such correlation could be seen in the present study by comparing the protein content in the different flours. A correlation between lower protein content and higher peak viscosity could only be seen in Bjertorp.

Some other factors that influences the peak viscosity are the amylose content (Nikolić et al. 2021) and alpha-amylase activity (Singh et al. 2016). Peak viscosity and amylose content were positively correlated according to the Serbian study. On the other hand, a negative correlation was seen between alpha-amylase activity and peak viscosity. Thus, a low peak viscosity could be dependent on a high alpha-amylase activity, high amylase to amylopectin ratio, but also higher content of lipids (Nikolić et al. 2021). It is believed that amylopectin increases the absorption of water and thus the viscosity, while amylose and lipids have an opposite effect. Amylose content was not analysed in the present study. However, peak viscosity and FN were positively correlated in the loading plot (Figure 5). FN is an indicator

of the amount of sprout damage and alpha-amylase activity. Therefore, alpha-amylase activity might have an effect on the peak viscosity.

## 5.2. Genetic and environmental effect on breadmaking properties and quality

According to Henriksson (2021), the mills in Sweden desires to find varieties that are similar to Julius. By looking at the score plot (Figure 6), it is only Bright cultivated in Svalöv that have similar properties as Julius. However, if looking at the parameters WAM and P being located at the same position in the loading plot (Figure 5), Julius and Bright are significantly different according to Table 4. However, the ANOVA did not distinguish between the two cultivation sites, meaning that all samples from the four different environments are considered. As all the obtained values influence the mean value, it will also influence the significance outcome. In order to confirm the abovementioned observation, an improvement of the study could have been to distinguish the two cultivation sites for all varieties when running the Tukey pairwise comparisons using two-way ANOVA.

As mentioned, environment 4 had a significantly higher water absorption capacity in comparison with the other environments. This is due to a significantly higher protein content. A higher protein content in the flour leads to an increased water absorption capacity (Khatkar 2005).

### 5.2.1. Bread volume

Hallfreda had a significantly lower wet gluten content and bread volume. According to Johansson et al. (2013), breadmaking potential is dependent on the gluten protein content and composition. As Hallfreda had a lower wet gluten content as well as yielding lower bread volume, it indicates that the gluten network is smaller in size and less complex than the other varieties. This is affected both by its genotype and the environment. Due to the importance of HMW-GS for the gluten network, it indicates that Hallfreda has a less favourable composition of HMW-GS. As the wet gluten content is lower, the gluten network will not be able to entrap as much CO<sub>2</sub> as other varieties, which will influence the bread volume negatively. This is confirmed in Figure 5, where wet gluten and volume are positively correlated. Furthermore, the ratio between gliadin and glutenin is dependent on environmental factors, such as nitrogen fertilisation and temperature (Johansson et al. 2013). Temperature could not have been affecting as the varieties were grown in the same cultivation sites. Thus, Hallfreda should not stand out as much as it does. However, Hallfreda had the highest average nitrogen input, influencing the glutenin content. This would result in a stronger wheat gluten (ibid.). Even though Hallfreda might

have more glutenin, the size of the subunits may be smaller and less complex in comparison with other varieties, which could result in a lower gluten content and thus bread volume.

### 5.2.2. Effect of cultivation site and nitrogen supply

By looking at Table 4, it was notable that environment 1 and 3 had the same average nitrogen supply. Additionally, the obtained values from the analysed parameters were not significantly different. There was no significant effect on the baking properties between the environments with low nitrogen. Thus, the environmental factor seems therefore to not have any effect on the baking quality for lower protein content. As the average nitrogen supply differs between environment 2 and 4, corresponding to the high protein content, it is more difficult to draw the same conclusion. However, by comparing the mean values between environment 1 and 2, as well as between 3 and 4, the baking quality only differed significantly in Bjertorp. This could be due to a significantly higher nitrogen supply in Bjertorp than in Svalöv (environment 2 vs. 4). In Svalöv, nitrogen input did not have any significant effect on the baking properties. Due to the differences in nitrogen supply between environment 2 and 4, there are limitation in this study. With the obtained results, there are difficulties to see whether the high nitrogen supply as well as the cultivation site Bjertorp have an effect on the baking quality. Now, results are only valid for the low nitrogen supply as well as for Svalöv. Therefore, the study design could have been improved.

Hallfreda had a significantly higher average nitrogen input for the four samples in comparison with the other varieties. Withal, Hallfreda had the lowest protein content in the grains and in the flour. Moreover, several other baking quality parameters were significantly different in comparison with the other varieties. In contrast, Julius and Festival required the lowest nitrogen input to reach a significantly equal protein content. The varieties were fertilised with different amount of nitrogen in order to obtain an equal protein content in the wheat kernels as Julius. As the nitrogen supply differed, the varieties might have varying amount of glutenin, which provides the dough with a greater gluten strength (Johansson et al. 2013). To be comparable in a better way, environment 2 and 4, as well as the varieties should have had the same average amount of nitrogen fertilisation. This in order to see, with certainty, whether nitrogen supply has an effect on the baking quality between the different environments and varieties.

### 5.3. Future research

Based on the present study, a larger universal study including replicates of the different analyses would be of interest. This in order to see variation between the same material to get a more trustworthy mean and to see how great the variance is. Likewise, inclusion of samples from several cultivation years would be interesting, to see how the climatic conditions affect the baking quality of different wheat varieties. Perhaps, different varieties are able to withstand varying conditions differently. In addition, research on how the same amount of nitrogen fertilisation influences the baking properties would be of interest.

To get a better understanding in the observed variation in peak viscosity, further analysis of amylose content and the ratio between amylose and amylopectin would be interesting. Analysing the glutenin content as well its composition of HMW-GS in the different varieties could also be of interest in the future.

Based on interest, future studies could focus on whether the observed patterns in the score plots and the scatter plot would appear if other varieties are being analysed. If so, there might be underlying reasons to explain.

## Conclusion

One important factor that has to be considered is the fact that some analyses were not replicated. Therefore, the possibility to draw conclusions with certainty is limited. However, the genetic factor seemed to have a greater effect on the analysed parameters, affecting the baking performance and quality. Keeping in mind, the samples were only from one cultivation year. Thus, no annual differences could be seen, where climatic conditions could have affected the result. Moreover, in order to have better accuracy in the statement that environment 4 is significantly different from environment 2 in the analysed parameters, it would have been preferred to have the same amount of nitrogen supply. In the present study, the samples were not chosen based on the nitrogen supply. Instead, a similar protein content to Julius, fertilised with low amount of nitrogen and high amount of nitrogen respectively, was desired.

This study aimed to investigate how bread quality is affected by winter wheat varieties (genotype) and environmental conditions. It was found that the varieties and the environments were contributing differently to the variation in analysed parameters. Breadmaking properties, such as peak viscosity of the flour, bread volume and dough tenacity seems all to be linked to variety, where differences in genotype affect the quality. Protein content of the flour was instead affected by the environmental conditions. For those samples that were cultivated with low nitrogen supply, the environmental factor did not have any effect on baking quality. For the samples cultivated in Svalöv, baking quality was not influenced by differences in nitrogen supply. Thus, the genetic effect seemed to be greater than the environmental effect on breadmaking properties and bread quality.

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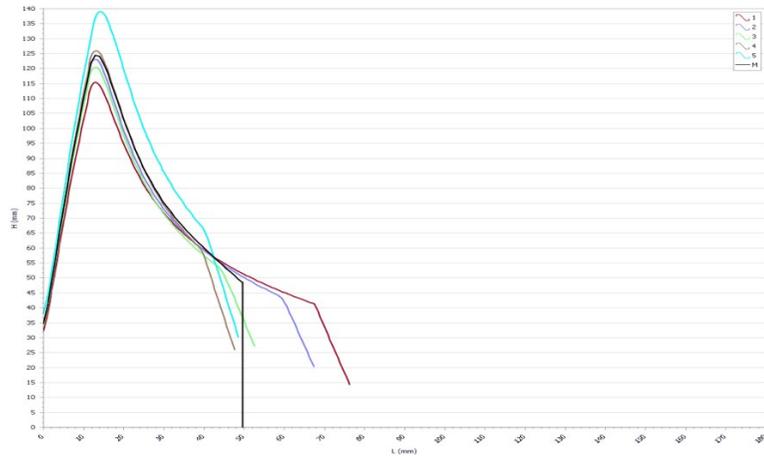
Finally, I would like to thank my family and friends for the biggest support during my time as a student at SLU. Thank you for always being there, for showing lots of care. Last but not least, thank you Erik for believing in me, for helping me move all around Sweden, for being my second home and for encouraging me with daily routines during this semester.

## Appendix 1 – Alveogram

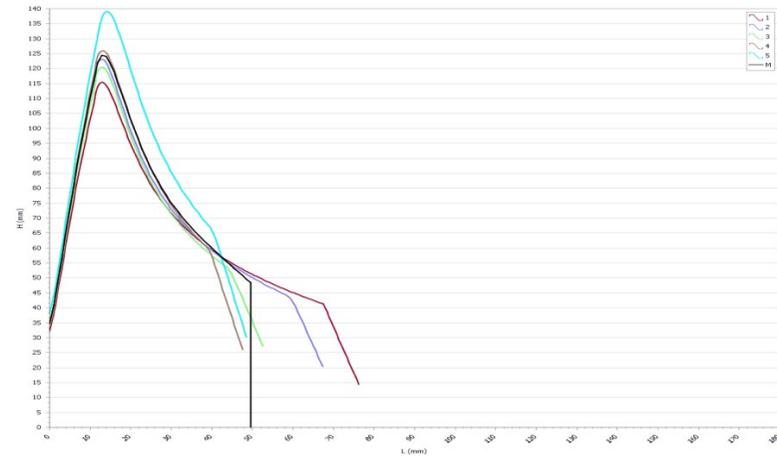
Below, all alveogram that were obtained during the alveograph operation are presented. To interpret the alveogram (see also Figure 2), the following abbreviations are described.

<b>Point</b>	<b>Unit</b>	<b>Description</b>
G		Swelling Index
H <sub>2</sub> O	%	Moisture content
Ie	%	Elasticity Index
L	mm	Length (Extensibility)
P	mmH <sub>2</sub> O	Height (Tenacity)
P/L ratio		
W	10 <sup>-4</sup> J	Area under the curve (Deformation energy = flour strength)

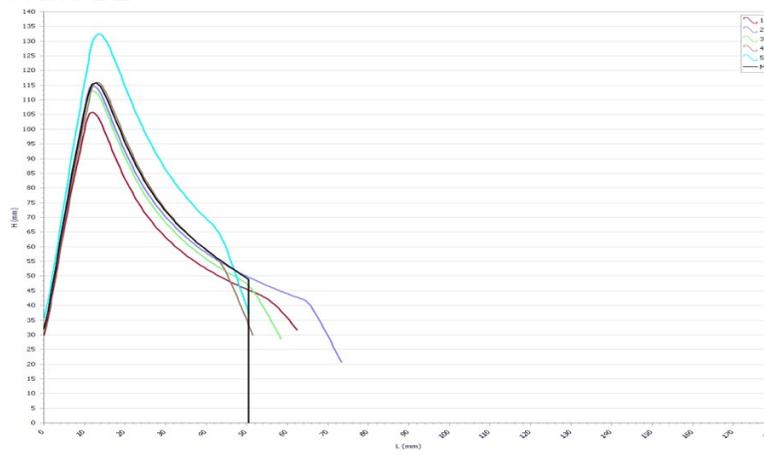
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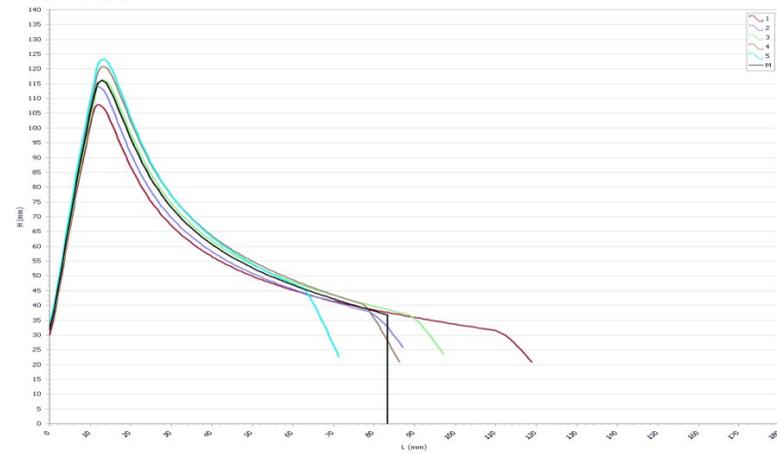
Julius SH



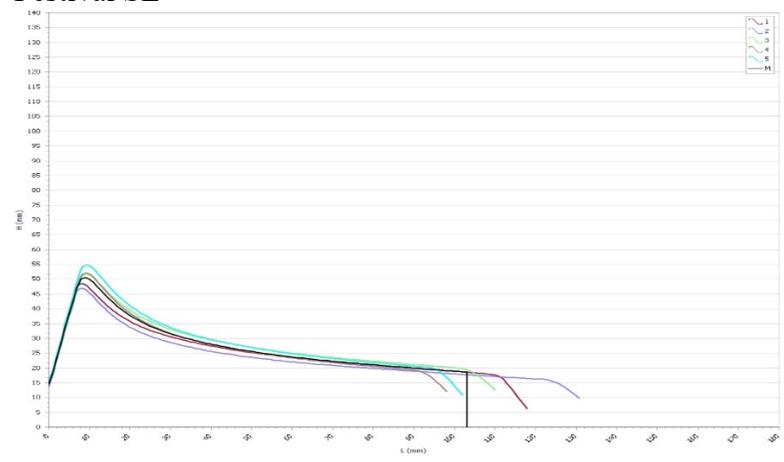
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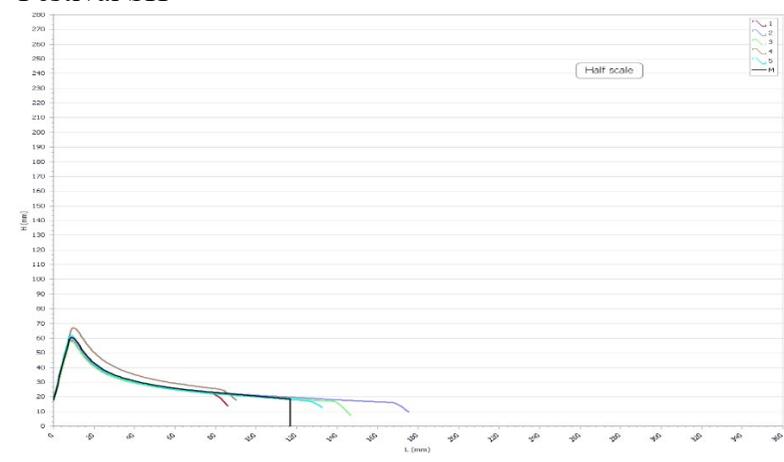
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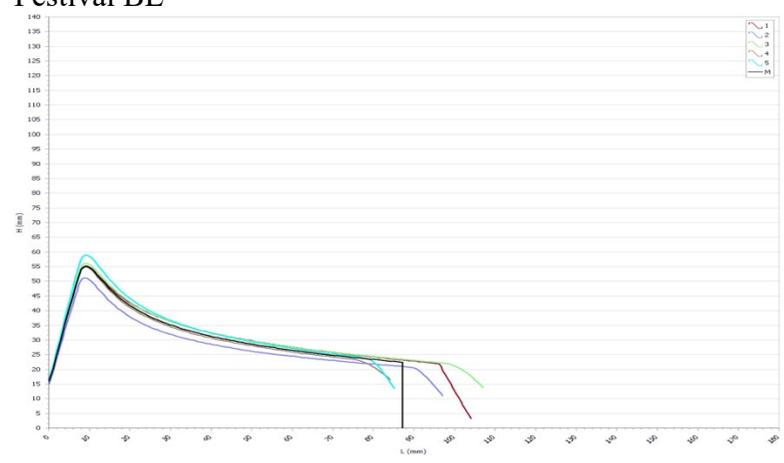
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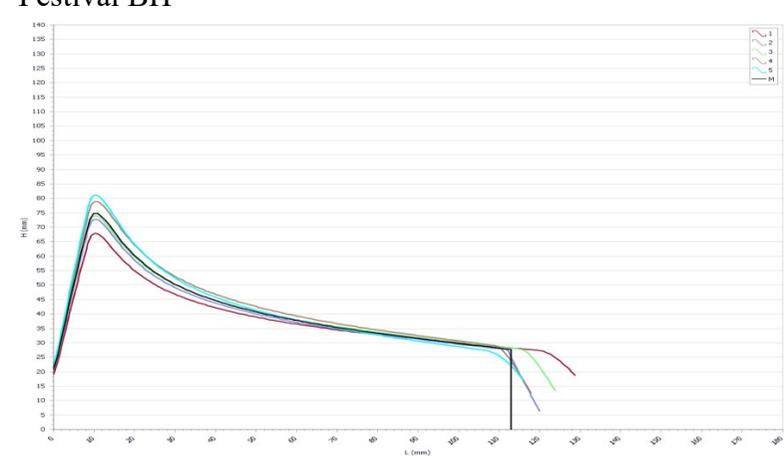
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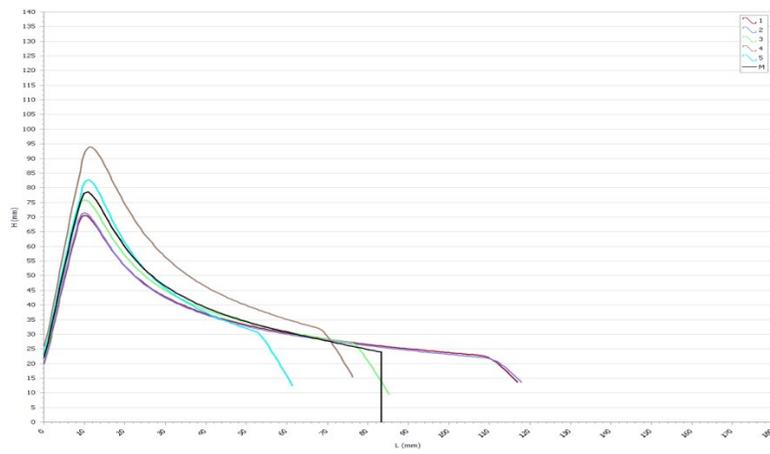
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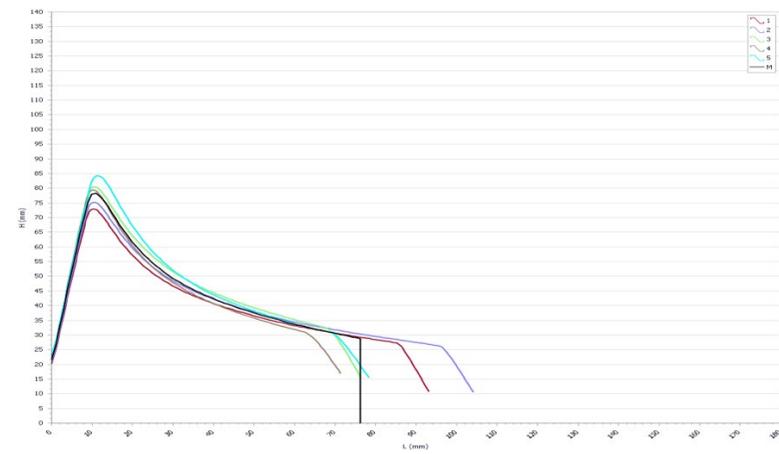
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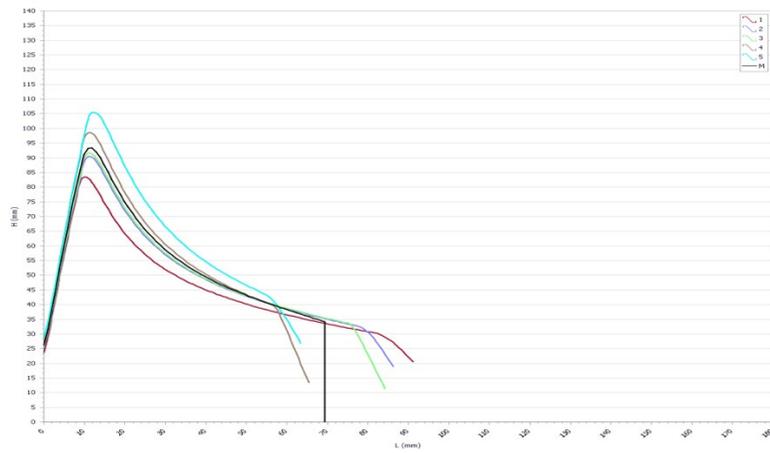
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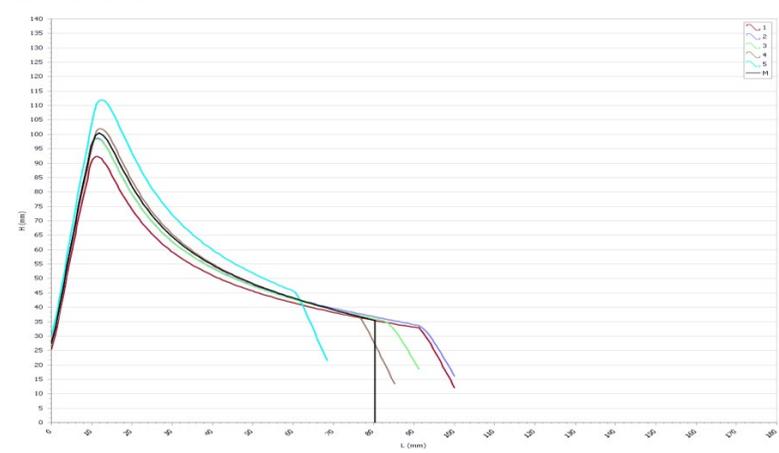
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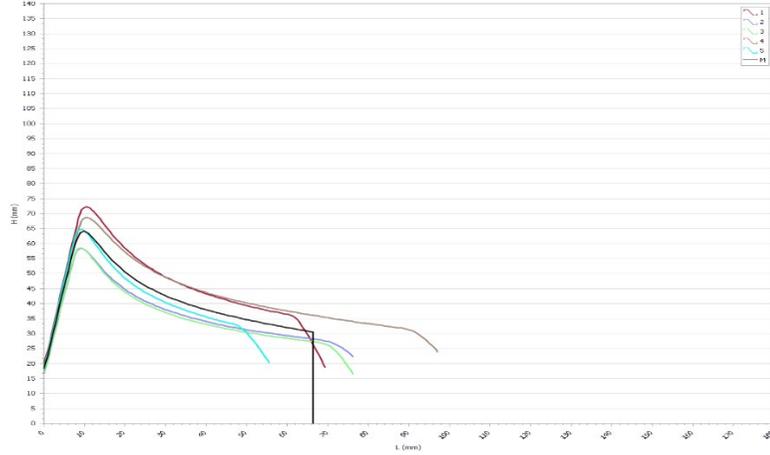
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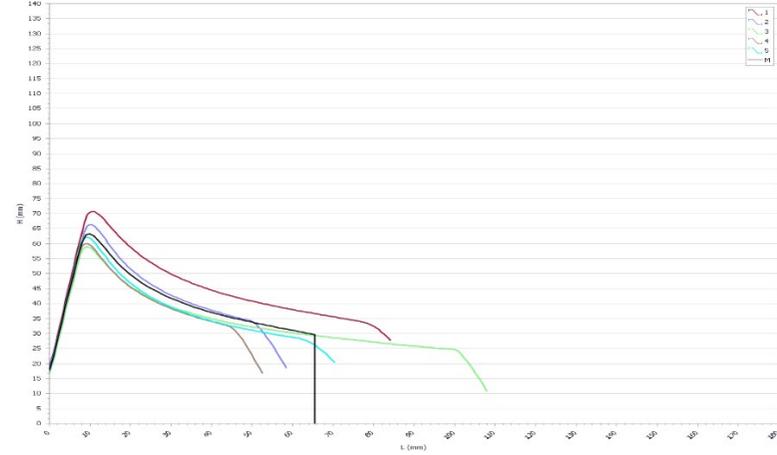
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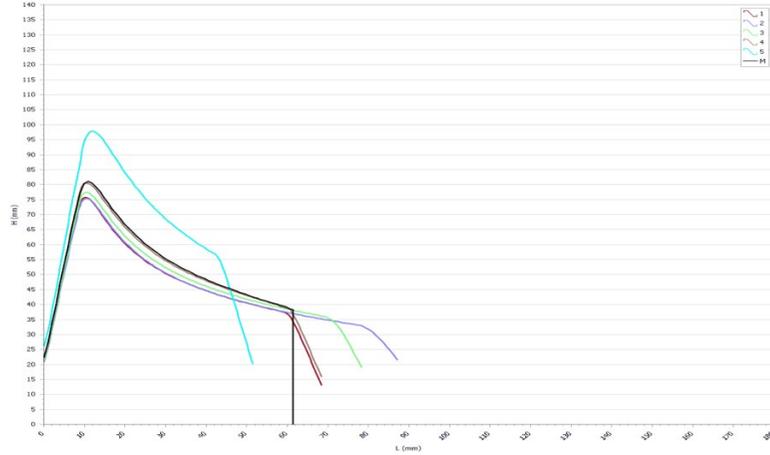
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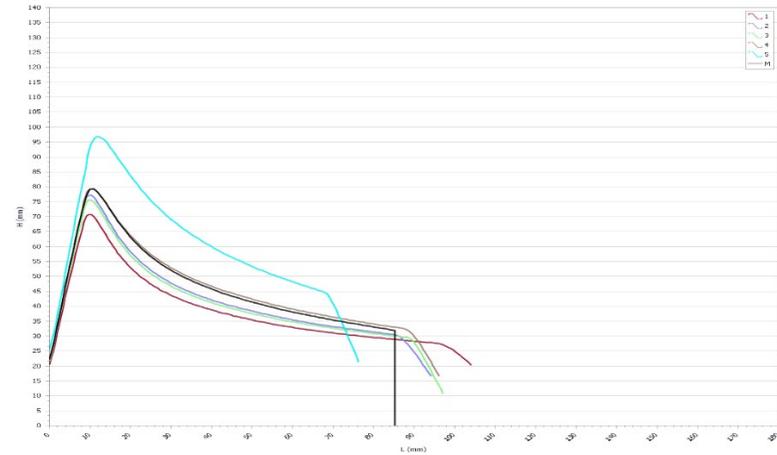
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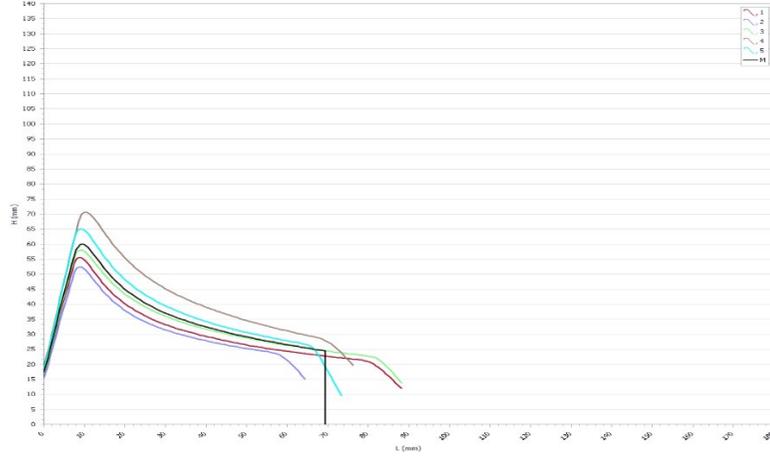
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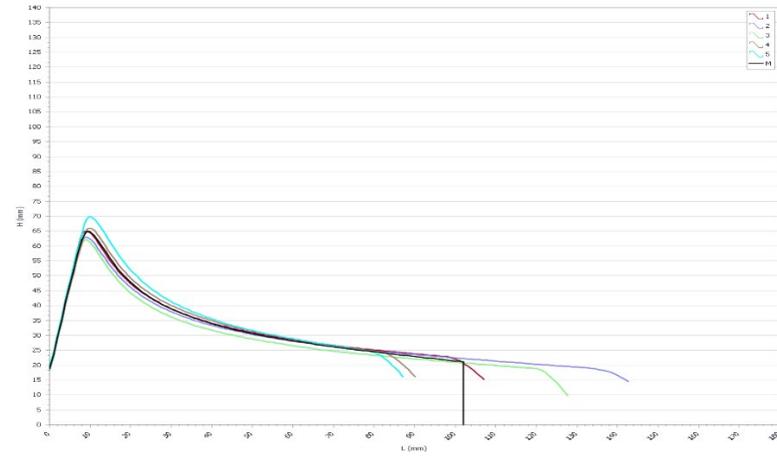
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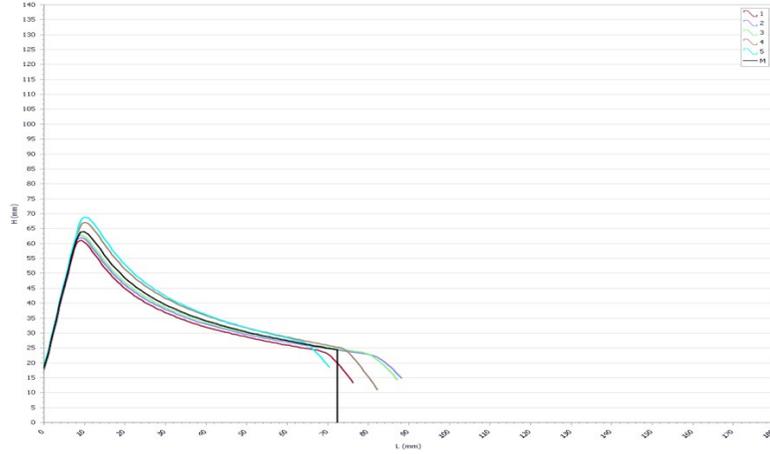
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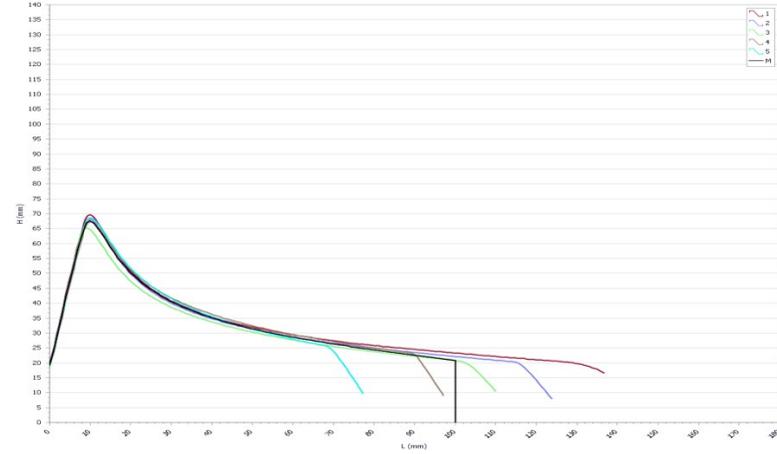
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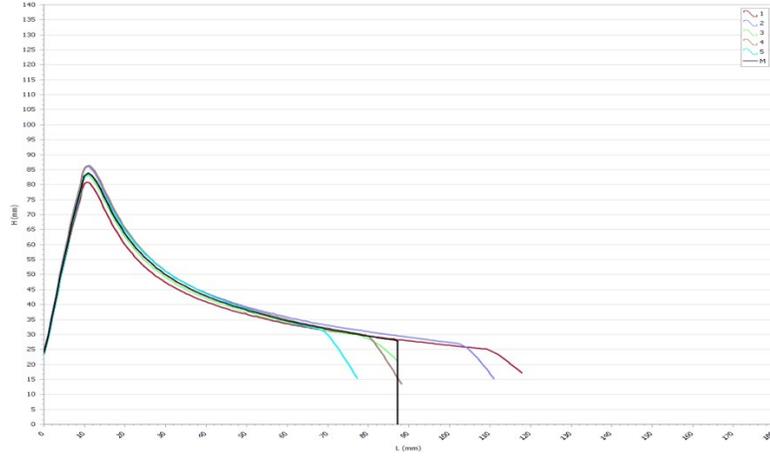
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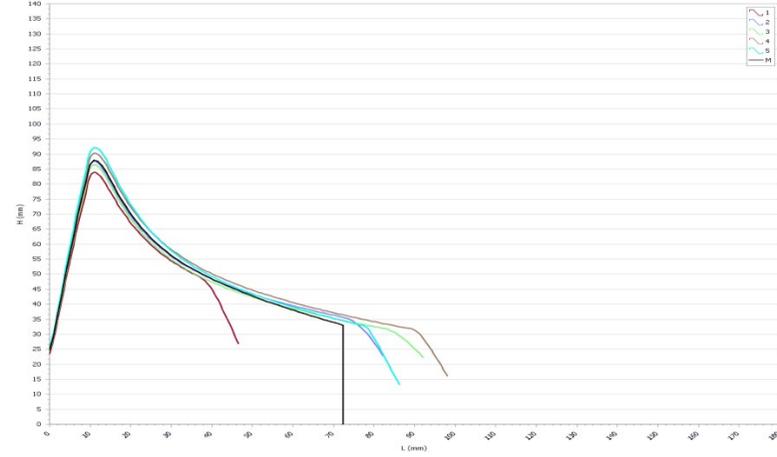
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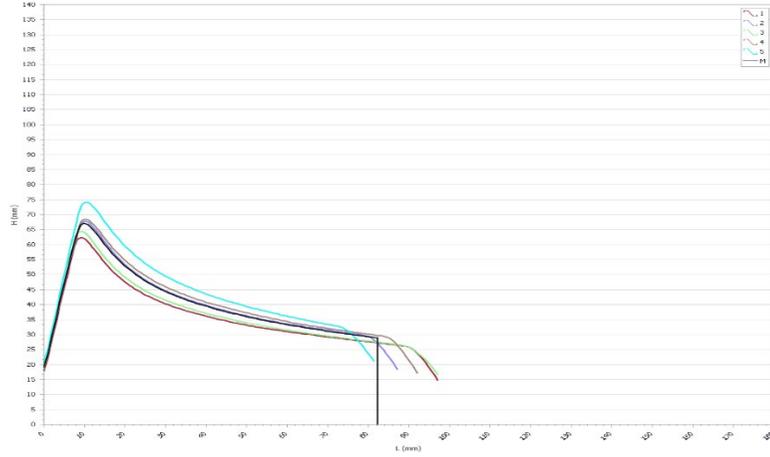
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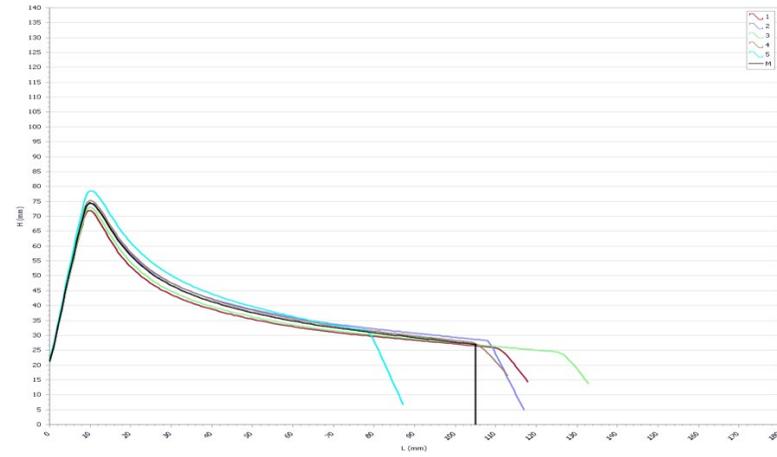
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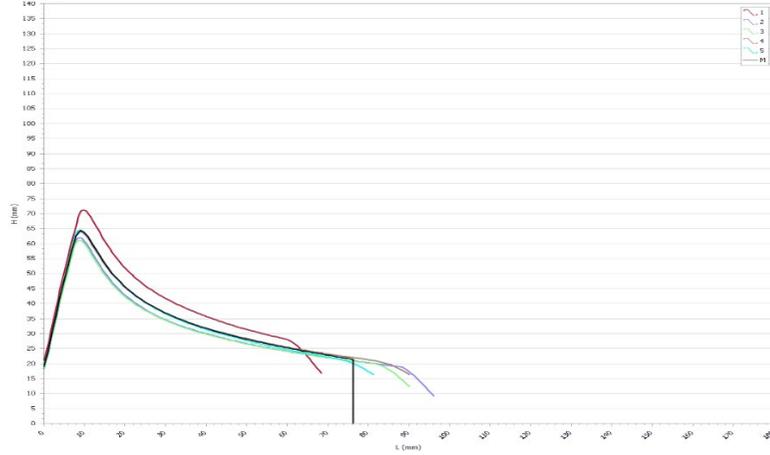
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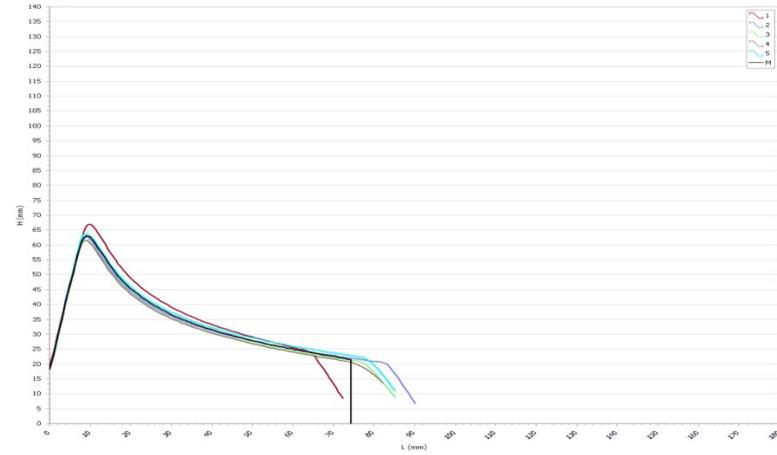
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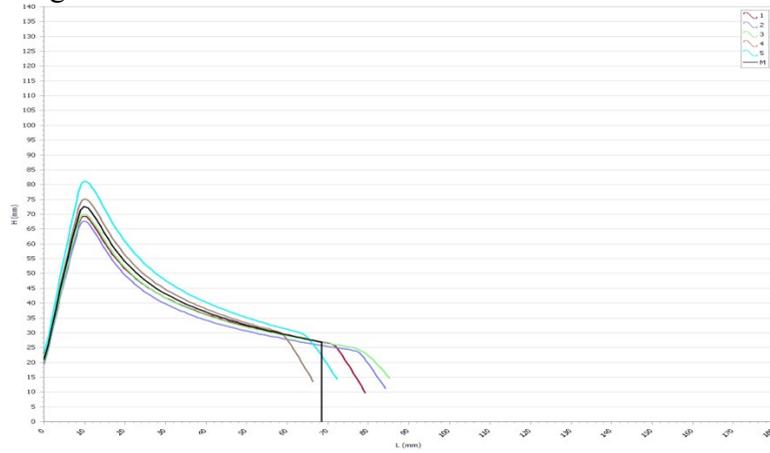
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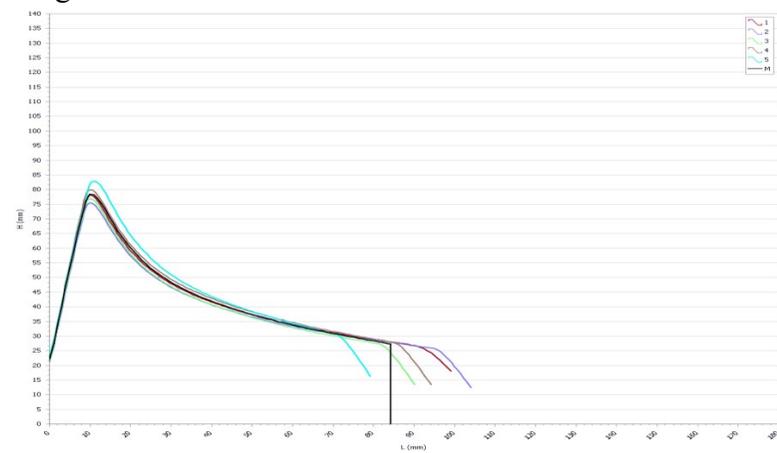
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Bright BL



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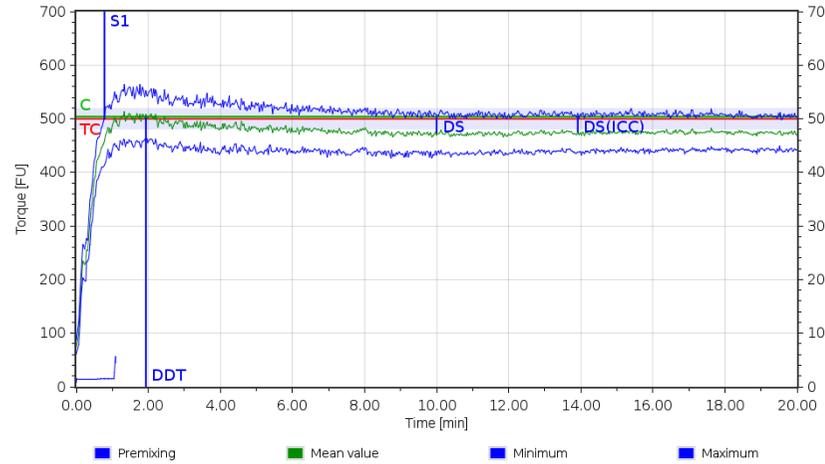


## Appendix 2 – Farinogram

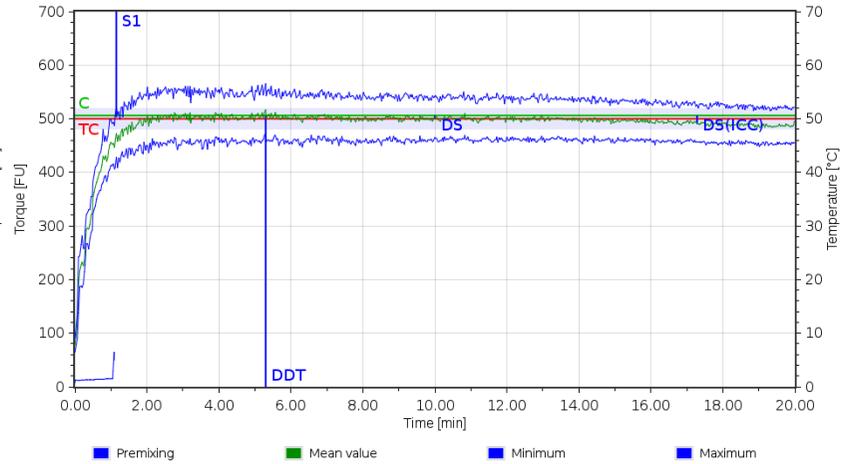
Below, all farinogram that were obtained during the farinograph operation are presented. To interpret the farinogram (see also Figure 3), the following abbreviations are described.

<b>Point</b>	<b>Unit</b>	<b>Description</b>
C	FU	Consistency
DDT	min	Development time
DS	FU	Degree of softening (10 min after beginning)
DS(ICC)	FU	Degree of softening (ICC / 12 min after max.)
S	min	Stability

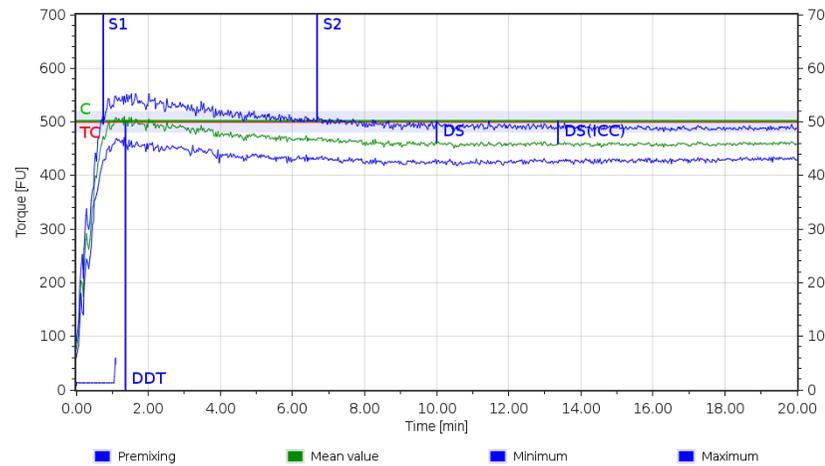
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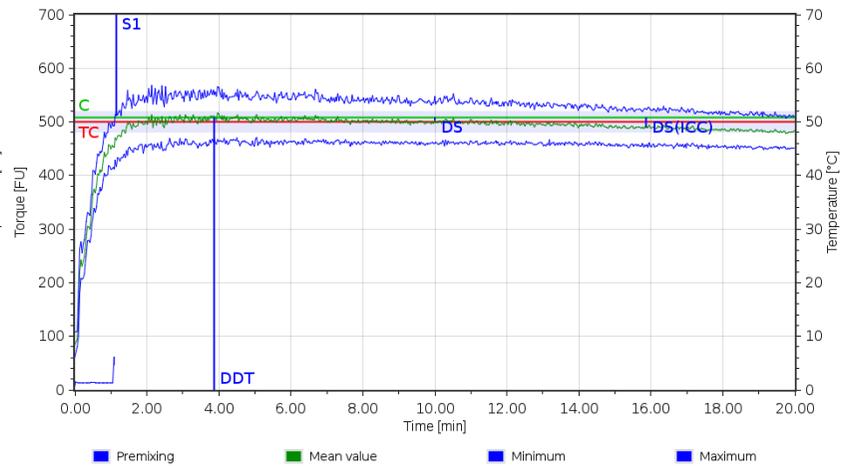
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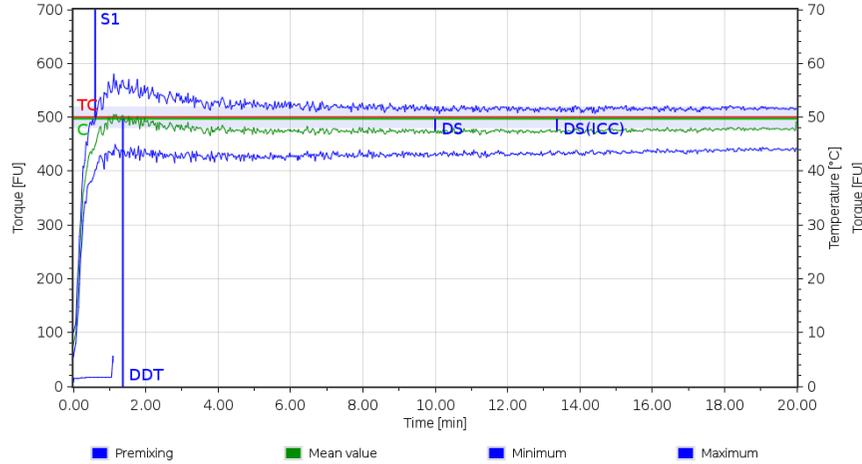
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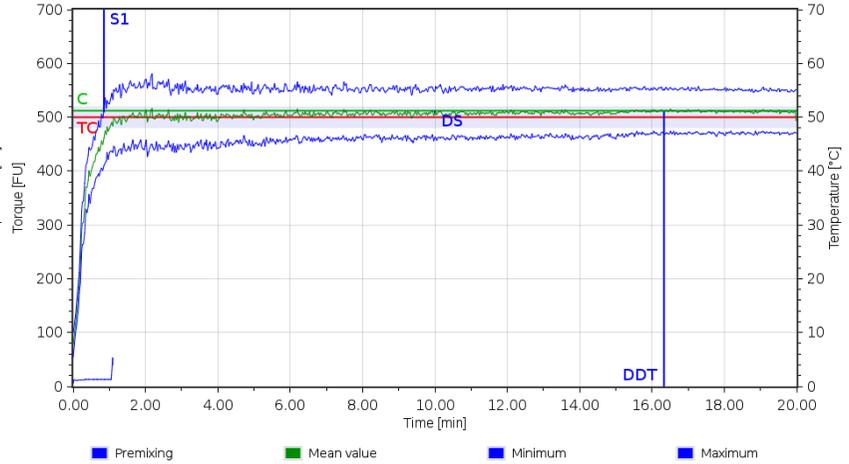
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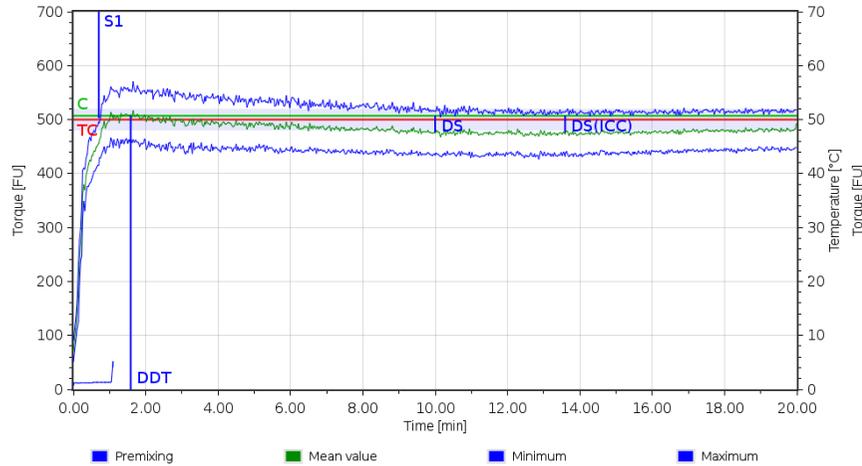
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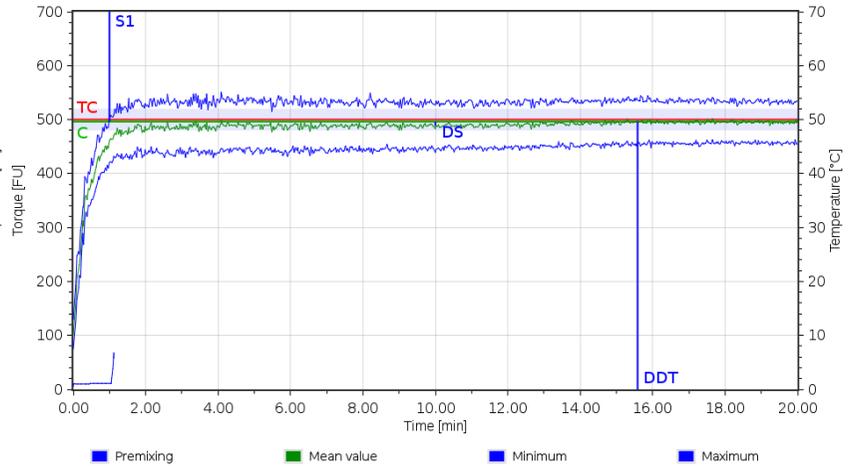
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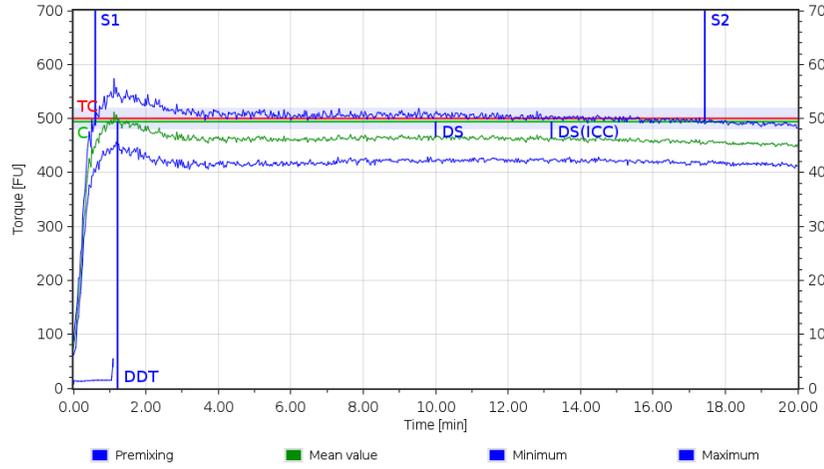
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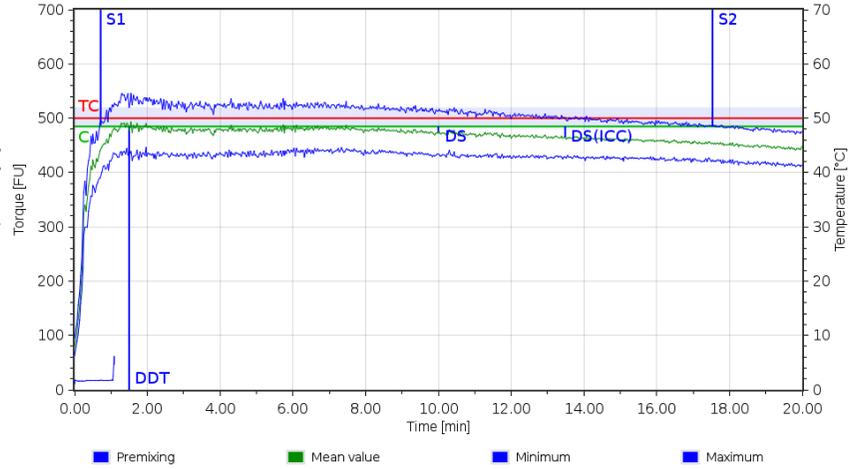
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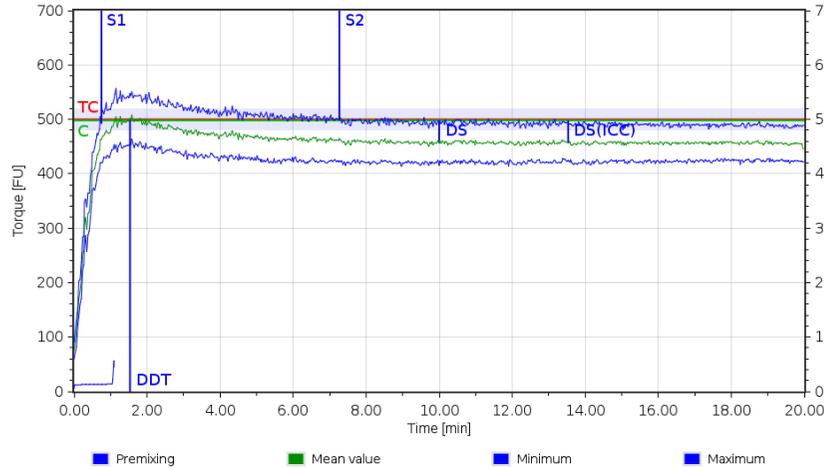
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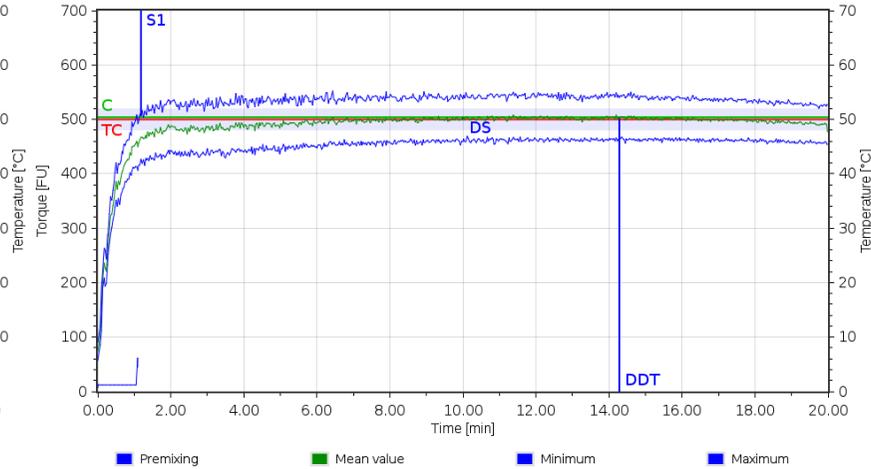
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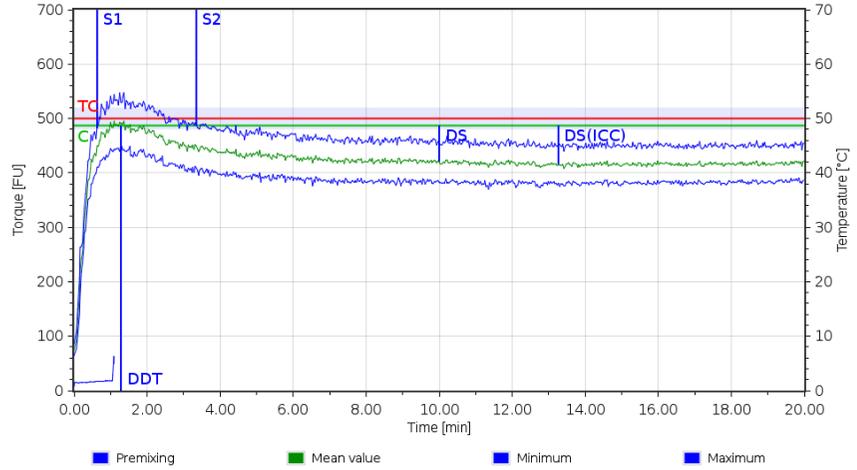
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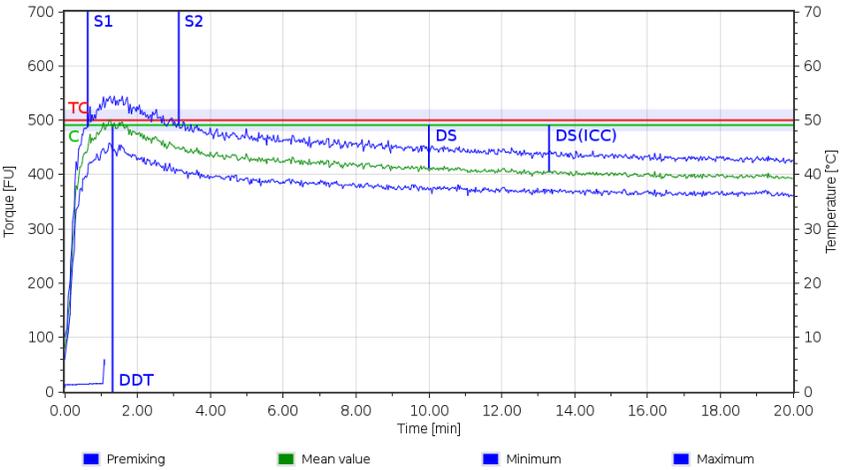
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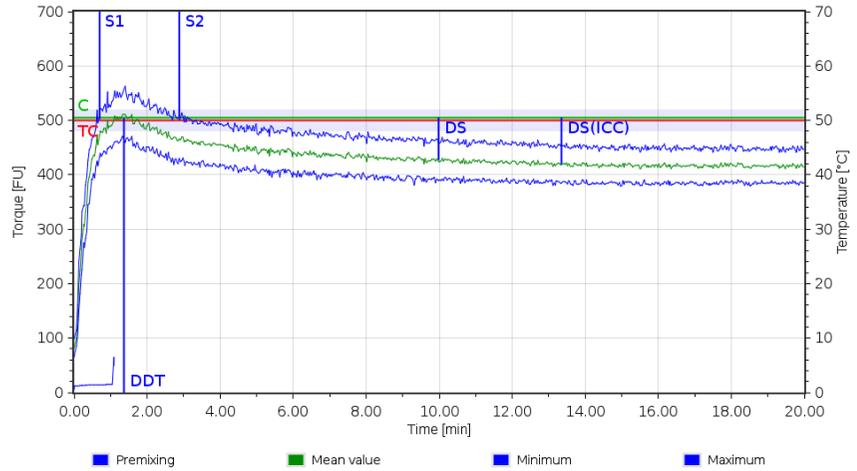
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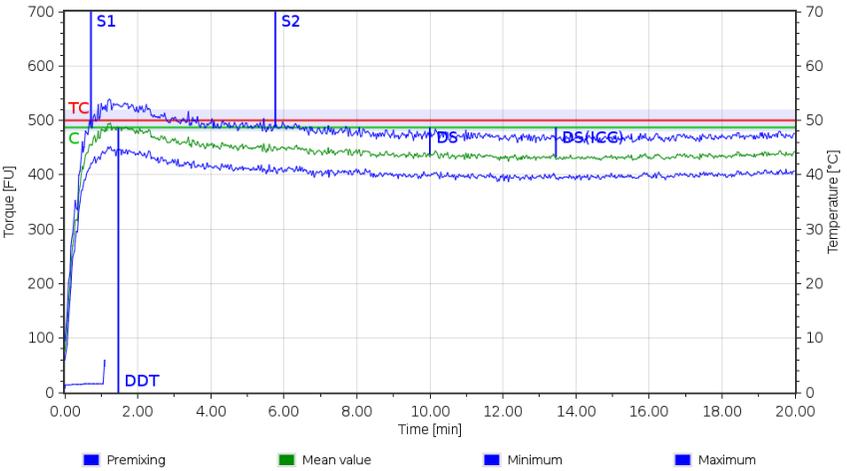
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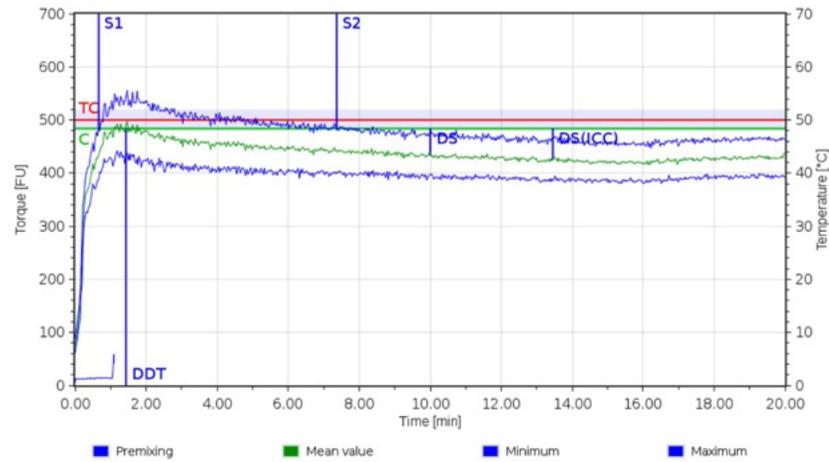
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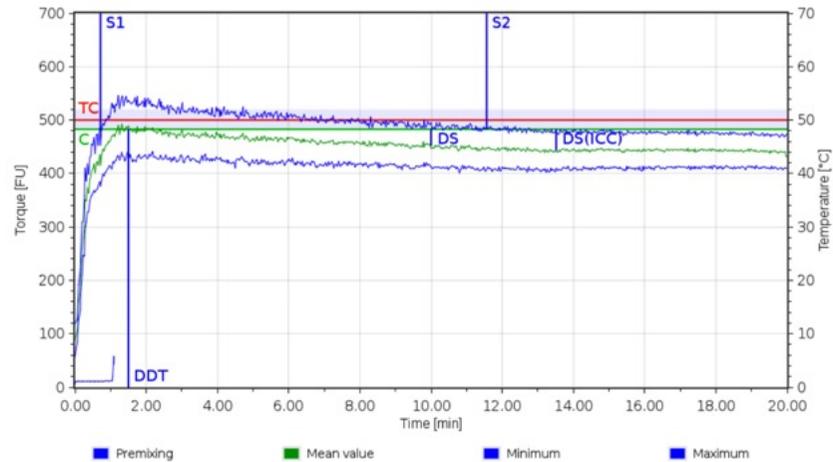
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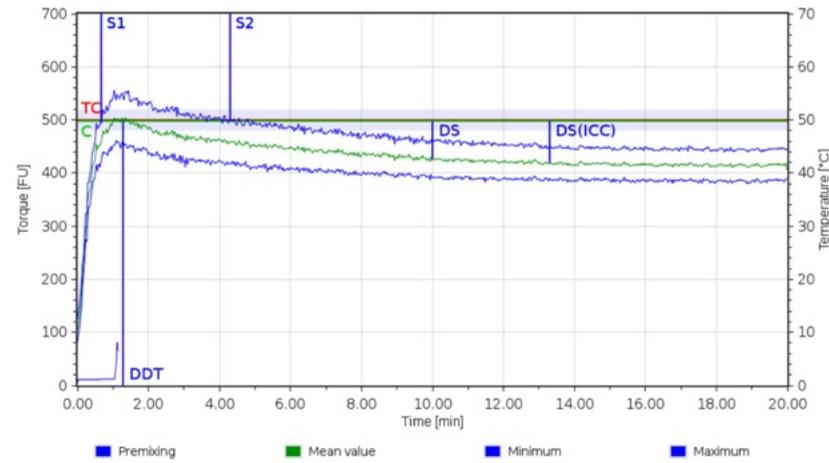
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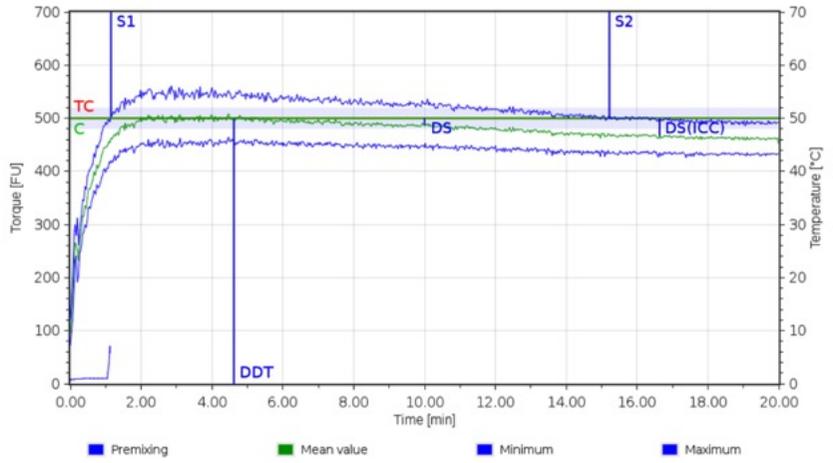
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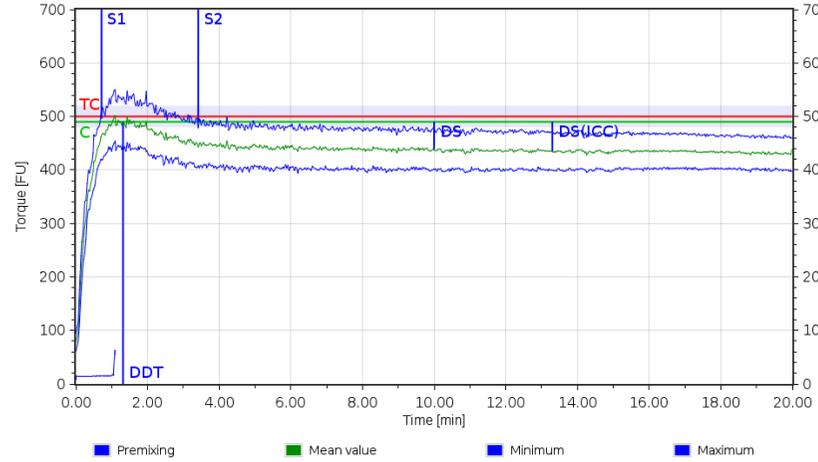
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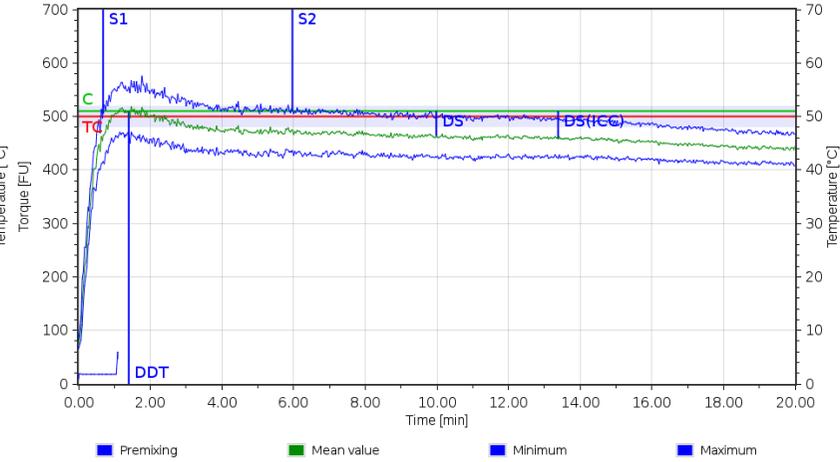
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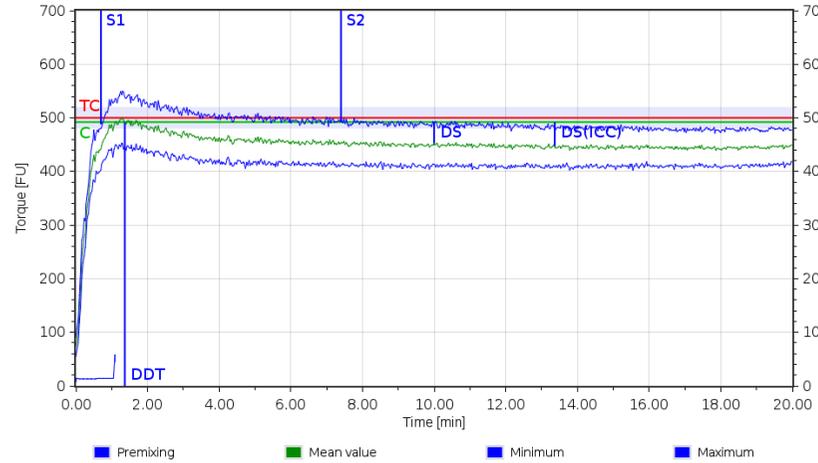
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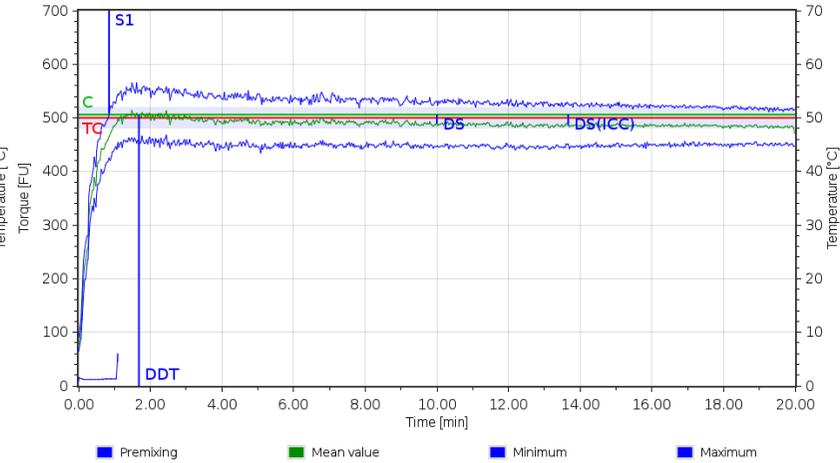
Norin SH



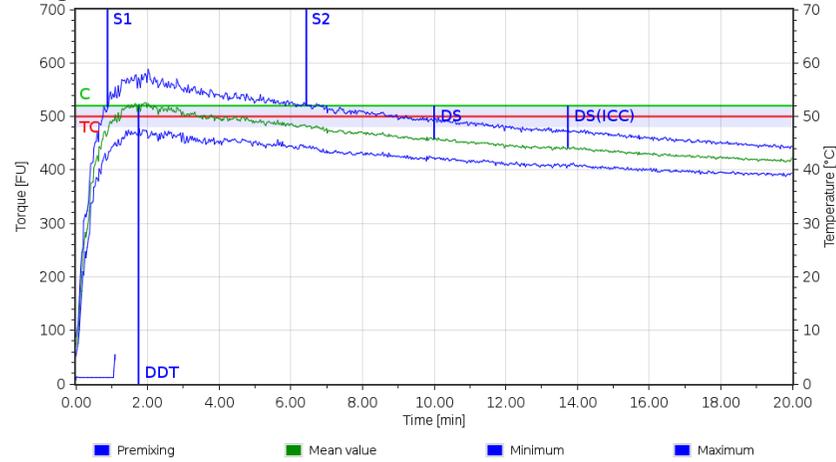
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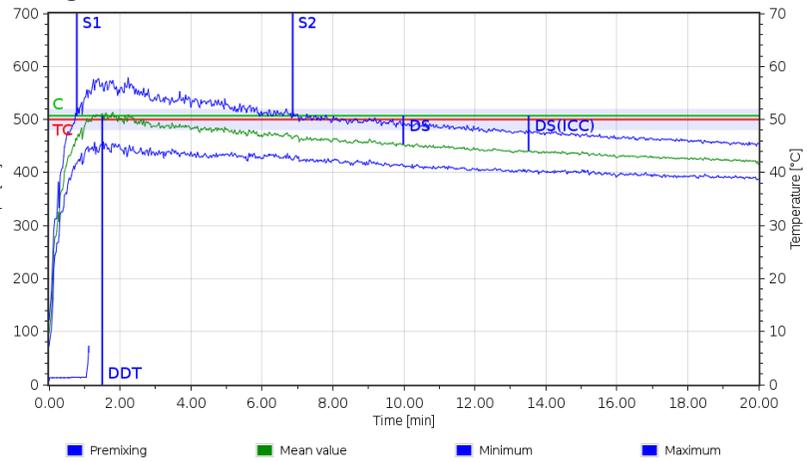
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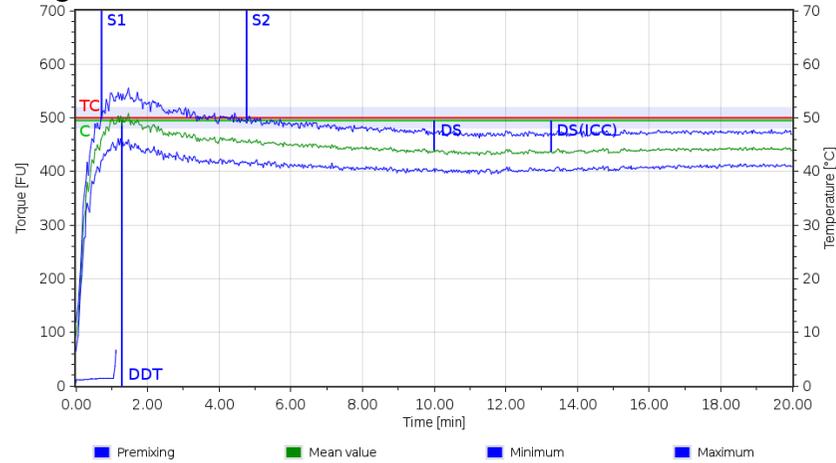
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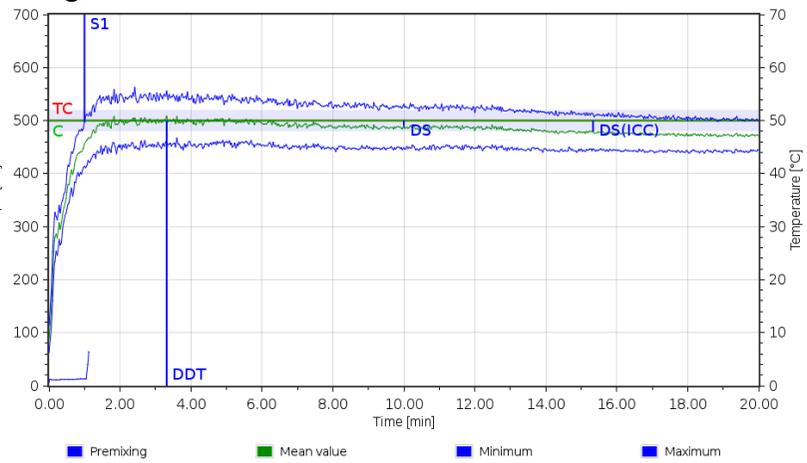
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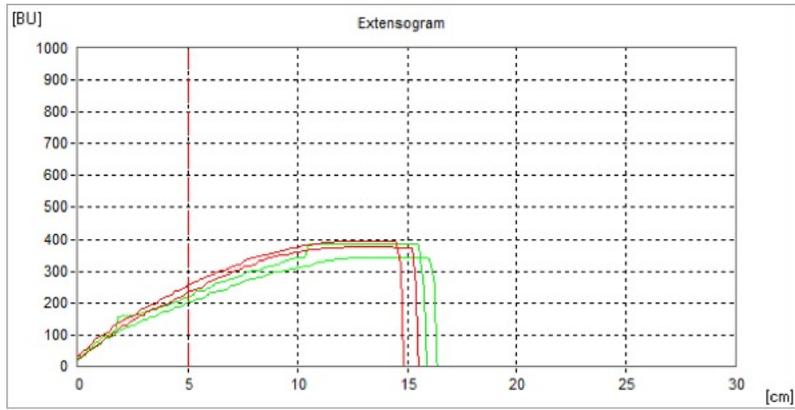


## Appendix 3 – Extensogram

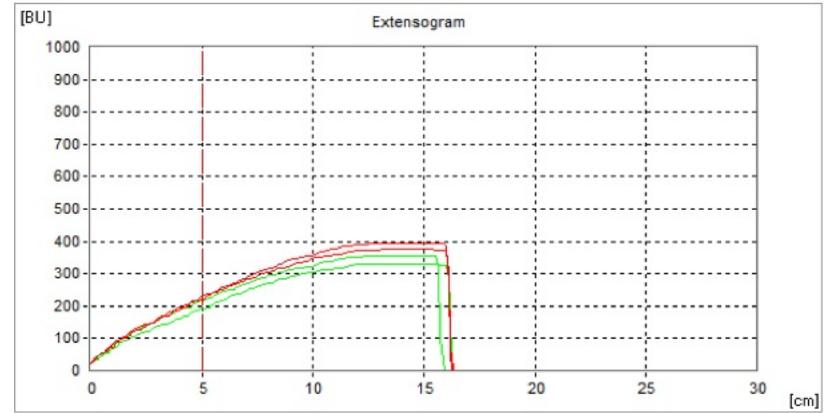
Below, all extensogram that were obtained during the extensograph operation are presented. To interpret the extensogram (see also Figure 4), the following abbreviations are described.

<b>Point</b>	<b>Unit</b>	<b>Description</b>
Energy	cm <sup>2</sup>	Area under the curve
Ext.	mm	Extensibility
Max.	BU	Maximum
RN		Ratio Number
RN Max.		Ratio Number (Max.)
RTE	BU	Resistance to Extension

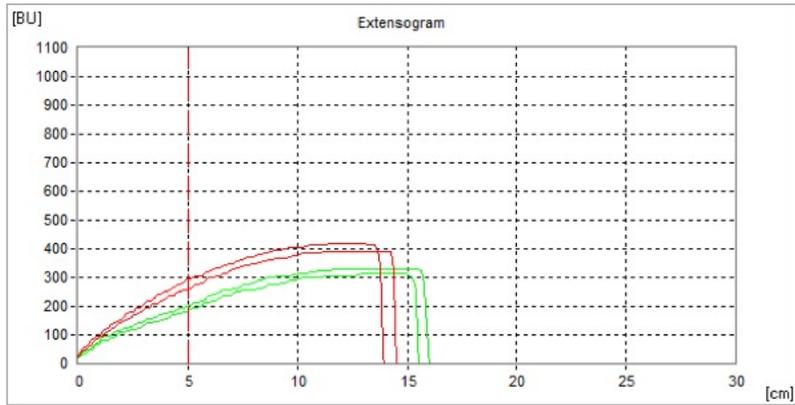
Julius SL



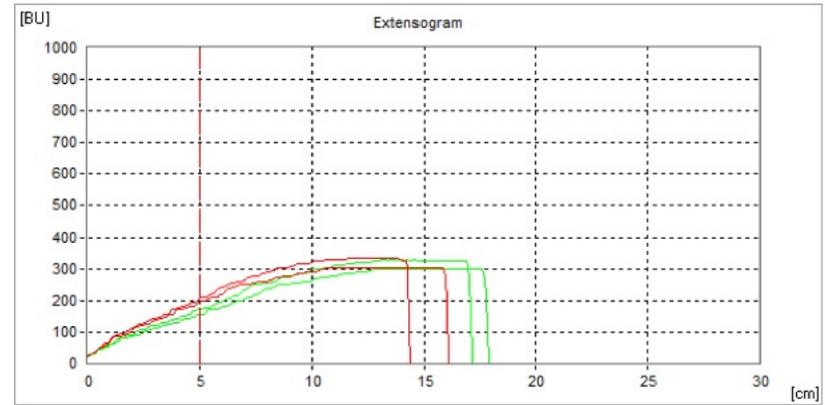
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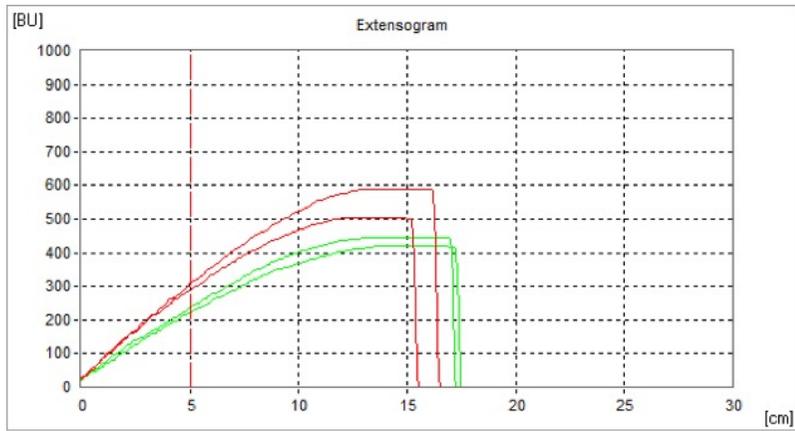
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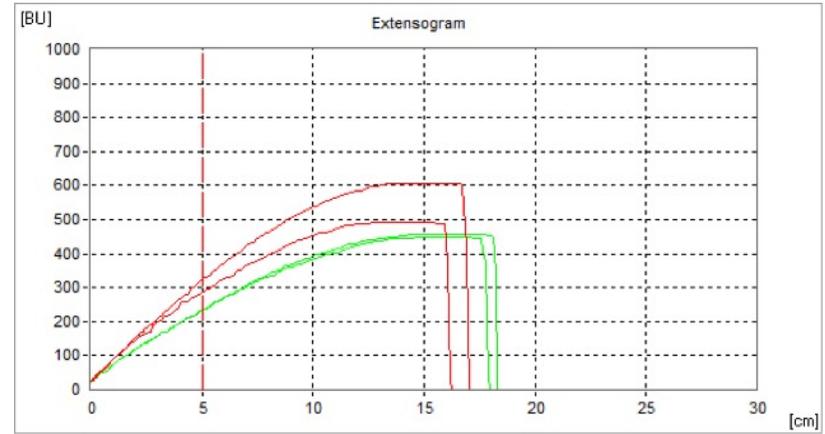
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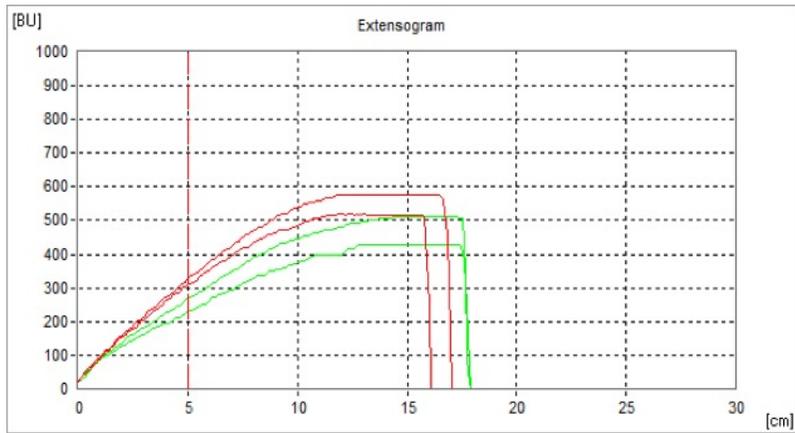
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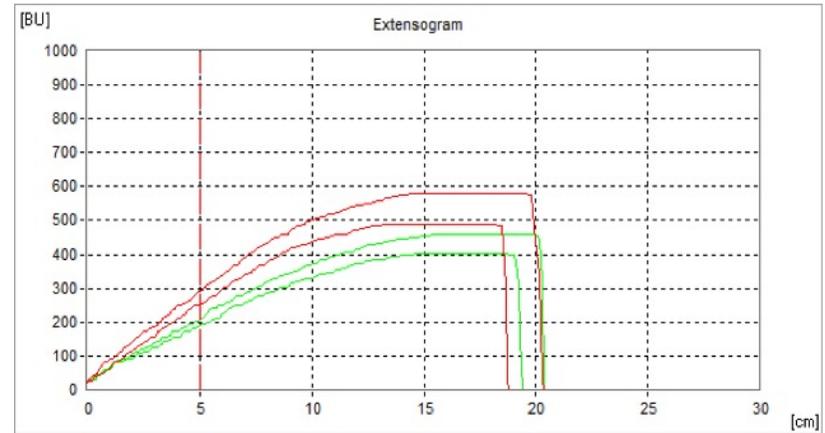
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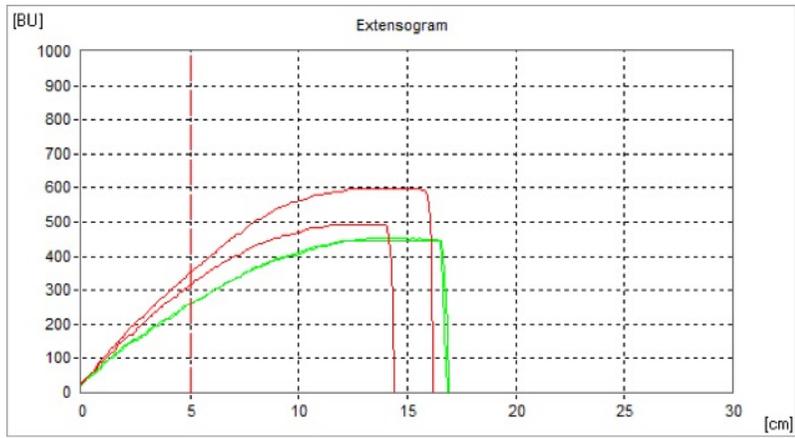
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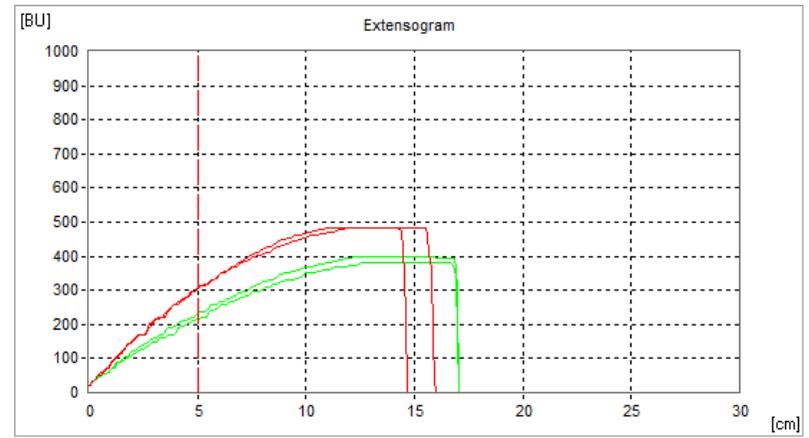
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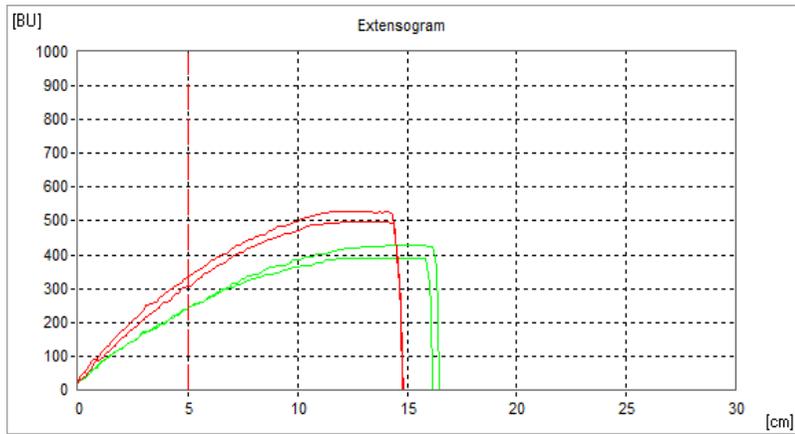
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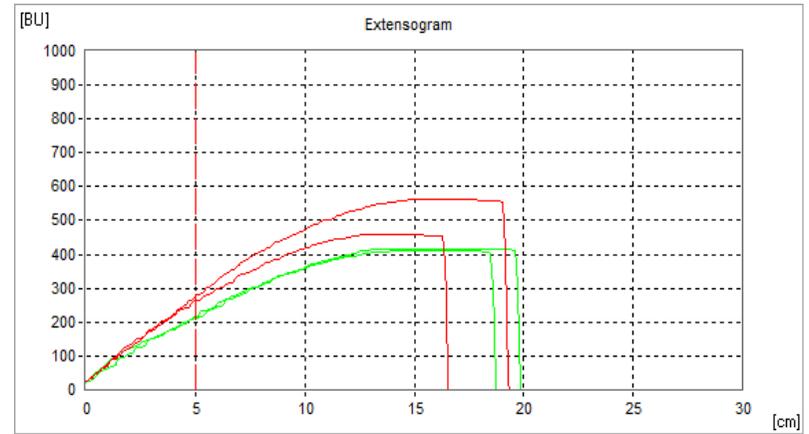
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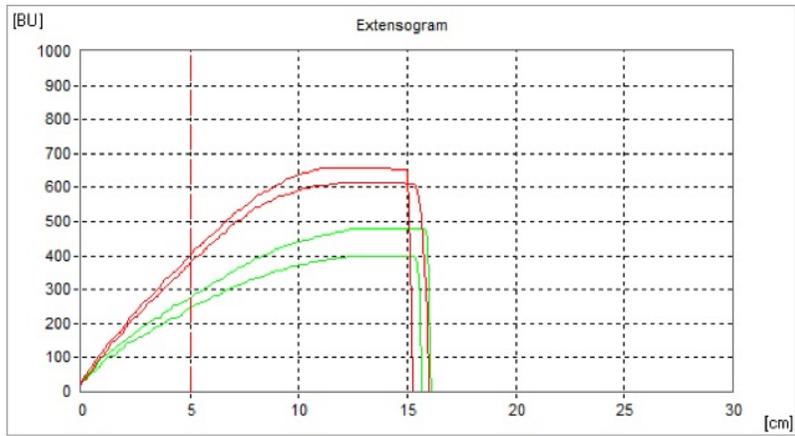
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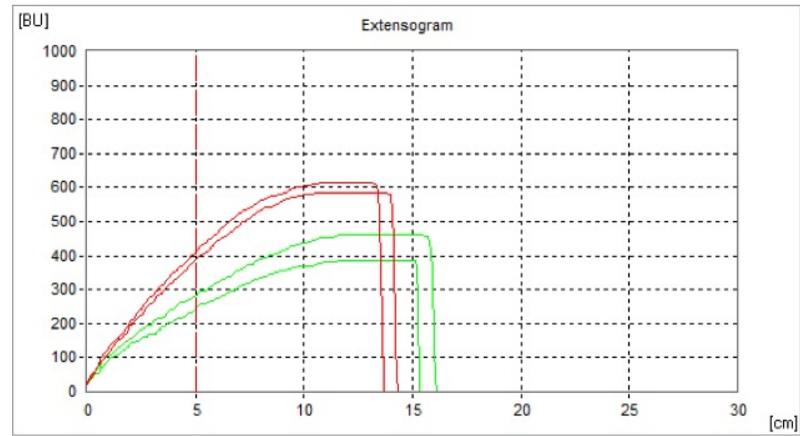
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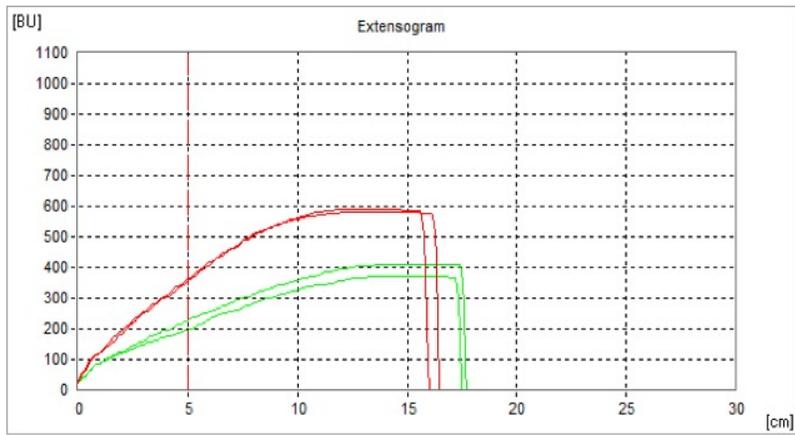
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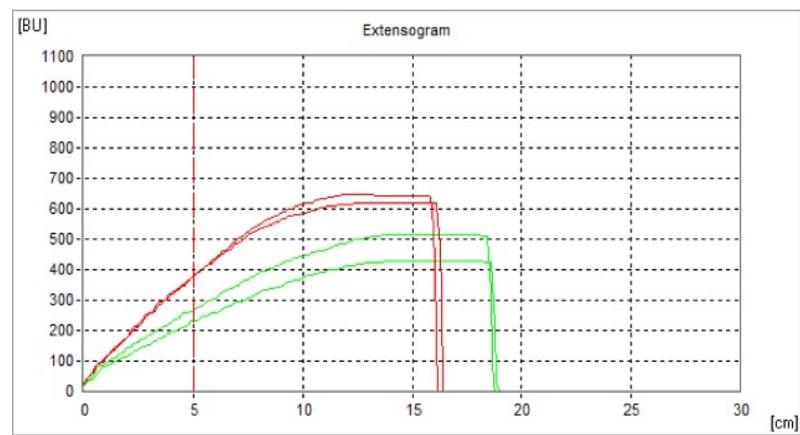
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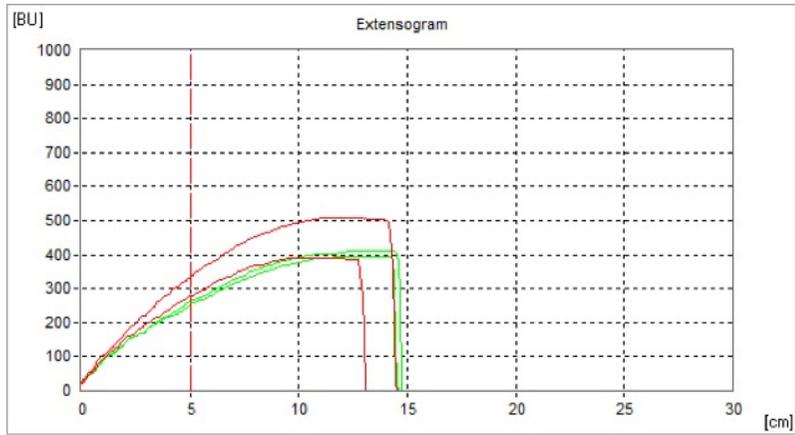
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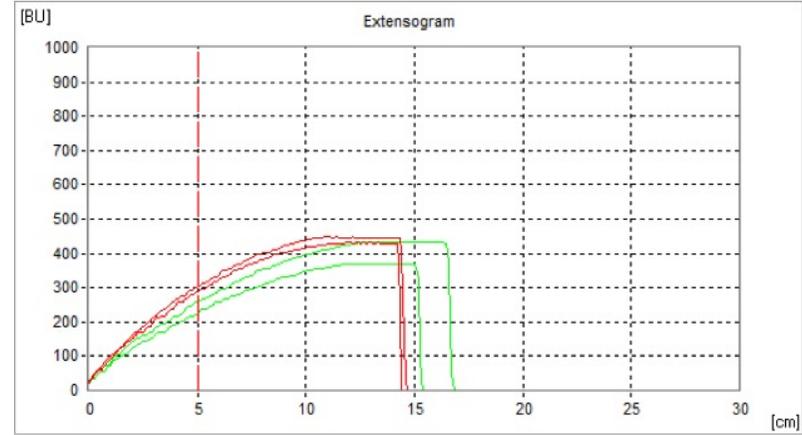
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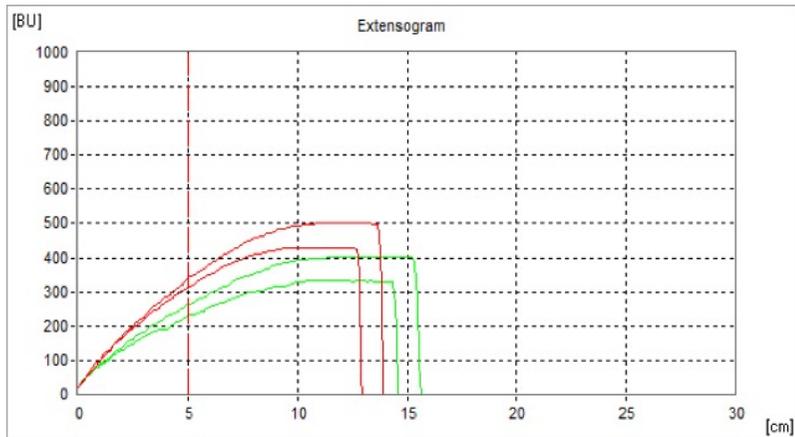
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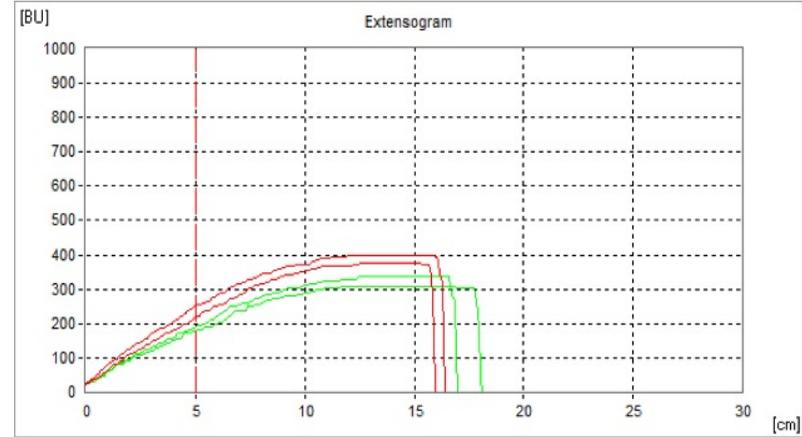
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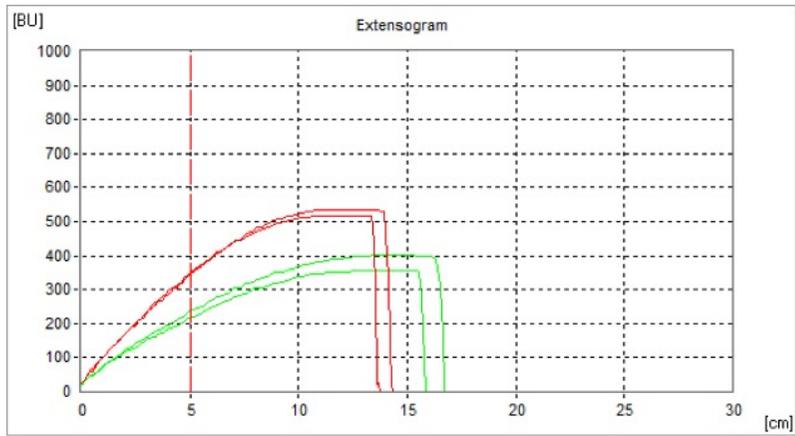
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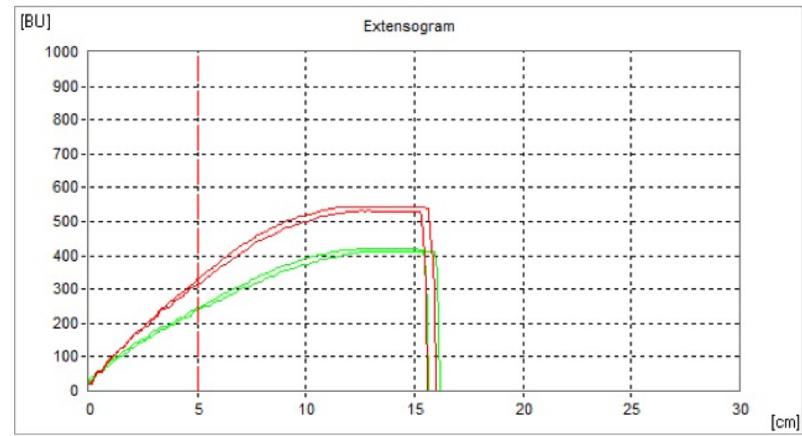
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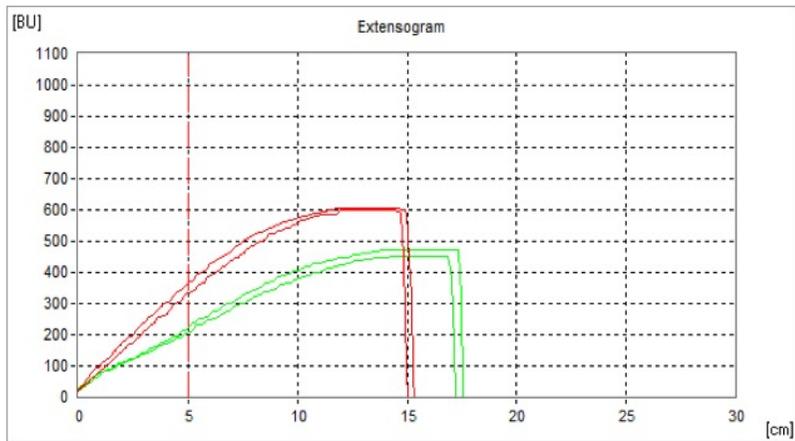
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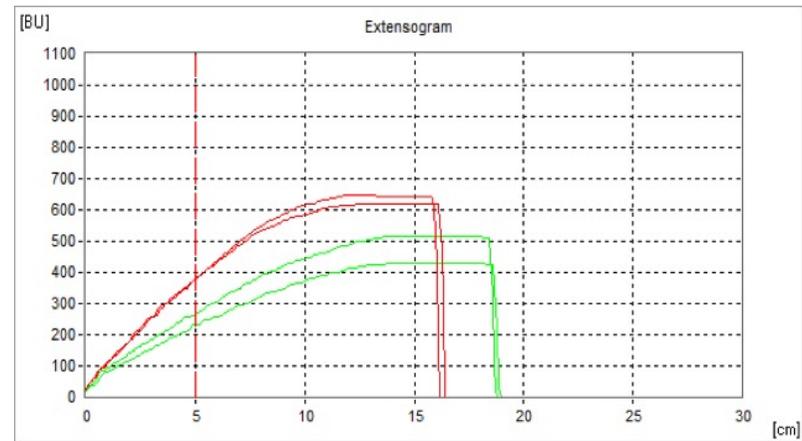
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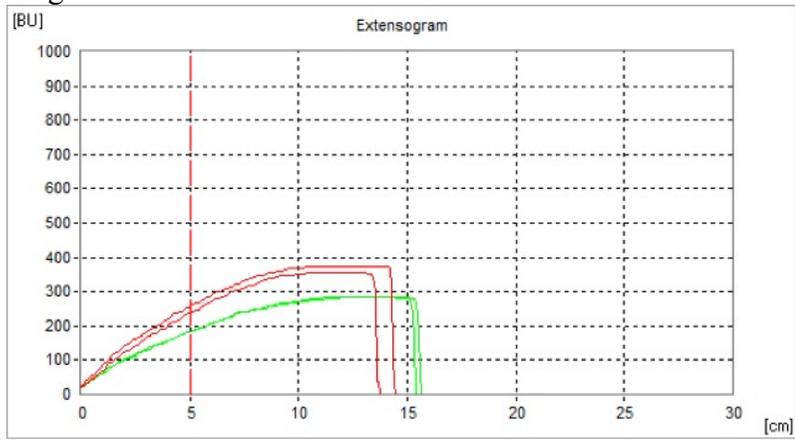
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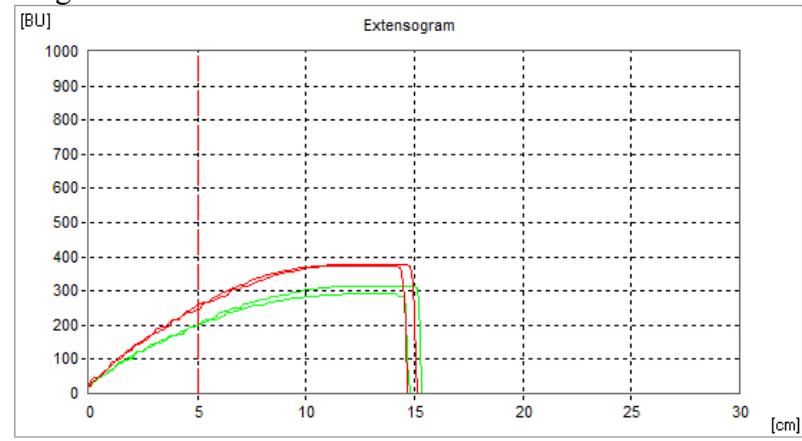
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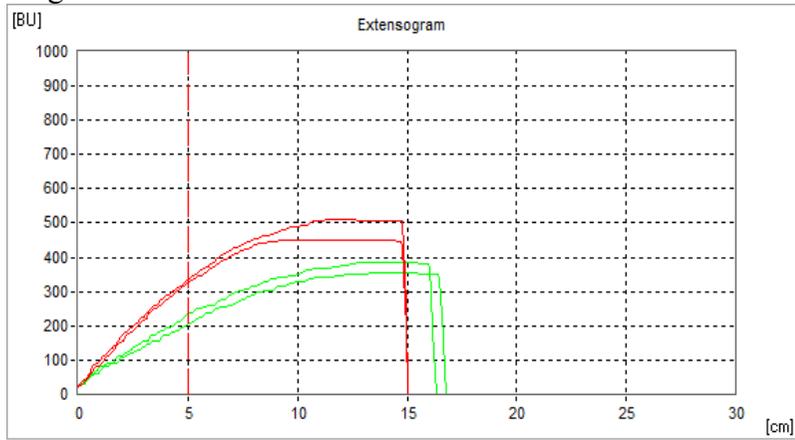
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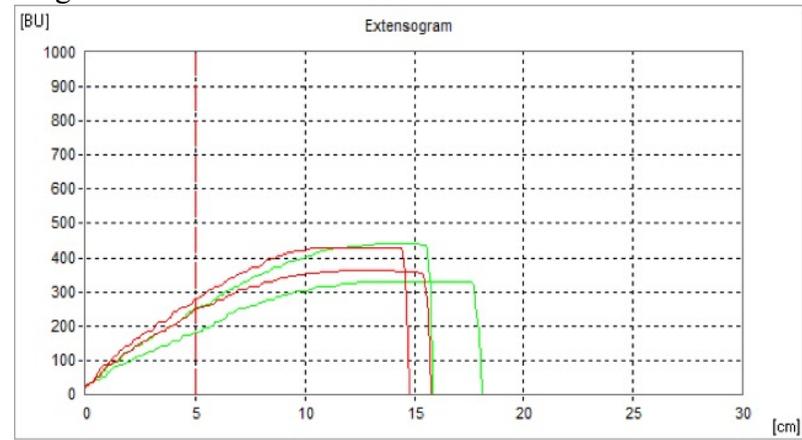
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Bright BL



Bright BH



## Appendix 4 – Popular scientific summary

### **A bag of wheat flour – how much can it vary?**

Flour, water, yeast and salt are the minimum ingredients needed to make bread. Still, the resulting bread quality, most apparently its volume, may vary greatly. How come that different flours have different breadmaking potential? Well, it is based on the genes of the wheat crop, but not only the genes. Environmental conditions, such as temperature, fertilisation of the crop, and *where* the crop is cultivated are all factors that will influence how well the wheat flour will bake.

Did you know that one bag of wheat flour, intended to be used for breadmaking, may contain wheat from several different varieties? Perhaps you ask yourself why? Is it because we cultivate different wheat varieties, or is it due to another reason? Well, it is due to both.

Wheat varieties have different properties. Otherwise, only one variety would be needed. The breeder has to take a lot of factors into consideration when developing a new variety. Primarily, the wheat plant has to tolerate the soil, being strong enough and withstand diseases. Later, farmers get paid based on protein content, explaining the importance of having a wheat variety with a high such. The kernels should also be condensed, meaning that they weigh much. They should also have a high falling number, which is a wheat quality indicator. Finally, wheat varieties also need to have good quality to enable breadmaking. As you hear, there are a lot of factors that you have to keep in mind.

Wheat flour mainly consists of starch and protein. Together with water, they can form a dough, owing to the formation of a gluten network. Different wheat varieties have different genomes, just as us humans. Genomes contains all the information needed to produce the wheat plant. This means that different varieties have different amount and composition of gluten proteins, based on their genome. External environmental conditions will also influence how big and complex the gluten network will be. Altogether, this will influence the baking quality.

This master thesis was performed in collaboration with Lantmännen. The aim was to investigate how baking quality of bread is affected by variety, cultivation site and nitrogen fertilisation. In this study, seven winter wheat varieties were used: Julius, Festival, Brons, Hallfreda, Kask, Norin and Bright. These were cultivated at two different sites in Sweden, Svalöv and Bjertorp, with varying amount of nitrogen fertilisation. This resulted in four different environments (two cultivation sites x two nitrogen supplies). All 28 samples were milled and a lot of different parameters were measured by using different analytical methods.

The obtained results from this study indicates that the varieties and the environments affect the baking quality parameters differently. Breadmaking properties, such as peak viscosity of the flour, bread volume and dough tenacity seemed all to be linked to variety. For example, Julius and Brons had a lower peak viscosity, whereas Norin had a higher one. Hallfreda had a lower wet gluten content and bread volume in comparison with all other varieties. Still, Hallfreda was fertilised with the highest amount of nitrogen. Flour protein content was instead affected by the environment. For those samples that were cultivated with a low nitrogen supply, cultivation site did not affect baking quality, and for the samples cultivated in Svalöv, baking quality was not affected by differences in nitrogen fertilisation.

The take home message from this study is that the baking quality of bread is affected both by the variety and the environment, but in different ways. By knowing the breadmaking potential of different flours, these can be blended into the flour bag to complement each other's properties. Because if the knowledge is scarce, baking quality between the bags of wheat flour will vary a lot.