



Sveriges lantbruksuniversitet  
Swedish University of Agricultural Sciences

Faculty of Natural Resources  
and Agricultural Sciences

# Barley starch, structure and properties

Korn stärkelse, struktur och egenskaper

*Jenny Eriksson*

**Barley starch, structure and properties**  
Korn stärkelse, struktur och egenskaper

*Jenny Eriksson*

**Supervisor:** Anna Källman, Department of Food Science, SLU, Uppsala

**Examiner:** Kristine Koch, Department of Food Science, SLU, Uppsala

**Credits:** 30hec

**Level:** Advanced A2E

**Course title:** Independent project/degree project in Food Science - Master's thesis

**Course code:** EX0425

**Programme/education:** Agronomist of food science

**Place of publication:** Uppsala, Sweden

**Year of publication:** 2012

**Title of series:** Publikation/Sveriges lantbruksuniversitet, Institutionen för livsmedelsvetenskap

**Serie no:** 362

**Online publication:** <http://stud.epsilon.slu.se>

**Key words:** Starch, Amylose, Amylopectin, Viscosity, Limit Dextrins



## Abstract

Starch is a naturally occurring substance in most plants and is used as energy storage. It is a component that mainly consists of amylose and amylopectin. Together they build a complex pattern which gives starch its specific properties. The proportion of amylose and amylopectin varies in different plants but generally starch consists of 25 % amylose and 75 % amylopectin. There have been discoveries of barley varieties containing 100 % amylopectin called “waxy starches” or “waxy barley” as well as varieties that have a high content of amylose, around 70 % and these are called “amylotypes”.

Amylopectin as well as amylose consists of  $\alpha$ -1, 4 linkage between  $\alpha$ -D-glucose. The great difference between them is the degree of branching. In amylopectin, 4 to 5 % of the glycosidic bonds are  $\alpha$ -1, -6 bonds. The average chain length is shorter than in amylose, but with more branching it is a larger molecule. With the different branching the molecule forms an intricate structure. The chains of amylopectin are believed to be organized in clusters which bind to each other with the assistance of a certain type of chains.

In this study six different barley varieties were investigated according to starch structure and properties. In order to fully characterize these samples, several different methods were used in a combination. Gel Permeation Chromatography was performed to separate the amylose from the amylopectin, and then the collected fractions were analysed with the Phenol-Sulphuric acid method. The properties of the samples were examined with RapidViscoAnalyser that measures the viscosity during a temperature increase and decrease with continuous stirring. The chain length distribution of amylopectin and its  $\alpha$ ,  $\beta$ -limit dextrins were studied with High Performance Anion Exchange Chromatography - Pulsed Amperometric Detection.

The amylose content varied between 0.6 % and 48 % in the different starches. The RVA demonstrated that the more amylose and fibres the samples contained the higher final viscosity. The High Performance Anion Exchange Chromatography - Pulsed Amperometric Detection showed no clear indication of difference between the samples and to establish the whole structure of these six barley varieties and their properties more research is necessary.

**Key Words:** Starch, Amylose, Amylopectin, Viscosity, Limit Dextrins

## Sammanfattning

Stärkelse är en naturligt förekommande substans i de flesta växter och det används för att lagra energi. Det är ett ämne som huvudsakligen består av amylos och amylopektin. Tillsammans bygger dessa komponenter ett komplext mönster som ger stärkelse dess specifika egenskaper. Andelen amylos och amylopektin varierar i olika växter, men i allmänhet består stärkelse av 25 % amylos och 75 % amylopektin. Det har upptäckts att det finns sorter av korn som innehåller 100 % amylopektin och kallas därför "vaxartad stärkelse" eller "vaxartad korn", men det finns även sorter som har en hög halt av amylos, ca 70 %, och dessa kallas "amylotypes".

Amylopektin och amylos består av  $\alpha$ -1, 4 bindningar mellan  $\alpha$ -D-glukos. Den stora skillnaden mellan dem är graden av förgreningar, amylopektin har 4 - 5 % glykosidbindningar som är  $\alpha$ -1, 6 bindningar. Den har en kortare kedjelängd än amylos, men med mer förgreningar är det en större molekyl. Med de olika förgreningarna hos molekylen bildas en komplex struktur. Kedjorna i amylopektin tros vara organiserade i kluster som binder till varandra med hjälp av en viss typ av kedjor.

I denna studie undersöks sex olika korn sorters stärkelsestruktur och egenskaper. För att till fullo karakterisera dessa prover används flera olika metoder i en kombination för att undersöka proverna så noggrant som möjligt. Gelfiltering utfördes för att separera amylos från amylopektin, och sedan undersöktes de insamlade fraktionerna med hjälp av Fenol-Svavelsyra metoden. Viskositeten undersöktes med RapidViscoAnalyser som mätte viskositeten vid en höjning och sänkning av temperatur under kontinuerlig omrörning. Slutligen genomfördes High Performance Anion Exchange Chromatography – Pulsed Amperometric Detection på både amylopektin och dess limit dextriner för att fastställa kedjelängdsfördelningen.

Andelen amylos i korn sorterna varierade kraftigt mellan 0,56 % och 48 %. RapidViscoAnalyser påvisade att om provet innehöll mer amylos och fibrer uppnåddes en högre slutgiltig viskositet. Resultatet av High Performance Anion Exchange Chromatography - Pulsed Amperometric Detection visade ingen tydlig indikation på skillnader mellan proven och för att kunna strukturbestämma dessa sex kornsorter och undersöka dess egenskaper närmare är mer forskning nödvändig.

**Nyckelord:** Stärkelse, Amylos, Amylopektin, Viskositet, Limit dextrin

# Table of contents

Abstract .....	4
Sammanfattning .....	5
Table of contents.....	6
Introduction.....	8
1. 1. Starch.....	8
1. 1. 1. Amylose structure.....	9
1. 1. 2. Amylopectin structure .....	9
1. 1. 3. Properties of starch .....	9
1. 2. Purpose.....	10
1. 3. Analytical methods.....	10
1. 3. 1. Gel Permeation Chromatography.....	10
1. 3. 2. Viscosity .....	10
1. 3. 3. Limit dextrin.....	10
Material and method .....	11
2. 1. Materials.....	11
2. 1. 1. Samples.....	11
2. 1. 2. Enzymes .....	11
2. 2. Method.....	12
2. 2. 1. Debranching.....	12
2. 2. 2. Gel Permeation Chromatography.....	12
2. 2. 3. Viscosity .....	12
2. 2. 4. Limit Dextrins.....	13
2. 2. 4. Anion-exchange chromatography with pulsed amperometric detection .....	13
Results .....	14
3. 1. Amylose content.....	14
3. 2. Viscosity .....	14
3. 3. $\Phi$ , $\beta$ -Limit Dextrins .....	17
Discussion .....	20
4. 1. Amylose content.....	20
4. 2. Viscosity.....	21
4. 3. Limit Dextrin .....	22
Conclusions.....	22
5.1. Research for the future .....	22
References.....	24
Appendix1.....	26



## Introduction

Barley (*Hordeum vulgare* L.) was one of the crops used in the beginning of the agriculture revolution. (Ullrich, 2011). It was one of the first crops to get domesticated, and have been used for thousands of years as both food and feed and is today the fifth most produced crop in the world. It has been shown to have equal quality properties as maize and wheat but can be produced where those crops cannot, mainly in cold and/or dry climate. Since barley is one of the oldest domesticated crops it has evolved in many different directions and thus the barley family contains a large diversity and therefore also a number of varieties. It is today the most widely adapted cereal grain.

For a long time barley has been known to bring health beneficial properties when consumed. (Ullrich, 2011). It has for example a preventing effect on heart diseases and a cholesterol lowering effect which is due to the soluble fibre,  $\beta$ -glucan. Barley also affects the human glucose metabolism positively by decreasing the rate of glucose absorption to the blood which gives a more stable blood sugar level as well as improve the colon integrity. The properties of barley make it a very interesting crop for food science! Today around 94 % of the global production of barley is mainly used for beer production, according to "Euromalt statistics" (Coceral, n.d.), and only 2 % is used for human consumption.

### 1. 1. Starch

Starch is a naturally occurring substance in most plants and is used as energy storage in green plants (Manelius, 2000). Starch granules are synthesized in the amyloplasts, and in barley each amyloplast contains one granule. (Delcour and Hoseneý, 2010). The amyloplasts are situated in the starchy endosperm of cereals such as barley (Manelius, 2000). The granules can vary in size and type. Barley has two types of granules, the lenticular granules and the smaller spherical that make up about 90% of the total amount of granules (Andersson, Fredriksson, Oscarsson Bergh, Andersson and Åman, 1998). These granules have a structure which consists of alternating semi-crystalline and amorphous growth rings and the semi-crystalline growth ring consists of the crystalline and amorphous lamella in amylopectin. The crystalline lamella consists of the  $\alpha$ -helix structure of amylopectin and the amorphous lamella consists of the branching points of amylopectin (Manelius, 2000).

Starch is a component that mainly consists of amylose and amylopectin. (Delcour and Hoseneý, 2010, Perez and Bertoft, 2010). Together they build a complex pattern which gives starch its specific properties. Both amylose and amylopectin are built up of glucose units which together form long and branched chains. All starch molecules have an individual hemiacetal group, which also is called "the reducing end". Monomers are then linked either with a  $\alpha$ -1, 4 or a  $\alpha$ -1, 6 bonds to this hemiacetal group. These different bindings give the starch molecule different structure, linear as the amylose or branched as the amylopectin.

The proportion of amylose and amylopectin varies in different plants but generally starch consists of 25 % amylose and 75 % amylopectin. (Delcour and Hoseneý, 2010). There have been discoveries of cereals as barley containing 100 % amylopectin called "waxy starches" or "waxy barley". There are also different varieties that have a high content of amylose, around 70 % and these are called "amylotypes".

Starch does not only contain amylose and amylopectin but also other components are present in small concentrations. (Delcour and Hoseneý, 2010). These minor components are lipids (lysophospholipids mostly in barley), phosphorus, protein and low concentrations of nitrogen.

### **1. 1. 1. Amylose structure**

Amylose is known as a linear structure with  $\alpha$ -1, 4 linkage between  $\alpha$ -D-glucose. (Delcour and Hoseneý, 2010) Nevertheless a part of the molecule has secondary chains attached with  $\alpha$ -1, -6 bonds.

The long chains of amylose can form helix structures, but also amylose-lipid complexes (Delcour and Hoseneý, 2010). Lipids can have an important impact on starch properties and they occur both inside the granule and on the surface (Copeland, Blazek, Salman and Chiming Tang, 2008).

### **1. 1. 2. Amylopectin structure**

Amylopectin as well as amylose consists of 1, 4 linked  $\alpha$ -D-glucose units. (Delcour and Hoseneý, 2010). The great difference between them is the degree of branching. Amylopectin has 4 to 5 % of the glycosidic bonds that are  $\alpha$ -1, -6 bonds, the glucose chains are shorter than in amylose but with more branching it is a larger molecule.

With the different branching the molecule forms an intricate structure. (Delcour and Hoseneý, 2010). To explain the pattern of the molecule it is divided in three types of chains of glucose units known as A-, B- and C-chains. There is only one C-chain in a molecule since it is the one containing the reducing end. The c-chain carries all other chains. B-chains can carry other B-chains or A-chains, whereas A-chains do not carry any other chains. The different chains are connected through  $\alpha$ -1-6-linkages. With these chains a randomized structural model is formed. This is a model more commonly known as the "Hizukuri model" or the "cluster model" and according to Hizukuri (1986) it demonstrates the relationship between the clusters in an amylopectin molecule.

In most investigations it is common to divide/distinguish A from B chains in starch, and include the C-chain with the B-chains (Bertoft, 2004). B-chains are also usually divided into subgroups according to length. The A-chains and the shorter B-chains are believed to form clusters which are held together by longer B-chains, B<sub>2</sub>-chains. (Bertoft, 2004). The cluster structure is built up of alternating amorphous and crystalline lamellae, where the branch points make up the amorphous lamella and the side chains the crystalline lamella, due to their ability to form stable double helices. It is the fact that amylopectin can take part in both crystalline and amorphous structures that is the reason of the alternating structure. The side chains branching of amylopectin are able to form  $\alpha$ -helices and forms the clusters and the crystalline lamella (Perez and Bertoft, 2010). The amorphous lamella is the space containing the branching points (see figure 1).

### **1. 1. 3. Properties of starch**

The starch products that humans consume have mostly been processed before consumption. The most common process that is used is heating in the presence of water (Copeland et al, 2008). All crops containing starch demonstrate different and unique properties in the presence of varying temperatures and varying gelatinization properties (Perry and Donald, 2000). These properties can depend on many different factors, for example the proportion of amylose, other nutrients present or the crystalline structure in the granule. Perry and Donald (2000) showed that for potato and maize starch in excessive water in room temperature the one factor that affected gelatinization directly was the redistribution of water during heating.

When starches are put in excess water and are exposed for heat, linear amylose is solubilised. (Delcour and Hoseneý, 2010). But if the temperature is increased even amylose with secondary chains, and higher molecular weight, can be solubilised. When heat is applied the starch granules lose their birefringence and crystallinity and the molecular order is lost. As long as amylose leaks out and more water is taken up by the granule the viscosity of the surrounding solution increases.

## **1. 2. Purpose**

The purpose of this investigation was to study the structure of amylopectin in six different varieties of barley. In order to fully characterize the samples several different methods were used in a combination in order to approach the solution from every possible way. One of these six samples was provided as a reference and is well characterized, normal and used for feed.

## **1. 3. Analytical methods**

Several commonly used methods for starch structure investigation have been used to characterize starch in these barley varieties. All samples were analysed in duplicate or triplicate.

### **1. 3. 1. Gel Permeation Chromatography**

Starch was debranched with the enzymes pullulanase and isoamylase. Then the sample was applied onto a Sepharose CL-6B (1\*90cm) column to separate amylose and amylopectin from each other according to size.

The method used to investigate the samples was the Phenol-Sulphuric acid method performed according to DuBois, Gilles, Hamilton, Rebers and Smith (1956). The method gives reliable estimations of the sugar content of pure solutions and it is a commonly used method for sugar mixtures and their methyl derivatives.

The amount of colour produced at a constant phenol concentration is proportional to the amount of sugar present in the solution. The sugar concentrations are plotted against the given absorbance and this generates a specific pattern showing the chain length distribution and separates the long from the short chains. The long chains were amylose and the short was amylopectin.

### **1. 3. 2. Viscosity**

To determine starches ability to form gel and increase the viscosity it is most advantage to investigate how starch reacts with heat and water. Since the solubility of the starch is directly depending on with temperature, the solubility does not increase with time but only with temperature (Delcour and Hosney, 2010).

During heat treatment the granules of starch are gelatinized. (Copeland et al, 2008). The granules lose their crystallinity and structural organization. As long as the granules of the starch are intact the viscosity is increasing and amylose is released into the water. (Delcour and Hosney, 2010). But as soon as the molecules of the soluble starch orient themselves in the direction of stirring and damage of fragile and swollen granules occur, the viscosity will decrease. During the following cooling a gel is formed by the disaggregated starch molecules, followed by a retrogradation that gradually forms into a semi-crystalline aggregate. (Copeland et al, 2008) These aggregates do not have the same form as the native granule.

According to Copeland et al (2008) all this can be examined with a RapidViscoAnalyser (RVA). It is a machine that measures the viscosity during a temperature increase and decrease with continuous stirring. By doing so pasting profiles and gelatinization behaviour is set for the different samples tested.

### **1. 3. 3. Limit dextrin**

As previously described the amylopectin molecule has an advanced and complex structure. (Delcour and Hosney, 2010, Perez and Bertoft, 2010). To know the internal structure of amylopectin it is necessary to degrade the molecules from the non-reducing ends. This is accomplished by the use of two different enzymes, phosphorylase and  $\beta$ -amylase.

The sample is treated twice with phosphorylase which is active until there are three glucose residues on a B-chain and four residues on an A-chain left (Bertoft, 1989). The  $\beta$ -amylase is then added twice in order to degrade maltose molecules until it reaches the branching point. As a result there is only one glucose residue left on the B-chain (Bertoft, 1989) and the A-chain have two left (Delcour and Hoseneay, 2010). This is called a  $\phi$ ,  $\beta$ -Limit Dextrin.

Anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was then performed on both the  $\phi$ ,  $\beta$ -Limit Dextrins and amylopectins of all barley varieties to determine the chain length distribution of the molecules.

## Material and method

### 2. 1. Materials

#### 2. 1. 1. Samples

Barley samples were provided by several different companies and were chosen with regards to the big genetic differences and the variation in amylose content, fibres and other compounds (see table 1). The Swedish agriculture university (SLU) provided barley variety "SLU 7" which was chosen because of its high  $\beta$ -glucan level and other dietary fibres. This is the barley variety with highest amount of fibres. SW Seed, Svalöv, Sweden (SW) provided two new barley varieties called SW 28708 and Karmosé, these varieties was chosen for their content of amylose. SW 28708 has a considerable low content and Karmosé have a high content. They also provided barley Gustav, which is well known feed variety of barley and is therefore used as a referee in this study. The Royal Veterinary and Agricultural University of Denmark (KVL) provided a barley variety called KVL 301, which have a low rate of  $\beta$ -glucan, but a high level of fibres. The Nordic genetic bank (NordGen), Svalbard, Norway (NGB) provided NGB 114602.

All barley varieties were grown during the same period and place in Chile during the summer 2010.

**Table 1. Summary of the different barley varieties chosen for this project and their special properties**

Sample	Total dietary fibre	Comments	Total $\beta$ -glucan	Starch content
NGB 114602	20.07	Rich of anthocyanin	medium	50.91
SLU 7	27.98	Shrunken endosperm	high	40.85
KVL 301	24.04	Mutant in Bomi	low	41.82
SW 28708	17.33	Waxy, close to 100% amylopectin, without husk	medium	49.83
Karmosé	22.13	High amylose content	medium	46.68
Gustav	17.91	Feed	medium	55.04

#### 2. 1. 2. Enzymes

The enzymes used in this study was Isoamylase, 210 U/mg, Pullulanase M1 (from *Klebsiella planticola*), 32 U/mg and  $\beta$ -amylase (Barley), 705 U/mg, all purchased from Megazyme, Wicklow,

Irland. Phosphorylase a, from rabbit muscle, 9 U/mg solids, purchased from Sigma-Aldrich, Steinheim, Germany.

## 2. 2. Method

### 2. 2. 1. Debranching

In order to determine amylose content 2 mg of starch was dissolved in 50µl of 90% Dimethyl sulphoxide (DMSO). This solvent dissolves starch by breaking the hydrogen bounds without degrading the structure (Han and Lim, 2003). It was diluted with 400µl warm water and at room temperature 50µl 0.1M acetate buffer pH 5.5 was added. Then it was incubated over night with 2µl pullulanase and 1 µl isoamylase. The sample was then heated to the point of destruction of the enzymes.

### 2. 2. 2. Gel Permeation Chromatography

The method was performed according to Laohaphatanaleart, Piyachomkwan, Sriroth and Bertoft (2010). The sample was debranched as previously described and 50µl 5M NaOH was added. Then 200µl of the sample were injected on the Sepharose CL-6B (GE Health care, Uppsala, Sweden) (1\*90cm) column. The sample was eluted with 0.5M NaOH at 0.43 ml/min. Fractions of 500µl was collected in calibrated test tubes with the Fraction collector, Gilson FC204, Middleton, WI, USA. Every second fraction was then analysed with the Phenol-Sulphuric acid method (DuBois et al, 1956).

0.5ml phenol and 2.5ml sulphuric acid was added to the test tubes and the samples were then shaken by a vortex. The tubes were then placed to cool down. Before measuring the absorbance the test tubes were once more shaken so a homogeneous mix was maintained. The tubes were wiped and then the absorbance was measured with a Biochrom WPA CO7500 Colorimeter, Cambridge, UK, at a wavelength of 490nm.

### 2. 2. 3. Viscosity

The method used in this experiment was conducted as the one by Zhou and Mendham (2004) but with minor modifications. The amount of sample varied between the different investigations, in the first test the same amount of flour was used (see table 2). In the second test the same starch content was used (see table 2) and in order to achieve this, the amounts of flour were corrected with consideration to dry matter and the given starch content. For calculations see appendix 1. A third test was conducted with four other starches from barley (table 4), these four varieties have previously been determined structurally in detail (Bertoft et al, 2011). They were examined with this method to improve the understanding of properties and to have something to compare the other six barley varieties with. The same amount of water was added in all, as in the tables 2 and 3. All samples were analysed in triplicate.

**Table 2. Experimental conditions for viscosity measurements with RVA of the six barley flours**

Barley	Amount barley <sup>a</sup> flour used in the first test (g)	Amount barley flour in the second test (g)	Water added (ml)
NGB 114602	3	2.4	25
SLU 7	3	3	25
KVL 301	3	2.9	25
SW 28708	3	2.5	25
Karmosé	3	2.6	25
Gustav	3	2.24	25

<sup>a</sup> the first experiment was conducted with the same amount of flour

<sup>b</sup> in the second test the flour amount was corrected with consideration to dry matter and the given starch content so the total amount of starch in the sample was the same for all barley flour tested

**Table 3. Experimental conditions of four different barley starches, during viscosity measurements with RVA**

Barley starch	Amount of starch (g)	Water added (ml)
Cinnamon	2.5	25
Cindy	2.5	25
SW49427	2.5	25
Glacier	2.5	25

The samples were weight out according to table 2 and 3. Then they were poured into a canister, water was added and the samples were vigorously stirred with a paddle before analysing. A Rapid Viscoanalyzer (RVA, Newport Scientific, Warriewood, NSW, Australia) was used to determine the pasting properties that the slurries attained (Zhou, 2004). The program defined by the manufacture (std1) had a running time of 13 min, during which the temperature was regulated. The starting temperature of 50°C was held for approximately 1min, and then it was raised to 95°C where it was held for 2.5min. Then cooled to the starting temperature of 50°C. The stirring varied as well, during the first 10sec the speed was 960rpm as the sample needed to be dispersed and then it was held at 160rpm continuously during the rest of the test period.

#### 2. 2. 4. Limit Dextrins

In order to perform this experiment amylopectin purified from the barley flour was used. 200mg from the isolated amylopectin is weight up and dissolved in 6 ml 90% DMSO by stirring in room temperature and 65ml of warm distilled water was added. The sample was then left to cool down and when that was achieved 7.2ml 1.1M NaPO<sub>4</sub>-buffer pH 6.8 and 3.4ml 2.8M Na-EDTA-solution was added as well. 1.8mg phosphorylase was dissolved in 18ml distilled water and added to the sample. Then the sample was left in room temperature with a constant stirring overnight.

The second day the sample was heated in order to destroy the enzyme. Then it was added to a filtering system, Omega 10 K membrane in Minimate™ TFF Capsule System (Pall Life Sciences, Ann Arbor, MI, USA) which was connected to a MasterFlex® Console drive (Cole-Parmer Instrument Company, Barrington, IL60010, U.S.A). The volume was first reduced to 10ml and 150ml distilled water was added. The next time the volume was reduced to 30ml and 150ml was added, this was repeated three times. During this time filtrate was collected in a beaker and the absorbance was measured using the Phenol-Sulphuric acid method (DuBois et al, 1956). There should be a measurably decrease of absorbance during the day, since sugars are removed by the filter.

The last time distilled water was added, the volume reduced to 10ml and was then pumped out in to a separate beaker. The sample cup was washed four times with 5ml water and a fine pipette was used to collect the sample stuck in the 3-way-tap. To the beaker containing the sample: 3.6ml 1.1M NaPO<sub>4</sub>-buffer pH 6.8 and 1.7ml 2.8M Na-EDTA-solution was added. 0.9mg phosphorylase was dissolved in 9ml distilled water and added last.

The following three days were conducted in almost the same way. The sample was heated up and filtrated as described above. Day 3 and 4 the sample were incubated with 100µl β-amylase and 3.5ml acetate buffer instead. On the last and fifth day of this experiment, the absorbance should reach zero as no maltose should be present in the sample. The sample was then freeze-dried.

#### 2. 2. 4. Anion-exchange chromatography with pulsed amperometric detection

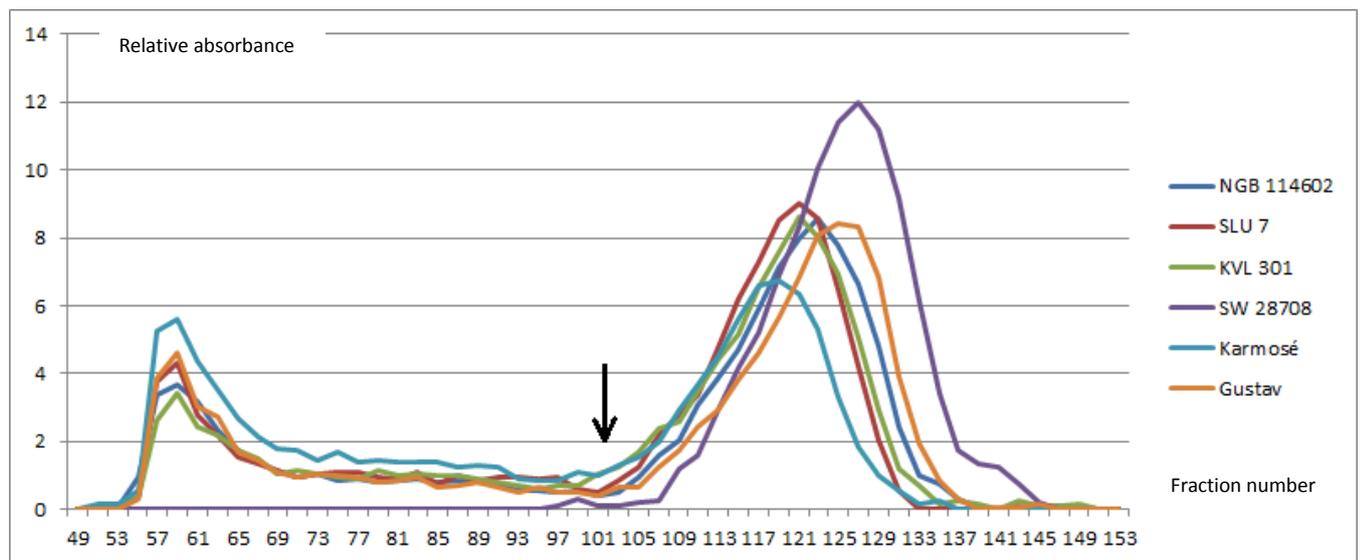
The investigation was performed according to Bertoft et al (2004). 2mg of the sample was weight out into a test tube and it was then debranched as previously described. Then 1,5ml distilled water was added so that the total volume of sample solution becomes 2ml. Then it was filtrated through a 0.45 µm Titan2 syringe nylon membrane filter (SUN-SRI, Rockwood, TN, USA) to a vial. The samples was analysed with HPAEC-PAD using a Dionex DX 500 system equipped with an ED 40 pulsed

amperometric detector (Dionex, Sunnyvale, CA, USA). Columns used were CarboPac PA-100, Guard colon, (4\*50mm) and a CarboPac PA-100, (4\*250mm) and samples eluted with 0.5 M NaOH and a gradient of 0.5M acetate. The PAD response was converted to carbohydrate concentration according to Koch, Andersson and Åman (1998).

## Results

### 3. 1. Amylose content

Before injected to the Sepharose CL-6B column the samples of barley starch were debranched with the enzymes pullulanase and isoamylase. These enzymes debranch the complex structure of amylopectin so that no branches are left. Amylose is the bigger molecule according to length since amylopectin naturally has a shorter chain length, and it is therefore eluted first (figure 2).



**Figure 1. Enzymatically debranched barley starch samples, using pullulanase and isoamylase, which have been seperated according to size using a Sepharose CL-6B gel. The amylose is eluted first, followed by amylopectin.**

The resulting profile from the Phenol-Sulphuric acid method (DuBois et al, 1956) have two peaks and in-between a distinct decline in absorbance which represent the separation of two different molecules (see figure 2). The first peak represents amylose content. The absorbance decline to a low level and then the amylopectin, which takes more time to get through the column, is eluted. The fraction that separates amylose from amylopectin was chosen to be 103 which represent the generally lowest absorbance in all samples.

No more amylose is to be expected to elute later than fraction 103. The amylose content was calculated as area under curve (table 4).

### 3. 2. Viscosity

The RVA measurements gave information about how the starch in the flour reacts with heat and water, during stirring. As demonstrated in Figure 3 the viscosity increases with the temperature rising to 95°C due to the gelatinization. (Delcour and Hosney, 2010). The loss of granular birefringence can also be called “pasting” and as the starch become disordered, soluble starch is released into the water and that is what causes the swelling and primarily the increase of viscosity. Pasting temperature was according to the instruction manual for the RVA (Newport Scientific, Warriewood, NSW, Australia) the temperature when the rate of increase in viscosity reaches 11.5 RVU in 0.2min

and is shown as the first increase of viscosity (see figure 3). When the viscosity is at its highest during heating it is called “peak viscosity” (Zhou and Mendham, 2004).

When the viscosity then decreases it is called “shear thinning” (Delcour and Hosenev, 2010). After reaching 95°C and held for 2.5 min the cooling to 50°C starts (Zhou and Mendham, 2004). The viscosity then increases again and that occurrence is called “setback”. The energy decrease as the temperature falls, and that gives rise to new hydrogen bonds and entanglement between the starch chains. This increase of the viscosity is shown in figure 3, 4 and 5.

First the same amount of barley flour was used in order to establish the most basic differences. To illustrate the differences between the barley flours during the processes that take place, values from the program are summarized in table 4.

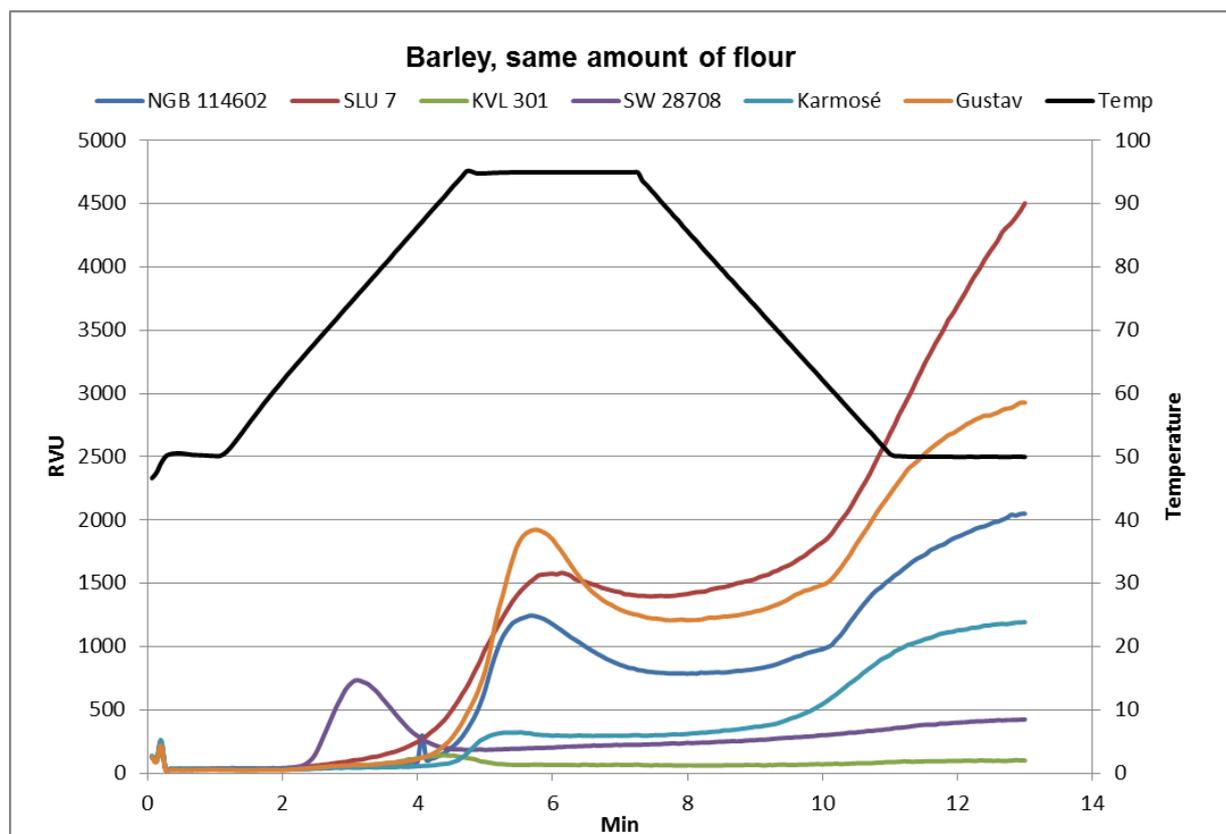


Figure 3. Result of the viscosity measurements with RVA using 2.5g barley flour to determine gelatinization capability and the viscosity of the slurry during heating and continuously stirring of the water.

Table 4. Summary of the result obtained from the program (defined by the manufacturer) during RVA to demonstrate the differences in properties and structure between the barley varieties

Barley flour	Amylose content (%)	Pasting temp (PT) (°C)	Peak viscosity (PV) (RVU)	Temp during PV (°C)	Set back (SB) (RVU)	Final viscosity (FV) (RVU)
NGB 114602	30.3	87	1245	95	1264	2050
SLU 7	32.7	76	1582	95	3107	4505
KVL 301	31.5	82	141	91	40	97
SW 28708	0.6	65	732	76	201	423
Karmosé	47.8	92	322	95	898	1193
Gustav	31.2	84	1924	95	1720	2928

To establish if the starch amount in barley was regulating the gelatinization and viscosity properties demonstrated a second test was to perform with the same measurement as previous but this time with the same amount of starch in all samples (figure 4). Detailed calculations are shown in appendix 1.

Like previously the values from the program in the RVA was summarized and put in a table to illustrate the differences between the barley flours during the processes that take place (table 5).

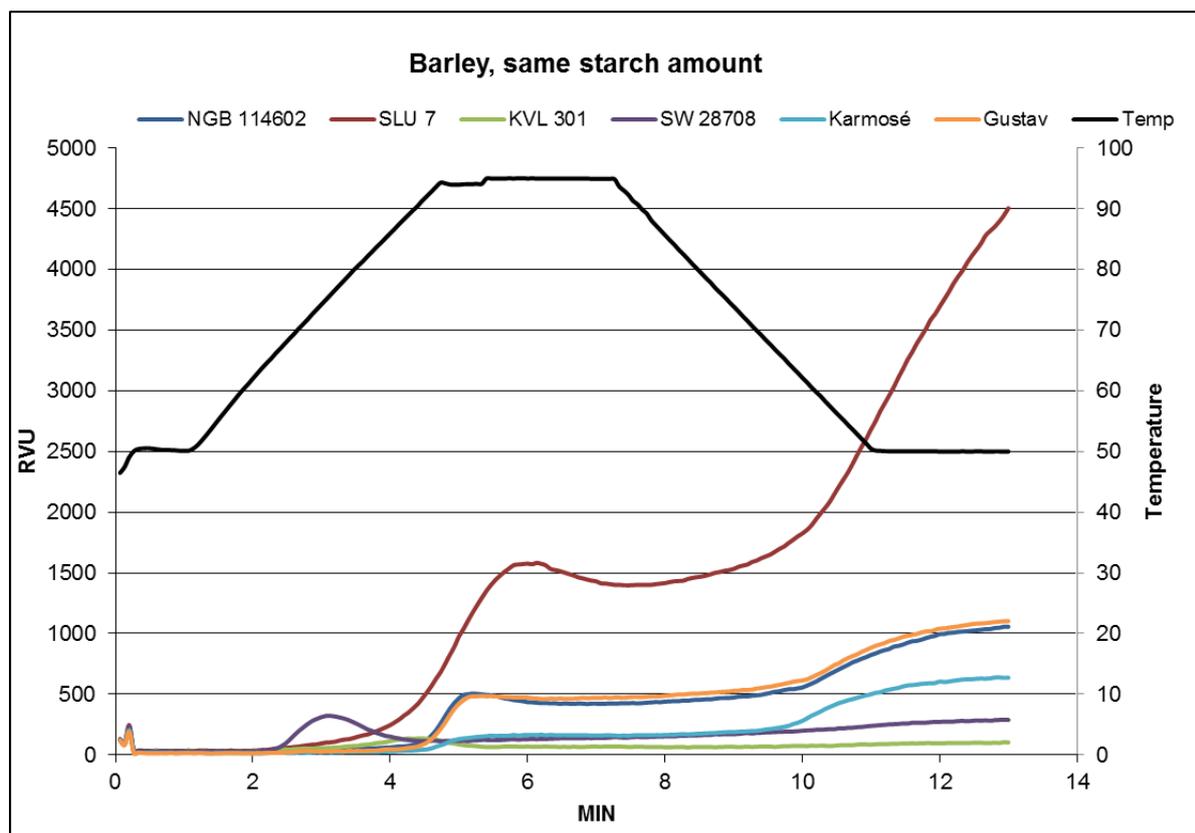


Figure 4. Result of the viscosity measurement with RVA using the corrected amount of barley flour with consideration to dry matter and the given starch content so that the total amount of starch was the same in all samples analysed

Table 5. Summary of the result from RVA over values from the program designed by the manufacturer to demonstrate the differences in properties between the barley varieties when the starch content in all samples used was corrected to the same amount

Barley flour	Amylose content (%)	Pasting temp (PT) (°C)	Peak viscosity (PV) (RVU)	Temp during PV (°C)	Set back (SB) (RVU)	Final viscosity (FV) (RVU)
NGB 114602	30.3	89	506	95	635	1056
SLU 7	32.7	74	1582	95	3108	4505
KVL 301	31.5	83	136	90	42	103
SW 28708	0.6	65	322	75	149	288
Karmosé	47.8	93	167	95	476	636
Gustav	31.2	87	487	90	634	1103

A third study was performed using the RVA with four varieties of barley starch which previously have been studied in detail by Bertoft et al (2011).

Values of importance are summarized in table 6.

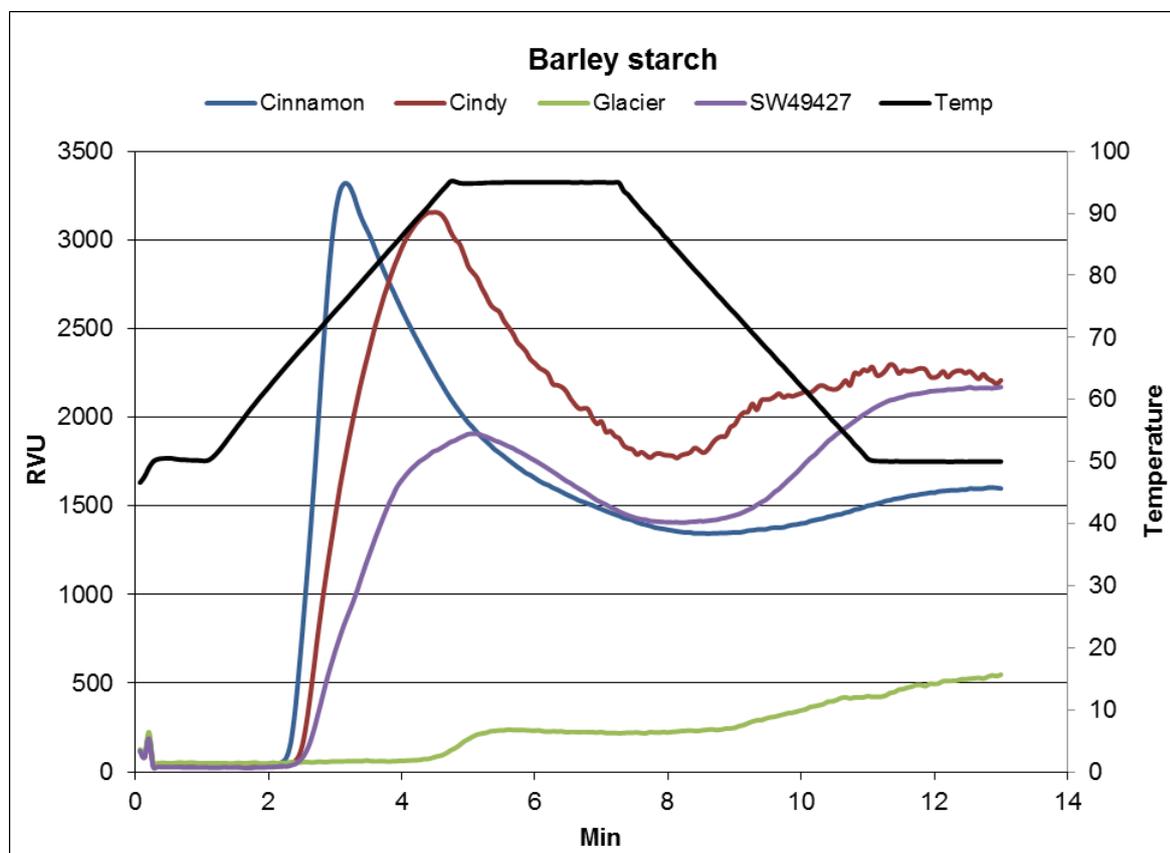


Figure 5. Result of the viscosity measurements with RVA using four starches of barley that previously been studied in detail by Bertoft et al (2011).

Table 6. Summary of the results from RVA over the values from the program designed by manufacture (std1) to demonstrate the differences in properties between the different barley starches<sup>a</sup>

Barley starch	Amylose content (%) <sup>b</sup>	Pasting temp (PT) (°C)	Peak viscosity (PV) (RVU)	Temp during PV (°C)	Set back (SB) (RVU)	Final viscosity (FV) (RVU)
Cinnamon	0.0	64	3317	76	255	1598
Cindy	10.2	66	3155	92	436	2208
Glacier	32.6	92	229	95	331	548
SW49427	3.7	66	1907	95	765	2170

<sup>a</sup> Barley starches have previously been studied in detail by Bertoft et al (2011)

<sup>b</sup> Values from Bertoft et al, 2011

### 3. 3. $\Phi$ , $\beta$ -Limit Dextrins

The limit dextrins were weight up after freeze-drying in order to establish the yield (table 8). In order to calculate both external and internal chain length distribution amylopectin and  $\Phi$ , $\beta$ -Limit Dextrins was analysed. Amylopectin and its limit dextrin were debranched with isoamylase and pullulanase. The result from HPAEC-PAD of the chain length distribution of the different amylopectin as well as in the limit dextrins achieved is presented as figure7 and 8. The PAD response was converted to carbohydrate concentration (Koch, Andersson and Åman, 1998) and then to be able to compare the

different samples and quantify the amounts of sample that the peaks represent calculations were made according to Bertoft, (2004).

When the samples then were analysed with HPAEC-PAD the chain length distribution could be calculated. The average chain length (CL) was calculated for both the limit dextrans and the amylopectins. The values of average CL varied between 17.6 and 18.2, which is not a big variation. Even though some differences can be seen in the figure, SLU 7 and Karmosé both have profiles that differ a little from the others. But according to the calculations no major difference was illustrated.

As previously described amylopectin consists of three different chains called A-, B- and C-chains (Delcour and Hosenev, 2010). Most commonly A- and B-chains are divided into two groups since they differ in structural properties, C-chains are included in the group of B-chains since they cannot be separated with this method. ACL is the chain length of A-chains and BCL is the chain length for B-chains (table8). Bfp and Afp is the fingerprint values those are the most characteristic for each type of starch. (Bertoft, 2004) Bfp are the summary of DP 3 to DP 7 and Afp is the summary of DP 6 to DP 8.

The B-chains are then divided into short (BS) and long (BL) chains according to length (table8). Limit value was calculated in order to establish external and internal chain length distribution (Table7). External chain length (ECL) is the chains outside the branching points that can form  $\alpha$ -helix. The internal chain length (ICL) is the length between the branching points. Smol% is the molar percentage short chains DP6 to DP37 in the amylopectin (table7).

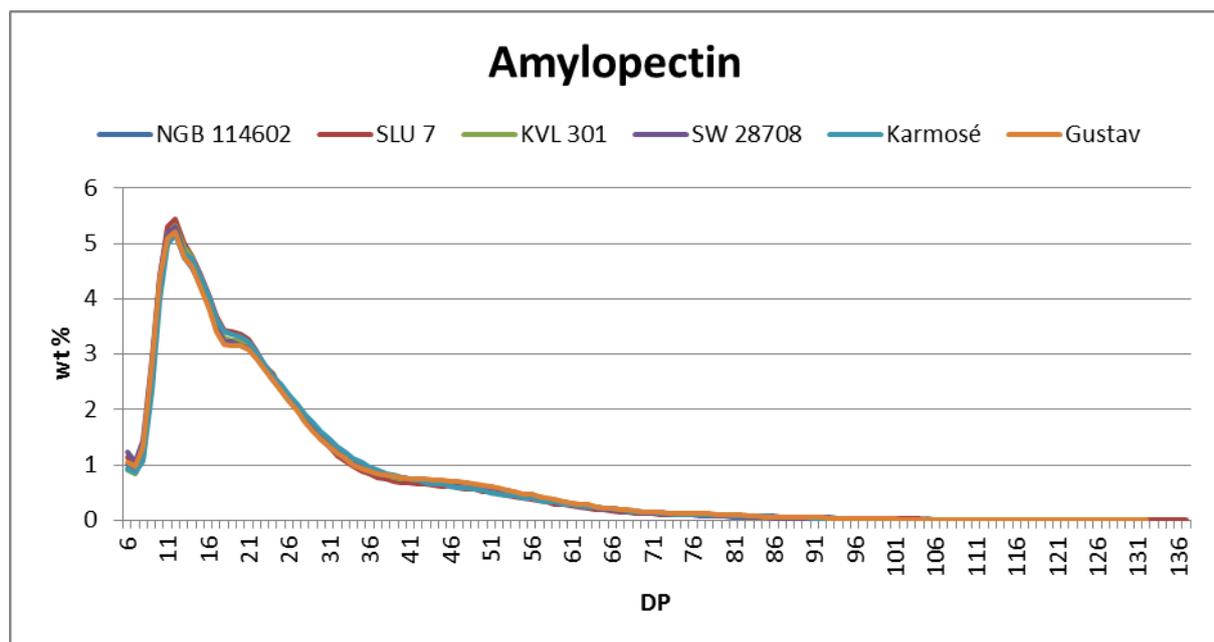


Figure 6. Result of HPAEC-PAD<sup>a</sup> on the samples of amylopectin, which was debranched with isoamylase and pullulanase, in order to establish the chain length distribution.

<sup>a</sup> The PAD response was converted to carbohydrate concentration according to Koch, Andersson and Åman (1998).

**Table 7. Summary of the results by HPAEC-PAD and the result of performed calculations<sup>a</sup> on the chain length distribution of amylopectin analysed**

Barley flour	CL <sup>1</sup>	SCL <sup>2</sup>	LCL <sup>3</sup>	ECL <sup>4</sup>	ICL <sup>5</sup>	Afp <sup>6</sup>	S mol% <sup>7</sup>
NGB 114602	18.1	15.7	51.4	12.9	4.2	8.3	93.4
SLU 7	17.7	15.7	51.	12.5	4.2	8.4	94.4
KVL 301	18.1	15.9	50.6	12.9	4.2	7.5	93.6
SW 28708	17.6	15.6	49.9	12.04	4.6	9.5	93.9
Karmosé	18.2	16.1	50.9	13.31	3.9	7.5	93.9
Gustav	17.9	15.6	51.3	13.1	3.9	8.6	93.4

<sup>a</sup> Calculations performed according to Bertoft (2004)

<sup>1</sup>  $\sum$  % corr area /  $\sum$  mol

<sup>2</sup>  $\sum$  % corr area DP 6 to 37 /  $\sum$  mol DP 6 to 37

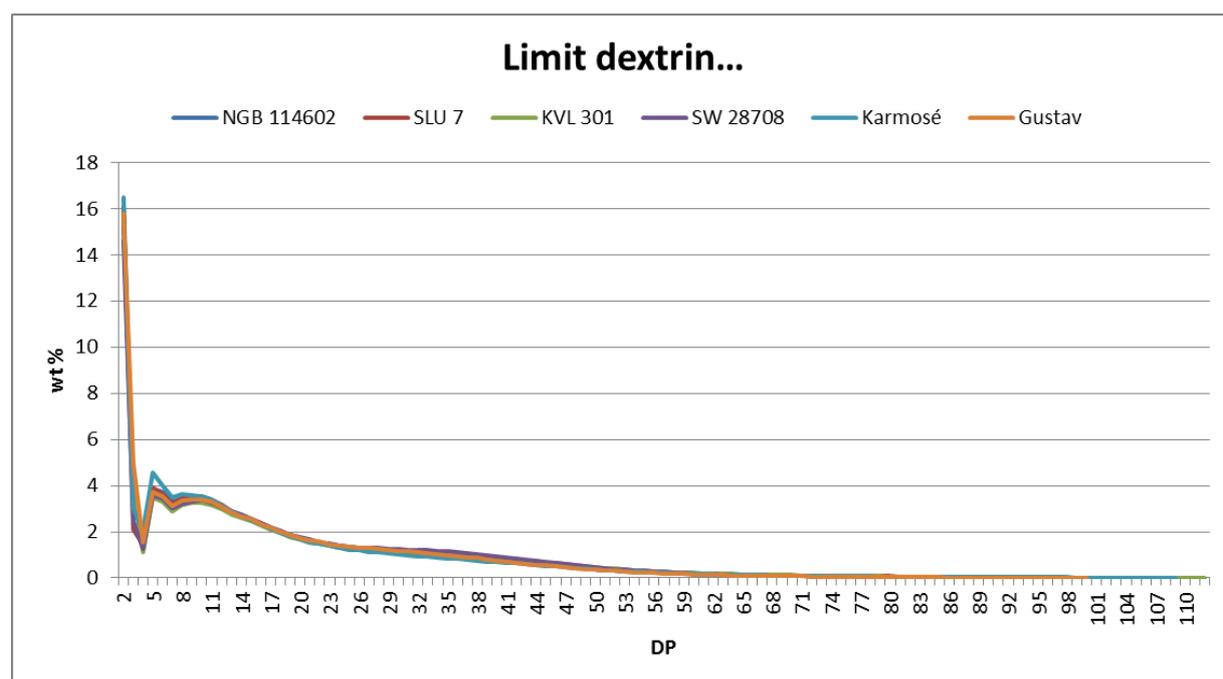
<sup>3</sup>  $\sum$  % corr area from DP 38 /  $\sum$  mol from DP 38

<sup>4</sup> CL \* (fb limit value /100) + 1, 5

<sup>5</sup> CL - ECL - 1

<sup>6</sup>  $\sum$  DP 6 - 8 mol%

<sup>7</sup>  $\sum$  DP 6 -37 mol%



**Figure 7. Result of performed HPAEC-PAD<sup>a</sup> on enzymatically debranched samples of Limit Dextrins, using isoamylase and pullulanase in order to establish the chain length distribution.**

<sup>a</sup> The PAD response was converted to carbohydrate concentration according to Koch, Andersson and Åman (1998).

**Table 8. Weight after filtrating and debranching amylopectin to limit dextrans, and summary on the results of HPAEC-PAD and performed calculations on the chain length distribution of the Limit Dextrin samples**

Barley flour	Limit Dextrin (mg)	CL	ACL <sup>8</sup>	BCL <sup>9</sup>	Bfp <sup>10</sup>	BS <sup>11</sup>	BL <sup>12</sup>	Limit value <sup>13</sup>
NGB 114602	44.3	6.7	51.1	48.9	22.6	44.7	4.2	62.9
SLU 7	54.8	6.71	54.1	45.9	19.5	41.8	4.1	62.03
KVL 301	52.5	6.7	52.9	47.2	21.7	42.7	4.4	62.9
SW 28708	35.9	7.1	51.8	48.2	20.2	43.4	6.4	59.8
Karmosé	38.4	6.4	52.6	47.5	22.9	43.9	3.5	64.95
Gustav	40.7	6.4	50.7	49.3	24.6	45.5	3.7	64.4

<sup>8</sup>mol % DP 2

<sup>9</sup> $\sum$  mol % from DP 3

<sup>10</sup> $\sum$  mol% DP 3 to 7

<sup>11</sup>Short B-chains,  $\sum$  mol% DP 3 to 27

<sup>12</sup>Long B-chains,  $\sum$  mol%, from DP 28

<sup>13</sup> $(CL \div AP - CL \div LD \div AP) / (CL \div AP * 100)$

## Discussion

The barley varieties chosen for this investigation were chosen for their differentially in carbohydrate composition, structure and other properties which were established in a first test.

### 4. 1. Amylose content

The amylose content was calculated as the area under the curve in figure 2 and varied between 0.56 % and 48 % (see table4). The fraction 103 was set as a separator between amylose and amylopectin since that fraction represent the lowest value between the eluted molecules in all samples. The same fraction was chosen for all of the six samples so the result could be compared between the samples.

It is possible that at fraction 103 some amylose is still in the column and it is even possible that some of the amylopectin have been eluted before this fraction. Therefore it is most important to have in mind that some overlap exists and that fraction 103 is not the exact divider between the two molecules. Though it gives a clear distinction of the division of two molecules varying in size. The method is commonly used and gives a precise value of the molecule amounts eluted and the rate between amylopectin and amylose.

In figure 2 two of the barley varieties stand out from the rest. Karmosé have a higher first peak then the rest of the samples, but then a low second peak. That profile is often seen with a high amylose and low amylopectin variety, which also is the case in this study. Karmosé had a amylose content of 48 % which is a rather high value compared to common values of 25 % amylose and 75 % amylopectin according to Delcour and Hosney (2010). SW28708 had no first peak and a high second peak, which is common for varieties with low amylose and high amylopectin content, also called "waxy". SW28708 had an amylose content of 0.56 % which is a very low value. This is clearly demonstrated in figure 2.

As seen in figure 2 the second peaks vary in height as well as in when it is eluted. The height demonstrates the amylopectin content of the different samples. During this investigation the fraction

collector changed the size of the drops collected, and different amounts of samples was therefore collected in the sets of test tubes. The profiles can because of this vary in eluted fraction.

There is no clear indication that the amount of starch alone affects the result of the RVA measurements. Others have shown that only temperature and the relocalization of water in the granule affects the gelatinization, not the amylose content of the different samples (Perry and Donald, 2000). Therefore more studies should be performed on these barley varieties for better understanding of the properties as well as the structural diversity and how it effect the viscosity and cooking properties.

## 4. 2. Viscosity

RVA was performed on all barley flours. Although the varieties are not chosen because of their differentially in starch components it is obvious that they have a lot of different properties when they are examined with RVA.

SLU 7 and Gustav are the two barley varieties that reach the highest viscosity, both at peak- and final-viscosity. SLU 7 was chosen because of its content of high  $\beta$ -glucan level and it is the variety with the highest total amount of fibres. That could be the explanation for why the viscosity doesn't decrease as much after peak viscosity, the fibres absorbs water and together with the solubilized amylose increase the viscosity. Gustav is the barley variety that is used as a reference in this test; it has a low content of fibre, but a higher content of starch then SLU 7. Therefore it has a larger decrease of viscosity after peak viscosity (figure3).

A result that was rather unexpected was that of KVL 301 (figure3 and 4), it had the lowest viscosity measurements of all the samples. The barley variety was chosen for its low rate of  $\beta$ -glucan and high level of fibres. Even though it had almost the same amount of starch as SLU 7 no viscosity was created during the RVA, the sample did not gelatinize at all. The largest difference between KVL 301 and SLU 7 was the amount of  $\beta$ -glucan (table1). But since it was whole flour examined, other components in the flour can influence the result.

SW 28708 is a variety that was chosen for its low content of amylose, which is why it has a peak viscosity at a very low temperature compared to the other barley flours. It also has a very low setback, mainly because there is no amylose that can leak out and give more room for water in the granule as well as increase the viscosity in the surrounding solution. With Karmosé the relationship is the opposite, it has a high content of amylose which mean that the viscosity do not increase until a lot of amylose have been solubilized and since there is not a great deal of amylopectin to bind water in the granule, hence the late and low peak viscosity.

NGB 114602 has some similarity with Gustav in both figure 3 and 4. It does not reach the same peak viscosity when the same flour amount is used, but when the same amount of starch is used the similarity is obvious. The most common relationship between amylose and amylopectin is a rate of 25 % amylose and 75 % amylopectin (Delcour and Hosenej, 2010) and these are the varieties with the amylose content closest to 25 % (table 4) the other samples can therefore be compared to these profiles.

When the flours were investigated with the same amount of starch (figure 4) it was clear that the starch amount in the samples affect the viscosity. All varieties decrease in viscosity but have the same profile, except for SLU 7 which is the only one that reaches about the same viscosity values as previously, see table 4 and 5. Probably because the same amount of flour is used for both tests (appendix1 for calculations). SLU 7 showed a remarkably high peak- and especially final-viscosity which indicates that more than the starch amount affects the viscosity, most likely the fibre content.

Four other starches of barley were also examined with this method. Cinnamon which have 0 % amylose and therefore an early, high peak viscosity and as expected a low setback and final viscosity since the starch chains forms new hydrogen bonds and recrystallize (Delcour and Hosney, 2010). SW 49427 has a slightly higher amylose content than Cinnamon, which gives it a lower peak viscosity but a higher final viscosity. Cindy and SW 49427 reach the same final viscosity and peak viscosity at the same time (table 6). The peak viscosity differs which is directly linked to the swelling of granules and which indicates that Cindy has a broader amorphous lamella which makes it possible for more water to penetrate the granule (Perry and Donald, 2000, Bertoft et al, 2011).

Glacier is the starch with the highest amount of amylose which is clearly demonstrated in figure 5. Its profile resembles the profile of Karmosé which also has the highest amount of amylose of the barley flour varieties. The viscosity increase after the granules lose their molecular structure and the amylose leaks out.

SW 28708 is the one of the barley varieties that reminds of Cinnamon since both are waxy, but as the flour have other compounds and therefore less amount of starch that affect it does not show a high peak viscosity like Cinnamon. The four starches that has been analysed with this method is not totally comparable with the barley flour even though they were examined with the same starch amount. The flours have other ingredients than starch since it is whole meal, which affects the results.

To have comparable results it would be interesting to have RVA on flour of Cinnamon, Cindy, SW 49427 and Glacier. As well as one of the starches from NGB 114602, SLU 7, KVL 301, SW 28708, Karmosé and Gustav.

### **4. 3. Limit Dextrin**

The limit dextrins and amylopectin examined with HPAEC-PAD resulted in very similar results of the six barley varieties (table 7 and 8). The chain length distribution did not vary that much between the samples, CL for the amylopectin varied between 17.7 and 18.2. CL for the Limit Dextrins varied between 6.4 and 7.1, which also is a narrow interval and no real difference can be found. A main reason for this is that the barley varieties were not chosen for different starch structure.

Many different factors can affect the result. The purity of the isolation is important to think about during the process, since there are many isolation steps performed. But as the results from the HPAEC-PAD are so similar it is most likely that the isolations have been done successfully.

## **Conclusions**

This study has provided new information regarding structure and properties on barley varieties. Amylose content was calculated and showed great variation as expected. The RVA method showed how different the barley varieties were and how much research there is left on this area and on these samples. The RVA investigation demonstrated that the starch has a great impact on viscosity and since most starch products for human consumption is heated it is an interesting area of research. A trend shown in all figures is the more amylopectin, the lower peak temperature and higher viscosity but it results in a low final viscosity. The more amylose and fibres the samples contained the higher final viscosity. The HPAEC-PAD showed no clear indication of difference between the samples and to establish the structure of these six barley varieties more research is necessary.

### **5.1. Research for the future**

More research should be done on the structure of starch, this study did not include enough investigations to determine any major differences in structure and therefore it could give valuable information to make closer studies.

There are a lot of things that can affect the results gained from this investigation. For example the structure and amount of the different starch granules, the amylose distribution in them, crystallinity and the water redistribution after gelatinization during RVA compared to before. These are also things which could differ in the different barley varieties. Therefore more research should be done on these samples to determine the structure as well as the properties!

## References

- Andersson, L., Fredriksson, H., Oscarsson Bergh, M., Andersson, R., and Åman, P. (1998). Characterisation of Starch from Inner and Peripheral Parts of Normal and Waxy Barley Kernels. *Journal of Cereal Science* 30 (1999) 165 – 171.
- Bertoft, E. (1989). Partial characterization of amylopectin alpha-dextrins. *Carbohydrate research* 189 (1989) 181 – 193.
- Bertoft, E. (2004). On the nature of categories of chains in amylopectin and their connection to the super helix model. *Carbohydrate Polymers* 57 (2004) 211-224.
- Bertoft, E., Källman, A., Koch, K., Andersson, R., and Åman, P. (2011). The cluster structure of barley amylopectins of different genetic backgrounds. *International Journal of Biological Macromolecules* 49 (2011) 441 – 453.
- Coceral. EUROMALT, The malting industry in the EU [online]. Available from: <http://www.coceral.com/cms/beitrag/10011989/248433>. [27 November 2011].
- Copeland, L., Blazek, J., Salman, H., and Chiming Tang, M. (2008). Form and functionality of starch. *Food Hydrocolloids* 23 (2009) 1527 – 1534.
- Delcour, J.A., and Hosney, R. C. (2010). *Principles of cereal science and technology* 3<sup>rd</sup> edition, AACC International Inc., St. Paul, Minnesota, USA. ISBN: 978-1-891127-63-2. p 25-30, 35-42.
- DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers P.A., and Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *American Chemical Society*, 1956, 28 (3), 350-356.
- Han, J.A., and Lim S.T. (2003). Structural changes of corn starches by heating and stirring in DMSO measured by SEC-MALLS-RI system. *Carbohydrate Polymers* 55 (2004) 265-272.
- Hizukuri, S. (1986). Polymodal distribution of the chain length of amylopectins, and its significance. *Carbohydrate Research* 147 (1986) 342 – 347.
- Koch, K., Andersson, R., and Åman, P. (1998) Quantitative analysis of amylopectin unit chains by means of high-performance anion-exchange chromatography with pulsed amperometric detection, *J. Chromatography A*. **800**, 199-206.
- Laohaphatanaleart, K., Piyachomkwan, K., Sriroth, K., and Bertoft, E. (2010). The fine structure of cassava starch amylopectin, Part 1: Organization of clusters. *International Journal of Biological Macromolecules* 47 (2010) 317–324.
- Manelius, R. (2000). *Enzymatic and acidic hydrolysis of native and modified starch granules*. Diss. Åbo: Åbo Akademi University. ISBN 952-12-0748-5.
- Perez, S., and Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch/Stärke* 2010, 62, 389-420.
- Perry, P.A., and Donald, A.M. (2000) SANS study of the distribution of water within starch granules. *International Journal of Biological macromolecules* 28 (2000) 31 – 39.

Ullrich, S. E. (2011). *Barley: Production, Improvement and Uses*. Chichester, West Sussex, UK; Ames, Iowa, USA: Wiley-Blackwell. ISBN: 978-0-8138-0123-0. p 3-14, 546–548.

Zhou, M. X., and Mendham, N. J. (2004). Predicting barley malt extract with a Rapid Viscoanalyser. *Journal of Cereal Science* 41 (2005) 31 – 36.

## Appendix 1

During this investigation of viscosity the barley flours were analysed both by using same amount of flour and same amount of starch.

To adjust the amount of flour in order to contain same amount of starch, calculations were made as follows:

A maximum amount of flour was set at 3g since the risk of lumps or overflow due to surplus of liquid increases. Dry matter was taken in consideration in the calculations.

The barley variety with the lowest starch concentration was to be in the highest amount, which was set to 3g. In all following calculations was the presuppose so that the rest of the barley varieties had the same amount of starch as SLU 7.

SLU 7

The amount of the sample was 3g  
Dry matter 94 %  
Starch concentration in flour 40, 85 %  
 $3g * 0,94 = 2,82$   
 $2,82 * 0,4085 = 1,152g$  starch (in 3g barley flour)

NGB 114602

1,152g starch was acquired  
Dry matter 93, 3 %  
Starch concentration in flour 50, 91 %  
 $1,152g \text{ starch} / 0,5091 = 2,263$   
 $2,263 / 0,933 = 2,425g$  barley flour was to be dissolved in 25ml water

KVL 301

1,152g starch was acquired  
Dry matter 93, 5 %  
Starch concentration in flour 41, 82 %  
 $1,152g \text{ starch} / 0,4182 = 2,755$   
 $2,755 / 0,935 = 2,946g$  barley flour was to be dissolved in 25ml water

SW 28708

1,152g starch was acquired  
Dry matter 93, 7 %  
Starch concentration in flour 49, 83 %  
 $1,152g \text{ starch} / 0,4983 = 2,312$   
 $2,312 / 0,937 = 2,467g$  barley flour was to be dissolved in 25ml water

Karmosé

1,152g starch was acquired  
Dry matter 93, 7 %  
Starch concentration in flour 46, 68 %

1,152g starch / 0,4668 = 2,468

2,468 / 0,937 = 2,634g barley flour was to be dissolved in 25ml water

Gustav

1,152g starch was acquired

Dry matter 93,4 %

Starch concentration in flour 55,04 %

1,152g starch / 0,5504 = 2,093

2,093 / 0,934 = 2,241g barley flour was to be dissolved in 25ml water