Carbohydrate Quality of Barley Products with Focus on β-Glucan

Susanne Djurle
Faculty of Natural Resources and Agricultural Sciences
Department of Molecular Sciences
Uppsala

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Abstract

Barley (*Hordeum vulgare* L.) has a high content of dietary fibre and especially of mixed-linkage (1→3),(1→4)-β-D-glucan (β-glucan). It is well documented that a high intake of dietary fibre promotes beneficial health effects e.g. lower risk of type II diabetes and cardiovascular disease. β-Glucan in oats and barley even has an approved EFSA health claim for maintaining normal blood cholesterol levels. Cereals are usually processed before consumption and it is therefore important to study not only raw materials, but also how they are affected by processing.

Six barley varieties with different starch and dietary fibre content and composition were followed from kernel via sifted flour to two products: extruded breakfast cereal and bread. The starch and dietary fibre content and composition were analysed in each step to determine how processing affected each variety. The difference between kernels and sifted flour was large, as expected since the outer part of the kernel containing mostly insoluble dietary fibres was removed. The varieties were affected mostly in the same way and differences in kernels were observed also in sifted flours.

Extrusion increased the extractability of arabinoxylan and β-glucan while decreasing the molecular weights and the contents. The molecular weight of arabinoxylan of one variety (SW 28708) was less affected by extrusion than the other varieties while another variety (KVL 301) had a considerably lower extractability of β-glucan in the extruded product than the other varieties.

Bread baking also increased the extractability of β-glucan and arabinoxylan while decreasing the molecular weights. There was however one variety (SLU 7) that maintained a higher molecular weight of β-glucan. Since molecular weight reduction during baking is a known problem this was studied further. The β-glucanase activity was similar in sifted flour of all barley varieties, but higher for the wheat flour used in this study. This implied that differences in β-glucan breakdown depend on structure or some inhibitory factor in SLU 7. Incubation with water in 37 °C also gave lower breakdown of β-glucan in SLU 7 compared to other varieties. β-Glucan generally consists of 90% cellotriosyl and cellotetraosyl units but the ratio between them was different for SLU 7 than for other varieties, which could be part of the explanation for the differences in β-glucan breakdown.

Keywords: barley, β-glucan, baking, extrusion, molecular weight, dietary fibre, resistant starch

Author's address: Susanne Djurle, SLU, Department of Molecular Sciences,
P.O. Box 7015, 750 07 Uppsala, Sweden

Dedication

To Lucas, Vidar and Oscar.

Wonder is the beginning of wisdom.
Socrates

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Susanne Djurle, Annica A.M. Andersson, Roger Andersson (2016). Milling and extrusion of six barley varieties, effects on dietary fibre and starch content and composition. *Journal of Cereal Science*, 72, 146-152

II Susanne Djurle, Annica A.M. Andersson, Roger Andersson. Effects of bread baking on barley dietary fibre, with emphasis on β-glucan and resistant starch. (Submitted)

III Susanne Djurle, Annica A.M. Andersson, Roger Andersson. β-Glucan resistance to degradation during bread baking. (Submitted)

IV Elisa De Arcangelis, Susanne Djurle, Annica A.M. Andersson, Emanuele Marconi, Maria C. Messia, Roger Andersson. Structure analysis of β-glucan in six barley varieties differing in starch and dietary fibre composition. (Manuscript)

Paper I is reproduced with the permission of the publisher.

The contribution of Susanne Djurle to the papers included in this thesis was as follows:

I Participated in planning the study together with industrial partners and supervisors, carried out most of the experimental work, participated in evaluating the results and was responsible for writing the manuscript.

II Participated in planning the study together with industrial partners and supervisors, carried out most of the experimental work, participated in evaluating the results and was responsible for writing the manuscript.

III Participated in designing the study together with supervisors, evaluating the results and was responsible for writing the manuscript.

IV Participated in designing the study together with the co-authors, assisted with the experimental work and participated in analysing the results and writing the manuscript.

Abbreviations

ANOVA	Analysis of variance
DP	Degree of polymerisation
dw	Dry weight
HPSEC	High performance size exclusion chromatography
M_{cf}	Calcofluor average molecular weight
MW	Molecular weight
M_w	Weight average molecular weight
NIR	Near infrared
β-Glucan	Mixed linkage (1→3,1→4)-β-D-glucan

1 Introduction

Cereals are staple foods all over the world but which cereal that is most used for human food in a particular country varies depending on climate and culture. Barley (*Hordeum vulgare* L.) has been cultivated possibly as far back as 17 000 years ago and it can adapt to a wider range of environmental conditions than any other cereal crop (Nilan & Ullrich 1993). Barley has been cultivated in Sweden for many years and was previously an important food crop but today it is mostly used for animal feed and brewing. Barley has gained more interest as a human food during recent years due to the high content of dietary fibre. The content can differ depending on variety and growth conditions but hulless barley contains 13.6–20.2% dietary fibre (Oscarsson et al. 1996), while wheat contains only 11.5–15.5% (Andersson et al. 2013).

Barley especially has an high content of mixed-linkage (1→3),(1→4)-β-D-glucan (β-glucan). The starchy endosperm cell walls contain approximately 70% β-glucan and approximately 20% arabinoxylan (Fincher 1975). This means that the β-glucan content is high throughout the starchy endosperm and not just located in the outer part of the kernel, as is the case for the majority of dietary fibre in most cereals.

Barley is also an interesting cereal to study since many varieties exists, from both spontaneous and induced mutation, and they have a wide range of polysaccharide composition. Barley is diploid, self-fertilised and easy to hybridise. Genetic studies of barley are also simplified due to that cultivated barley only has 7 chromosome pairs, and they are relatively large (Nilan & Ullrich 1993).

1.1 Dietary fibre

Simply put, dietary fibre is polysaccharides that are not digested in the small intestine in human. A more precise definition has been debated since the term was coined by Hipsley in 1953 (Hipsley 1953). In 2008 the European Commission agreed on a definition to be used within the European union (Commission Directive 2008/100/EU):

> "Fibre" means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:
>
> - edible carbohydrate polymers naturally occurring in the food as consumed;
> - edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence;
> - edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence.

Also included are non-carbohydrate compounds (e.g. lignin) if these are closely associated with dietary fibre, but not if they are extracted and later added to foods.

A similar definition has also been decided by CODEX Alimentarius, which is part of the World Health Organisation and Food and Agriculture Organisation and sets guidelines for national regulatory authorities. The largest difference is that CODEX Alimentarius leaves it to each country to decide whether to include polymers with 3-9 monomers or not (CODEX 2009; CODEX 2016).

In plants, polysaccharides serve several functions: they give structure, act as an energy reserve and are water binding. Polysaccharides include both starch that is mostly digestible to humans and dietary fibre according to the above definition. The structure of a polysaccharide depends on the units in the polymer but also how they are linked together. Cellulose, amylose, amylopectin and β-glucan are all built from D-glucose units but different linkages give them distinctly different properties. Polysaccharides with only one type of monomer are called homopolysaccharides, while polysaccharides with more than one type of monomer are called heteropolysaccharides. The number of monosaccharide units in a polymer can be an important factor for the function and is usually depicted as degree of polymerisation (DP).

In humans dietary fibre can provide many health benefits, such as lower risk of cardiovascular disease, type II diabetes and colorectal cancer (Lattimer & Haub 2010; Mudgil & Barak 2013). Recommended intake of dietary fibre varies between countries but is usually 25-35 g/day though many people have an intake lower than that (Buttriss & Stokes 2008). To increase the intake of dietary fibre more whole grain cereals can be included in the diet, or normally consumed wheat products can be replaced by similar products made from cereals with a higher dietary fibre content, e.g. barley.

1.1.1 β-Glucan

β-Glucan is an highly interesting dietary fibre due to its health benefits. The European Food Safety Authority has approved a health claim which states that β-glucan contributes to maintaining normal blood cholesterol levels. This claim can be used for foods that contain at least 1 g β-glucan (from oats, oat bran, barley, barley bran, or from mixtures of these sources) per portion and at least 3 g/day (Commission Regulation (EU) No 432/2012). It does not specify anything regarding molecular weight of the β-glucan included, but it has been shown that higher molecular weight β-glucan has a greater cholesterol-lowering ability than low molecular weight β-glucan (Wolever et al. 2010).

β-Glucan is an unbranched homopolysaccharide built from β-D-glucopyranose units that are linked with (1→3)-glycoside and (1→4)-glycoside linkages. Each (1→3)-glycoside linkage is usually separated by two or three (1→4)-glycoside linkages (Figure 1). Approximately 10% of β-glucan consists of structures with four or more (1→4)-glycoside linkages between (1→3)-glycoside linkages. The proportion of different linkages affects the structure and thereby the function of β-glucan. Each (1→3)-glycoside linkage induces a kink in the structure, making it more flexible and soluble, while segments with only (1→4)-glycoside linkages may mimic cellulose and be more insoluble (Delcour & Hoseney 2010). Other regular segments can also form more insoluble structures, for example can several consecutive cellotriosyl units form a helix and create cross-linkage (Böhm & Kulicke 1999; Izydorczyk & Dexter 2008).

The structure of β-glucan can be studied by lichenase hydrolysis. Lichenase cleaves (1→4) linkages that are adjacent to a (1→3) linkage, thereby generating oligomers in a specific manner. The relation between different oligomers can then give information on the molecular structure of the polymer. Different cereals generally have different ratios of DP3/DP4, with wheat, barley and oats having 4.5, 3.3 and 2.2, respectively (Cui et al. 2000).

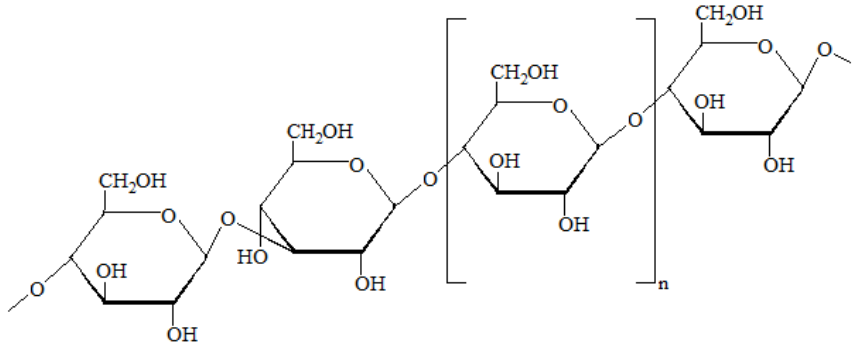

Figure 1. Schematic structure of β-glucan, where n equals 1 or 2 in 90% of the polymer and is larger than 2 in 10% of the polymer.

1.1.2 Arabinoxylan

Arabinoxylan is a heteropolysaccharide built from arabinose and xylose residues. It has a backbone of (1→4) linked β-D-xylose residues, of which some are substituted with α-L-arabinose residues. The substitutions are usually either at O-3 or at O-3 and O-2 in wheat and rye, while barley also has monosubstitution at O-2 (Izydorczyk & Dexter 2008; Saulnier & Quemener 2009). The substitution pattern and ratio of arabinose to xylose affect the structure, and thereby the physical properties of the molecule (Vinkx & Delcour 1996). The substitution pattern is non-random, with highly substituted regions usually followed by regions of less substitution (Izydorczyk & Dexter 2008). It seems that in water-extractable arabinoxylan usually half of the xylose residues are substituted, while non-water extractable arabinoxylan exists as low, intermediate and highly substituted (Vinkx & Delcour 1996). The ratio of arabinose to xylose in arabinoxylans from barley also varies substantially depending on location. The hull can have a ratio of 1:9 while in the starchy endosperm it is 1:3, but the ratio also depends on the extraction method (Newman & Newman 2008).

1.1.3 Small constituents

There are other dietary fibre components in barley which constitute a smaller fraction, e.g. fructan and cellulose.

Cellulose is a linear, insoluble homopolysaccharide similar to β-glucan but with only (1→4)-glycoside linkages. This makes the polymer form a flat, ribbon-like structure that enables cellulose to interact via numerous hydrogen bonds and form fibrous bundles (BeMiller & Huber 2008). Cellulose provides structure to plants and is an abundant molecule in nature, but only a small constituent in barley kernels.

Fructan is built from β-D-fructose units with (2→6) and (2→1)-fructosyl linkages and usually has a terminal α-D-glucose residue (Verspreet et al. 2015). There are several types of fructan structures depending on the origin, but fructan in barley consists of a branched structure with both (2→6) and (2→1)-fructosyl linkages (Verspreet et al. 2015). The main function of fructan is as an energy reserve for the plant, but it can also play a role as a membrane stabiliser and stress tolerance mediator (Van den Ende 2013). Several methods for measuring total dietary fibre content in foods do not include fructan, since it is soluble and usually removed during sample preparation. This means that fructan often has to be analysed separately and added to the total dietary fibre content.

1.2 Starch

Starch occurs in plants in particles called granules (BeMiller & Huber 2008), which are insoluble in cold water but take up water, swell and disrupt when heated. When cooled, some of the molecules partly re-associate and crystallise, which is called retrogradation. Starch is composed of two polymers, the mostly linear amylose and the highly branched amylopectin. Amylose consists of mainly (1→4) linked α-D-glucopyranosyl units, and can adopt a helical shape. Many of the hydroxyl groups are on the outside of the helix, making it hydrophilic, while the inside is hydrophobic due to high prevalence of hydrogen atoms. Amylopectin is one of the largest molecules in nature and can have a molecular weight up to 5×10^8 g/mol. As amylose, it consists of only α-D-glucopyranosyl units, but it is highly branched and have both (1→4) and (1→6) linkages. Starches from most plants contain approximately 25% amylose, but there are high amylose varieties and low amylose (waxy) varieties of many cereals, including barley (BeMiller & Huber 2008). In some studies, the composition of starch has been linked to the content of β-glucan. High amylose varieties and low amylose varieties have a higher content of β-glucan than varieties with normal starch (Andersson et al. 2008; Izydorczyk et al. 2000; Xue et al. 1997).

Amylose retrogradation is much faster than retrogradation of amylopectin. The amylose can be fully retrograded by the time baked goods have cooled to room temperature, while amylopectin retrogradation takes longer. The rate of retrogradation depends on several factors, including amylose/amylopectin ratio, temperature, presence of other interacting molecules and starch concentration (BeMiller & Huber 2008).

1.2.1 Resistant starch

Resistant starch is the part of starch that is not digestible in the human small intestine, which makes it a dietary fibre. Englyst et al. (1992) identified different types of resistant starch. Type I is physically inaccessible starch, type II is resistant starch granules (structurally resistant) and type III is retrograded amylose formed during processing. The amount of resistant starch type III formed during a process is influenced by several factors, where the water content and cooling conditions are important (Sajilata et al. 2006). The content of amylose in the starch is also highly influential and a high content leads to higher formation of resistant starch, as shown by Berry (1986) for maize and by Björck et al. (1990) for barley.

1.3 Processing

Most cereals are processed before consumption, e.g. by bread baking, cooking or extrusion. The effect on dietary fibre and starch depends on the process chosen, with some important factors being the water content, enzyme activity, shearing forces, heat and time of processing. The choice of raw material also affects the result, since different raw materials respond differently to processing.

1.3.1 Extrusion

Extrusion uses high temperature, high pressure and high shear forces to change the structure of the raw material. It is applied to make different breakfast cereals and snacks from cereal grains (Eastman et al. 2001). In extrusion, the processing parameters can be varied and different pressure, water addition, temperature, screw speed and shape of the screws and die can be used to affect the product. The temperature is normally in the range 90-180 °C and the pressure can regulate whether the product expands at the die or not. A typical effect of extrusion is higher extractability of dietary fibres (Comino et al. 2016; Østergård et al. 1989; Sharma & Gujral 2013; Vasanthan et al. 2002).

1.3.2 Baking

Baking of bread is popular world-wide and bread can be made from a range of different cereals. In the Western world, white wheat bread is mostly consumed. It has large volume and desirable structure, but contains low amounts of dietary fibre. Therefore bread with whole grain wheat or made from other cereals can be consumed instead to increase dietary fibre intake.

Since bread baking is a wide-spread practice, the process and product can differ considerably and thereby the effect on different ingredients. The yeast activity, fermentation conditions, kneading and mixing, enzymes and resting time are some factors that can influence the carbohydrates. Typical effects are degradation of fructan, increase in extractability of dietary fibres and formation of resistant starch.

Bread baking also decreases the molecular weight of β-glucan, due to endo β-glucanase in wheat and barley flour (Andersson et al. 2004; Flander et al. 2007). Several methods have been tested to minimise the β-glucan breakdown, and some reduction can be achieved by reducing mixing and fermentation time, increasing the particle size (Andersson et al. 2004; Åman 2004; Rieder et al. 2015) or possibly using sourdough instead of yeast (Gamel et al. 2015).

2 Background - BarleyFunFood

This thesis work was part of the thematic research project BarleyFunFood run by the Faculty of Natural Resources and Agricultural Sciences at the Swedish University of Agricultural Sciences. The general aim was to increase understanding of polysaccharide biosynthesis in cereals and to produce cereals with improved carbohydrate composition for further use in the industry.

Six different barley varieties were chosen to be included in the work in the BarleyFunFood projects. These were: Gustav, NGB 114602, SLU 7, KVL 301, SW 28708 and Karmosé (Table 1).

Table 1. *Characteristics of barley varieties*

Barley variety	Characteristics
Gustav	Feed, reference variety
NGB 114602	Anthocyanin rich, similar to Gustav
SLU 7	High β-glucan, high fructan, shrunken endosperm
KVL 301	Low β-glucan, low fructan
SW 28708	Low amylose (waxy), hulless
Karmosé	High amylose

These varieties were chosen based on a selection process that started by collecting 250 barley varieties from gene banks, different universities and commercial breeders all over the world. These were screened with near infrared (NIR) techniques and 20 barley varieties with different NIR spectra and differences in previously documented traits were chosen and grown in Vilcún, Chile, during summer 2008/09. The cultivation properties and carbohydrate composition of the 20 barley varieties were characterised and a principal component analysis was carried out (Figure 2). This revealed that the 20 barley varieties could be grouped into five clusters, and one variety from each cluster was chosen to be included in BarleyFunFood based on known traits. The reference cultivar Gustav, in the same cluster as NGB 114602, was also included, making six varieties in all.

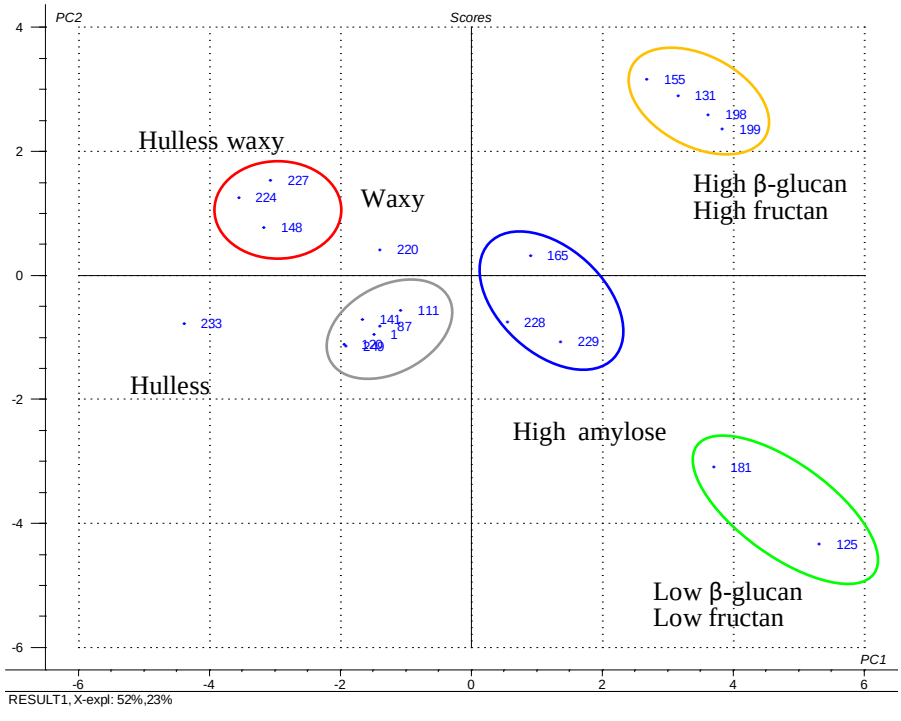

Figure 2. Principal component analysis plot of 20 selected barley varieties, with each number representing one variety.

During the evaluation and selection of varieties, some traits seemed to be associated. For example, there was a correlation between β-glucan content and fructan content, whereby varieties with a high content of β-glucan also had a high content of fructan (Figure 3) which has not been shown previously.

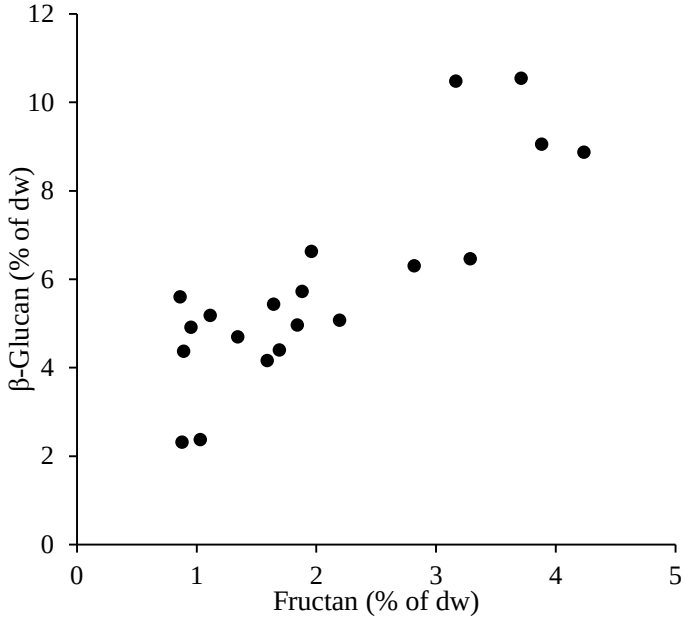

Figure 3. β-Glucan and fructan content (% of dry weight) in the 20 barley varieties screened.

3 Objectives

The overall aim of this thesis was to study the carbohydrate quality of six different barley varieties in order to determine whether some are more suited for certain products than others and to increase knowledge on how cereal carbohydrates are affected by processing.

Specific objectives of this thesis were to:

- Determine the content and composition of dietary fibre and starch in kernels, sifted flour, extruded product, bread crumb and bread crust (Papers I and II).
- Evaluate whether differences in kernels were evident in end products (Papers I and II).
- Evaluate whether different barley varieties respond differently to processing (Papers I, II and III).
- Study β-glucanase activity in barley flour and in mixtures of barley flour and wheat flour (Paper III)
- Study the molecular structure of β-glucan in different barley varieties to look for structural differences (Paper IV).

4 Materials and Methods

4.1 Sample materials and products

Six different barley varieties (see Table 1) were used in this thesis: Gustav, NGB 114602, SLU 7, KVL 301, SW 28708 and Karmosé. They were grown in Svalöv, Sweden, during the summer of 2012.

A 30 kg portion of kernels of each variety was milled in a laboratory mill (Laboratoriums-Mahlautomat model MLU 202, Gebruder Bühler Maschinenfabrik, Uzwill, Switzerland), producing six fractions of sifted flour and two bran fractions from each variety. The sifted fractions were pooled, extensively characterised (Papers I, III and IV) and used for making products, while the bran fractions were saved and only analysed to a limited extent (Paper I).

In Paper I, the sifted flour was used to make an extruded breakfast cereal (Figure 4). This was done in a twin-screw extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, U.K.) with addition of 0.8% salt (based on fresh flour weight). The flour feeding rate was 50 g/min and water addition was adjusted to give an expanded product.

In Paper II, breads were made with 50% sifted barley flour and 50% refined wheat flour ("Extra bageri vetemjöl", kindly provided by Lantmännen) (Figure 5). The crust and crumb were separated 1 cm below the surface, freeze-dried and analysed individually. Breads with 25% barley flour were also made for β-glucan analysis.

Before analysis, a centrifugal mill with a 0.5 mm sieve (Retsch, Hann, Germany) was used to mill the kernels, the bran, the extruded product, the bread crumb and the bread crust.

In Papers III and IV, the sifted flour was used as is. In Paper III, several refined wheat flours purchased at local supermarkets were also tested.

Figure 4. Extruded product made from 100% barley flour.

Figure 5. Breads made with 50% barley flour and 50% wheat flour.

4.2 Methods

All samples were analysed at least in duplicate and results are reported as average values on a dry weight (dw) basis after drying at 105 °C for 16 h. Detailed descriptions of established methods are available in the references, while methods modified during this work are described briefly below and more extensively in Papers I-IV.

4.2.1 Dietary fibres

The dietary fibre content and composition of the kernels, sifted flour, extruded product and bread crust and crumb were analysed (Papers I and II). Total dietary fibre content was analysed as neutral sugar residues according to Theander et al. (1995) (AOAC Method 994.13), with modifications by Andersson et al. (1999) for analysis of extractable and non-extractable constituents separately. β-Glucan content was determined with a mixed-linkage beta-glucan kit (K-BGLU; Megazyme, Bray, Ireland) according to McCleary & Codd (1991) (AOAC Method 995.16). Extractable β-glucan content and calcofluor molecular weight distribution of β-glucan were analysed by high performance size exclusion chromatography (HPSEC) according to Rimsten et al. (2003), but with a calcofluor concentration of 0.0025%. This technique excludes molecules smaller than 10^4 g/mol (Munck 1989), but it is highly specific for β-glucan and the fluorescence response is independent of molecular weight (Rimsten et al. 2003). Molecular weight of arabinoxylan was analysed by HPSEC coupled to multiple angle laser light scattering and refraction index detectors according to Andersson et al. (2009). Arabinoxylan with a retention time of 14.4-22.0 min was included in the results. Fructan content was analysed with an assay kit: K-FRUC (Megazyme, Bray, Ireland) according to McCleary et al. (1997) (AOAC Method 999.03). To remove

galactosyl-sucrose oligosaccharides a pre-treatment step with α-galactosidase was included. The method was modified as follows (Paper I): the extraction step was scaled down to 100 mg sample accurately weighed into a glass tube and 10 mL preheated deionised water for 20 min at 80 °C. The filtration step was replaced with centrifugation: 1 mL was centrifuged for 15 min at 10 600 × g and the supernatant was then used instead of the filtrate for analysis.

4.2.2 Starch

Starch and amylose content were analysed for the kernels, sifted flours, extruded products and bread crust and crumb. For sifted flours, bread crumb and crust and the extruded product, resistant starch content was also analysed (Papers I and II).

Starch content was determined with thermostable α-amylase according to Åman et al. (1994), while amylose content was analysed according to Chrastil (1987), but with solubilisation and lipid removal according to Morrison & Laignelet (1983). The resistant starch content was analysed with an assay kit from Megazyme (K-RSTAR; Megazyme, Bray, Ireland), according to McCleary & Monaghan (2002) (AOAC Method 2002.02).

4.2.3 Enzymatic activity

β-Glucanase activity in the sifted flour was measured in two ways (Paper III). First, it was determined with a kit from Megazyme (K-MBGL 03/11, Bray, Ireland) according to McCleary & Shameer (1987), but with an incubation time of 4 h instead of 10 min to account for the low activity in flours compared with malt for which the kit was developed. Second, the enzyme activity was measured as the effect on β-glucan molecular weight and extractability during incubation. Barley flour and different flour mixtures were incubated with water for 30 min at 37 °C and then the molecular weight and extractability were measured according to Rimsten et al. (2003) as described previously.

To further evaluate the activity of wheat enzymes on β-glucan from different barley varieties, two extracts of each barley flour were made. The endogenous enzymes in the barley flour were inactivated by addition of 50% ethanol and placing in a boiling water bath. After washing with 50% ethanol, the pellet was dispersed in $CaCl_2$-solution (0.30 mg/mL) with α-amylase and placed in a boiling water bath for 1.5 h to degrade starch. After cooling and centrifugation, the first extract (“Solution1”) was taken from the supernatant. In another portion of the supernatant, the polymers were precipitated by addition of ethanol (to obtain 80% ethanol) and cooled. The pellet was

dissolved in water to form the second extract ("Solution 2"). These extracts were then added to wheat flour and incubated for 30 min at 37 °C and thereafter the molecular weight and extractability were measured by HPSEC according to Rimsten et al. (2003) as described previously.

4.2.4 Structural characterisation

The structure of β-glucan was studied in different fractions (Paper IV). Two extractions were made from the sifted flour of each barley variety, giving three samples (Figure 6). The first extraction was done with water and α-amylase in a boiling water bath. After centrifugation, the supernatant was collected and precipitated with ethanol (60%), giving sample 1 (WE). The pellet from the first extraction was incubated in 50mM NaOH and the supernatant was neutralised and precipitated with ethanol (60%), giving sample 2 (NaE). The third sample was the pellet from the NaOH incubation (Res). The samples were dispersed in 20 mM NaH_2PO_4-buffer and incubated with lichenase at 50 °C for 1 h. The samples were then analysed with high performance anion exchange chromatography. The chromatograms generated were used to analyse the lichenase digestion of β-glucan in the different samples, in order to detect differences in the molecular structure in different varieties and fractions (WE, NaE and Res).

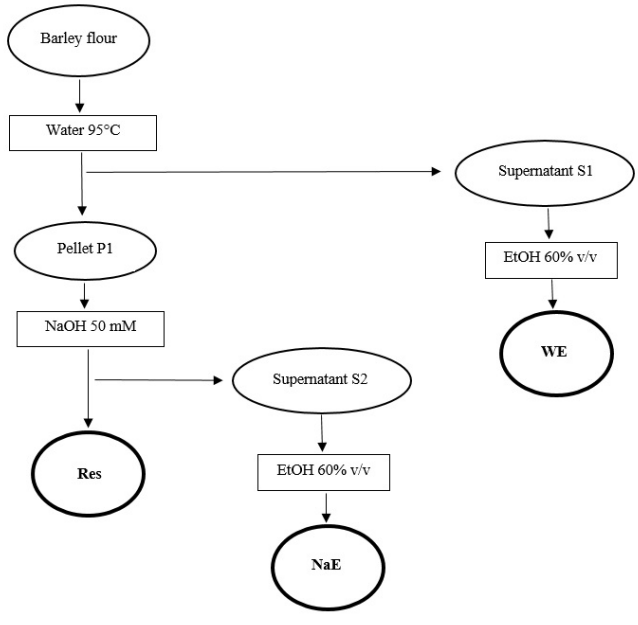

Figure 6. Extraction procedure for analysis of the molecular structure of β-glucan in barley flours.

5 Results and discussion

In this section, results from all papers are presented and discussed together. For details and specific values, please see the corresponding paper: Paper I for milling and extrusion, Paper II for bread baking, Paper III for enzyme activity and Paper IV for molecular structure of β-glucan.

5.1 Total dietary fibre

The difference in total dietary fibre content was large between whole kernels and sifted flour. The hulless variety SW 28708 was affected less, while SLU 7, which has a shrunken endosperm, was affected more than the other varieties, as could be expected. A large proportion of dietary fibre, in particular insoluble dietary fibre, is removed with the bran fractions. This leads to a lower content of total dietary fibre in sifted flour than in kernels, and also higher extractability.

The total dietary fibre content in both kernel and sifted flour was highest for SLU 7, despite the large decrease between kernels and sifted flour of this variety. The total dietary fibre content was lowest in SW 28708 kernels but, due to the smaller decrease in content for SW 28708, it was NGB 114602 that had the lowest content in the sifted flour.

Extrusion gave a small decrease in total dietary content for all varieties. SLU 7 had the highest content of total dietary fibre in the extruded product (16%), while NGB 114602 had the lowest content (8%). The extractability of total dietary fibre was increased by extrusion. KVL 301 had the lowest extractability in the flour and also in the extruded product (34% and 44%, respectively), while the other varieties had extractabilities of 43-57% in flour and 51-60% in the extruded product.

The total dietary fibre content was not significantly affected by baking. SLU 7 had the highest and NGB 114602 the lowest content in the flour

mixture, bread crumb and bread crust. The extractability of total dietary fibre showed a small decrease during baking, but was in the same range for flour mixture (37-53%), bread crumb (36-49%) and crust (36-51%).

Figure 7 shows the total dietary fibre content in the kernels, sifted flour, extruded product, bread crumb and bread crust for all varieties. SLU 7 had the highest content of total dietary fibre in all samples. The content in the kernels, and even more so the content in the sifted flour, was largely reflected in the content in the products (Figure 7).

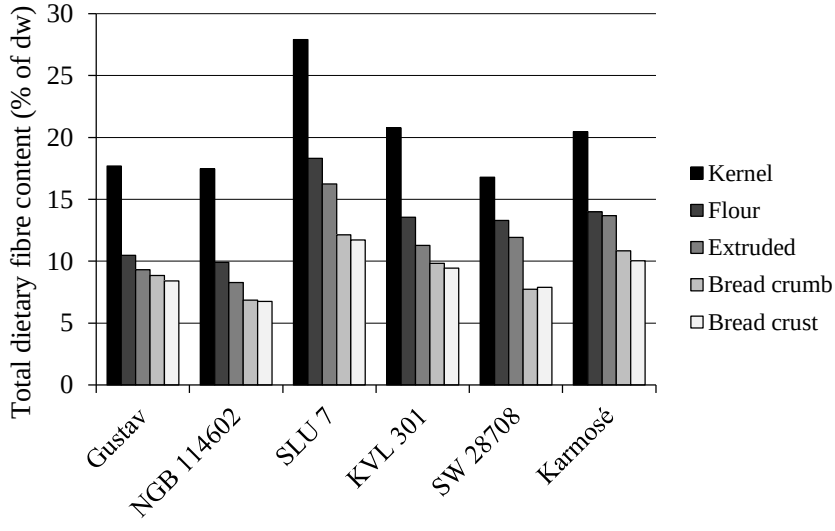

Figure 7. Total dietary fibre (% of dw) in kernel, sifted flour, extruded product, bread crumb and bread crust of the six barley varieties. Note that the bread was baked with 50% barley flour and 50% refined wheat flour.

5.2 Arabinoxylan

Arabinoxylan content was lower in the sifted flour than in kernels. An interesting observation was that KVL 301 had the highest content of arabinoxylan in kernels and sifted flour, despite having an average content of total dietary fibre. The extractability of arabinoxylan was higher in sifted flour than in kernels, but the molecular weight distribution was similar.

Arabinoxylan followed a similar trend as total dietary fibre and showed a small decrease in content and an increase in extractability by extrusion. The arabinoxylan content was not significantly affected by baking, which is contradictory to findings in other studies reporting a decrease (Andersson et al. 2009; Knez et al. 2014; Westerlund et al. 1989). SLU 7 and KVL 301 had the highest content of arabinoxylan in the flour mixture, bread crust and crumb, while NGB 114602 had the lowest. The extractability of arabinoxylan was significantly increased by baking ($p<0.05$) as previously reported (Comino et al. 2016; Westerlund et al. 1989) and the average molecular weight of arabinoxylan decreased significantly ($p<0.05$). NGB 114602 differed from the other varieties in that it had the lowest content of arabinoxylan, but one of the highest extractabilities.

5.3 β-Glucan

β-Glucan content was highest in SLU 7 kernels and sifted flour, making this variety a good candidate for high β-glucan products. As for total dietary fibre the extractability of β-glucan was expected to be higher in the sifted flour than in kernels and this was the case for all varieties except SW 28708. SW 28708 had a lower content of β-glucan in the sifted flour than in kernels, a pattern found for all varieties, but was the only one with a higher content of non-extractable β-glucan in the sifted flour than in kernels. Analysis of the outer and inner bran fractions of SW 28708 and Gustav revealed that the pattern was clearly different in SW 28708, with a high content of extractable β-glucan in the inner and outer bran of SW 28708 (Figure 8). SW 28708 is a hulless variety while the other varieties studied are hulled, which can explain some of the difference. However, it also means that the inner and outer bran of SW 28708, with their high content of extractable β-glucan, could be used for food production with less processing than needed for hulled varieties, where the bran and hull must first be separated.

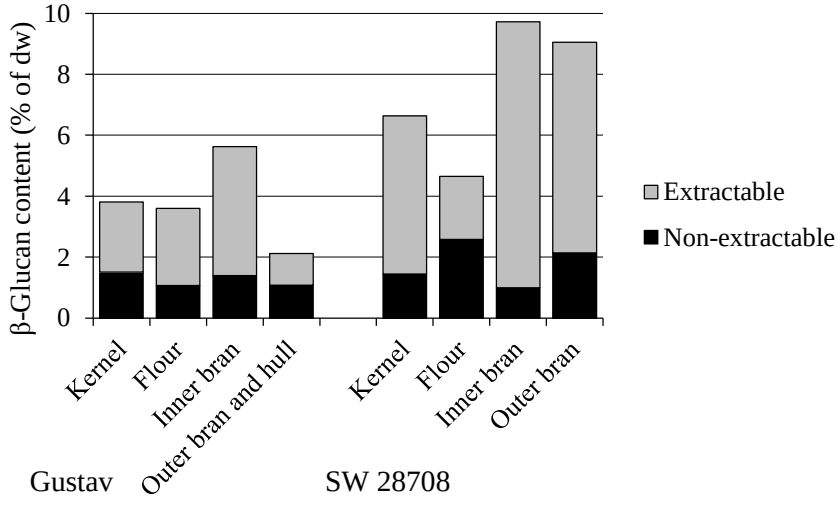

Figure 8. β-Glucan content (black non-extractable, grey extractable) in kernels, sifted flour, inner and outer bran (and hull) of the barley varieties for Gustav and SW 28078 (% of dw). Reproduced from Djurle et al. (2016) (Paper I), with permission from Elsevier.

SLU 7 also had the highest content of β-glucan in the extruded product. The extractability of β-glucan after extrusion was high (92-98%) for all varieties except KVL 301, which had an extractability of 76%. KVL 301 was also the variety with the lowest content of β-glucan in the extruded product. An increase in the extractability of β-glucan by extrusion has been shown in many previous studies (Comino et al. 2016; Sharma & Gujral 2013; Vasanthan et al. 2002) and high extractability of β-glucan is often desired in health-promoting food products, along with high molecular weight. The average molecular weight was decreased by extrusion and the molecular weight distribution showed a shift to lower molecular weight (Figure 9).

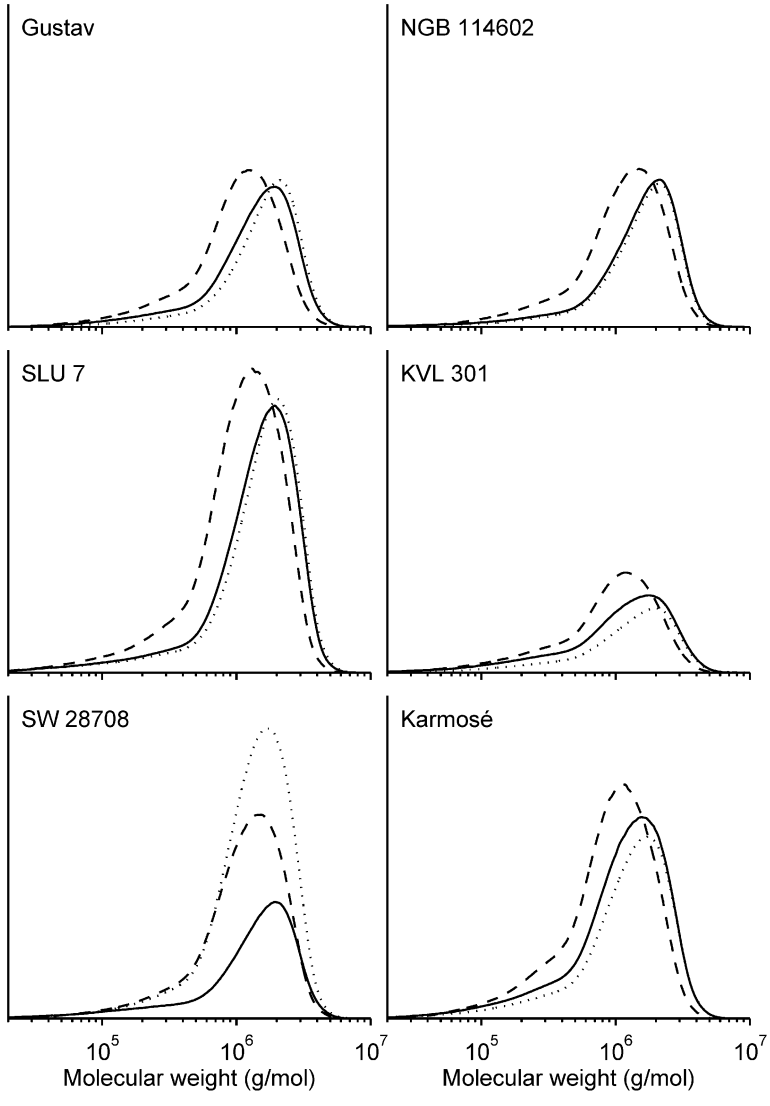

Figure 9. Molecular weight distribution of β-glucan in kernels (dotted line), sifted flour (continuous line) and extruded product (dashed line) of the six barley varieties. Area under the distribution curve represents content of extractable β-glucan. Reproduced from Djurle et al. (2016) (Paper I), with permission from Elsevier.

There was a small but significant ($p<0.05$) decrease in β-glucan content caused by bread baking. Previous studies have reported a decrease (Choi et al. 2011) or no significant change (Andersson et al. 2009; Blandino et al. 2013; Koletta et al. 2014). SLU 7 had the highest content of β-glucan in bread crust and crumb, while the other varieties had a similar content (Figure 10).

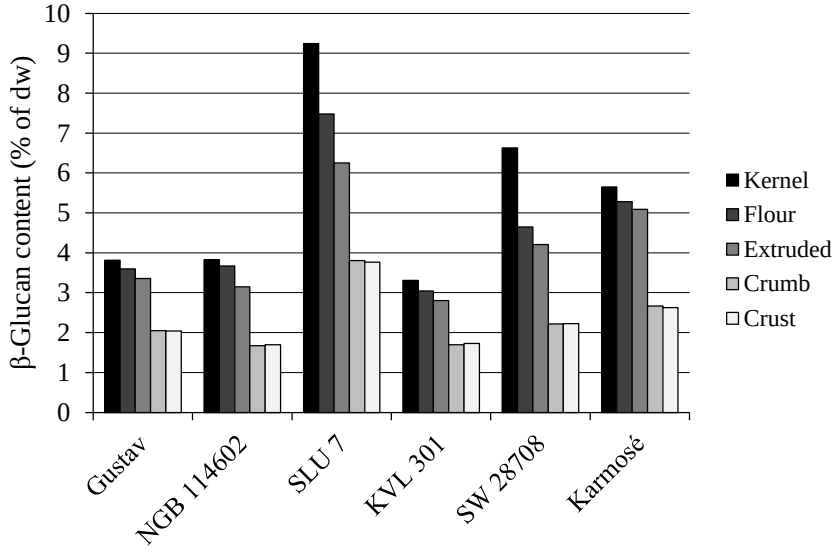

Figure 10. β-Glucan content (% of dw) in in kernels, sifted flour, extruded product, bread crumb and bread crust of the six barley varieties. Note that the bread was baked with 50% barley flour and 50% refined wheat flour.

Baking increased the extractability of β-glucan significantly ($p<0.05$), an effect that has also been shown in other studies (Andersson et al. 2004; Comino et al. 2016). The difference in extractability between crumb and crust was small for all varieties except KVL 301, where the crumb had a noticeably higher extractability than the crust (97% and 76%, respectively). Most studies analyse the crumb and crust together, so to the best of my knowledge this difference has not been seen previously.

There was a significant ($p<0.05$) decrease in average molecular weight of β-glucan caused by bread making. This has also been reported in many previous studies (Andersson et al. 2004; Cleary et al. 2007; Ronda et al. 2015). This decrease is a problem, since high molecular weight β-glucan has greater cholesterol-lowering ability than low molecular weight β-glucan (Wolever et al. 2010). In Paper II, it was found that one of the varieties, SLU 7, maintained a higher average molecular weight during baking than the other varieties. SLU 7 had an average molecular weight of 0.86×10^6 and 0.79×10^6 g/mol in the bread crumb and crust, respectively, while the other varieties had 0.36-0.50×10^6 and 0.36-0.47×10^6 g/mol, respectively. The molecular weight distribution of β-glucan before and after baking showed breakdown for all

varieties, but that of SLU 7 was different, with two distinct peaks (Figure 11). The smaller of these two peaks corresponded well with the peaks in the other varieties, while the higher peak represented β-glucan that was not as affected by bread baking as the β-glucan in the smaller peak and in the other varieties.

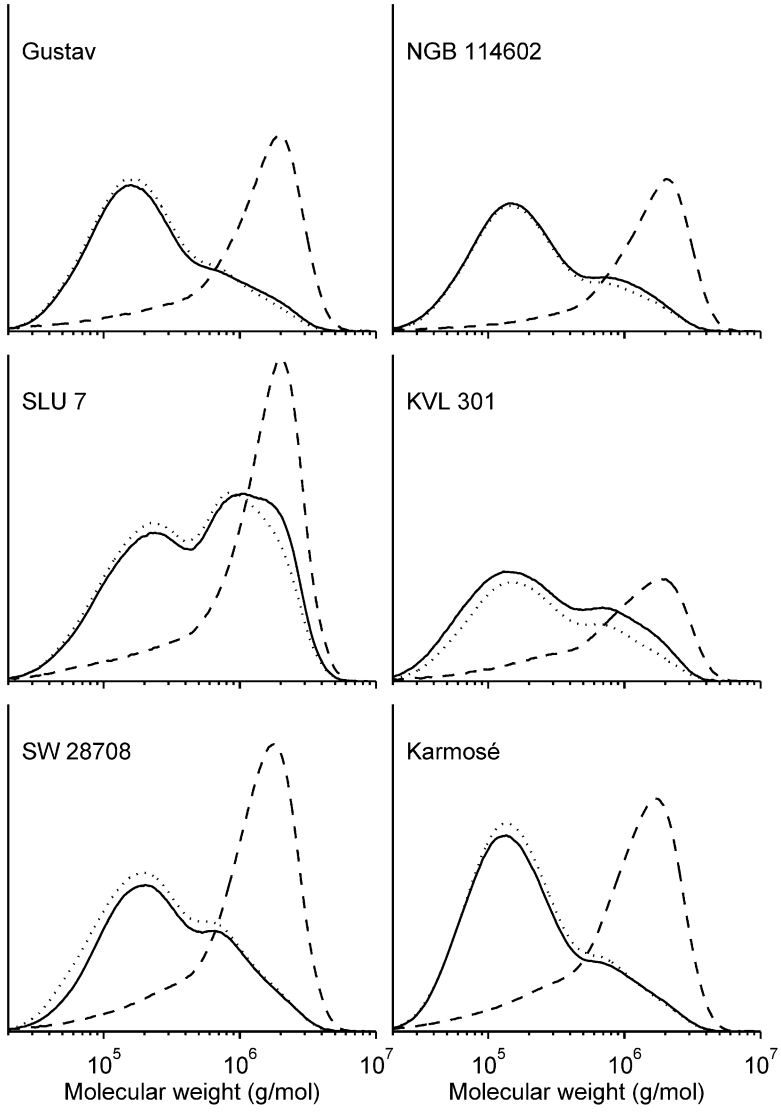

Figure 11. Molecular weight distribution of β-glucan in the flour mixture (dashed line), bread crumb (continuous line) and bread crust (dotted line) of the six barley varieties. Area under the distribution curve represents content of extractable β-glucan.

To determine whether SLU 7 displays the same behaviour when included in bread in lower proportions, bread with 25% barley flour and 75% wheat flour was also analysed. This bread gave a similar molecular weight distribution curve with more of the higher peak than in other varieties, but more breakdown than in 50% bread (Figure 12).

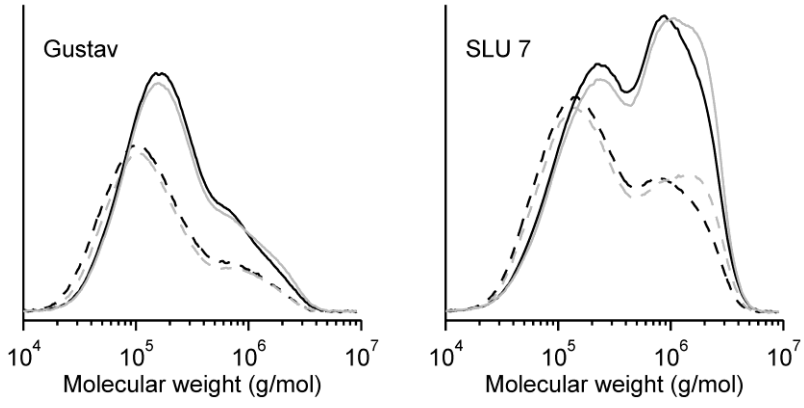

Figure 12. Molecular weight distribution of β-glucan in bread crumb (gray) and bread crust (black) in 50% barley bread (continuous line) and 25% barley bread (dashed line) of Gustav and SLU 7. Area under the distribution curve represents content of extractable β-glucan.

5.3.1 β-Glucanase activity

β-Glucanase activity analysed according to McCleary & Shameer (1983) was similar for all barley varieties, but higher for wheat flour. A large part of the degradation of β-glucan therefore depends on the enzymes from wheat flour and differences in degradation cannot be explained by different β-glucanase activity in the different barley varieties. β-Glucanase activity was also measured as the effect on molecular weight and content of extractable β-glucan during incubation with water at 37 °C, with the purpose of mimicking baking. This was not directly correlated with results obtained for enzyme activity analysed according to McCleary & Shameer (1983) but instead the largest decrease in average molecular weight was found in Gustav, SW 28708 and Karmosé, which also had the highest extractability after incubation. This can possibly be explained by the fact that the method by McCleary & Shameer (1983) only includes soluble substrate, while the incubation method includes both soluble and non-soluble substrates.

The average molecular weight in barley flour and flour mixtures of barley and wheat was lower after incubation for all varieties. The molecular weight

distribution shifted to lower molecular weight, but it also displayed three populations after incubation (0.25, 0.65 and 2.0 × 10^6 g/mol, respectively), which indicates non-random action of the enzymes.

SLU 7 maintained a higher average molecular weight and in an incubated flour mixture with only 25% barley flour and 75% wheat flour the β-glucan population with the highest molecular weight was still clearly visible for SLU 7, while in the corresponding mixtures for the other varieties there were only small amounts left. SLU 7 also seemed to be able to inhibit the breakdown of β-glucan from Gustav, since the molecular weight distribution of the mixtures was more similar to that of SLU 7 than that of Gustav, and since the mixture had higher average molecular weight than could be expected from simple linear regression, taking concentrations of SLU 7 and Gustav into account.

Extracts of SLU 7 and Gustav flour were made to try to isolate a β-glucanase inhibitory factor in SLU 7, but this was unsuccessful. Therefore the most likely explanation is that the structure of the β-glucan in SLU 7, combined with its high content, is the key to the lower degradation.

5.3.2 Structure characterisation of β-glucan

Sequential extraction of β-glucan was performed and the fractions were digested by lichenase giving oligosaccharides, most with a DP of 3-9. The DP3/DP4 ratio was higher for SLU 7 than for the other varieties in all fractions and significantly different from all but SW 28708, which had the second highest ratios. Higher DP3/DP4 ratios for waxy than non-waxy genotypes have been reported previously (Lazaridou & Biliaderis 2007). The relation between different oligosaccharides affects the conformation of the β-glucan chain, and the β-glucan structure of SLU 7 could therefore be different than the structure of the other varieties. A higher DP3/DP4 ratio can give multiple intermolecular associations that lower solubility (Böhm & Kulicke 1999). The higher DP3/DP4 ratio in SLU 7 may therefore indicate a more compact structure, which may have hindered the action of β-glucanase during bread making, resulting in lower β-glucan breakdown.

SW 28708 was most similar in oligosaccharide pattern to SLU 7. In Figure 11, two populations of β-glucan are visible in SW 28708 bread, although not with the same amount of the high molecular weight population for SW 28708 as for SLU 7. This indicates some similarities between the two varieties, in structure and in behaviour during baking. The feature was more pronounced in SLU 7 which, combined with the high content of β-glucan, makes SLU 7 a highly interesting variety to study.

5.4 Starch

The content of starch followed the opposite trend to the content of total dietary fibre and was higher in the sifted flour than in kernels for all varieties. SW 28708 had the smallest and SLU 7 the largest difference, as also found for total dietary fibre. Gustav and NGB 114602 had the highest content of starch in the kernels and in sifted flour. No difference in the amylose content in starch was observed between kernels and sifted flour.

Extrusion did not have an effect on the starch content or the content of amylose in the starch for any of the varieties tested. Resistant starch was found in flour and extruded product of the high amylose variety Karmosé, but only traces were found in the other varieties. The formation of resistant starch during extrusion is highly dependent on the conditions applied. Previous studies have shown no formation (Østergård et al. 1989), lower content in the extruded product than the raw material (Faraj et al. 2004; Murray et al. 2001) as well as formation of resistant starch (Huth et al. 2000).

Bread baking decreased the starch content significantly ($p<0.05$). The average content in the flour mixture was 67.7%, while bread crumb contained 55.8% and bread crust 56.2%, on average. The content of resistant starch was increased by bread baking ($p<0.05$). The content was higher in bread crumb than in bread crust, which is line with previous findings (Johansson et al. 1984; Englyst et al. 1983), and was probably due to the higher moisture content in the bread crumb, enabling resistant starch formation. Karmosé had the highest content of resistant starch in the bread, while SW 28708 had the lowest. This is also in line with previous studies that have shown that higher amylose content gives greater formation of resistant starch (Berry 1986; Björck et al. 1990), as Karmosé had the highest amylose content and SW 28708 the lowest.

5.5 Small dietary fibre constituents

The content of fructan was similar in kernel and sifted flour for all varieties, which indicates it was evenly distributed in the kernel. Cellulose, on the other hand, was unevenly distributed with a higher content in kernels than in sifted flour, meaning it was mostly located in the outer parts of the kernel.

Fructan is usually degraded during baking (Andersson et al. 2009; Knez et al. 2014) and only a small amount of fructan was left in the crumb and crust of bread made from the six varieties studied here (0.3-0.7%). This is due to the action of the enzyme invertase in baker's yeast (*Saccharomyces cerevisiae*) (Nilsson et al. 1987; Verspreet et al. 2013). Several studies have reported an increase in total dietary fibre content by bread baking (Johansson et al. 1984; Siljeström & Asp 1985) but it is crucial to note if the authors of different

studies have include fructan or not, since older studies usually don't. Since total dietary fibre is a value including several components, some might increase and some decrease, giving no change in the sum. In relation to bread making, the inclusion of fructan in total dietary fibre might lead to a decrease since most fructan is usually degraded. Resistant starch is usually formed during bread baking, but the level is highly dependent on the amylose content in the starch and other factors mentioned previously. Therefore, there will be an increase in total dietary fibre if the formation of resistant starch is greater than the degradation of fructan, and vice versa. There might also be changes in other constituents that affect the sum of total dietary fibre, but the balance between resistant starch formation and fructan degradation is important for deciding whether there will be an increase or decrease in total dietary fibre content by baking.

6 Main findings

- The differences in composition seen in kernels were largely reflected in the products. SLU 7 had the highest content of total dietary fibre in kernels and in both products, while NGB 114602 had the lowest total dietary fibre content in the flour and both products. SLU 7 also had the highest content of β-glucan in kernels and both products, while KVL 301 and NGB 114602 had the lowest content of β-glucan in kernels and both products.

- The extractability of β-glucan was higher in the outer parts of the kernel than in the starch endosperm for SW 28708, which was unexpected. The content of β-glucan was also higher in the outer part.

- Fructan seems to be evenly distributed in the kernel.

- The different varieties followed the same trends in response to processing but not always to the same degree, which can be used to optimise new food products.

- SLU 7 contains β-glucan that was more resistant to degradation during bread baking and could possibly protect β-glucan in mixtures with other barley varieties from degradation.

- Barley β-glucan was affected by β-glucanase from both wheat and barley during baking.

- SLU 7 had a higher DP3/DP4 ratio in β-glucan than other varieties, which could explain differences in β-glucan breakdown during bread baking and incubation.

7 Future research

In this thesis, the aim was to analyse different barley varieties throughout different food processing operations. Although some interesting findings were made, there is always more research to be done and more angles to explore. Some areas for future research are to:

- Further analyse the structure of β-glucan in SLU 7 compared with other barley varieties. New discoveries of differences in molecular structure could further explain the behaviour of β-glucan and β-glucanases during baking.

- Expand the molecular structure study to include more varieties and possibly find varieties with similarities to SLU 7, or that have other interesting characteristics.

- Explore the genetic differences in SLU 7, to find the genes responsible for the differences in β-glucan structure. Could these genes be transferred to other crops?

- Conduct further baking tests with SLU 7 and optimise the baking process to preserve β-glucan. There are many variables to explore, including sourdough, different proportions of barley flour, different particle sizes and fermentation time. The aim should be a SLU 7-bread that has β-glucan with high molecular weight and high enough content of β-glucan to use the EFSA health claim.

- If time and money had allowed, a sensory analysis of the different barley breads would have been included in this thesis. As part of the development of the SLU 7-bread, a sensory panel is essential to yield not only a healthy

bread but also a bread that people want to eat, as otherwise it will not help promote a healthier society.

- Explore the possibilities of the outer milling fractions of SW 28708 that had a high content of β-glucan with high extractability. This is an interesting raw material for further study.

- Study the microstructure of the kernel of the different barley varieties, to see if there are any differences. It would also be interesting to look at the sifted flours to determine the particle size distribution.

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