Characterisation of Dietary Fibre in Cereal Grains and Products

Emphasis on Triticale and Rye

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Cover: Triticale and rye grains
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Abstract

Utility of cereals is mainly defined by their composition. High content of extractable dietary fibre (DF) with retained molecular features may be desired for human consumption to derive certain benefits associated with DF. In contrast, low amount of extractable DF with degraded molecules will give higher feed value to cereals intended for animal feed.

This thesis investigated the composition of DF in cereals, particularly triticale and rye products. Processing effects on extractable DF components, e.g. arabinoxylan (AX), β-glucan and fructan, were also examined. The structure of AX and β-glucan in triticale, barley and tritordium and the rheology of triticale extracts as influenced by content and extractability, molecular size and structure of AX were analysed.

DF in triticale spanned a relatively narrow range (13-16%), with significant cultivar and location effects. Unfavourable growing conditions resulted in significantly lower molecular weights of AX, β-glucan and fructan in triticale. On the whole, triticale DF profile was more similar to wheat than rye. Among rye products, porridge had the highest DF content (23%) with retained molecular weight, followed by crisp breads (17.8%) and soft breads (12.6%). AX appeared to be more stable during processing, while β-glucan was more labile to endogenous enzymes. Substitution pattern of AX fragments released after enzymatic hydrolysis demonstrated less branching in triticale grown under unfavourable weather conditions. The molar proportion of cellotriosyl units of barley β-glucan had a strong positive correlation with the total content. Viscoelastic properties of triticale extracts varied between locations and cultivars. β-Glucan appeared to make a negligible contribution to triticale extract rheology, which was mainly influenced by structural features of AX rather than extractable content or molecular size.

The knowledge presented here will be useful for consumers of rye products when assessing processing-generated changes in DF content and composition. The cereal industry will be able to redefine processing parameters and DF labelling based on new facts. Broad variation in DF chemistry of triticale will provide more options for farmers and feed manufacturers to select cultivars best suited for animal feed formulation.

Keywords: Rye, triticale, dietary fibre, processing, arabinoxylan, β-glucan, fructan, enzymatic fingerprinting, rheology

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Dedication

To my parents for their endless prayers.

Words, without power, is a mere philosophy.
M. Iqbal

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 Rakha, A., Åman, P., Andersson, R. (2011). Dietary fiber in triticale grain. Variation in content, composition and molecular weight distribution of extractable components. *Journal of Cereal Science* doi:10.1016/j.jcs.2011.06.010.

II Rakha, A., Åman, P., Andersson, R. (2010). Characterisation of dietary fibre components in rye products. *Food Chemistry* (119), 859-867.

III Rakha, A., Åman, P., Andersson, R. (2011). How does the preparation of rye porridge affect molecular weight distribution of extractable dietary fibers? *International Journal of Molecular Sciences* (12), 3381-3393.

IV Rakha, A., Saulnier, L., Åman, P., Andersson, R. (2011). Enzymatic fingerprinting of arabinoxylan and β-glucan in triticale, barley and tritordium grains. (*Submitted manuscript*).

V Rakha, A., Åman, P., Andersson, R. (2011). Rheological properties of aqueous extracts from triticale grains. (*Submitted manuscript*).

Papers I-III are reproduced with the permission of the publishers.

The contribution of Allah Rakha to the papers included in this thesis was as follows:

I Carried out major part of analytical work, participated in evaluation of results and was responsible for writing the manuscript.

II Participated in the collection of samples, planning of experimental work and evaluation of results. Was responsible for analytical work and for writing the manuscript.

III Participated in the designing and planning of the experimental work and evaluation of results. Was responsible for major part of the analytical work and for writing the manuscript.

IV Participated in the planning of the experimental work and evaluation of results. Was responsible for major part of analytical work and for writing the manuscript.

V Participated in the planning of the experimental work and evaluation of results. Was responsible for analytical work and for writing the manuscript.

Abbreviations

A/X	Arabinose/xylose
ADF	Acid detergent fibre
ANOVA	Analysis of variance
AX	Arabinoxylan
AXOS	Arabinoxylan oligosaccharides
BG_3	3-*O*-β-Cellobiosyl-D-glucose
BG_4	3-*O*-β-Cellotriosyl-D-glucose
BG_5	3-*O*-β-Cellotetraosyl-D-glucose
BG_6	3-*O*-β-Cellopentaosyl-D-glucose
DF	Dietary fibre
DP	Degree of polymerisation
FEH	Fructan *exo*-hydrolase
GLM	General linear model
GOS	Gluco-oligosaccharides
HPAEC	High performance anion exchange chromatography
HPLC	High performance liquid chromatography
HPSEC	High performance size exclusion chromatography
M_{cf}	Calcofluor average molecular weight
M_n	Number average molecular weight
M_w	Weight average molecular weight
M_w/M_n	Polydispersity index
NDF	Neutral detergent fibre
PAD	Pulsed amperometric detection
PC	Principal component
PCA	Principal component analysis
PLS	Partial least square
RS	Resistant starch
RVA	Rapid Visco Analyzer

WE-AX	Water extractable arabinoxylan
X	Xylose
XX	Xylobiose
β-Glucan	(1→3) (1→4)-β-D-Glucan
δ	Phase angle
G*	Complex modulus
G′	Elastic modulus
G″	Viscous modulus

1 Introduction

1.1 Dietary fibre definition

Since the inception of the dietary fibre (DF) hypothesis by Burkitt, Trowell and colleagues in 1970s, our appreciation of DF and its relationship with health has led to a strong focus on biochemical and nutritional characterisation of DF in subsequent decades. The term 'dietary fibre' was coined by Hipsley (1953) and subsequently adopted and defined by Trowell (1972) as:

> "The residue derived from plant cell walls that is resistant to hydrolysis by human alimentary enzymes".

Since then, there was no universally accepted regulatory definition of DF until 2009, when CODEX finally adopted it (Phillips & Cui, 2011). Thus the most updated definition of DF by CODEX states:

> "Dietary fibre means carbohydrate polymers[1] with ten or more monomeric units[2], which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories:
>
> - Edible carbohydrate polymers naturally occurring in the food consumed.
> - Carbohydrate polymers which have been obtained from food raw materials by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.
> - Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.
>
> [1]When derived from a plant origin, dietary fiber may include fractions of lignin and/or other compounds when associated with polysaccharides in the plant cell walls and if these compounds are quantified by the AOAC gravimetric analytical method for dietary fibre analysis: Fractions of lignin and the other compounds (proteic fractions, phenolic compounds, waxes, saponins, phytates,

cutin, phytosterols, etc.) intimately "associated" with plant polysaccharides in the AOAC 991.43 method. These substances are included in the definition of fibre insofar as they are actually associated with the poly- or oligo-saccharidic fraction of fibre. However when extracted or even re-introduced in to a food containing non digestible polysaccharides, they cannot be defined as dietary fibre. When combined with polysaccharides, these associated substances may provide additional beneficial effects (pending adoption of Section on Methods of Analysis and Sampling).

[2]Decision on whether to include carbohydrates from 2 to 9 monomeric units should be left to national authorities."

A similar definition has been introduced in the EU by the European Commission under Commission Directive 2008/100/EC. However, one important difference is that this definition states that all carbohydrates with degree of polymerisation (DP) ≥ 3 should be included in the DF definition, and is therefore not optional for EU member states. Currently, both CODEX and the European Commission are working on a list of approved methods which can be used for the determination of DF content or content of individual DF components.

In countries such as Finland, Norway, New Zealand, Australia and Sweden, fructan and fructo-oligosaccharides have been allowed as part of DF in nutrition labelling for quite some time. However, many manufacturers do not include fructan in their DF labelling and are thus reporting low DF values which may result in a marketing disadvantage. Others often tend to use the enzymatic gravimetric methods AOAC 985.29 or AOAC 991.43 for DF measurements and combine these with spectrophotometric method (AOAC 999.03) of fructan analysis. Total DF calculated by combining the values of both analyses performed in separate runs is slightly overestimated, since fructan with longer DP precipitates during the ethanol precipitation step of the gravimetric method (Ku *et al.*, 2003). With the introduction of the CODEX definition of DF, where inclusion of carbohydrates with DP ≥ 3 is mandatory for EU member states, a strong urge resulted in a new method to analyse total DF including resistant starch (RS) and non-digestible oligosaccharides in one run (McCleary *et al.*, 2010). This method combines the key attributes of AOAC Official Methods of Analysis 985.29, 991.43, 2001.03 and 2002.02 and might help to harmonise DF labelling across EU.

1.2 Importance of dietary fibre

DF plays an important role in our daily nutrition. The typical Western diet contains < 20 g/day DF, which is considerably lower than the minimum

recommended for adults (25 g/day) by the European Food Safety Authority (EFSA, 2010). Major health benefits associated with optimum intake of DF include stool bulking, increased satiety and reduced risk of developing type 2 diabetes and coronary heart disease (Kendall *et al.*, 2010; Buttriss & Stokes, 2008). These health effects are attributed to the major DF components such as arabinoxylan (AX), mixed linkage (1→3) (1→4)-β-D-glucan (β-glucan), fructan, cellulose, RS, etc. and are governed by both quantity and quality of these components (Wood, 2010; Regand *et al.*, 2009; Tosh *et al.*, 2008). In animal feeding, however, high intake of DF, particularly soluble AX and β-glucan, has anti-nutritional effects. It may lower the feed value and reduce the weight gain (Choct *et al.*, 1992). Apart from their nutritional role, DF components also make a significant contribution to the rheological, technological and organoleptic properties of plant foods. For example in cereals, DF influences starch pasting properties (Santos *et al.*, 2008) and interferes with the starch-gluten matrix (Autio, 2006). Furthermore, higher inclusion of DF, particularly cell wall components, results in increased farinographic water absorption, the extent of which depends on characteristics of the fibre. Products enriched with DF are usually darker in colour and may lack consumer appeal. Polyphenol oxidase present in fibre-rich outer layers of the grain may lead to discolouration (Quinde-Axtell *et al.*, 2006). A brief account of major DF components is presented in the following sections.

1.3 Dietary fibre in cereals

Cereal grain consists of different tissues varying in composition and properties (Figure 1). For example, a typical wheat grain contains about 3% germ, 83% starchy endosperm and 14% peripheral layers (Barron *et al.*, 2007; Hemery *et al.*, 2007). Cell walls from the outer grain tissues are generally thick and play a protective role in grain. These cell walls are composed of cellulose and complex xylans and may contain a significant quantity of lignin. However, cell walls of aleurone and starchy endosperm are predominantly composed of AX and β-glucan, and smaller amounts of cellulose, heteromannans, protein and esterified phenolic acids (Hemery *et al.*, 2007; Saulnier *et al.*, 2007; Fincher & Stone, 1986). The cell walls of cereal grains are thus a major source of soluble and insoluble DF in our daily diet.

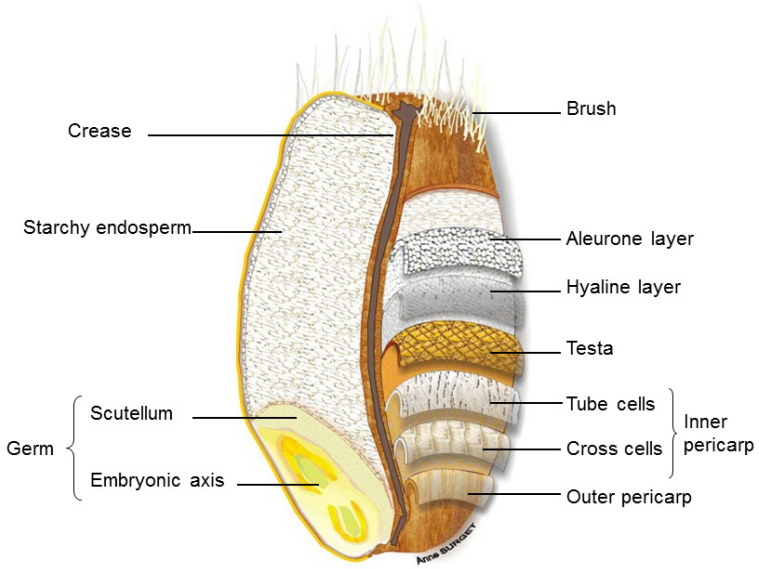

Figure 1. Histological composition of wheat grain. Adopted from Surget and Barron (2005).

Rye (*Secale cereale* L.) is the second most commonly used grain for bread production after wheat (Bushuk, 2001). Rye is winter hardy and has modest requirements regarding soil and climate, which makes it an interesting alternative for more sustainable production. It is a traditional food cereal in Northern and Eastern Europe and adds diversity to the bread and breakfast cereal markets in these regions. Triticale (X *Triticosecale* Wittmack), a man-made cereal, was developed by combining A & B genome of durum wheat (*Triticum turgidum* L.) and R genome of rye (*Secale cereal* L.) (Varughese *et al.*, 1996). The objective of triticale breeding was to merge the baking and yield potential of wheat with the adaptability of rye to almost all geographical ranges (McGoverin *et al.*, 2011; Bender, 2006; Varughese *et al.*, 1997). Thus under optimum growing conditions the yield potential of triticale is nearly the same as that of wheat, while it outperforms wheat in marginal environments (Varughese *et al.*, 1996). Triticale currently has limited utilisation, primarily as an animal feedstuff, but can also be used in baking by supplementing with wheat due to its low gluten content (McGoverin *et al.*, 2011). Triticale is a good source of protein with better biological value owing to its high lysine content, which is generally the limiting amino acid in cereals (Varughese *et al.*, 1996).

Both rye and triticale are important cereals in Sweden, with annual production of 218,900 MT and 255,400 MT respectively (FAO, 2009). In Northern and Eastern Europe, rye is mostly consumed in the form of bread or porridge. Contrary to rye, major usage of triticale is animal feed. Among different cereals of commercial importance, rye has the highest content of DF, ranging from 18-22% (Andersson *et al.*, 2009) or 15-18% when fructan is not included in DF calculations. The major components of rye DF are AX, fructan and mixed linkage β-glucan, with contents ranging from 8.0-12.1%, 4.5-6.4%, and 1.3-2.2%, respectively (Boskov Hansen *et al*., 2003). Triticale is normally intermediate in DF profile to its rye and wheat parents, with DF content ranging from 10-15% (Picolli da Silva & de Lourdes Santorio Ciocca, 2005; Pettersson & Åman, 1987). AX (≈7%) constitutes about half the total DF in triticale, while β-glucan (≈0.6%) is present in relatively smaller amounts (McGoverin *et al.*, 2011). The DF content of cereals is summarised in Table 1.

Table 1. *Dietary fibre content*[a] *(% of edible weight) of different cereals as reported by USDA National Nutrient Database SR 23 (USDA, 2010).*

Cereal	Dietary fibre (%)
Rye	15.1
Triticale	14.6
Wheat	12.2
Dehulled oats	10.6
Dehulled barley	10.1
Millet	8.5
Corn	7.3
White sorghum	6.3
Brown rice	4.6

[a]Dietary fibre content analysed by enzymatic-gravimetric method 985.29 of the AOAC. In the case of rye, the method was 991.43.

1.4 Major dietary fibre components

1.4.1 Arabinoxylan

AX, a non-starch polysaccharide, constitutes 60-70% of endosperm cell walls in most cereal grains, with the exception of the endosperm cell walls of oats and barley (≈20%) and rice (40%) (Stone, 2006; Fincher & Stone, 1986). The backbone of the AX molecule consists of (1→4)-linked β-D-xylopyranosyl residues, which may be either mono-substituted by α-L-arabinofuranosyl residue at O-2 or O-3 position or di-substituted at O-2 and O-3 (Figure 2). The mono-substitution at O-2 is rare in wheat and rye but more frequent in barley

(Saulnier & Quemener, 2009; Izydorczyk & Dexter, 2008; Gruppen *et al.*, 1992; Viëtor *et al.*, 1992). The substitution pattern may vary depending on cultivar and location of AX in the grain (Bonnand-Ducasse *et al.*, 2010; Izydorczyk & Dexter, 2008; Saulnier *et al.*, 2007). For example, AX in intermediate layers (inner pericarp, testa and nucellar tissue) has very low A/X ratio (0.07) compared with that in outer pericarp (1.15). Degree and pattern of substitution by arabinose residues along the xylan backbone and degree of polymerisation of the xylan chain are important determinants of the structural heterogeneity and physico-chemical properties of AX (Vinkx & Delcour, 1996). For instance, AX with decreasing arabinose substituents becomes less soluble, since the unsubstituted xylan chains tend to associate and form stable, insoluble aggregates (Andrewartha *et al.*, 1979). However, highly branched AX in plant matrix can be difficult to extract due to possibly stronger embedment in cell walls or interactions with other compounds (Cyran *et al.*, 2003). The extent of branching also controls the action of pentosanases and highly branched AX molecules are less prone to enzymatic degradation (Ordaz-Ortiz *et al.*, 2005; Vinkx & Delcour, 1996).

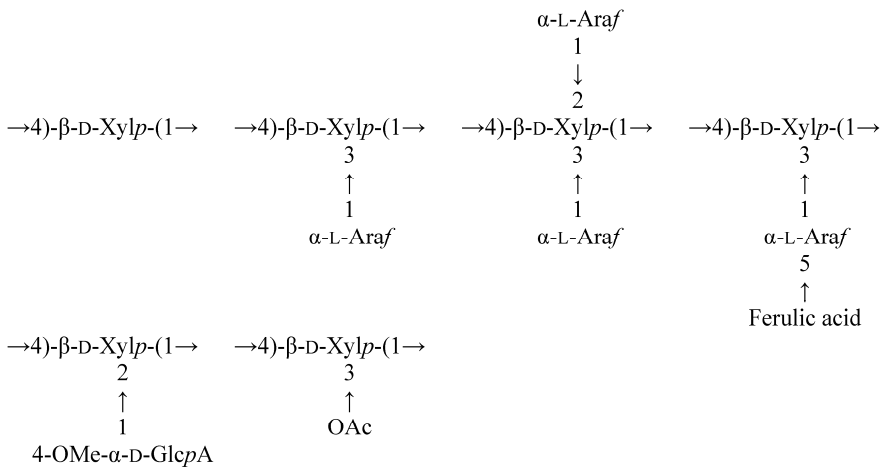

Figure 2. Main structural features of arabinoxylan.

The structure of water extractable AX (WE-AX) is slightly different from that of water unextractable AX solubilised by alkaline extraction (Gruppen *et al.*, 1993). The alkali-extracted AX is believed to have higher molecular weight and slightly higher A/X ratio due to more arabinose branching (Gruppen *et al.*, 1992; Fincher & Stone, 1986). For instance in rye alkali-extractable AX, it has been reported that about 50% of the xylose residues are unsubstituted, 30% mono-substituted and 20% di-substituted (Verwimp *et al.*, 2007; Cyran *et al.*,

2004). However, the relative proportions of mono-substituted and di-substituted xylose residues in WE-AX may depend on extraction temperature. In a study by Cyran *et al*. (2003) on rye cultivar Amilo, the level of O-3 mono-substituted xylose residues decreased from 44% to 19% and di-substituted xylose residues increased from 10% to 19% with an increase in extraction temperature from 4 °C to 40 °C. Compared with rye WE-AX, wheat WE-AX (n = 6) comprises 61-68% unsubstituted, 10-17% O-3 mono-substituted and 19-28% di-substituted xylose residues (Skendi *et al.*, 2011).

The strong positive correlation (r = 0.96) between the proportion of di-substituted xylose residues and A/X ratio in WE-AX indicates that the variation in A/X ratio is mainly controlled by di-substitution (Ordaz-Ortiz & Saulnier, 2005). Interestingly, the A/X ratio of WE-AX is correlated negatively (r = -0.73) with the proportion of mono-substituted xylose residues. The extent of arabinose substitution may influence the end-use quality of the flour, since wheat flours with good bread-making properties have a higher proportion of di-substitution (Cleemput *et al.*, 1993). Apart from solubility and molecular weight distribution, the rheological properties of AX also depend strongly on its fine structure (Cyran & Ceglinska, 2011; Bach Knudsen & Lærke, 2010). Both molecular weight and structure influence the hydrodynamic volume of the polymer, and thereby viscosity (Courtin & Delcour, 2002). Furthermore, variation in extract viscosity (of rye grains) is more dependent on the extent of di-substitution than mono-substitution (Bengtsson *et al.*, 1992). This underscores the importance of AX structural features for its various functional properties. The AX content of major cereals is summarised in Table 2 and can reach up to 12% (of the dry matter) in rye grain (Boskov Hansen *et al.*, 2003). Huge variability in molecular weight of AX is reported in the literature, mainly because of different methods of extraction and analysis (Bach Knudsen & Lærke, 2010). For instance, Girhammar and Nair (1992) reported weight average molecular weight (M_w) of 7.70×10^5 g/mol in rye AX using gel permeation chromatography, while Andersson *et al* . (2009) reported 20.0×10^5 g/mol M_w using size exclusion chromatography coupled with light scattering and refractive index detection.

Table 2. *Arabinoxylan content (% of dry matter) of major cereals.*

Cereal (n)	Arabinoxylan content (%)	Reference
Barley (7)	4.2-5.4	(Izydorczyk, 2010)
Oats (16)	4.1-14.5	(Åman, 1987)
Rye (45)	8.0-12.1	(Boskov Hansen *et al.*, 2003)
Triticale	3.4-5.2	(Henry, 1985)
Wheat (26)	4.4-6.9	(Gebruers *et al.*, 2010b)

1.4.2 β-Glucan

β-Glucan is an important cell wall polysaccharide in oats and barley (Cui & Wang, 2009). It constitutes about 75% of starchy endosperm cell walls and about 26% of aleurone walls in barley (Fincher & Stone, 1986). However, starchy endosperm cell walls of wheat contain only 20% β-glucan, whereas the proportion goes up to 29% in aleurone cell walls (Stone, 2006). β-Glucan in oats is more concentrated in the sub-aleurone layers, while in barley and rye it is evenly distributed across the starchy endosperm (Cui & Wang, 2009). In wheat, β-glucan is probably concentrated in the sub-aleurone cell walls, since debranning is useful to produce bran fractions enriched with β-glucan (Cui & Wang, 2009; Dexter & Wood, 1996).

Chemically, cereal β-glucan is a linear homopolymer of β-D-glucopyranosyl residues linked mostly by 2-3 consecutive (1→4) linkages (≈70%) interrupted by a single (1→3) linkage (≈30%), as demonstrated in Figure 3 (Wood, 2001). So far, no evidence exists for more than one adjacent (1→3) linkage in the β-glucan chain (Izydorczyk & Dexter, 2008). The structural features of cereal β-glucan construed after lichenase (EC 3.2.1.73) hydrolysis and subsequent analysis of oligomers released reveal that over 90% of the polymer consists of 3-*O*-β-D-cellobiosyl-D-glucose (trisaccharide unit, DP 3) and 3-*O*-β-D-cellotriosyl-D-glucose (tetrasaccharide unit, DP 4) (Cui & Wang, 2009). The remaining part contains longer sequences with 5-20 consecutive (1→4)-linked β-D-glucopyranosyl residues (Izydorczyk *et al.*, 1998a; Izydorczyk *et al.*, 1998b). In water-soluble β-glucan from barley, only oligomers up to DP 13 have been detected, with DP 5, 6 and 9 being predominant among oligomers of DP > 4 (Izydorczyk *et al.*, 1998a). In contrast, the alkali-extractable part with DP > 4 contains much longer sequences with DP as long as 20 and DP 9 being the principal oligomer (Izydorczyk *et al*., 1998b). It is believed that long cellulose-like sequences of (1→4)-linked β-D-glucose residues may form strong internal or external aggregation through hydrogen bonding and make β-glucan less soluble (Lazaridou & Biliaderis, 2007; Izydorczyk *et al*., 1998a). The ratio of trisaccharides to tetrasaccharides forms a fingerprint of the particular grain and is different for each cereal. It is usually highest for wheat (3.7-4.8), followed by barley/rye (2.7-3.6) and oats (1.7-2.4) (Wood, 2010). The molar ratio of DP3/DP4 is also an important determinant of functional properties of β-glucan, such as solubility (Cui *et al.*, 2000). Higher molar proportion of the trisaccharides, i.e. higher ratio of DP3/DP4, offers a greater prospect of consecutive cellotriosyl units, thereby resulting in a more regular structure of β-glucan, which makes it less soluble (Wood, 2010; Izydorczyk & Dexter, 2008). Higher molar ratio of trisaccharides to tetrasaccharides in wheat can

thus possibly be one reason for lower solubility of wheat β-glucan compared with that of oats and barley. Further differences in trisaccharide to tetrasaccharide ratio can be found in different grain tissues. β-Glucan from outer grain layers (pericarp, aleurone) exhibits much higher DP3/DP4 ratio compared with that originating from starchy endosperm cell walls (Izydorczyk & Dexter, 2008).

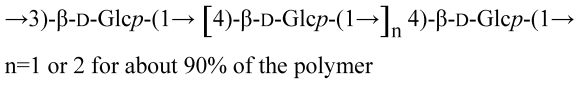

Figure 3. General structure of cereal mixed linkage (1→3) (1→4)-β-D-glucan.

β-Glucan content in different cereals varies and is about 0.4% in triticale (Henry, 1985) and up to about 10% in barley (Izydorczyk, 2010) (Table 3). Solubility/extractability of β-glucan in different cereals varies greatly and β-glucan from oats and barley is classified as more soluble DF (Wood, 2010). Furthermore, the extractability of β-glucan is dependent on method of extraction used. Normally 70-75% of β-glucan in oats is extractable by hot water, but only 10-20% of that in rye (Wood, 2010). Similar to content and extractability, huge diversity in molecular weight of β-glucan in different cereals has been observed. In general, molecular weight of β-glucan in cereals follows the trend oats > barley > rye > wheat (Cui & Wang, 2009). Lazaridou and Biliaderis (2007) reported molecular weight ranges of 0.65-31.0 × 10^5 g/mol in oats, 0.31-27.0 × 10^5 g/mol in barley, 0.21-11.0 × 10^5 g/mol in rye and 2.1-4.9 × 10^5 g/mol in wheat. The discrepancies in molecular weight of β-glucan in cereals may originate from varietal and environmental factors (Ajithkumar *et al.*, 2005). Furthermore, the variation in β-glucan molecular weight among different cultivars of the same cereal is driven mostly by environmental factors. Molecular weight of the polymer reported in the literature may also vary depending on method of extraction and determination used. Significant degradation may take place if endogenous or microbial β-glucanases are not inactivated properly or if the extraction is carried out under alkaline or acidic conditions at high temperature (Wood, 2010; Lazaridou & Biliaderis, 2007). Molecular weight of the polymer also differs in various botanical parts of the grain. For example, β-glucan in barley flour has higher molecular weight than that in shorts and bran, with bran being the lowest (Zheng *et al.*, 2011).

Table 3. *β-Glucan content (% of dry matter) of major cereals.*

Cereal (n)	β-Glucan content (%)	References
Barley (39)	2.4-8.3	(Holtekjølen *et al.*, 2006)
Oats (121)	2.2-4.2	(Åman & Graham, 1987)
Rye (55)	1.3-2.2	(Boskov Hansen *et al.*, 2003)
Triticale	0.4-0.7	(Henry, 1985)
Wheat (26)	0.5-1.0	(Gebruers *et al.*, 2010b)

1.4.3 Fructan

Fructan including fructo-oligosaccharides (DP 3-9) is the soluble DF with prebiotic properties (Gibson *et al.*, 2004). Fructan, known to selectively stimulate the growth of probiotics, is resistant to digestion and absorption in the upper gut and is fermentable by intestinal microflora. Fructan is reported to modulate the immune system beneficially by altering the structure and composition of mucosa and microflora, thus improving the ability of the individual to better respond to certain intestinal infections (Lomax & Calder, 2009; Roberfroid, 2007). The role of fructan in enhancing mineral absorption, particularly Ca, and in the bone health of adolescents is well documented (Alexiou & Franck, 2008; Roberfroid, 2007). The increased Ca absorption in turn reduces the risk of osteoporosis. The potential of fructan in reducing the risk of colon cancer is being investigated, with some positive results in animal models (Roberfroid, 2007; Hughes & Rowland, 2001).

Fructan is widespread in bacteria and flowering plants and to a lesser extent in algae and liverworts (Hendry, 1993). About 45,000 species (15% of the total angiosperm flora) contain fructan. They are distributed in roots, bulbs, seeds, stems and leaves as storage carbohydrates. Among the grasses, members of the sub-family Pooideae of the family Poaceae, which includes cereals of major economic importance, i.e. rye, wheat, barley and oats, store fructan (Hendry, 1993). Rye contains the highest amount of fructan among cereals (up to 6.4%) as visible from Table 4 (Boskov Hansen *et al*., 2003). However, the main industrial sources of fructan are chicory roots (*Chicorium intybus*) and tubers of Jerusalem artichoke (*Helianthus tuberosus*). The role of fructan in plants during abiotic stresses has been reviewed extensively (Livingston *et al*., 2009; Valluru & Van den Ende, 2008; Vijn & Smeekens, 1999). Fructan is reported to protect plants from freeze injury and drought stress. In dicots, fructan acts as a long-term storage carbohydrate in underground organs, while in monocots it acts as a short-term storage carbohydrate in leaves, stems and roots (Valluru & Van den Ende, 2008).

Table 4. *Fructan content (% of dry matter) in major cereals.*

Cereal (n)	Fructan content (%)	Reference
Barley	1.6	(Huynh *et al.*, 2008)
Oats (121)	0.1	(Åman, 1987)
Rye (25)	4.5-6.4	(Boskov Hansen *et al.*, 2003)
Triticale (80)	0.2-1.5	(Pettersson & Åman, 1987)
Wheat (19)	1.5-2.3	(Huynh *et al.*, 2008)

Plant fructan is a polymer of β-D-fructofuranosyl residues with or without a terminal glucose residue (Valluru & Van den Ende, 2008; Vijn & Smeekens, 1999) (Figure 4). Fructan exhibits great structural diversity and variable chain length, with DP ranging from three to few hundred fructose residues (Ritsema & Smeekens, 2003). However, in general plant fructan has a DP range of 30-50. Based on chemical structure, fructan is divided into five categories (Vijn & Smeekens, 1999):

1. Inulin-type fructan consists of linear (2→1)-linked β-D-fructosyl residues attached to the fructosyl moiety of sucrose. It is mainly present in dicotyledonous plants, particularly chicory (*Chicorium intybus*) and Jerusalem artichoke (*Helianthus tuberosus*). The shortest inulin molecule is a trisaccharide 1-kestose (1-K), also called isokestose. Inulin-type fructan obtained from chicory roots is most widely studied.
2. Levan or phlein is a linear fructan consisting of (2→6)-linked β-D-fructosyl residues. 6-Kestose (6-K) is the shortest levan molecule. Levan is usually present in some grasses, e.g. *Dactylis glomerata* (Bonnett *et al.*, 1997) and *Poa secunda* (Wei *et al.*, 2002).
3. Mixed levan or graminan-type fructan consists of a (2→6)-linked β-D-fructosyl residues chain with (2→1)-linked branches attached to the fructosyl moiety of sucrose. Bifurcose is the shortest molecule in this category. Mixed levan is present in most of the plant species belonging to the order Poales. Members of the Poaceae family, e.g. wheat and barley, are typical examples of plants containing graminan-type fructan (Bonnett *et al.*, 1997).

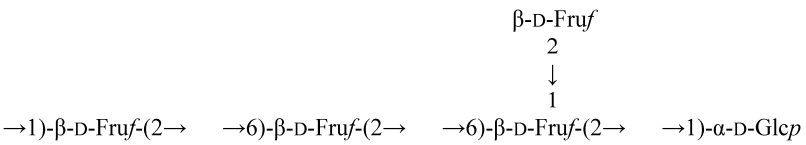

Figure 4. General structure of fructan.

4. Levan neoseries fructan is the polymer of (2→6)-linked β-D-fructosyl chains attached to C1 and C6 of the glucose moiety of sucrose. This type of arrangement results in fructose chains on both sides of the glucose residue. Levan neoseries are present in few plant species of the order Poales, e.g. oats (Livingston *et al.*, 1993).
5. Inulin neoseries have (2→1)-linked β-D-fructosyl chains attached to C1 and C6 of the glucose moiety of sucrose. Neokestose is the smallest molecule of this category. Inulin neoseries are present in plants of the family *Liliaceae*, e.g. onion (*Allium cepa*) and asparagus (*Asparagus officinalis*) (Shiomi, 1989).

1.4.4 Other components

Resistant starch (RS) is regarded as part of DF in the new definition of CODEX (Phillips & Cui, 2011), since it is not digested by the enzymes present in the upper gut of humans and is readily available to colonic microflora (Englyst & Macfarlane, 1986). Based on its origin, RS is divided into four distinct sub-classes (Cummings & Stephen, 2007; Englyst & Macfarlane, 1986). RS1 is physically inaccessible starch because of its presence in whole or partly milled kernels. RS2 is resistant to digestion because of its compact granular structure, e.g. starch present in raw potato and banana. RS3 is formed naturally during retrogradation of gelatinised starch and hence is considered important after processing of cereals. RS4 is a chemically modified form of starch and is formed by derivatisation of starch through cross-linking and/or substitution.

Cellulose, the most abundant macromolecule on land, is a linear polymer of (1→4)-linked β-D-glucopyranosyl residues with anhydrocellobiose as the repeating unit (O'Sullivan, 1997; McNeil *et al.*, 1984). Due to its linearity, cellulose associates strongly with itself through hydrogen bonds, resulting in tightly packed compact aggregates called microfibrills. These microfibrills are surrounded by a matrix of other cell wall components such as lignin and hemicelluloses, making the compound highly insoluble. The cellulose content of cereal grain cell walls varies according to the tissue of origin and cereal species (Fincher & Stone, 1986). Cellulose is abundant in secondary cell walls, whereas it comprises 20-30% of dry matter in most primary cell walls (McNeil *et al.*, 1984). In endosperm cell walls of wheat and barley, cellulose accounts for only few percent of the total walls, while in the outer, lignified layers it may comprise up to 20% of the cell walls (Fincher & Stone, 1986). Its content in rye grain varies from 1-3% (Liukkonen *et al.*, 2006).

Associated compounds such as lignin when analysed together with other constituents are also included in the CODEX definition of DF. However, once

isolated and added back, they would not be considered part of DF (EFSA, 2010). Lignin is a polymer of phenyl-propanoides and is deposited in secondary thickened cells walls (Vanholme *et al.*, 2010). Lignin is regarded as a structural component of plants and is strongly bound to the heteroxylans. The content of Klason lignin in rye grain usually varies from 1-3% (Boskov Hansen *et al.*, 2003).

1.5 Processing and dietary fibre

Processing is a prerequisite to make food palatable and is considered important for the content, composition and bioavailability of nutrients (Slavin *et al*., 2000). Some of the process-induced modifications are favourable, while others can be regarded as undesirable for the nutritional quality of food. The importance of judicious processing was laid down a long time ago by Hippocrates when he said:

> "And this I know, moreover, that to the human body it makes a great difference whether the bread be fine, or coarse; of wheat with or without the hull, whether mixed with much or little water, strongly wrought or scarcely at all, baked or raw—and a multitude of similar differences…….. Whoever pays no attention to these things, or, paying attention, does not comprehend them, how can he understand the diseases which befall a man?" (Hippocrates, 400 BC).

The physiological functions attributable to DF are normally related to its viscosity (Lazaridou & Biliaderis, 2007). The viscosity of carbohydrate polymers such as β-glucan is interpreted from their extractable content, molecular size and structural features (Wood, 2010; Cui & Wang, 2009; Mälkki & Virtanen, 2001). According to investigations by Regand *et al*. (2009), 73% of the bioactivity of β-glucan in attenuating the peak blood glucose response is explained by molecular weight × content. Keeping this in mind, EFSA (2009) placed certain preconditions when permitting health claims pertaining to β-glucan. In order to carry the claim regarding maintenance of normal blood cholesterol level, the foods should provide at least 3 g/day of β-glucan from oats, oat bran, barley, barley bran or from mixtures of *non-processed* or *minimally processed* β-glucan in one or more servings. These restrictions make sense, since previous studies show that β-glucan with reduced molecular weight does not demonstrate physiological benefits in human subjects (Tosh *et al.*, 2010; Tosh *et al.*, 2008). The reduction in molecular weight or content of fibre polymers can be due to endogenous or microbial enzymes, temperature, pH, yeast fermentation, mixing, etc. (Andersson *et al.*, 2009; Tiwari & Cummins, 2009; Poutanen, 2008; Åman *et al.*, 2004; Andersson *et al.*, 2004; Boskov Hansen *et al.*, 2002). Accordingly,

products prepared through various processes such as baking, extrusion, porridge cooking, malting, etc. may end up in variable content, extractability and molecular weight and consequently bioactivity. The processing effects on DF components are governed by the rigour of processing and the susceptibility of the DF components. Food matrix is also an important consideration when assessing the process-induced modifications in DF components (Regand *et al.*, 2009). Compared with bread, porridge normally retains high molecular weight of polymers such as β-glucan (Åman *et al.*, 2004). Consequently, the efficacy of various foods to exert physiological benefits differs greatly (Regand *et al.*, 2009). Oat porridge and granola, for example, were most efficient in attenuating the peak blood glucose response owing to high β-glucan peak molecular weight and viscosity, whereas in the same study bread and pasta exhibited significant depolymerisation of β-glucan and showed lowest bioactivity. Knowledge of process-induced changes on DF content and composition is therefore of the utmost importance in preparing healthy food products.

2 Objectives

The overall objective of this work was to characterise the DF profile of rye and rye products and triticale grains. Rye is important for human consumption, while the major usage of triticale is in animal feed. A high content of preserved DF in rye-based products is considered beneficial for its physiological functions and processing is normally intended to retain its bioactivity. Conversely, for animal feed a lower amount of DF with low extractability and degraded molecules is desired, since this will result in low viscosity in the animal gut. The viscosity of triticale or rye extract depends to a large extent on its DF content, composition, molecular weight distribution and structural features of major soluble components, namely AX and β-glucan.

Specific objectives of the work were to:

- Characterise the DF of triticale cultivars grown at different locations in Sweden (Paper I).
- Study the content and composition of DF in rye-based crisp breads and soft breads (Paper II).
- Investigate the impact of porridge preparation on the molecular weight distribution of extractable DF components (Paper III).
- Compare the structural features of AX and β-glucan in different cereals and determine the impact of growing conditions and genetic variability (Paper IV).
- Study the rheology in extracts of triticale cultivars grown at different locations and establish the relationship between viscoelastic properties and the chemistry of DF (Paper V).

3 Materials and methods

This section describes the materials used and methods applied to obtain the results compiled in this thesis. Only a brief description of materials and methods is presented in this section, as they are described in detail in Papers I-V. However, methods dealing with relative DP distributions of fructan and enzymatic fingerprinting of AX and β-glucan structural features underwent certain modifications and are presented in detail. The method for studying the rheological properties of triticale flour was developed during the study and a thorough account is given here.

3.1 Materials

3.1.1 Cereal grains and products

Eight triticale cultivars (SW 168, Fidelio, SW 137B, Dinaro, SW 383A, DED 145/02, Talentro, Cando) were grown at two locations in Sweden: Svalöv (55°56'N, 13°6'E) and Kölbäck (58°27'N, 15°15'E). Two of these cultivars (Fidelio and Cando) were also grown at a third location, Haga (59°36'N, 17°2'E). Wheat (cv. Harnesk) and rye (cv. Ottarp) reference cereals were grown at Svalöv and Kölbäck (Papers I, IV & V). Twelve crisp breads, eight soft breads and two extruded rye products were obtained from commercial outlets in Sweden. Three milling streams, i.e. inner endosperm, outer endosperm and bran, were obtained from Lantmännen AB, Stockholm, Sweden (Paper II). Commercial whole meal rye flours used for preparation of porridge and rye flour slurries were purchased from a local supermarket (Paper III). Twenty barley lines were obtained from the Nordic Gene Bank (NGB) of the Nordic Genetic Resource Centre, Sweden (1-6), the Swedish University of Agricultural Sciences (SLU), Sweden (7, 8), SW Seed AB, Svalöv, Sweden (9-17) and the Royal Veterinary and Agricultural University (KVL), Denmark

(18-20) (Paper IV). Five tritordium breeding lines were obtained from Agrasys S.L. Parc Cientific de Barcelona.

3.1.2 Chemicals and enzymes

The chemicals used were of analytical grade and were tested to suit the specific protocol. Most of the enzymes used were obtained from Megazyme (Bray, Ireland) and their purity and specific activity was tested by the supplier.

3.2 Preparation of porridge

A full factorial design with two levels of flour (50 and 35 g) and salt (0 and 1 g) and four levels of rest time (0, 20, 40, 60 min) before cooking was used in experiment one of Paper III. The centre points of salt (0.5 g) and flour (42.5 g) with all four levels of rest time were carried out in triplicate to account for experimental error. In experiment two, the effect of incubation temperature (20, 35, 45, 55 °C) and pH (4.5, 6.2) on molecular weight of AX was investigated.

The experimental porridges were prepared according to the design using a fixed amount of water (180 ml). The water-soaked ingredients were incubated at 20 °C and stirred for 10 seconds every 5 min to ensure uniform mixing and action of the endogenous enzymes. After cooking for 7 min on a hot plate at intermediate setting, the porridges were transferred to an aluminium container, covered and immediately frozen at -20 °C (experiment one, Paper III). In experiment two, which was aimed to investigate the effect of temperature and pH, rye flour slurries were prepared using 10 g flour and 27 ml water or sodium acetate buffer (0.5M, pH 4.5). The slurries were incubated at the required temperature for 1 h and stirred for 10 seconds every 5 min. After 1 h the slurries were immediately frozen at -20 °C.

3.3 Sample preparation

The grain samples were cleaned to remove impurities. The soft breads, porridges and rye flour slurries were freeze-dried for 72 h. All the grain samples, crisp breads, soft breads, extruded products, porridges and rye slurries were milled with a cyclone sample mill (Retsch, Hann, Germany) to pass through a 0.5 mm screen.

3.4 Analytical methods

All analytical measurements were carried out at least in duplicate and the deviation between two analytical replicates was usually about 5%.

3.4.1 Physical and chemical measurements

Certain physical and chemical analyses were performed on triticale grains (Paper I). Bulk density was recorded by Infratec^{TM} 1241 (Foss, Denmark) equipped with a test weight module. Thousand kernel weight was measured by the International Seed Testing Association (ISTA) method (ISTA, 2006). Grain protein content measurements were performed on an NIT instrument (Infratec^{TM} 1241 Grain Analyser, Foss, Denmark). Crude fat content was measured using the standard gravimetric method on a Tecator instrument (Tecator AN 301, 2001). Starch content was analysed enzymatically according to Åman *et al*. (1994). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed according to AOAC Official Method (2002.04) and AOAC Official Method (973.18), respectively (AOAC, 2005). Maltose and sucrose contents were measured according to the method described by Sullivan and Carpenter (1993).

3.4.2 Quantification of dietary fibre components

Analyses of DF and its constituents were carried out in Papers I-V. Total, extractable and unextractable DF were quantified by the Uppsala method (Theander *et al.*, 1995) subsequently modified by Andersson *et al*. (1999) for separate measurement of extractable and unextractable DF. AX content was calculated from the arabinose, xylose, and galactose values obtained through the Uppsala method, assuming an arabinose/galactose ratio of 0.69 in extractable arabinogalactan (Loosveld *et al.*, 1997). Total β-glucan was quantified by an enzymatic method as detailed by McCleary and Codd (1991), while extractable β-glucan was measured by taking into account the area under the curve during molecular weight determinations (Rimsten *et al.*, 2003). Fructan content in grain and product samples was measured according to the method described by McCleary *et al*. (1997) using the enzymatic assay kit K-FRUC (Megazyme, Bray, Ireland). The samples were treated with α-galactosidase (Megazyme, Bray, Ireland) for removal of raffinose-type oligosaccharides, which may interfere with fructan analysis.

3.4.3 Molecular weight determinations

Molecular weight distributions of AX, β-glucan and fructan were determined in Papers I-III & V. Arabinoxylan weight-average molecular weight (M_w) and number-average molecular weight (M_n) were measured by high performance

size exclusion chromatography (HPSEC) coupled with multiple angle laser light scattering and refractive index detectors (Wyatt Technology, Santa Barbara) as described by Andersson *et al*. (2009). The molecules eluting in the range 15-22 ml were taken into consideration to calculate M_w and M_n since they could be reliably measured. β-Glucan molecular weight distribution and Calcofluor average molecular weight (M_{cf}) of β-glucan were determined using size exclusion chromatography with Calcofluor detection according to the method described by Rimsten *et al*. (2003) with a Calcofluor concentration of 0.0025%. With this detection technique, molecules smaller than 10,000 g/mol are excluded from calculations since these shorter fragments cannot be analysed with precision (Munck, 1989).

Fructan molecular weight distribution

Fructan molecular weight distribution was analysed by high performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD). The fructan was extracted in 80% ethanol and the extract was treated with amyloglucosidase to remove the maltooligosaccharides. Later on, the solution was divided into two aliquots and one was treated with fructanase. Finally, both aliquots were filtered and run on HPAEC (Figure 5).

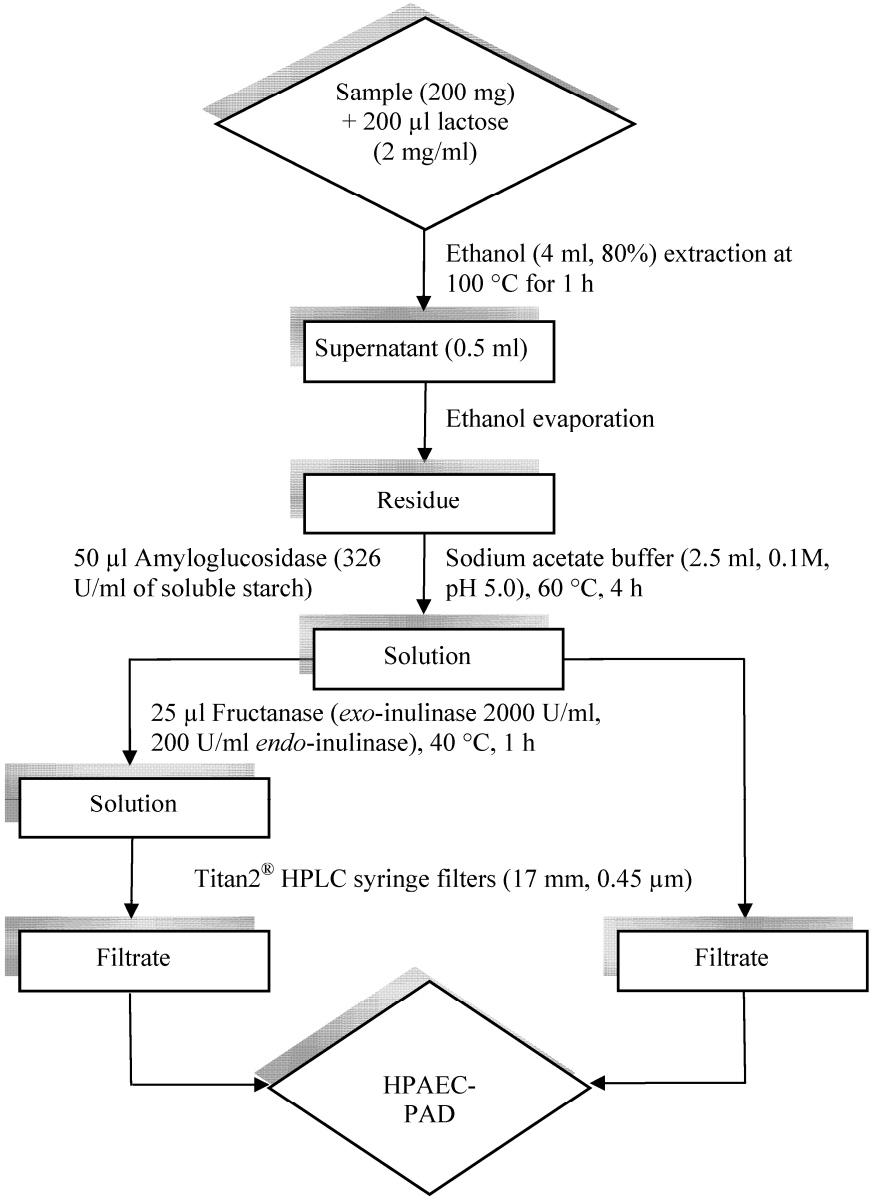

Figure 5. Flow diagram of fructan molecular weight distribution analysis.

The samples were analysed on Dionex DX 500 Chromatography System (Sunnyvale, C.A, USA) equipped with a GP 40 gradient pump. The eluent was 150 mM NaOH (A) at 1.0 ml/min with a gradient of 150 mM NaOH containing 500 mM $NaCH_3COO$ (B); 0-10 min linear gradient 0-20% eluent B; 10-30 min linear gradient 20-100% eluent B; and 30-40 min isocratic at 100% B. Between

runs, the system was flushed with 100% A for 5 min. Chromatography was carried out with a CarboPac^{TM} PA-1 (4 × 250 mm, P/N 35391) anion exchange column pre-fitted with a CarboPac^{TM} PA-1 guard column (P/N 43096). Detection was carried out by ED40 PAD. The relative distribution (% basis) of different DP ranges was calculated based on the area under the peaks. The area was calculated by subtracting the area of chromatogram without fructan from that with fructan present (Figure 6).

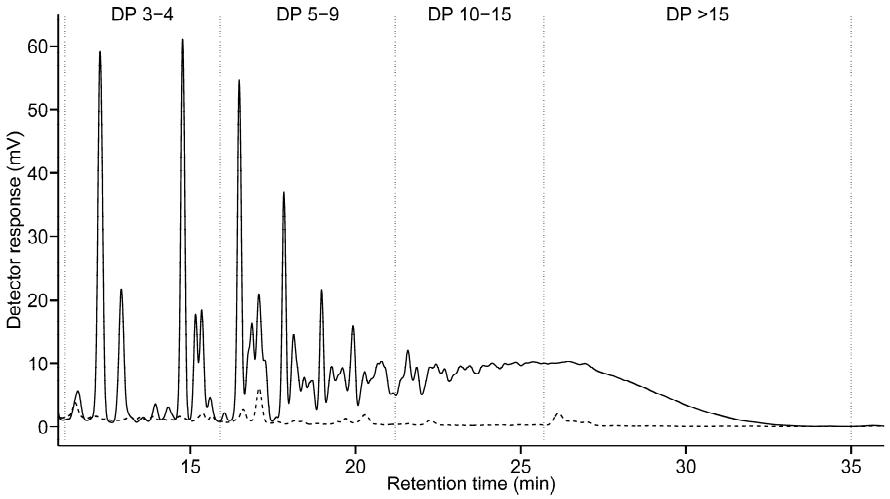

Figure 6. Fructan DP distribution profile of a typical rye grain. The relative area under the chromatogram was calculated by subtracting the area under the fructan chromatogram (——) from the area obtained after fructanase treatment (·····).

3.4.4 Enzymatic fingerprinting of arabinoxylan and β-glucan

Enzymatic fingerprinting of AX and β-glucan in cereal grains was performed in Paper IV according to the method described by Saulnier and Quemener (2009) with slight modifications. Finely milled sample (500 mg) was boiled with 80% ethanol to inactivate the enzymes. The residue was dried and treated with *endo*-xylanase and lichenase to hydrolyse the AX and β-glucan, respectively, and subsequently run on HPAEC to measure the fragments released (Figure 7).

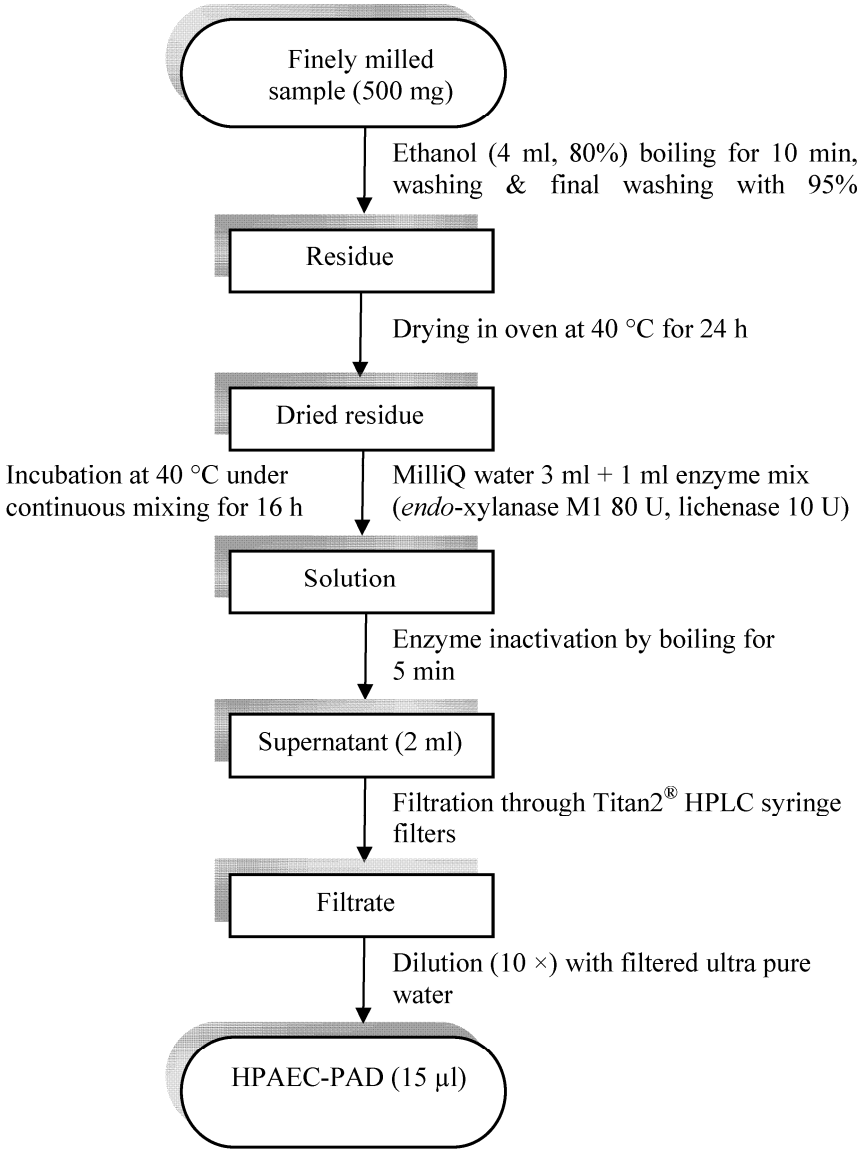

Figure 7. Flow diagram of arabinoxylan and β-glucan enzymatic fingerprinting.

The aliquots of the supernatant were analysed on HPAEC equipped with TSP EC2000 PAD. The AX and β-glucan fragment released after enzymatic hydrolysis by *endo*-xylanase M1 and lichenase, respectively, were identified by running standards. The peak areas were normalised for AX and β-glucan fragments. The major gluco-oligosaccharides (GOS) produced after lichenase hydrolysis are 3-*O*-β-cellobiosyl-D-glucose (BG_3) and 3-*O*-β-cellotriosyl-D-

glucose (BG_4), with small quantities of 3-*O*-β-cellotetraosyl-D-glucose (BG_5) and 3-*O*-β-cellopentaosyl-D-glucose (BG_6) (Saulnier & Quemener, 2009; Tosh *et al.*, 2004). The longer cellulose-like sequences with DP > 6 were not analysed in this study. Lichenase, a specific *endo*-(1→3) (1→4)-β-D-glucan 4-glucanhydrolase, cleaves only β-(1→4) glycosidic linkages of an O-3 substituted unit. The treatment of WE-AX with an *endo*-xylanase from *Trichoderma viride* generated xylose (X), xylobiose (XX) and a series of arabinoxylan oligosaccharides (AXOS) up to DP 9, as identified in Figure 8. Since *endo*-1→4-β-xylanase M1 belongs to family 11 of the glycoside hydrolases, it requires at least three unsubstituted xylopyranosyl units in the xylan backbone to split the glycosidic bonds (Bach Knudsen & Lærke, 2010). For nomenclature of different AXOS, see the paper by Fauré *et al*. (2009).

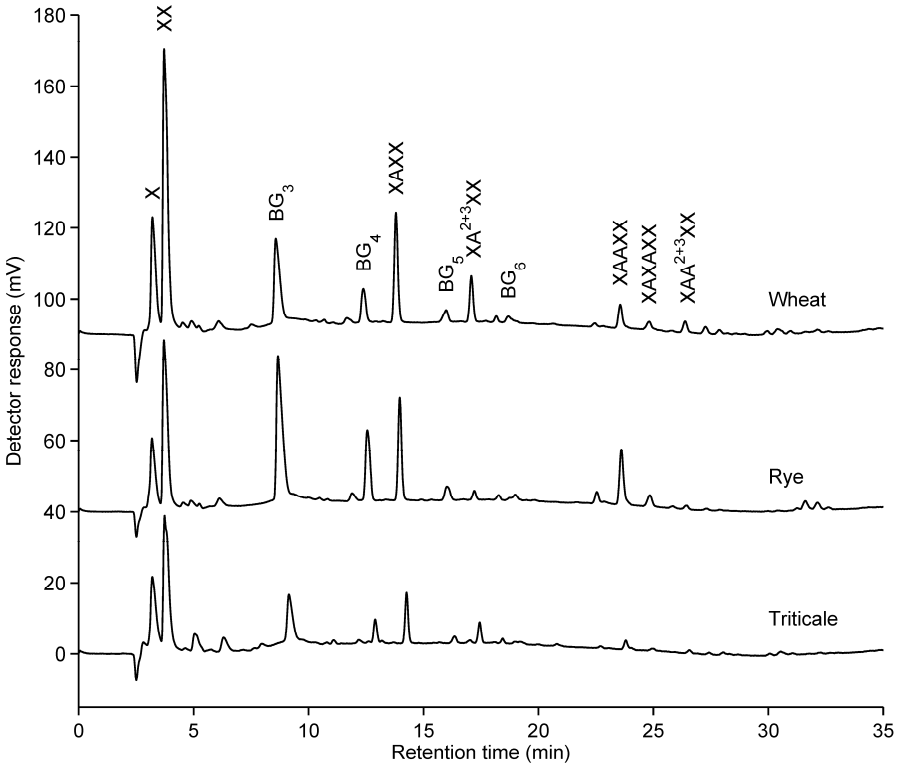

Figure 8. Arabinoxylan and β-glucan fragments released after enzyme treatment. Arabinoxylan oligosaccharides containing letter (A) without any superscript denotes xylose residue with arabinose substituent at position three, while A^{2+3} denotes xylose residue with arabinose substituents at positions two and three.

3.4.5 Rheological measurements

The viscoelastic properties of triticale grains (Paper V) were measured with a new method developed by the author using a rheometer. In brief, the polymers were extracted with boiling water in the presence of thermostable α-amylase to degrade starch, precipitated with 80% ethanol and re-dissolved in a minimum quantity of water to carry out the rheological measurements (Figure 9).

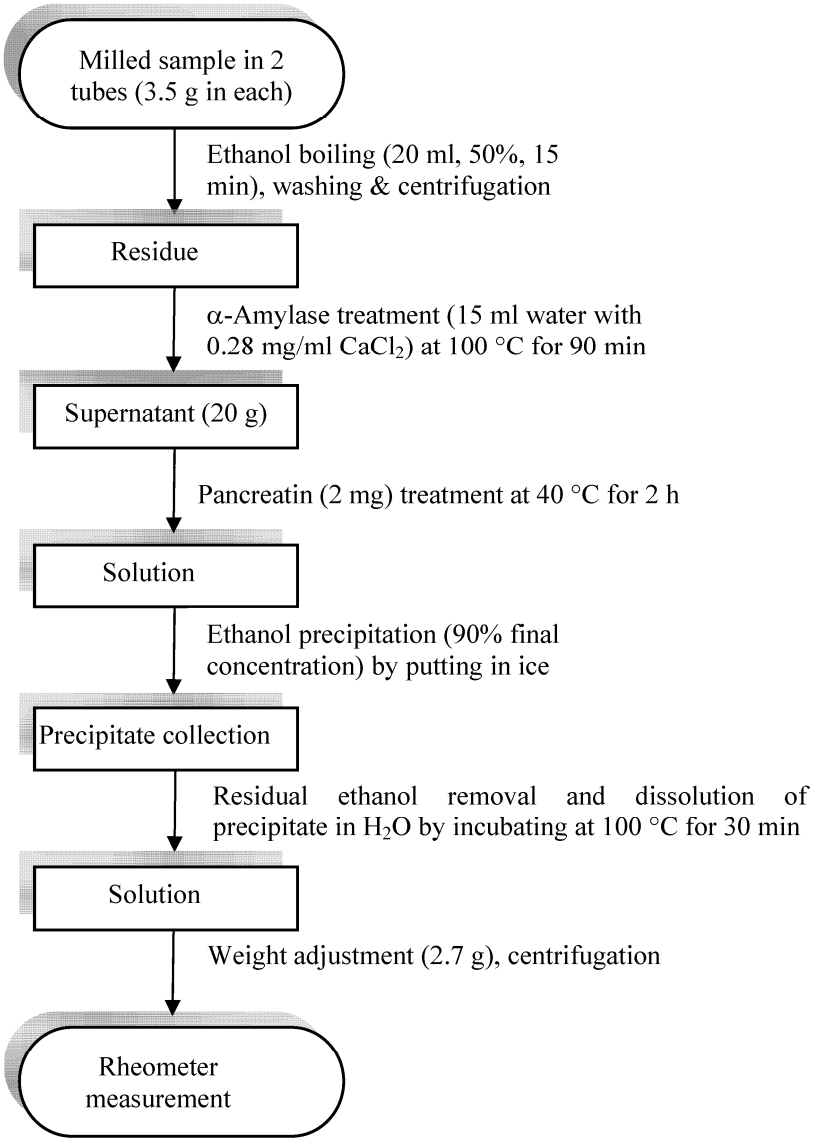

Figure 9. Flow diagram of rheological measurements.

The oscillation test was performed on a Bohlin C-VOR rheometer using constant strain (0.04) and running the frequency sweep (logarithmic) with minimum angular frequency of 0.6283 rad/s and maximum angular frequency of 31.42 rad/s. The above-mentioned strain value lies in the linear viscoelastic region and was obtained by running the amplitude sweep experiment. Cone and plate (CP4/40) type measuring geometry was used, with cone diameter 40 mm and cone angle 4°. The number of test points (samples) was set to 10 (upward only). All measurements were carried out at 25 °C.

3.5 Statistical methods

The results obtained in Paper I were analysed for cultivar and location effects with two-way analysis of variance (ANOVA) by Statistical Analysis System Software 9.2 (SAS Institute, Cary, NC). The significance level was set to $p < 0.05$. Tukey's pair-wise comparisons were made to test the differences between cultivars. Simple linear correlation (Pearson correlation) was carried out between selected parameters.

The data obtained in Paper III were analysed by Minitab 15 statistical software (Minitab Inc., State Collage, PA, USA). The responses from the designed experiment (experiment one) were analysed by regression using the Generalized Linear Model (GLM) procedure with factors defined as covariates. The contents of DF components from experiment one were analysed by one-way ANOVA with the factor resting time. The effect of temperature on the molecular weight of AX (experiment two) was evaluated by the GLM procedure with pH and temperature as factors and temperature defined as covariate.

The data obtained in Paper IV were analysed by Principal Component Analysis (PCA) using The Unscrambler X 10.0.1 (CAMO Software AS, Norway) software. The similarity maps drawn from score plots helped to group samples with comparable features and loading plots were useful to find the relationship between variables. Different conclusions drawn from PCA regarding location effects on the structural features of triticale grain were verified by ANOVA using SAS 9.2.

The results from Paper V were analysed by Partial Least Square (PLS) regression to find the power of different structural and compositional features of DF components to predict viscosity. In this case the software used was The Unscrambler X 10.0.1 (CAMO Software AS, Norway). The location effects on various viscoelastic properties were analysed by ANOVA with SAS 9.2.

4 Results and discussion

4.1 Dietary fibre composition

This section mainly deals with the content and composition of DF and the molecular weight distribution of major DF components, namely AX, β-glucan and fructan in triticale cultivars (n = 8) grown at two locations in Sweden (Svalöv and Kölbäck). Data on rye (cv. Ottarp) and wheat (cv. Harnesk) reference cultivars grown in parallel are also presented and compared with triticale (Paper I). The three rye milling streams (inner endosperm, outer endosperm and bran) studied in Paper II are also discussed. The second part of this section describes the above-mentioned DF parameters in rye products, which were crisp breads (n = 12), soft breads (n = 8), an extruded breakfast cereal, an extruded crisp bread (Paper II) and rye porridge (Paper III).

Although the main focus in triticale characterisation was on DF content and composition, certain physical and chemical parameters important for grain usage were also analysed. In these parameters, location effects were clearly evident, since bulk density (average 745 g/l), 1000 kernel weight (average 56.7 g) and starch content (average 66.5%) were significantly higher in cultivars grown at Svalöv compared with Kölbäck (702 g/l, 46.4 g and 63.5%, respectively). These results were supported by the appearance of grains, with the Kölbäck samples having shrivelled kernels. However, crude protein (average 14.9%), maltose (1.5%), sucrose (1.5%) and ADF (3.4%) contents were significantly higher in cultivars grown at Kölbäck than in those grown at Svalöv (average contents of 13.0%, 0.4%, 1.1% and 3.1%, respectively). Higher rainfall during the growing season at Kölbäck might have prompted the endogenous enzymes activity in kernels, resulting in degradation of carbohydrates and thus leading to higher amount of sugars and lower starch content. Sprouting in the ears, particularly under humid conditions, is a well documented phenomenon in triticale grains (Martinek *et al.*, 2008; Sodkiewicz,

1999). Location effects on other parameters such as crude fat and NDF content were non-significant. Strong positive correlations of bulk density with starch content (r = 0.83) and 1000 kernel weight (r = 0.81) were observed. The slight inverse relationship observed between protein and starch content (r = -0.59) is in agreement with previous findings by Glatthar *et al*. (2005). Compared with reference wheat and rye, the protein content of triticale was higher, which underscores its feeding value, while the starch content of triticale was more similar to wheat than rye, which had a lower content (Paper I).

4.1.1 Dietary fibre content in grains

Total DF content (including fructan) of triticale varied from 13.2% to 16.0% (average 14.6%) (Figure 10). A significant effect of location on total DF content of triticale grains was evident, with cultivars grown at Kölbäck (average 14.7%) having significantly higher contents than those grown at Svalöv (average 14.5%). These location effects were again explained by unfavourable growing conditions at Kölbäck resulting in shrivelled grains. Slightly higher values reported here compared with those in the literature (Picolli da Silva & de Lourdes Santorio Ciocca, 2005; Pettersson & Åman, 1987) can be explained by the fact that fructan is considered part of DF in the calculations. The DF content of triticale is very similar to that of the reference wheat cultivar (13.8%) but much lower than that of the reference rye cultivar (20.2%). Among rye milling fractions studied in Paper II, inner endosperm contained the lowest (11.8%) amount of DF, outer endosperm an intermediate amount (21.8%) and bran the highest amount, 37.7%.

The major component of triticale, rye and wheat DF was AX, which comprised 46.0%, 42.6% and 45.3%, respectively, of the total DF (Table 5). The AX extractability in triticale cultivars grown at Svalöv (average 13.8%) was significantly lower than for Kölbäck (15.2%). These values are considerably lower than those observed for reference rye cultivar (29.5%) or commonly reported for rye AX (26-40%) in the literature (Andersson *et al.*, 2009; Boskov Hansen *et al.*, 2003). The content of β-glucan in triticale cultivars was quite low and varied from 0.5-1.0%, which is similar to that in the reference wheat (0.6%) but far lower than that in the reference rye cultivar (1.9%). β-Glucan extractability of triticale cultivars grown at Svalöv (average 15.3%) was not significantly different from that of those grown at Kölbäck (average 15.1%). Considering the major usage of triticale in animal feed, low amounts of extractable AX and β-glucan are important for its feed value, since the extract viscosity of triticale is proportional to the amount of water extractable non-starch polysaccharides (Pettersson & Åman, 1987). The soluble fraction of DF forms a viscous mass in the animal gut and thereby

lowers nutrient absorption when included in animal feed (Józefiak *et al.*, 2004). To counteract this problem, rye- and triticale-based feed formulations for monogastric animals often include enzyme supplementation to lower the viscosity and improve the feed to weight gain ratio (Józefiak *et al.*, 2007). Fructan was also present in significant quantities in triticale, rye and wheat grains, with average contents of 2.3%, 4.4% and 1.8%, respectively. These values of fructan content in triticale are higher than the average value of 0.8% (n = 80) reported by Pettersson and Åman (1987), but similar to the value (2.2%) mentioned by Huynh *et al*. (2008).

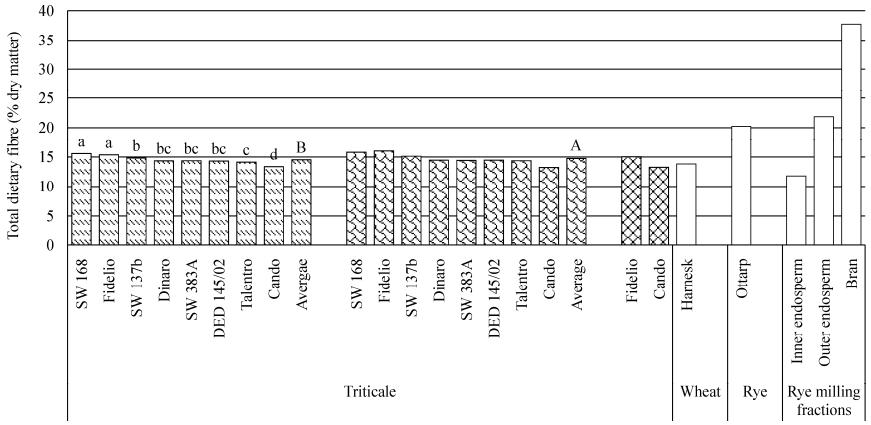

Figure 10. Total dietary fibre content of triticale cultivars grown at Svalöv ( ), Kölbäck ( ) and Haga ( ), wheat and rye reference cultivar (Paper I), and milling fractions of rye (Paper II). Triticale cultivars carrying different letters (lower case) are significantly different from each other $p < 0.05$). The different letters (upper case) on the average values of two regions show location effect.

Table 5. *Average content (% of dry matter) of arabinoxylan (AX), β-glucan, fructan, cellulose + resistant starch (RS) and Klason lignin and β-glucan extractability in triticale cultivars, a rye and wheat reference cultivar, rye milling fractions, and rye products.*

	AX[a]	β-Glucan content	β-Glucan extractability[b]	Fructan	Cellulose + RS[c]	Klason lignin
Grains						
Triticale[1] (n = 18)	6.7	0.7	15.0	2.3	2.1	1.5
Svalöv[d] (n = 8)	6.7[B]	0.7[A]	15.3[A]	2.2[A]	2.1[A]	1.5[A]
Kölbäck[d] (n = 8)	6.9[A]	0.6[B]	15.1[A]	2.3[A]	2.1[A]	1.6[A]
Rye[1]	8.6	1.9	11.4	4.4	2.2	1.7
Rye inner endosperm[2]	4.4	1.5	26.0	3.4	1.2	0.2
Rye outer endosperm[2]	9.3	3.4	17.0	4.6	2.1	0.5
Rye bran[2]	18.2	4.4	16.0	5.0	4.3	3.0
Wheat[1]	6.2	0.6	18.6	1.8	2.2	1.6
Products						
Crisp breads[2] (n = 12)	7.8	1.9	29	2.8	2.4	1.3
Soft breads[2] (n = 8)	5.4	1.2	35	1.6	2.5	0.6
Extruded crisp bread[2]	4.1	0.9	47	1.4	1.1	0.7
Breakfast cereal[2]	5.3	2.3	54	2.8	1.5	0.8
Rye porridge without resting[3]	8.6	2.3	17	3.9	4.5	2.4
Rye porridge rested for 60 min[3]	8.6	2.3	24	4.1	4.4	2.6

[a]AX was calculated by subtracting the arabinose fraction that is part of arabinogalactan and assuming that the arabinose/galactose ratio in extractable arabinogalactan is 0.69.
[b]β-Glucan extractability values were obtained from molecular weight determinations (Rimsten *et al.*, 2003).
[c]Only cellulose in the case of grains or milling fractions. Cellulose + RS values were calculated by subtracting the β-glucan from total glucose value obtained through the Uppsala method (Theander *et al.*, 1995).
[d]Values within columns for triticale cultivars grown at Svalöv and Kölbäck and followed by different letters (upper case) are significantly different from each other ($p < 0.05$).
[1]Paper 1; [2]Paper II; [3]Paper III

4.1.2 Dietary fibre content in products

Rye products studied in Paper II varied widely in their composition and processing (labelling information). Whole grain rye flour was the major ingredient of crisp breads, but a few of them contained some wheat flour. By contrast, wheat flour was present in most of the soft breads as a major ingredient, followed by rye flour. An appreciable amount of oat flour (30%) was present in the extruded breakfast cereal.

Among the rye products studied, there was a wider variation in total DF content (including fructan) of soft breads (range 7.9-17.5%) compared with crisp breads (range 13.0-19.8%) (Paper II), as shown in Figure 11. Average DF content in crisp breads (17.8%) was considerably higher than that in soft breads

(12.6%). The key reason for lower DF content in soft breads is the dilution effect due to addition of wheat flour for gluten development. As discussed previously, the DF content of wheat is significantly lower than that of rye. The two extruded products, i.e. breakfast cereal and extruded crisp bread, contained 14.3% and 9.4% DF, respectively. When comparing the DF content listed on the product label against the analysed value, surprisingly many manufacturers were reporting lower values, probably due to non-inclusion of fructan in DF calculations (Paper II). Rye porridge (Paper III) cooked with or without resting was higher in DF content (23%) compared with the rye products studied in Paper II. Formation of RS of type III (about 2.5%) contributed towards the high total DF content of porridge.

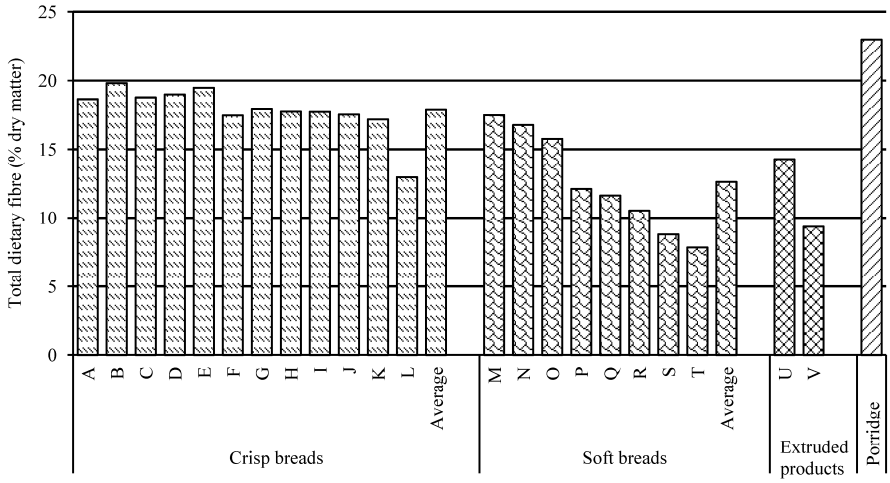

Figure 11. Total dietary fibre content of crisp breads, soft breads, extruded products (Paper II) and rye porridge (Paper III).

Similarly to rye grain, AX was the major component of DF in rye products studied in Paper II and its average content in crisp breads (7.8%) and soft breads (5.4%) was lower than that in porridge (8.6%) (Table 5). Rye products are a good source of β-glucan in the diet in Eastern and Northern Europe, since they are consumed in larger quantities than products made from barley and oats, the cereals with the highest β-glucan content. The β-glucan content of breakfast cereal and porridge was 2.3%, followed by crisp breads (average 1.9%) and soft breads (average 1.2%). The presence of whole grain oat flour in breakfast cereal explains the high β-glucan content, while in soft breads addition of wheat flour lowers the β-glucan content. Fructan, a soluble DF component, is present in appreciable amounts in rye-based products. The

fructan content of crisp breads (average 2.8%) was considerably higher than that of soft breads (average 1.6%) or extruded crisp bread (1.4%), while porridge had a similar content to rye grain (≈4%). In a study by Whelen *et al.* (2011), the amount of fructan in rye bread (3.3%) was more than double that in wheat breads (0.9-1.6%). The average content of cellulose and RS (analysed together) in crisp breads and soft breads was similar (≈2.5%) and lower than observed in porridge (4.5%). However, the relatively wider variation in cellulose and RS content in soft breads (range 0.7-4.1%) compared with crisp breads (range 1.7-3%) can be attributed to diversity of ingredients and processing conditions. Klason lignin, a non-carbohydrate part of DF, was present in smaller amounts. The huge variability in the content of DF components in rye products can partly be attributed to variation in ingredients, since contents may vary in different cereals, within cultivars of a cereal and botanical fractions of the grain (Huynh *et al.*, 2008; Boskov Hansen *et al.*, 2003; Karppinen *et al.*, 2003). Other major factors in variable DF composition can be diversity of processing in different products (Andersson *et al.*, 2009; Karppinen *et al.*, 2003), an issue discussed later in this thesis.

4.1.3 Molecular weight profile of dietary fibre in grains

The M_w and M_n of AX in triticale cultivars grown at Svalöv (average 15.4 × 10^5 g/mol and 6.4 × 10^5 g/mol, respectively) was significantly higher than that in cultivars from Kölbäck (average 14.3 × 10^5 g/mol and 4.9 × 10^5 g/mol, respectively) (Table 6). This can also be observed in AX molecular weight distribution profiles, where triticale grown at Kölbäck had a shift towards low molecular weight fractions (Figure 12). The average M_w values observed in triticale here were higher than those (5.7 × 10^5 g/mol) measured by Girhammar and Nair (1992) using gel permeation chromatography. Triticale M_w values were lower than rye M_w values observed in this study (17.2 × 10^5 g/mol) and those reported previously (20.0 × 10^5 g/mol) by Andersson *et al.* (2009). Polydispersity index (M_w/M_n) of AX was significantly higher for triticale grown at Kölbäck (3.0) than Svalöv (2.4), reflecting the broader AX molecular weight distribution at Kölbäck. Degree of arabinose substitution on the xylan backbone (A/X ratio) in triticale was similar to reference rye and was not affected by location. However, the reference wheat cultivar carried slightly less arabinose branching. Significant location effects on M_{cf} of β-glucan in triticale were also evident (Table 6). The M_{cf} of cultivars grown at Svalöv (average 3.8 × 10^5 g/mol) was more than double that of those grown at Kölbäck (average 1.7 × 10^5 g/mol). These results are evident from β-glucan molecular weight distribution profiles of triticale (Figure 12). Cultivars grown at Kölbäck had a narrow and unimodal molecular weight distribution of β-glucan, whereas in

cultivars grown at Svalöv, a relatively broader and bimodal distribution was observed, with a considerable fraction of high molecular weight β-glucan. The low M_{cf} values of triticale at Kölbäck and lack of a high molecular weight fraction can probably be attributed to pre-harvest enzymatic (endogenous) action. Triticale cultivars grown at Svalöv were significantly higher in long-chain (DP > 9) fructan molecules than those from Kölbäck, while the reverse was true for short-chain (DP 3-9) fructan (Table 6).

Table 6. *Weight average (M_w) and number average (M_n) molecular weight of arabinoxylan (g/mol), polydispersity index (M_w/M_n), arabinose/xylose (A/X) ratio, Calcofluor average molecular weight (M_{cf}) of β-glucan (g/mol) and relative distribution (%) of fructan with degree of polymerisation (DP) 3-9, > 9 in triticale cultivars, a rye and wheat reference cultivar, rye milling fractions, and rye products.*

	Arabinoxylan				β-Glucan	Fructan	
	$M_w \times 10^5$	$M_n \times 10^5$	M_w/M_n	A/X	$M_{cf} \times 10^5$	DP 3-9	DP > 9
Grains							
Triticale[1] (n = 18)	11.5	5.7	2.7	0.62	2.9	81	19
Svalöv[a] (n = 8)	15.4^A	6.4^A	2.4^B	0.62^A	3.8^A	79^B	21^A
Kölbäck[a] (n = 8)	14.3^B	4.9^B	3.0^A	0.63^A	1.7^B	83^A	17^B
Rye[1]	17.2	15.5	1.1	0.59	7.7	59	41
Rye inner endosperm[2]	10.7	3.7	2.9	0.66	13.3	49	51
Rye outer endosperm[2]	11.0	5.5	2.0	0.65	13.1	48	52
Rye bran[2]	13.8	7.2	1.9	0.50	8.6	52	48
Wheat[1]	11.8	4.9	2.4	0.53	4.1	74	26
Products							
Crisp breads[2] (n = 12)	9.9	3.3	3.1	0.53	3.7	49	51
Soft breads[2] (n = 8)	13.3	4.1	3.3	0.49	3.0	47	53
Extruded crisp bread[2]	6.9	2.6	2.7	0.58	10.6	69	31
Breakfast cereal[2]	9.5	7.0	1.4	0.53	15.0	58	42
Rye porridge without resting[3]	14.3	9.6	1.5	0.57	8.9	50	50
Rye porridge rested for 60 min[3]	14.0	9.3	1.5	0.57	4.3	51	49

[a]Values within columns for triticale cultivars grown at Svalöv and Kölbäck and followed by different letters (upper case) are significantly different from each other ($p < 0.05$).
[1]Paper 1; [2]Paper II; [3]Paper III

This degradation of AX, β-glucan and fructan can possibly be attributed to endogenous enzymes. The presence of endogenous xylanases and β-glucanases in cereals is well established (Andersson *et al.*, 2003; Boskov Hansen *et al.*, 2002; Rasmussen *et al.*, 2001; Bonnin *et al.*, 1998). The activity of these endogenous enzymes is affected by both environmental and genetic factors (Gebruers *et al.*, 2010a). Falling number is one estimate of enzyme activity and in this study the falling number values of most cultivars were below the detection limit of the instrument. Low falling number values for triticale compared with wheat and rye have previously been reported by Erekul and Köhn (2006). Humid conditions with high rainfall were featured during the growing season at Kölbäck, which might have triggered enzyme activity and degradation of AX and β-glucan.

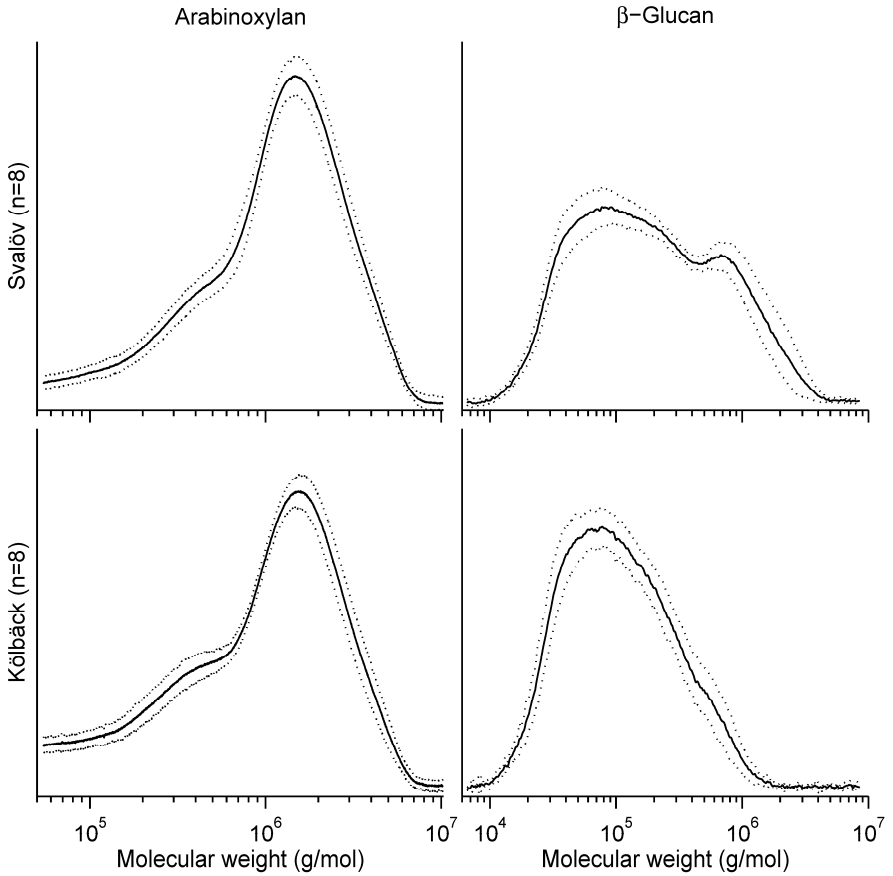

Figure 12. Average molecular weight distributions of arabinoxylan and β-glucan in triticale cultivars (n = 8) grown at two locations in Sweden. The dotted lines around the molecular weight distributions show the standard deviations.

This investigation revealed genotypic and environmental variation in triticale DF profile. Some cultivars were found to be stable and were not affected by the quite different growing conditions in the two regions. These cultivars can be ideally suited for large-scale production of triticale across Sweden and will not require immense modifications in feed formulations depending on growing region. However, yield must be considered along with DF profile when assessing the potential of any cultivar for mass production. On the whole, triticale appeared to be more similar to wheat than rye in its DF profile.

4.1.4 Molecular weight profile of dietary fibre in products

Average molecular weight of AX, β-glucan and fructan in rye products is presented in Table 6. Soft breads AX exhibited exceedingly broad variation in M_w values (range 8.0×10^5-22.2×10^5 g/mol) compared with crisp breads AX (range 8.5×10^5-11.0×10^5 g/mol) (Paper II). Relatively wider ranges were also observed for M_n and polydispersity index in soft breads. High polydispersity index means that the molecular weight distribution in soft breads spans over a wide range. This broad variation in AX molecular weight parameters of soft breads can be explained by diversity of ingredients and processing. Rye porridges contained AX with high M_w (average 14.0×10^5 g/mol), which is similar to M_w commonly observed in rye flour (Paper III). In contrast to the high average values of AX molecular weight parameters in soft breads, average M_{cf} values of β-glucan were relatively higher for crisp breads (3.7×10^5 g/mol) than soft breads (3.0×10^5 g/mol). The highest M_{cf} values, however, were observed for breakfast cereal (15.0×10^5 g/mol), followed by extruded crisp bread (10.6×10^5 g/mol). The porridge made without resting (8.9×10^5 g/mol) or with 60 min resting (4.3×10^5 g/mol) also retained β-glucan molecular weight better than most of the crisp breads or soft breads. Average fructan DP distributions were similar in crisp breads and soft breads, while extruded crisp bread had a higher level of fructan with relative DP 3-9 (69%). The porridges were similar to the rye base flour in their fructan DP distribution. Processing- and ingredient-related variation within crisp breads and soft breads on fructan DP distribution was present and is discussed in detail in the following section, along with AX and β-glucan molecular weight distribution profiles.

4.2 Processing effects on dietary fibre and its components

In this section, processing-generated variation in content, extractability and particularly molecular weight distribution of extractable DF components, i.e. AX, β-glucan and fructan, is discussed. Results from Paper II, which deals with

different rye-based products prepared by different processes, and Paper III, which investigates the impact of different variables of porridge making on molecular weight distribution of extractable DF, form the basis of the discussion.

The content of total DF may increase or decrease depending upon type of processing. Other than ingredient-related variation, the wide range of DF content in rye crisp breads and soft breads (Paper II) can partly be attributed to processing. During bread making, when rigorous processing is involved, total content of DF is said to be decreased along with a change in relative proportion of soluble DF (Boskov Hansen *et al.*, 2002). That study reported an almost 25% decrease in the content of total DF during rye bread making, while the relative proportion of water extractable DF increased from 23% in rye whole grain meal to 31% in dough samples. During porridge making, the amount of total DF increased from 20.0% in rye flour to 23.0% in porridge, mainly due to RS formation of type III (retrograded amylose) (Paper III). The extent of RS formation is dependent on processing conditions and ingredients (Dewettinck *et al.*, 2008). This can also be seen from our rye products study, where wider variation in content of RS + cellulose (analysed together) was observed in soft breads (0.7-4.1%) compared with crisp breads (1.7-3.0%) (Paper II). The formation of RS takes place during re-crystallisation of gelatinised starch (Englyst & Macfarlane, 1986).

4.2.1 Arabinoxylan

AX is the major DF component in cereals and is prone to processing-related changes. The fluctuation in AX content in the crisp breads studied in Paper II was not large and the majority of them contained about 8% total AX. This is probably due to more uniform ingredient composition and processing conditions. In contrast, huge variability was observed in the AX content of soft breads (range 3.1-8.5%), probably reflecting diverse ingredients and processing. Processing-related changes in AX content and molecular weight have been the focus of many previous studies (Dornez *et al.*, 2008; Boskov Hansen *et al.*, 2002; Cleemput *et al.*, 1997; Rouau, 1993). During bread making, the total content of AX may decrease, while WE-AX increases (Boskov Hansen *et al.*, 2002). The different steps in bread making, i.e. mixing, fermentation/proofing and baking, are an important determinant of the fate of AX in bread loaves (Dornez *et al.*, 2008). Observations by Cleemput *et al.* (1997) support AX solubilisation during bread making, since 7-12% of the water unextractable AX became soluble after mixing and this increased up to 30% after baking. In the porridge study (Paper III), the total content of AX remained unchanged during porridge preparation with increasing rest time,

addition of salt or amount of flour in the recipe. However, a very small but significant (p = 0.002) increase in the content of extractable AX was apparent during 1 h resting at 20 °C before cooking. As reported previously, AX solubilisation can result from mechanical or enzymatic effects (Dornez *et al.*, 2008; Cleemput *et al.*, 1997). Since not much work input was involved during porridge making other than occasional mixing, the prospects of mechanical solubilisation are limited. More variability in the A/X ratio of soft breads (range 0.31-0.56) compared with crisp breads (range 0.50-0.55) might also have originated from the bread making process (Paper II). Earlier, Rouau *et al*. (1994) demonstrated a decrease in A/X ratio after mixing and fermentation.

Processing effects on molecular weight distribution of extractable AX were visible in crisp breads and soft breads (Paper II). The wide range in M_w of soft breads (8.0×10^5 - 22.0×10^5 g/mol) compared with crisp breads (8.5×10^5 - 11.0×10^5 g/mol) can partly be related to processing. The AX molecular weight distribution profiles of a sample of crisp bread, soft bread, extruded breakfast cereal, whole meal rye flour and porridge cooked with or without resting are presented in Figure 13. The differences in AX molecular weight distribution of crisp bread and soft bread are conspicuous. The sample of crisp bread does not show significant degradation, while the soft bread has a visible low molecular weight fraction. This concurs with the results of Andersson *et al*. (2009), where limited degradation in AX molecules was observed during crisp bread baking, while soft bread with sour dough displayed notable degradation. In bread making, AX hydrolysis may occur during mixing or fermentation and result in an increase in the proportion of low molecular weight fraction, with concomitant loss of long-chain fraction (Andersson *et al.*, 2009; Cleemput *et al.*, 1997). Those authors ascribed these changes in molecular weight distribution of AX during bread making to endogenous enzymes. High M_w in some of the soft breads might be the result of free radical-mediated oxidative cross-linking of AX molecules through feruloyl groups and formation of aggregates (Cleemput *et al.*, 1997; Markwalder & Neukom, 1976). In extruded breakfast cereal there was a very narrow distribution of AX molecular weight, with no signs of processing-related degradation, underscoring the usefulness of extrusion for processing.

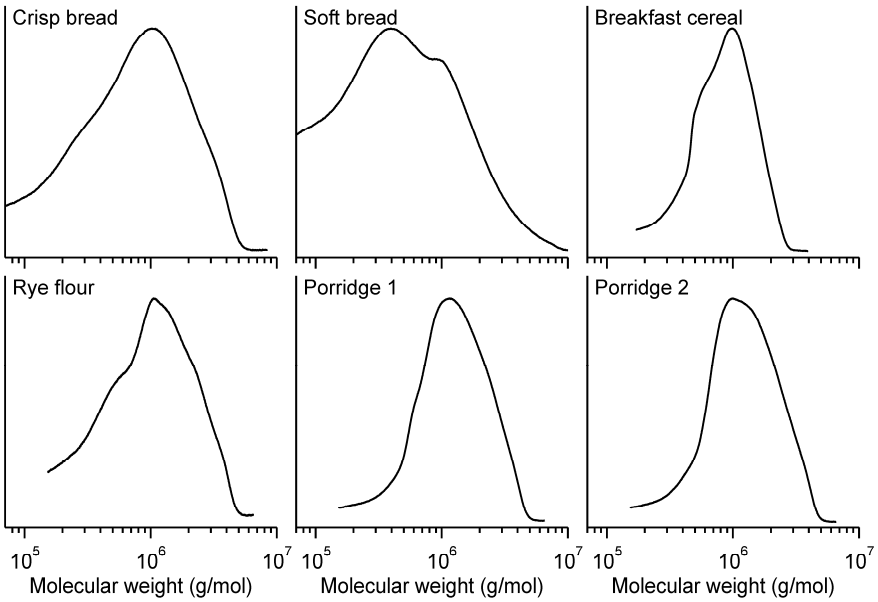

Figure 13. Arabinoxylan molecular weight distributions of crisp bread, soft bread, breakfast cereal, rye flour and porridges cooked without resting (1) and after 60 min resting (2).

During porridge preparation (Paper III), the effects of rest time and the amount of salt and flour in the recipe on M_w and M_n of AX were non-significant. The slight increase observed in AX M_w of porridges (on average 14.0×10^5 g/mol) compared with flour (12.0×10^5 g/mol) might be due to increased AX solubility of high molecular weight fraction or perhaps oxidative cross-linking. Likewise, AX molecular weight distribution profiles of porridges cooked with or without resting were very similar and only slight differences from base flour were observed (Figure 13), indicating stability of AX during resting. This non-action of xylanases on AX during 1 h resting was presumed to be due to non-optimal temperature (20 °C) and pH (6.2) during porridge making. A second experiment was performed to investigate the impact of temperature and pH on M_w by incubating rye flour slurries at different temperature (20, 35, 45, 55 °C) and pH levels (4.5, 6.2). The results showed a significant decrease in M_w due to incubation temperature ($p = 0.012$), while pH resulted in a non-significant ($p > 0.05$) decrease. Previous findings by Rasmussen *et al*. (2001) on temperature and pH optima of endogenous AX-degrading enzymes native to rye confirm this assumption. The optimum temperature for major endogenous enzymes in cereals responsible for the breakdown of AX molecules, i.e. *endo*-(1→4)-β-D-xylanase (EC 3.2.1.8), α-L-arabinosidase (EC 3.2.1.55) and β-D-xylosidase (3.2.1.37), is 40, 60 and 70 °C respectively, while the optimum pH is ≈4.5. In

contrast to porridge, pH of dough (particularly sourdough) is ≈4.5, which makes it more prone to AX degradation by xylanases. Furthermore, bread proofing is carried out at 40-45 °C which is suited to yeast (Cauvain, 2003). This temperature is also optimum for cereal *endo*-(1→4)-β-D-xylanase, which hydrolyses (1→4)-β-D-xylosidic linkages internally, resulting in a decrease in chain length.

Apart from endogenous enzymes, xylanase activity is also contributed from microbes present on the surface of the grain (Dornez *et al*., 2009; Dornez *et al*., 2008). During milling, the majority of these enzymes end up in the shorts or bran fraction of the roller mill and partly in flour streams. However, these microbial xylanases can be avoided to a great extent by debranning or surface treatment of the grain (Dornez *et al*., 2009). The presence of xylanase inhibitors such as *Triticum aestivum* xylanase inhibitor (TAXI)-type and xylanase inhibitor protein (XIP)-type may also provide stability to AX against microbial xylanases (Goesaert *et al*., 2004; Debyser *et al*., 1999). Therefore, the degree of AX degradation might be determined by pre-processing of the grain. The level and type of the enzymes in cereal grain also depend on multiple factors such as grain species, cultivar, tissue, growing environment, etc. (Dornez *et al*., 2009).

4.2.2 β-Glucan

The content, extractability and molecular weight distribution of β-glucan may change during processing (Tiwari & Cummins, 2009). In our bread study (Paper II), most of the crisp breads contained similar β-glucan content (≈2%), which is comparable to that commonly reported for rye flour (1.3-2.2%) (Boskov Hansen *et al*., 2003). Likewise, in the porridge study, the content of β-glucan remained unchanged during 1 h incubation at 20 °C before cooking and during cooking (Table 5, Paper III). This agrees with observations by Rose *et al*. (2010) during jet cooking of barley flour, Åman *et al*. (2004) during cooking of oat-based porridge and Jaskari *et al*. (1995) during hydrothermal treatment of oat bran at 95 °C for 20 min. Looking at the present results and previous studies, one could speculate that hydrothermal treatment, i.e. cooking, boiling, pasteurisation or baking, in itself does not result in loss of β-glucan. β-glucan is still quantifiable by the enzymatic method even though it is degraded during bread making or porridge preparation due to the activity of endogenous enzymes native to cereal grains (Andersson *et al.*, 2008).

Bread making may improve β-glucan extractability, as the polymer was more extractable in the soft breads (average 35%) and crisp breads (average 29%) studied in Paper II (Table 5) compared with typical whole grain rye (11%) or wheat flour (19%) (Paper I). An earlier study by Andersson *et al*.

(2008) also demonstrated an increase in β-glucan extractability during bread making. Both mixing and fermentation facilitated β-glucan release from the insoluble matrix, while baking itself did not improve extractability. However, the highest β-glucan extractability was observed in extruded breakfast cereal (54%), followed by extruded crisp bread (47%) (Paper II). Small amounts of oats and barley flour might have contributed to the high extractability of β-glucan in breakfast cereal and extruded crisp bread, respectively. However, a previous study by Vasanthan *et al*. (2002) also demonstrated increased extractability of β-glucan in barley flour during extrusion cooking. The increase in extractability was dependent on processing conditions, mainly temperature and moisture. Further support for increased extractability during extrusion can be derived from the findings of Beck *et al*. (2009) and Tosh *et al*. (2010). During porridge preparation, extractability of β-glucan also increased significantly (p = 0.016) due to rest time before cooking of porridge (Paper III). The extractability of rye flour used in the porridge study was 14% which went up to 17% in porridge cooked without resting and 24% in porridge rested for 60 min before cooking. This is equivalent to a 41% increase in β-glucan extractability over 1 h resting. Consequently, hydrothermal treatment aimed to open up the physical barriers for water absorption also rendered β-glucan more soluble. It can therefore be summarised that the polymer normally becomes more soluble during processing, probably due to hydrolysis of the polymer to short-chain molecules, which are easily extractable.

Studying molecular weight distribution is a more sensitive way to track processing-related degradation of the β-glucan. During bread making, β-glucan degradation results in decreased M_{cf} and a shift towards low molecular weight fraction. The average M_{cf} of crisp breads (3.7×10^5 g/mol) was relatively high compared with that of soft breads (3.0×10^5 g/mol) (Table 6). This difference can be seen from the β-glucan molecular weight distribution profile of a crisp bread and soft bread sample (Figure 14). Apart from inclusion of wheat in most of the soft bread, processing also contributed towards low molecular weight of β-glucan. Presence of sourdough did not seem to influence the β-glucan degradation, confirming similar observations reported by Åman *et al*. (2004). However, Andersson *et al*. (2009) observed a slightly decreasing proportion of high molecular weight β-glucan fraction in the order of air-leavened rye crisp bread, sourdough rye bread and yeast-leavened rye crisp bread. Mixing and fermentation of the dough are the most critical steps during bread making and result in a significant decrease in M_{cf}, whereas yeast and water content had non-significant effects (Andersson *et al.*, 2008; Andersson *et al.*, 2004). Those authors also noted that differences in M_{cf} values of dough and bread loaves were non-significant, suggesting a negligible role of baking on β-glucan M_{cf}.

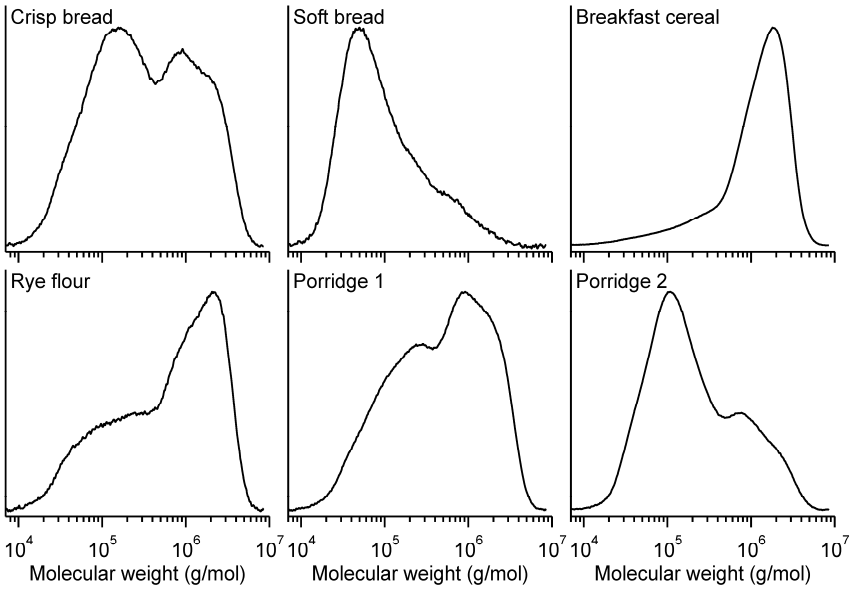

Figure 14. β-Glucan molecular weight distribution of crisp bread, soft bread, breakfast cereal, rye flour and porridges cooked without resting (1) and after 60 min resting (2).

Compared with crisp breads or soft breads, the two extruded products retained the β-glucan molecular weight to a greater degree (Table 6, Paper II). The distribution profile of breakfast cereal showed very narrow β-glucan molecular weight distribution with no apparent signs of degradation (Figure 14). The breakfast cereal had the highest M_{cf} (15.0×10^5 g/mol), followed by extruded crisp bread (10.6×10^5 g/mol). These results support earlier findings by Åman *et al*. (2004), where extruded oat product showed a monomodal distribution profile and β-glucan retained high molecular weight compared with other commercial oat-based foods. Despite the high shear and temperature used during extrusion cooking, the time is relatively short, which keeps β-glucan molecules intact (Tosh *et al.*, 2010; Beck *et al.*, 2009). It can therefore be assumed that extrusion is a comparatively better processing technique when the objective is to prepare products with retained β-glucan M_{cf}.

During porridge preparation, the M_{cf} of β-glucan was affected significantly by rest time ($p < 0.001$), amount of salt ($p = 0.043$) and flour ($p = 0.019$), as shown in Figure 15. The average M_{cf} value of porridge prepared without any rest time was 8.9×10^5 g/mol, which decreased by 51% to 4.3×10^5 g/mol during 1 h resting before cooking. The small but significant decrease in M_{cf} observed with the addition of salt might be attributable to increased endogenous enzyme activity. However, no plausible explanation can be given for the small decrease in M_{cf} with increasing amount of flour. The decrease in

β-glucan molecular weight may not be from cooking itself, since previous findings support stability of the polymer during porridge preparation, provided that endogenous enzymes are not activated (Åman *et al.*, 2004; Beer *et al.*, 1997). This degradation of β-glucan can rather be attributed to endogenous β-glucanases native to cereal grain or β-glucan hydrolases of microbial origin (Andersson *et al.*, 2008; Flander *et al.*, 2007; Mälkki & Virtanen, 2001; Zhang *et al.*, 1997). When flour is hydrated, *endo*-β-glucanases become active and cut the polymer randomly, resulting in a sharp decrease in average molecular weight of β-glucan. The molecular weight distribution profile of rye flour showed quite a high proportion of long-chain fraction (Figure 14). A sharp decline in long-chain fraction was evident in porridge cooked without resting, indicating rapid action of endogenous β-glucanases. By looking at the distribution profiles of porridges cooked with increasing rest time (Paper III) and previous results from Andersson *et al*. (2008), it is logical to conclude that most of the enzyme action takes place within the first few minutes.

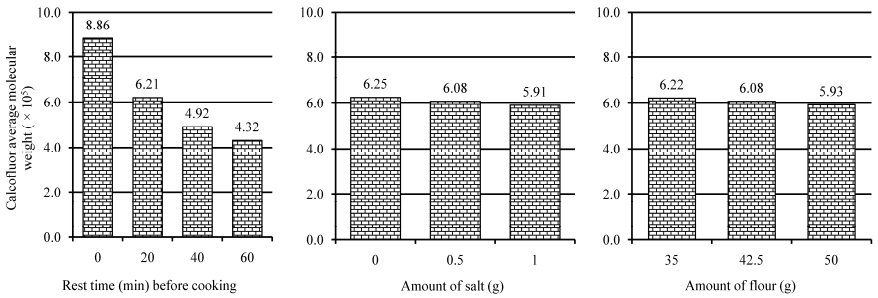

Figure 15. Main effects of rest time before cooking, amount of salt and amount of flour in the recipe of rye porridge on Calcofluor average molecular weight of β-glucan.

Endogenous enzyme activity varies among cultivars, and flours with high endogenous enzyme activity end up with lower M_{cf} in bread loaves (Andersson *et al.*, 2009; Andersson *et al.*, 2008). Further differences in the activity of endogenous enzymes can be observed in various botanical parts of the grain and it is high in aleurone and sub-aleurone layers (Andersson *et al.*, 2003). Depolymerisation of β-glucan can be carried out by four classes of β-glucanases, namely (1→4)-β-glucan 4-glucanohydrolase (cellulase, E.C. 3.2.1.4), laminarinase (E.C. 3.2.1.6), (1→3) (1→4)-β-glucan 4-glucanohydrolase (lichenase, E.C. 3.2.1.73) and (1→3)-β-glucan glucanohydrolase (*endo*-(1→3)-β-glucanase, E.C. 3.2.1.39) (Kanauchi & Bamforth, 2008; Poutanen, 1997).

4.2.3 Fructan

The wide range of fructan content in rye breads (0.8-4.0%) may be partly associated with processing (Paper II). Some crisp breads (baked without yeast) contained as much fructan as commonly observed in whole grain rye flour (4.1%, n = 18) (Andersson *et al.*, 2009). On the other hand, soft breads, the majority of which contained yeast and sourdough, were quite low in fructan compared with crisp breads. Apart from diverse ingredients, large variations in the fructan content of soft breads may arise from processing. Observations by Andersson *et al*. (2009) substantiate this claim of a processing-originated decrease in fructan content in rye breads. The initial content of 5% fructan in rye grain (cv. Kaskelott) decreased by 6% in air-leavened crisp bread, 32% in yeast-leavened crisp bread and 62% in sourdough rye bread. It is evident that crisp bread baked without yeast had nearly as much fructan as the grain, while in sourdough bread the majority of fructan was degraded, findings confirmed by our observations (Paper II). The relatively low pH of sourdough might aid fructan degradation, since low pH is favourable for hydrolysis of fructan (Matusek *et al.*, 2009; Franck, 2002). While following the pattern of fructan degradation during various steps of rye bread making, Boskov Hansen *et al*. (2002) found a 26% decrease in freshly prepared dough which subsequently went up to 34% in dough after proofing and 45% in bread crumb. This degradation of fructan during rye bread making has been ascribed to baker's yeast and yeast of sourdough by Fretzdorff and Welge (2003). Those authors further stated that final fructan content in bread loaf is dependent on fermentation and baking time. In another observation by Meyer and Peters (2009), yeast with low invertase activity resulted in only 13% loss of inulin-type fructan, compared with 37% by high invertase activity yeast in the bread study.

In porridge making, where no yeast is involved, the fructan content remained unaffected with increasing rest time before cooking or cooking time (2, 10, 20 min) (Paper III). This implies that cereal fructan is quite stable to heating at cooking temperature in the presence of water. Similar observations were made by L'Homme *et al*. (2003) for fruit matrices such as banana puree, where fructan showed no signs of degradation during cooking for 30 min at 80-110 °C. Therefore it is tempting to assume that pasteurisation, cooking or boiling under normal conditions will not render fructan degraded (Franck, 2002). In contrast, dry heating of inulin (from chicory) at 195 °C for 60 min can result in almost 100% degradation and form new products of fructose anhydrides (Böhm *et al.*, 2005). However, those authors observed negligible degradation at 100 °C and heating for 60 min under water-free conditions.

Our results illustrate the preference of yeast for short DP fructan, since breads containing yeast had smaller relative proportions of DP 3-4 and DP 5-9 (Paper II). This fact can be visualised from fructan distribution profiles of crisp bread baked without yeast, soft bread baked with yeast and sourdough, and extruded breakfast cereal (Figure 16). The fructan DP distribution of crisp bread was very similar to that of rye flour studied in Paper III, while soft bread showed a significantly lower relative proportion of short DP fructan than crisp bread. The extruded breakfast cereal, which also lacked yeast, contained a high proportion of DP ≤ 9. This predisposition of yeast towards low DP fructan has been reported previously (Meyer & Peters, 2009; Praznik *et al.*, 2002). In breads containing inulin-type fructan from Jerusalem artichoke, fructo-oligosaccharides of DP 3-4 have proven to be extremely labile to yeast fermentation compared with long DP inulin (Praznik *et al.*, 2002).

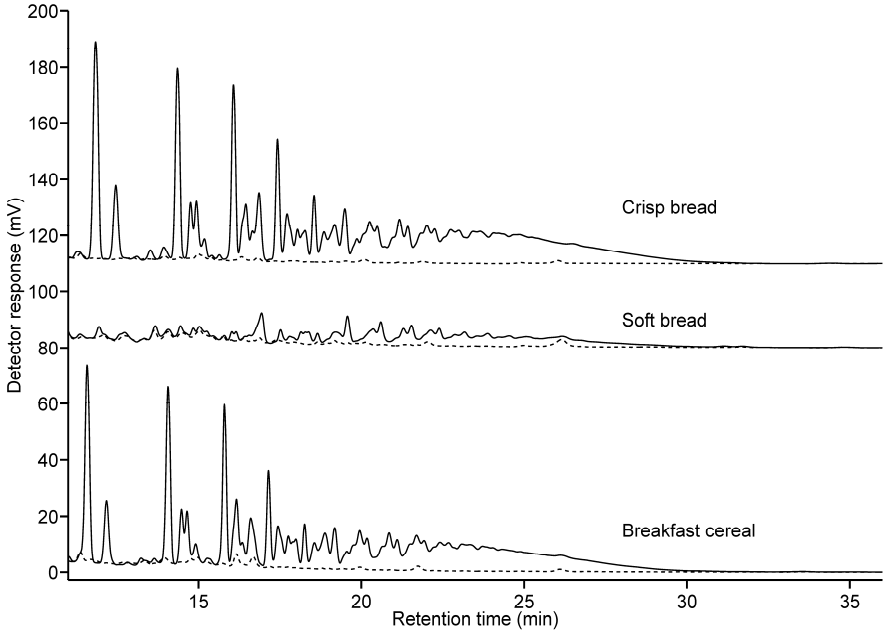

Figure 16. Fructan DP distribution profiles of crisp bread, soft bread (baked with yeast and sourdough) and breakfast cereal (extruded). Dashed line shows DP distribution after fructanase treatment.

In the porridge experiment, the effect of variable rest time before cooking and amount of salt and flour on the relative DP distribution of fructan was non-significant (Paper III). The chromatograms of flour and porridges rested for 60 min were very similar, indicating stability of fructan during rye porridge making. Thus, endogenous fructan *exo*-hydrolases (FEHs), if present, appear to

have very low activity. The complexity of linkages in cereal fructan and the specificity of FEHs might provide an explanation for the stability of fructan to endogenous enzymes in rye (Chalmers *et al.*, 2005; Kawakami *et al.*, 2005). Another explanation for the non-action of endogenous enzymes during resting could be the sub-optimal temperature and pH (20 °C and 6.2, respectively), since the optimum temperature and pH for FEH activity is around 40 °C and 4.5-5.5, respectively (Van den Ende *et al.*, 2003; Marx *et al.*, 1997). This stability of fructan to endogenous enzymes was also witnessed in the bread study (Paper II), where the crisp breads made without yeast had very similar fructan profile to the rye grain. This is an important consideration for modern processing of rye products, where the aim is to avoid losses of physiological functions associated with DF components. However, no previous study targeting fructan degradation by endogenous enzymes during processing could be found in the literature.

In brief, the physiological behaviour of different products can vary depending upon the extent of degradation of DF components. Rye products may contain as much DF as grain or even more, depending upon the type and extent of processing. Crisp breads normally contain significantly higher DF than soft breads and may retain molecular weight better. Compared with breads, extruded products retain molecular weight with a concurrent increase in solubility, while porridge is least degraded in extractable DF components. Cereal porridge or extruded products can therefore be more efficient in providing the physiological benefits associated with DF.

4.3 Enzymatic fingerprinting of arabinoxylan and β-glucan

Structural features of AX and β-glucan are considered important for their functional properties (Cui & Wang, 2009; Vinkx & Delcour, 1996). Interpretation of structural features determined through enzymatic hydrolysis of AX and β-glucan showed significant diversity in triticale, barley and tritordium grains (Paper IV).

4.3.1 Enzymatic hydrolysis of arabinoxylan

Here the focus is on structural features of triticale AX only, since AX constitutes about 50% of the DF in triticale, while most of the AX in barley is unextractable with water (Viëtor *et al.*, 1992) and very low relative area was observed under the AX peaks in barley. PCA of relative proportions of AX fragments from triticale cultivars grown at two different locations is presented in Figure 17. Principal component (PC) 1 of score plot separates the cultivars along two growth regions and is driven by fragments X and XX. The cultivars

grown at Kölbäck were significantly higher ($p < 0.05$) in fragment X (average 24.4%) compared with those grown at Svalöv (average 20.4%), as is visible from concurrent look at score and loading plot. Cultivar DED 145/02 grown at Svalöv appears to be an odd sample, with features more similar to cultivars from Kölbäck. Conversely, triticale cultivars grown at Svalöv were significantly higher in mono-substituted and di-substituted AXOS with the exception of $XA^{2+3}XX$ and $XA^{2+3}A^{2+3}XX$, where non-significant differences were observed (Paper IV). The relatively higher proportion of branched fragments in cultivars grown at Svalöv is not supported by A/X ratio of total AX which was similar for cultivars grown at Svalöv (average 0.62) and Kölbäck (average 0.63). Earlier work by Ordaz-Ortiz *et al*. (2005) has demonstrated a strong positive correlation between A/X ratio and proportion of di-substituted xylose residues in wheat WE-AX ($r = 0.96$). One reason for the lack of correlation between A/X ratio and extent of substitution in this study can be different extraction conditions, since ≈ 70% of the AX is extractable in this experiment (Ordaz-Ortiz & Saulnier, 2005). Along the PC2 of loading plot, the separation of cultivars is based on extent of substitution. The cultivars falling on the negative end of PC2 have more mono-substitution compared with those lying on the positive end of PC2. The variation along PC2 is thus genetically controlled. A/X ratio of total AX seems to have little meaning in explaining the substitution pattern visible in this plot since it lies on the origin of PC2. The first two PCs accounted for 67% of the variation in data.

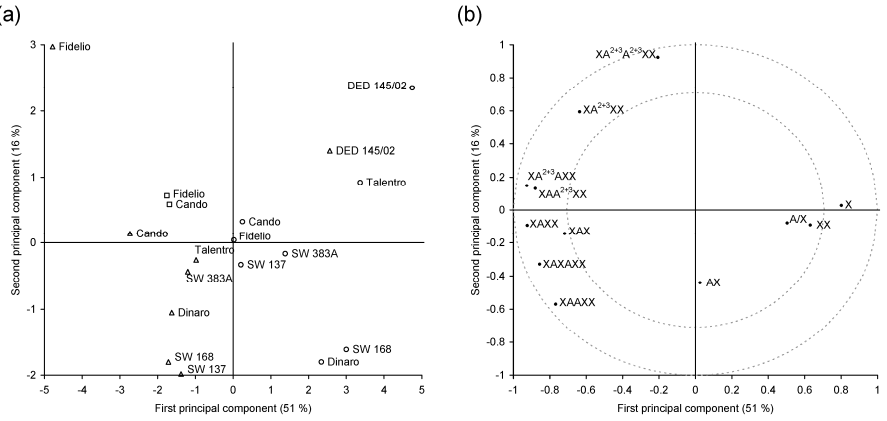

Figure 17. Score plot (a) and loading plot (b) from PCA of AX content, A/X ratio and AX fragments (X, XX & AXOS) released after enzymatic hydrolysis. AXOS containing letter (A) without any superscript denotes xylose residue with arabinose substituent at position three, while A^{2+3} denotes xylose residue with arabinose substituents at positions two and three. (Δ = triticale at Svalöv, ○ = triticale at Kölbäck, □ = triticale at Haga)

4.3.2 Enzymatic hydrolysis of β-glucan

β-Glucan is an important component of endosperm cell walls of barley and most of it is water soluble (Cui & Wang, 2009). Lichenase hydrolysis of water-soluble β-glucan in barley yields fragments of DP up to 13 (Izydorczyk *et al.*, 1998a), while the water-insoluble part of β-glucan may yield much longer cellulose-like sequences up to DP 20 (Izydorczyk *et al.*, 1998b). However, in this experiment we analysed GOS up to DP 6. Among the oligomers released BG_3 and BG_4 are most predominant and molar ratio of BG_3/BG_4 forms the fingerprint of a particular cereal (Cui & Wang, 2009). Among cereals studied in this experiment, molar ratio of BG_3/BG_4 ranged from 2.3-3.3 in barley, 2.5-3.4 in triticale and 2.8-3.4 in tritordium (Paper IV). Likewise molar proportion of BG_3+BG_4 in twenty barley cultivars/breeding lines ranged from 93.8-95.6% (average 95%). These values appear to be slightly higher than those observed by Irakali *et al*. (2004) for barley β-glucan, where 91-92% of the total oligomers consisted of BG_3+BG_4. The slightly higher values observed in our study are due to the fact that we accounted for oligosaccharides up to DP 6 and normalised the relative area under each peak. Molar proportion of trisaccharides and tetrasaccharides accounted for 88.3% in triticale and 86.5% in tritordium. Both ratio and molar proportion of trisaccharides and tetrasaccharides are important for physico-chemical properties such as solubility (Cui & Wang, 2009). β-Glucan extractability of cereals studied was found to be positively correlated (r = 0.75) with proportion of trisaccharides and tetrasaccharides. In contrast, a slight negative correlation of extractability with DP 5 (r = -0.79) and DP 6 (r = -0.57) was observed. No apparent correlation of BG_3/BG_4 with β-glucan extractability (r = -0.25) could be established. The ratio of BG_3/BG_4 is influenced by both genetic and environmental factors (Izydorczyk & Dexter, 2008). Barley genotypes with waxy starch are reported to exhibit higher BG_3/BG_4 ratio (3.1-3.2) compared with normal starch (2.5-2.9) or high amylose barley (2.8-3.1) (Storsley *et al.*, 2003). In our results, waxy barley had higher BG_3/BG_4 ratio (2.8-2.9) than normal barley line (2.3) but lower than some other lines (3.1-3.2).

A strong positive correlation (r = 0.86) between β-glucan content and molar proportion of BG_3 was observed in barley (Figure 18). Barley lines 3, 7, 19 and 20 contain the highest β-glucan content (8.9-10.5%) and stretch out on the top corner of the correlation plot. The two lines (2, 18) lying on the bottom of the plot contain the least amount of β-glucan (2.3%, 2.4%, respectively).

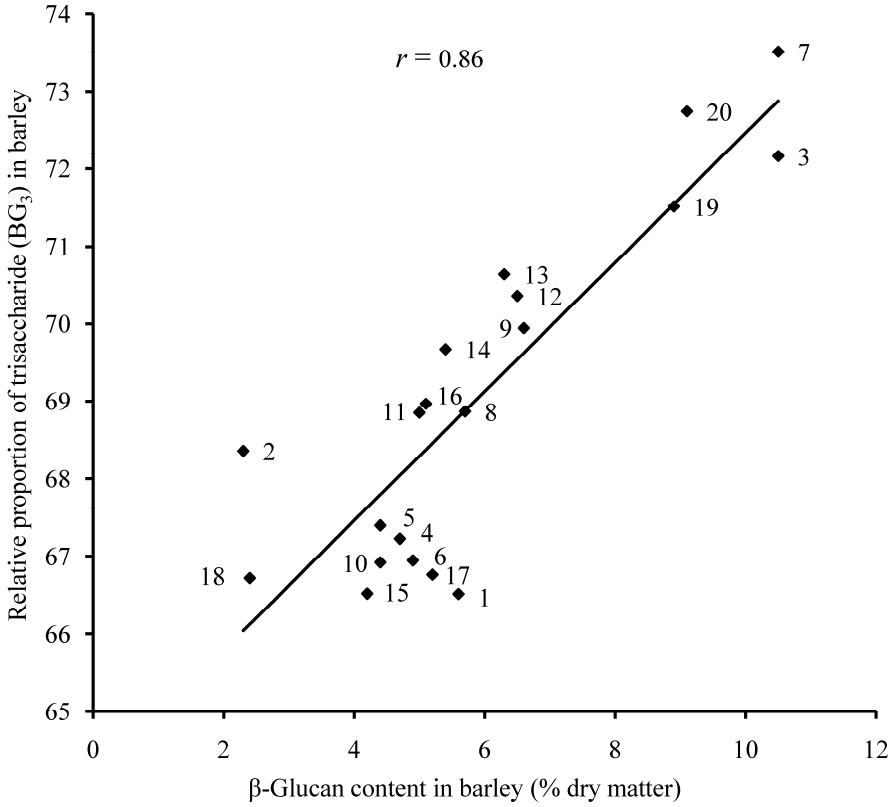

Figure 18. Correlation between β-glucan content and relative proportion of trisaccharide (BG_3) released after lichenase hydrolysis.

4.4 Rheological properties of triticale

Since the primary aim of triticale breeding is animal feeding, viscosity measurement forms an integral part of feed value assessment. Viscoelastic properties of triticale cultivars grown at different locations were measured by a method developed during the study (Paper V).

The rheology of flour slurries or extracts can be studied with a Rapid Visco Analyser (RVA) or rheometer. At first, trials of triticale viscosity measurements were conducted using an RVA-based method. After extensive experimentation to find the optimum amount of water, flour, hydrolytic enzymes and time; 6 g triticale flour was mixed with 25 ml water containing 100 µl α-amylase. The slurry was made to run at 95 °C at certain constant shear for a specific time and subsequently at lower temperatures to treat with xylanase and lichenase and estimate the contribution of AX and β-glucan towards triticale viscosity. However, constraints such as avoiding interference

by endogenous enzymes and sedimentation of the sample at the bottom of canister forced us to abandon this protocol and look towards rheometer-based methods.

For rheometer measurements, the flour was first boiled with 50% ethanol to inactivate the endogenous enzymes. Subsequently, the extractable DF polymers were extracted by boiling with water (90 min) in the presence of thermostable α-amylase. However, the maximum viscosity of the extract achieved was not high enough to be measured with sufficient accuracy using the cup and bob or cone and plate geometries of our instrument. Therefore the extract viscosity needed to be scaled up. This could have been achieved by either vacuum evaporation (Anttila *et al.*, 2004) or by precipitating the polymers and re-dissolving in minimum required volume to measure viscoelastic properties by the cone and plate method. We opted for the latter technique because of feasibility and were able to reach the required concentration. The viscoelastic properties of the triticale cultivars and a reference wheat cultivar were characterised by their elastic modulus (G′), viscous modulus (G″), complex modulus (G*) and phase angle (δ). The relationship between frequency (Hz), δ (°) and modulus (Pa) is presented in Figure 19.

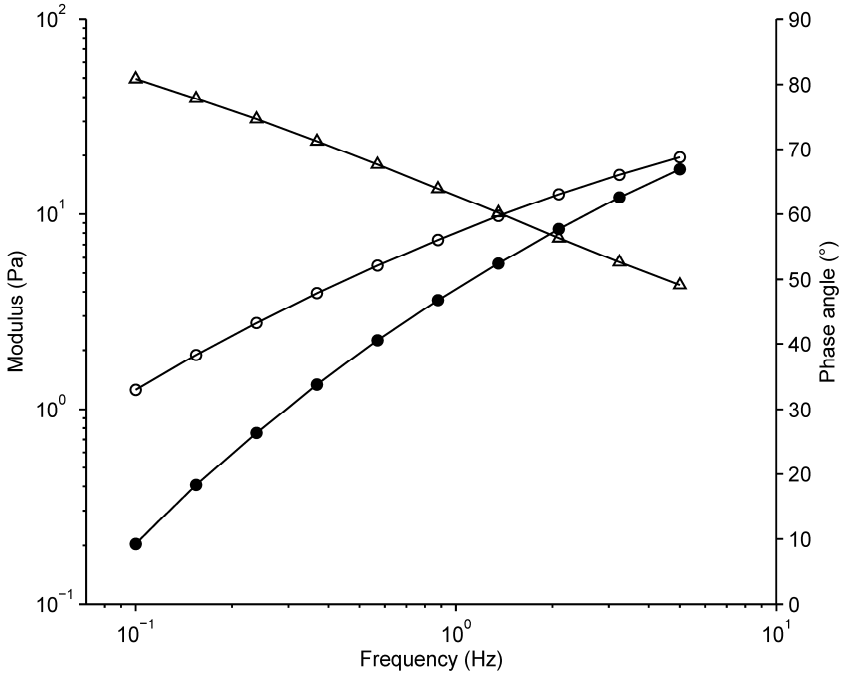

Figure 19. Relationship of frequency with modulus (viscous modulus = ○, elastic modulus = ●) and phase angle (Δ) in extract from triticale cultivar Talentro.

Triticale cultivars grown at Svalöv were significantly higher in G′ (average 9.0 Pa) and G″ (average 12.7 Pa) at 2.1 Hz than those grown at Kölbäck, with average values of 3.1 Pa and 5.6 Pa, respectively (Paper V). Significant location effects were also evident on G* at 2.1 Hz, as values for cultivars at Svalöv (average 15.6 Pa) were more than double those for cultivars at Kölbäck (average 6.4 Pa). It is worth remembering that G* is a measure of stiffness of polymer solution and, together with δ, defines G′ and G″. Large variability in G* values (at 2.1 Hz) of triticale cultivars (range 1.6-39.7 Pa) is visible in Figure 20. Triticale cultivar Fidelio had the highest G* at all locations and appeared very viscous during sample preparation. The δ of Fidelio grown at Svalöv was lower than for other cultivars, which follows the fact that materials having $\delta < 45°$ are solid-like and those with $\delta > 45°$ are liquid-like. However δ was not significantly different in the two growing regions. Because of interaction between location and cultivars, Tukey's pair-wise comparison was unable to confer significance on genetic differences among triticale cultivars which were quite obvious (Paper V). The wheat reference cultivar (cv. Harnesk) tested in this study resembled some of the triticale cultivars, e.g. Dinaro, DED 145/02, Cando, in its G* value (Figure 20) and other viscoelastic properties at 2.1 Hz (Paper V). On the other hand, the rye reference cultivar (cv. Ottarp) used in this project gave too viscous an extract to handle under the test conditions.

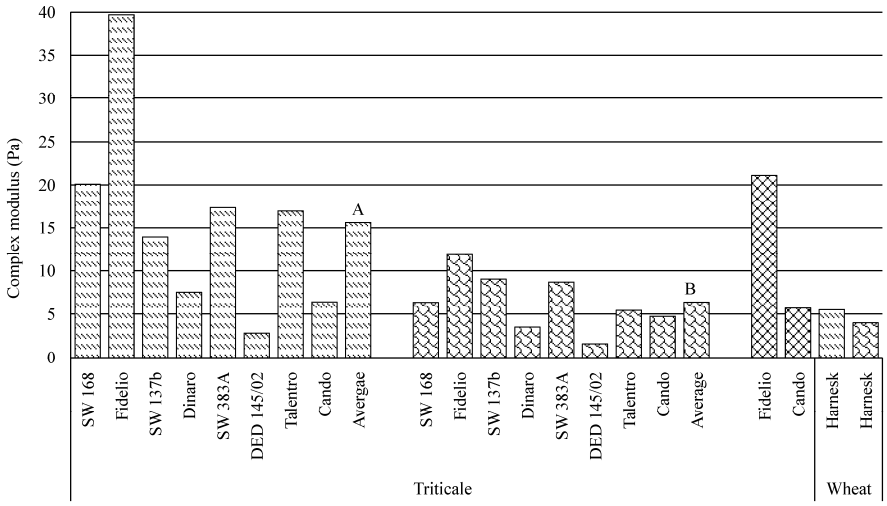

Figure 20. Complex modulus (at 2.1 Hz) of extracts from triticale grown at Svalöv ( ), Kölbäck ( ) and Haga ( ) and wheat reference cultivar grown at Svalöv ( ) and Kölbäck ( ). The different letters (upper case) on the average values of two regions show significant location effects ($p < 0.05$).

The relationship of G* (at 2.1 Hz) with content and extractability, molecular weight and structure of AX and β-glucan was studied by running PLS regression. When both AX and β-glucan were included as predictors, the model was capable of explaining 91% of the variation in G* at 2.1 Hz with three PLS factors and a small (0.11 Pa) root mean square error of prediction (RMSEP). Since AX is the major polysaccharide accounting for about 50% of the total DF in triticale while β-glucan is only a minor component (Paper I), G* modelling by AX chemistry was preferred. Not surprisingly, after removal of β-glucan content, extractability, molecular weight and structure, the regression power of the model was similar to that containing β-glucan. Subsequently, the contribution of AX content and extractability, molecular size and fine structure to G* values was assessed by taking one variable group out at a time and evaluating the regression model. With the removal of content and extractability parameters of AX, model prediction power was slightly decreased (R^2 = 0.87, RMSEP = 0.13 Pa). Dependence of triticale viscosity on content of water-extractable pentosans is already well known (Pettersson & Åman, 1987). A positive correlation between the content of WE-AX and extract viscosity has also been reported for wheat (Martinant *et al.*, 1999). Molecular size of AX made a similar contribution to the regression model (R^2 = 0.87, RMSEP = 0.13 Pa) as the content and extractability of AX. The very low correlation between M_w and G* (r = 0.45) also indicates the small contribution of molecular size to the modelling of extract rheology. These results are not in complete agreement with those reported by Ragaee *et al*. (2001), where a strong association (r = 0.84) between extract viscosity and high molecular weight AX fraction was established in rye. However, different experimental conditions might make the comparisons inconclusive. AX structure seems to have greater influence on extract rheology than water-extractable content and molecular size parameters. After removing structure from the regression model, the model regression power dropped significantly (R^2 = 0.70, RMSEP = 0.20 Pa).

High dependence of G* on structural features could also be established from the fact that triticale cultivars grown at Svalöv were low in AX content and extractability but were generally higher in branched AXOS. The A/X ratio of total AX had no correlation with G*, while a slight negative correlation between A/X ratio of soluble AX and G* (r = -0.65) was found. The results reinforce the previous findings that AX with low substitution (A/X ratio) results in higher extract viscosities (Ragaee *et al.*, 2001; Martinant *et al.*, 1999). This might be due to cross-linking of unsubstituted xylan chains resulting in gelation. However, it conflicts with the study by Andrewartha *et al*. (1979), where viscosity decreased with the partial removal of arabinose substituents.

It can be concluded that the viscoelastic properties of triticale cultivars tested varied between locations and cultivars. Some of the cultivars tested were very similar to wheat in their viscoelastic properties of extracts, while others had much higher values. The G* of triticale was mainly governed by the structural features of AX.

5 Conclusions

This work focused on characterisation of DF in grains (mainly triticale) and rye products. Triticale has its major use as an animal feed, while rye is an integral part of bread and breakfast cereals across Sweden. Both genetic and environmental factors contributed to the variation in DF content and composition of triticale. Some of the cultivars, such as SW 383A and Dinaro, were more stable in their DF content at both locations. More pronounced effects of location were observed on molecular weight distribution of extractable DF components, particularly β-glucan, since cultivars grown at Kölbäck (with high rainfall) showed notable degradation. The DF profile of triticale was quite similar to that of wheat, but it had a considerably lower amount of total and extractable DF than rye. The greater similarity of triticale to wheat than to rye may reflect the fact that it carries two genome proportions from its wheat parent (A & B genome) and only one from rye (R genome).

DF content in rye-based products varied widely based on ingredients and processing. Crisp breads containing whole meal rye flour as a principal ingredient were very similar to the grain in their DF composition. In contrast, soft breads having more diverse ingredients and processing had low average DF content. Rye porridge had the highest DF content and contained considerably more DF than the grain, primarily due to formation of RS of type III.

Processing of cereals is aimed to make them palatable with simultaneous retention of nutrient bioactivity. This demands careful selection of processing parameters to retain maximum benefits associated with DF. This study showed that content, extractability and molecular weight distribution of DF components were affected by processing. In general, extractability of AX and β-glucan increased during processing, molecular weight decreased and content remained more or less unchanged. Fructan appeared to be labile to yeast fermentation. Among the three extractable DF components examined here, β-

glucan was much more sensitive to endogenous enzymes-related degradation. Complexity of linkages in AX and fructan might be one reason for their stability against endogenous enzymes. Among the products studied, processing effects on molecular weight distribution of extractable DF components were more pronounced in crisp breads and soft breads than extruded products and rye porridge.

Structural features of AX and β-glucan analysed by enzymatic fingerprinting were quite different in triticale, barley and tritordium. Triticale cultivars grown at two locations differed in AX fragments released after *endo*-xylanase hydrolysis. Cultivars grown at Svalöv were generally high in substituted fragments, whereas those at Kölbäck were significantly higher in fragment X. However, the substitution pattern across the two regions was not related to A/X ratio. Relative proportion of β-glucan fragments released after lichenase hydrolysis varied in different cereals. Molar proportion of trisaccharides and tetrasaccharides was highest in barley, followed by triticale and tritordium. Among the barley genotypes studied, waxy barley had a higher molar ratio of BG_3/BG_4 compared with normal starch barley, but not the highest. The slight negative correlation observed between β-glucan extractability and GOS of DP 5 and DP 6 was logical. β-Glucan content and molar proportion of BG_3 had a strong positive correlation.

Viscoelastic properties of triticale varied with location. Cultivars grown at Svalöv were significantly higher in G′, G″ and G* than those at Kölbäck. Inter-cultivar variation was also quite marked, as some of the cultivars were very viscous and others were equally or even less viscous than wheat. Major variation in the viscosity of triticale was explained by structural features of AX and not so much by extractable content or molecular size of AX. β-Glucan, being less significant in triticale, does not contribute towards its extract rheology. The huge diversity in viscoelastic properties of the triticale cultivars grown in Sweden gives more options for farmers and feed manufacturers to design triticale-based feed formulations based on end-use quality.

6 Main findings

- Triticale DF content and composition was affected by growing location and cultivar.
- Triticale grown at a location with high rainfall resulted in higher DF content and lower molecular weight of AX, β-glucan and fructan.
- Triticale was more similar to wheat than to rye in its DF profile.
- Rye crisp breads were considerably higher in their DF content (average 17.8%) than soft breads (average 12.6%), while porridge contained up to 23% DF.
- Rye products prepared without fermentation retained high molecular weight of extractable DF components.
- Short-chain fructan was readily degraded in breads baked with yeast.
- Barley and tritordium generally had more branched AXOS than triticale.
- Triticale grown at a location with high rainfall had a smaller relative proportion of branched AXOS.
- Relative proportion of BG_3 had a strong positive correlation (r = 0.86) with β-glucan content in barley.
- Rheology of triticale extracts was mainly controlled by structural features of AX rather than extractable content or molecular size.

7 Future prospects

The thesis sought to thoroughly characterise the DF profile of triticale and also to explore the effects of processing on extractable DF components in rye products. The detailed account of triticale DF characterisation was intended to better describe its utility in feed, while the study on rye products was intended to help better define their end-use quality. However, certain unanswered questions demand further work, as listed below.

- Quantification of unsubstituted, mono-substituted and di-substituted xylan proportions in triticale.
- Characterisation of structural features of various cereal products consumed routinely, and their correlation with viscosity.
- Determination of yeast preference for fructan with different linkages.
- Studying the relationship between AX structural features of triticale cultivars tested with their feed value and exploration of the food perspectives of triticale.
- Further investigation into the relationship of viscosity with degree and pattern of AX substitution, since at one hand viscosity correlated negatively with A/X ratio of WE-AX and at the other hand cultivars with a high level of highly branched AXOS were more viscous.

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