Barley malt products
for improved intestinal health

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Barley is a good source of dietary fibre, particularly β-glucan and arabinoxylan, to increase formation of short-chain fatty acids (SCFAs). Butyric and propionic acid are involved in the preservation of colonic barrier function, thus decreasing the risk of inflammation. Previous studies have shown that malting barley increased formation of butyric acid in caecum of rats and had abundance of butyrate-producing bacteria than unmalted barley. However, this differed between malts, probably due to the differences in fibre functional characteristics.

The aim of this work was to investigate the impact of barley variety and malting conditions on the functional properties of the dietary fibre in barley malt products, with the overall aim of improving intestinal health. Focus was given to β-glucan functional properties, but also arabinoxylan. The influence of these fibres on substrate delivery to the colon, SCFAs formation, microbiota composition and gene expression was investigated.

In the first study, rats fed commercial barley malts had a higher level of butyric and propionic acids in the caecum and portal serum than those fed control diets (cellulose). β-glucan with broader molecular weight seemed to better increase the caecal SCFA formation. In addition, the malts contributed to improved mucosal barrier function and inflammatory state by decreasing mRNA expression of tight junction protein and toll-like receptors in the small intestine and distal colon. However, malt with high amounts of advanced glycation end-products seemed to attenuate the effect on occludin (tight junction protein) in the small intestine.

To evaluate the possibility of using malting to tailor functional characteristics of the fibre, three barley varieties were malted using different temperatures and levels of lactic acid in the steeping water. The extent to which barley components changed depended on the variety. Steeping at 35°C with 0.4% lactic acid preserved soluble fibre and β-glucan content better, but not the soluble arabinoxylan, compared with steeping at 15°C and without lactic acid. However, no changes in β-glucan molecular weight (> 10 kDa) were observed.

β-glucan molecular weight analysed with AF4 at a wider molecular weight range (> 2 kDa), was shown to be affected by both variety and processing (steeping or mashing) to different extents. Addition of proteolytic enzymes shifted the β-glucan of high molecular weight to a lower molecular weight range, suggesting that proteins are involved in the structure of β-glucan, which might result in an apparently higher molecular weight.

After passage through a dynamic gastrointestinal in vitro model (TIM-1), it was observed that β-glucan molecular weight gradually decreased in the different barley products investigated. The longer in vitro transit time of soluble fibre and β-glucan was related to their high content and/or molecular weight, which also seemed to be linked to a higher degree of fermentation in an in vivo model (rats).

In the last study it was investigated the differences of microbiota composition in rats fed barley malt products. Rats fed malt products had higher microbiota diversity (negatively associated with ulcerative colitis and obesity) than those fed barley extracts rich in arabinoxylan or β-glucan, and control. Malts contributing with a higher content of β-glucan increased the abundance of Lactobacillus and Blautia and tended to increase butyric acid, whereas soluble arabinoxylan increased Akkermansia and propionic acid in the caecum. By mixing barley products (brewers' spent grain and malt) it was possible to modulate the microbiota into an intermediary abundance of taxa, with slight increase of butyric acid compared with malt alone.

In conclusion, malting seems to be a potential processing method for tailoring barley composition to promote intestinal health. Both the selection of barley variety and the processing conditions affected the composition of malt products. The resulting barley products with a high content of soluble fibre, β-glucan and soluble arabinoxylan, enhanced colon fermentation, microbiota composition and, to some extent, the SCFA formation.

Key words: Barley, malt, steeping, β-glucan, β-glucan molecular weight, arabinoxylan, short-chain fatty acids, microbiota

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Abstract

There is increasing evidence that dietary fibre may protect against diseases in the colon such as inflammatory bowel diseases and colon cancer. Barley as a good source of dietary fibre, especially β-glucan and arabinoxylan, has the potential to be fermented by the microbiota into short-chain fatty acids (SCFAs). SCFAs are important substrates for the colonocytes for maintenance and functioning of the colon; butyric acid and, to some extent, propionic acid are involved in the preservation of colonic barrier function, thus decreasing the risk of colonic inflammation. Previous studies have shown that barley malt increased butyrate-producing bacteria and formed more butyric acid and in caecum of rats than unmalted barley. However, this outcome varied between malts, probably due to the differences in fibre solubility, type, content and molecular weight.

The aim of this work was to investigate the impact of barley variety and malting conditions on the functional properties of the dietary fibre in barley malt products, with the overall aim of improving intestinal health. Focus was mostly given to β-glucan functional properties, but also arabinoxylan. The influence of these fibres on substrate delivery to the colon, SCFAs formation, microbiota composition and gene expression was investigated.

In the first study, rats fed commercial barley malts had a higher level of butyric and propionic acids in the caecum and portal serum than those fed control diets (cellulose). β-glucan with broader molecular weight seemed to better increase the caecal SCFA formation. In addition, the malts contributed to improved mucosal barrier function and inflammatory state by decreasing mRNA expression of tight junction protein and toll-like receptors in the small intestine and distal colon. However, malt with high amounts of advanced glycation end-products seemed to attenuate the effect on occludin (tight junction protein) in the small intestine.

To evaluate the possibility of using malting to tailor functional characteristics of the fibre, three barley varieties were malted using different temperatures and levels of lactic acid in the steeping water. The extent to which barley components changed depended on the variety. Steeping at 35°C with 0.4% lactic acid preserved soluble fibre and β-glucan content better, but not the soluble arabinoxylan, compared with steeping at 15°C and without lactic acid. However, no changes in β-glucan molecular weight (> 10 kDa) were observed.

By analysing β-glucan with AF4 at a wider molecular weight range (> 2 kDa), although not quantitatively, it was possible to observe that β-glucan molecular weight distribution was affected by both variety and processing (steeping or mashing) to different extents. Addition of proteolytic enzymes shifted the β-glucan of high molecular weight to a

lower molecular weight range, suggesting that proteins are involved in the structure of β-glucan, which might result in an apparently higher molecular weight.

After passage through a dynamic gastrointestinal *in vitro* model (TIM-1), it was also observed that β-glucan molecular weight gradually decreased in the different barley products investigated. The longer *in vitro* transit time of soluble fibre and β-glucan was related to their high content and/or molecular weight, which also seemed to be linked to a higher degree of fermentation in an *in vivo* model (rats).

In the last study it was investigated whether the microbiota composition differed in rats fed barley malt products. Rats fed malt products had higher microbiota diversity (negatively associated with ulcerative colitis and obesity) than those fed barley extracts rich in arabinoxylan or β-glucan, and control (fibre-free). Malts contributing with a higher content of β-glucan increased the abundance of *Lactobacillus* and *Blautia* and tended to increase butyric acid, whereas soluble arabinoxylan increased *Akkermansia* and propionic acid in the caecum. By mixing barley products (brewers' spent grain and malt) it was possible to modulate the microbiota into an intermediary abundance of taxa, with slight increase of butyric acid compared with malt alone.

In conclusion, malting seems to be a potential processing method for tailoring barley composition to promote intestinal health. Both the selection of barley variety and the processing conditions affected the composition of malt products. The resulting barley products with a high content of soluble fibre, β-glucan and soluble arabinoxylan, enhanced colon fermentation, microbiota composition and, to some extent, the SCFA formation.

Popular science summary

A healthy colon is important for the prevention of many diseases, not only those in the colon but also affecting the entire body. In westernised societies there has been a sharp increase in many lifestyle diseases, including inflammatory bowel diseases and cancer, that has been significantly related to a low consumption of dietary fibre. Dietary fibre is comprised of a group of undigested food components present in cereals, vegetables and fruits that reach the colon. A well-known effect of fibres is the reduced risk for constipation but, apart from this, they can be utilised by the gut bacteria as an energy source and produce short-chain fatty acids (SCFAs). Among the SCFAs, the formation of butyric acid is especially important for maintenance of intestinal barrier function, thus decreasing the risk of various diseases. The beneficial effects of dietary fibres are highly dependent on their characteristics, such as composition, content, solubility and size.

With the purpose of using malt products to improve intestinal health, the work in this thesis investigated the changes of the physico-chemical properties of dietary fibre in barley, between different variety and processing conditions.

Barley is a cereal mainly used to produce beer containing interesting dietary fibres such as β-glucan and arabinoxylan, with potential to improve intestinal health. However, different characteristics of the fibre can produce different results: in one study with barley, the formation of butyric acid increased significantly after malting, while in another study there was no increase. This difference was attributed to the physico-chemical characteristics of the dietary fibre, thus by modifying them it may be possible to control and improve the nutritional effects. In this respect, BSG (brewer's spent grain), a brewery by-product, is another interesting product for use as a fibre-rich food ingredient, as it is available at low cost, and its use would contribute to reduction of waste from breweries.

In a first study, the maintenance of intestinal health by intake of barley malts was evaluated. Rats that were fed commercial malts with different fibre characteristics had an improved barrier function compared to rats fed control diets. However, high amounts of advanced glycation end-products (harmful compounds formed at high temperature between amino acids and reducing sugars) in the malts were shown to attenuate some of the beneficial effects. Malts with a wider β-glucan size distribution seemed to increase the formation of butyric and propionic acid in caecum and blood than malts with a narrower size distribution.

To evaluate the possibility of modifying the fibre characteristics, whole grain barley was malted at different steeping (soaking) conditions, the first step of the malting process. Barley steeped at 35°C with 0.4% lactic acid had a higher content of soluble fibre and β-glucan than malt prepared at traditional steeping conditions (15°C without lactic acid).

The extent of modification depended largely on the barley variety, probably due to differences in the physico-chemical structure.

The size of β-glucan molecules seems to be important for the SCFA formation. The β-glucan size distribution in the malts was dependent, to different extents, on barley variety and steeping conditions. By mashing malt to produce BSG, the β-glucan size distribution was also affected but to a lesser degree.

The physico-chemical characteristics of the fibre reaching the colon for microbial degradation are difficult to evaluate with *in vivo* models. Therefore, a dynamic gastrointestinal *in vitro* model (TIM-1) was used to simulate the digestion of barley malts and BSG in the stomach and small intestine. It was observed that the passage though the *in vitro* stomach and small intestine was slower with malts with a high content of soluble fibre and long β-glucans, and this was also linked with a higher degree of fermentation in rats. A gradual decrease of β-glucan size was also observed during the *in vitro* digestion. This was probably due to proteins being degraded by enzymes present in the stomach and small intestine contributing to the release of β-glucan bound to proteins. Interestingly, in another study, β-glucan size also decreased after removing proteins in the barley products, suggesting that proteins are associated with β-glucan.

In the last study, it was investigated whether barley products could change microbiota composition in rats. Malts and BSG increased microbiota diversity (linked to better health) compared with extracts rich in arabinoxylan or β-glucan, or a fibre-free diet. Between the malts and BSG, those with a high β-glucan content seemed to increase the abundance of *Lactobacillus* and *Blautia,* a butyric acid producer. On the other hand, soluble arabinoxylan increased the number of *Akkermansia* and propionic acid formation. By mixing barley malt with BSG it was possible to modulate the microbiota and SCFA formation into an intermediate composition.

In conclusion, changing the barley malt fibre to obtain malts with a higher content of soluble fibre, β-glucan and soluble arabinoxylan seems to be a possible approach to improve intestinal health.

List of Papers

Paper I – Barley malt increases hindgut and portal butyric acid, modulates gene expression of gut tight junction proteins and Toll-like receptors in rats fed high-fat diets, but high advanced glycation end-products partially attenuate the effects

Yadong Zhong, **Cristina Teixeira**, Nittaya Marungruang, Watina Sae-Lim, Eden Tareke, Roger Andersson, Frida Fåk and Margareta Nyman

Food & Function (2015), 6: 3165–76

Paper II – Effects of variety and steeping conditions on some barley components associated with colonic health

Cristina Teixeira, Margareta Nyman, Roger Andersson and Marie Alminger

Journal of the Science of Food and Agriculture (2016), 96: 4821–4827

Paper III – Analysis of β-glucan molar mass from barley malt and brewer's spent grain with asymmetric flow field-flow fractionation (AF4) and their relation to proteins

Claudia Zielke*, **Cristina Teixeira***, Huihuang Ding, Steve Cui, Margareta Nyman and Lars Nilsson

*equally responsible first authors

Carbohydrate Polymers (2017), 157: 541–549, doi: 10.1016/j.carbpol.2016.10.045

Paper IV – Application of a dynamic gastrointestinal *in vitro* model combined with an *in vivo* model (in rats) to predict the digestive fate of barley dietary fibre and evaluate potential impact on hindgut fermentation

Cristina Teixeira, Margareta Nyman, Roger Andersson and Marie Alminger

Submitted

Paper V – Barley products of different fibre composition selectively change microbiota composition in rats

Cristina Teixeira, Olena Prykhodko, Marie Alminger, Frida Fåk Hållenius and Margareta Nyman

Manuscript

The author's contributions

Paper I – The author analysed and interpreted the results on the content and chemical properties of β-glucan in the barley products and took part in writing the manuscript.

Paper II – The author was involved in the study design, performed the experiments and analyses, evaluated the results and was responsible for writing the manuscript.

Paper III – The author was involved in the study design, performed the experimental work, apart from the measurements and data processing of the AF4 measurements, and was involved in the analysis of data and writing the manuscript.

Paper IV – The author was involved in the study design, coordinated and performed the experiments and analyses, examined and evaluated the results, and was responsible for writing the manuscript.

Paper V – The author was involved in the study design, performed the experiments and analyses apart from the processing of raw sequence data and bioinformatic analyses, performed the evaluation of results and was responsible for writing the manuscript.

Abbreviations

AF4	Asymmetric-flow field flow fractionation
AGE	Advanced glycation end-products
AX	Arabinoxylan barley extract
BG	β-glucan barley extract
β-glucan	Mixed-linkage (1-3,1-4)-β-D-glucan
BSG	Brewers' spent grain
CD	Crohn's disease
dRI	Differential refractive index
FL	Fluorescence
IBD	Inflammatory bowel disease
MALS	Multi-angle light scattering detection
Phytate	Myo-inositol hexakisphosphate, InsP6
SCFA	Short-chain fatty acid
TIM	TNO Gastro-intestinal model
TLR	Toll-like receptor
UC	Ulcerative colitis
ZO-1	Zonula occludens-1

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Contents

Introduction

The increasing global prevalence of cardiovascular diseases, obesity and type-2 diabetes is well-known to be strongly associated with a low consumption of dietary fibre along with high intakes of energy-dense food. Not as evident is the relation between diet and the increase of colonic diseases such as inflammatory bowel diseases and colon cancer, which have also been linked to imbalanced composition of microbiota and their metabolites. Dietary fibre is the main substrate for the colon microbiota, that can be converted into short-chain fatty acids (SCFAs), and some of these may improve the gut barrier function. Cereal grains, especially whole grains, are important sources of dietary fibre, thus contributing not only to maintain a healthy gut, but also to improve systemic health.

Barley is one of the most extensively produced and well adapted crops with a high content of dietary fibre, especially β-glucan and arabinoxylan. Both have been linked with an increased formation of butyric and propionic acid in rodents and associated with anti-inflammatory effects. Butyric acid, followed by propionic acid, are the main sources of energy for the various functions of the colonocytes, preserving the mucosal barrier function from an influx of pathogens and other inflammatory compounds. It is desirable that SCFAs are abundant along the colon and that they reach the distal colon, where most colonic diseases occur. Previous studies having shown that malting barley increased the formation of butyric acid in the hindgut of rats, although to different extents with various malts. The variations were attributed to the fibre content, proportion of fibre and molecular weight of soluble fibre, which may be due to the properties of the barley variety and the selected processing conditions during the malting. Brewer's spent grain is a fibre and protein-rich by-product consisting of residues of malted barley from the beer brewing process and produced in large quantities. Until recently, the main application of BSG has been limited to animal feed but there is an increasing interest in investigating the possibility of converting BSG into value-added food products with potential effects on colonic health.

The work presented in this thesis aims to increase the understanding of the role of dietary fibre and the functional properties of barley malt and BSG, produced in different malting conditions and with selected varieties, on their potential to improve intestinal health. Such information could be useful for the design of barley-based food with specific health effects.

Objectives

The purpose of this work was to investigate the impact of barley variety and processing conditions on the composition and functional properties of the dietary fibre in barley malt products, with the overall aim of improving intestinal health.

For this purpose:

Barley malts of different AGE and β-glucan content were examined in relation to SCFA formation, barrier function and inflammatory state in the hindgut of rats fed high-fat diets (Paper I).

Different barley varieties were malted in different steeping conditions to evaluate the impact on fibre functional properties and the ability to modulate malts with potential beneficial effects for intestinal health (Paper II).

β-glucan molecular weight distribution of barley malt products was assessed with a methodology showing a broad range of molecular weight (> 2 kDa) to better understand the impact of different processing conditions and barley variety (Paper III).

The ability of barley malt products to retain functional properties, and their potential impact on hindgut fermentation was evaluated during gastrointestinal digestion of selected barley malt products in a dynamic *in vitro* model. Comparison was made with analysis of faecal excretion and caecal content from a study with an *in vivo* rat model (Paper IV).

The effects of barley-derived products processed in different conditions on the microbiota composition and SCFA formation were evaluated with an *in vivo* model on rats (Paper V).

Background

Barley

Barley (*Hordeum vulgare* L.) is one of the most ancient cereal crops produced on a global scale, with at least 10 000 years of domestication, having possibly started in the Fertile Crescent, east and central Asia (Ullrich, 2014). For the last ten years its production has reached 140 Mt a year, with approximately 60% produced in Europe (FAO, 2015). Barley's initial use as human food declined with the increase in wheat and rice consumption, leading to a large proportion of barley crops being used for beer production and animal feed (Newman & Newman, 2008). However, it still remains a principal food source in some Himalayan regions, Ethiopia and Morocco (Baik & Ullrich, 2008).

Its genetic diversity makes barley a crop well-adapted to different environmental conditions; it can be cultivated towards the poles as well as hot deserts, and at higher altitudes than other crops (Andersson, et al., 2008), but not in hot and humid environments mainly because of diseases. The different barley varieties can be classified according to the growing season (winter or spring), morphology (two- or six-row, hulled or hulless), end-use (food, feed or malt), and composition (e.g. varying content of amylose, β-glucan and lysine).

Morphological structure and chemical composition of barley

The barley grain can be roughly divided into five parts: husk (or hull), pericarp and testa (bran), aleurone, starchy endosperm and embryo (Figure 1, left). The starchy endosperm is the main part of the grain (~73%) followed by the husk (~13%), aleurone (~5%), pericarp and testa (~3%), and embryo (~3%) (Hockett, 2000; Newman & Newman, 2008).

The husk protects the grain against abrasion and inhibits gases and water exchange. The testa together with the pericarp provides a selective inward permeability from external solutes as well as the outward diffusion of soluble substances. High in flavonoids, the testa is also involved in the protection against biological and environmental stress (Skadhauge, et al., 1997). The aleurone is the outermost layer of the endosperm cells, composed of three cell layers in barley, while other grains have only one layer that

releases enzymes to mobilise nutrients to the embryo (Briggs, 1998). The starchy endosperm is composed of cells packed with starch grains embedded in a protein matrix, making it the main source of nutrients for the developing embryo (Jadhav, et al., 1998; Newman & Newman, 2008). The cell walls of both aleurone and starchy endosperm contain arabinoxylan and β-glucan, with aleurone richer in arabinoxylan (67–71%) and the endosperm richer in β-glucan (~70%) (Bacic & Stone, 1981; Fincher, 1975). Smaller amounts of cellulose, other polysaccharides and structural proteins also make up part of the intricate cell wall matrix (Forrest & Wainwright, 1977; Lazaridou, et al., 2008a). The embryo is rich in readily metabolised substances such as soluble sugars and lipids necessary to initiate growth, and gibberellin hormones to trigger degradation of macromolecules and mobilisation of the nutrients from the starch endosperm to the embryo (Briggs, 1998; Jadhav, et al., 1998; Newman & Newman, 2008).

Differences in barley composition depend greatly on both variety and growing conditions. Hulless varieties, for example, have a lower fibre content due to the absence of the outer layer, and barley grains grown at high temperature and precipitation tend to have an increased arabinoxylan and starch content, while hot and dry conditions tend to increase β-glucan content (Lazaridou, et al., 2008b; Swanston, et al., 1997). Starch, dietary fibre and protein are the main components of the barley grain (Figure 1, right) of which the dietary fibres in particular have the potential to promote health.
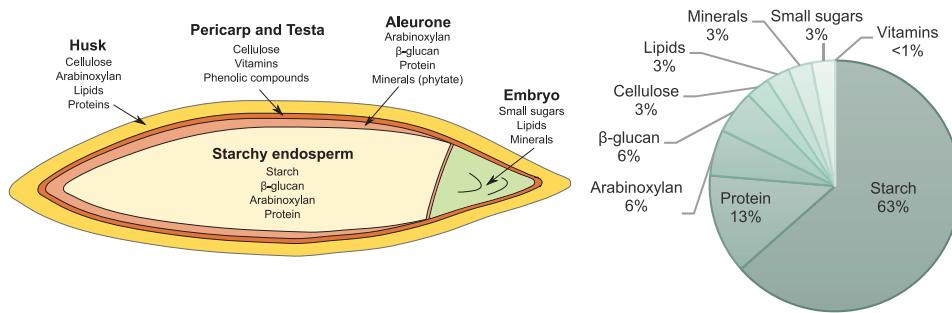
Figure 1.
Hulled barley grain with the most abundant components in each structure of the grain (left) and average proximate composition (right) of barley grain as reported in Baik and Ullrich (2008); Jadhav, et al. (1998); Newman and Newman (2008); Ullrich (2011).

Dietary fibre

Dietary fibres are defined by Codex Alimentarius Commission as "carbohydrate polymers with ten or more monomeric units, which are neither digested nor absorbed in the small intestine" (…) where "decision on whether to include carbohydrates from 3 to 9 monomeric units should be left to national authorities" (Codex Alimentarius Commission, 2009). Further, these types of carbohydrates are considered dietary fibre if they occur naturally in the food as consumed and are obtained from food raw materials or produced synthetically, providing all have demonstrated beneficial physiological effects. Other non-carbohydrate substances associated with the polysaccharides, but not isolated, can also be included in the definition of dietary fibre (e.g. lignin, protein fractions, and phenolic compounds).

Dietary fibre can be classified either as soluble (e.g. some hemicelluloses and pectin) or insoluble (most types of hemicelluloses, celluloses and lignin), or as fermentable or non-fermentable. Insoluble fibres are usually fermented to a lower degree than soluble fibres, thus the fermentative effects are usually related to soluble fibres, and non-fermentative effects to insoluble fibres. However, the classification of soluble and fermentable fibres is not interchangeable and both fibres can share these characteristics to different extents. Moreover, fibres of high molecular weight tend to be fermented more slowly than those of low molecular weight (Eswaran, et al., 2013).

Barley dietary fibre and health aspects

Previously assumed to provide only faecal bulk and prevent constipation, dietary fibre can also affect physiological functions in the colon and human metabolism, and suggested to decrease the development of colonic diseases such as colorectal cancer (Kumar, et al., 2012; Mantovani, et al., 2008). The mechanisms to which fibres exert physiological effect can be divided into fermentative and non-fermentative (Rose, et al., 2007). Some non-fermentative effects include the ability to increase faecal bulk, decrease colonic transit time, and bind toxic compounds and bile acids. The ability to increase viscosity of the digesta may delay gastric emptying, thus increasing satiety, while in the small intestine, may delay the absorption of glucose and lipids, helping to regulate their levels in the blood. In the long term these properties can prevent or control weight loss, type-2 diabetes and heart disease. In contrast, the passage in the colon with non-fermentable fibres is more rapid than without these types of fibres due to their bulking effect, thus reducing the period of contact of harmful agents with the mucosa, and possibly a decreased risk of colon cancer (Rose, et al., 2007). A reduced conversion of primary bile acids to harmful secondary bile acids is also thought to be due to binding with fibre (Kern, et al., 1978). Bile acids that are bound with fibre are excreted in faeces, interrupting the normal enterohepatic recirculation and decreasing blood cholesterol.

Fermentative effects of dietary fibre are related to the short-chain fatty acids (SCFAs) produced from microbiota fermentation of some types of fibre reaching the colon (Rose, et al., 2007). SCFAs contribute to the maintenance of the colonic function by serving locally as nutrients for the colonocytes' metabolic functions such as growth, differentiation and mucosa proliferation, colonic mobility, blood flow, pH, and absorption of electrolytes and nutrients (Cummings, et al., 1986; den Besten, et al., 2013), cholesterol and glucose metabolism (Anderson & Bridges, 1984; Hara, et al., 1999). An increased nutritional condition of the colon mucosa may prevent against the genesis of colonic disease as a compromised barrier function increases and the risk of influx of inflammatory or pathological substances decreases, such as lipopolysaccharides (LPSs). LPSs are secreted from dead gram-negative bacteria, occurring early in the progress of e.g. insulin resistance and type-2 diabetes and provoking strong immune responses (Cani, et al., 2007). Butyric acid and to some extent propionic acid have been particularly emphasised in this context, as they have been associated with anti-inflammatory, anti-tumorigenic and anti-microbial effects (Tan, et al., 2014).

Arabinoxylan, β-glucan and cellulose are the most common non-starch polysaccharides in barley considered to be dietary fibre, but some starches, referred to as resistant starch, may also be present. Different types of indigestible oligosaccharides are also found in small amounts, such as fructo-, galacto-, and arabinoxylo-oligosaccharides.

β-glucan

β-glucan is one of the main components of the cell walls of barley, partially soluble and with a molecular weight varying between 0.45 and 1.8×10^6 Da (Andersson, et al., 2008; Irakli, et al., 2004). It consists of a linear chain of three or four β-(1,4) glucose residues separated by a single β-(1,3) linkage. Variations in molecular weight, ratio of glycosidic linkages (β-(1,4)/β-(1,3)) and intermolecular interactions influence the solubility, viscosity and gelling properties (Izydorczyk & Dexter, 2008; Lazaridou, et al., 2004).

Barley's potential to promote health was recognised in 2005 by FDA (FDA, 2006) and EFSA (EFSA, 2011a) as a consequence of β-glucan consumption. These claims were first linked to the maintenance of a normal blood cholesterol and prevention of coronary heart disease, and later claims were added linking β-glucan consumption to reduction of postprandial glycaemic response and improvement of bowel function (EFSA, 2011b).

Some of β-glucan's known health benefits are associated with its high molecular weight and its ability to form viscous solutions in the small intestine, aiding in the regulation of glucose and lipid blood levels (Keenan, et al., 2007; Othman, et al., 2011; Wilson, et al., 2004). β-glucan of low molecular weight (50-400 kDa) has also demonstrated beneficial effects on blood cholesterol in humans (Keenan, et al., 2007), an ability to function as a potential immuno-stimulator in mice with a molecular weight as low as 2 kDa (Tanioka, et al., 2011) and to reverse inflammatory markers' levels in rats with

LPS-induced enteritis (Wilczak, et al., 2015), indicating that the effects are mediated via the colon. Furthermore, the sensorial properties are improved with the incorporation of low molecular weight β-glucan in food compared with high molecular weight β-glucan (Keenan, et al., 2007). A broad molecular weight of β-glucan could be beneficial, to ensure various benefits and the presence of fibre along the whole colon, particularly in the distal colon, where most colonic diseases arise. However, there are limitations in measuring the β-glucan of low molecular weight in complex mixtures. Calcofluor forms a complex, specific to β-glucan, that allows its detection by fluorescence, but this complex is not formed quantitatively with β-glucan molecules below 10 kDa (Jørgensen & Aastrup, 1988; Kim & Inglett, 2006).

Arabinoxylan

Arabinoxylan is a hemicellulose formed by a linear backbone of β-(1,4) linked xyloses, with xylose residues unsubstituted, mono- or di-substituted typically by arabinose residues (Izydorczyk, 2014), and less commonly by uronic acids and oligomeric chains of xylose, galactose or glucose (Izydorczyk & Dexter, 2008). The arabinose residues attached to the xylan chain can also be esterified to hydroxycinnamic-, ferulic- and *p*-coumaric acids (Smith & Hartley, 1983). Arabinoxylan is the main component of barley aleurone cell walls (Bacic & Stone, 1981), but is also found in the endosperm cell walls and in small amounts in the hull (Gupta, et al., 2010; Izydorczyk & Dexter, 2008). The determination of cereal arabinoxylan molecular weight is often difficult, since extraction and purification procedures steps can partially degrade the polymers. Barley arabinoxylans extracted with saturated Ba(OH)2 ranged from 0.64×10^6 to 2.22×10^6 (Storsley, et al., 2003).

The viscosity of arabinoxylan solution has also been related to a slowing down of the gastric emptying rate and a reduction of small intestine mobility as observed for other viscous fibres (Torsdottir, et al., 1991). It is thus possible that arabinoxylan has similar physiological effects to β-glucan as regards satiety, and regulation of cholesterol and glucose blood levels (Broekaert, et al., 2011; Lopez, et al., 1999; Lu, et al., 2000). As with β-glucan, the possibility cannot be excluded that the effects are mediated via fermentation in the colon. Arabinoxylan-oligosaccharides and xylooligosaccharides are products of arabinoxylan enzymatic hydrolysis during, for example, food processing or fermentation in the colon (Broekaert, et al., 2011). These oligosaccharides have been linked to the selective growth of some beneficial *Bifidobacteria* spp. and *Lactobacillus brevis*, with decrease of *Escherichia coli*, and most *Clostridia* (Rycroft, et al., 2001). Furthermore, arabinoxylan and its oligosaccharides have been shown to normalise stool water and defecation frequency (Tateyama, et al., 2005), to have anti-inflammatory properties (Ogawa, et al., 2005), and to have antioxidant effects probably due to the presence of ferulic acid (Ou, et al., 2007).

Starch

Starch in barley consists of a mixture of large and small granules found in the starchy endosperm. The amylose content in relation to amylopectin determines whether the starch in the grain is classified as waxy (< 1% amylose), normal (20−30%) or high-amylose (> 40%). High-amylose starch has gelling properties and is less susceptible to enzymatic hydrolysis than waxy starch; this might be related to the correlation with high amounts of resistant starch. Resistant starch is fermented by the microbiota and increases the levels of short-chain fatty acids in the colon (Newman & Newman, 2008; Ullrich, 2011), but different types of resistant starch can be fermented to varying degrees resulting in different amounts of SCFAs. Some resistant starches are known to increase the production of butyrate; however, it has also been shown that the fermentation of resistant starch from different sources (potato and maize) yielded dissimilar proportions of butyrate (Ferguson, et al., 2000; Nofrarias, et al., 2007).

Other barley components and their health aspects

Proteins

Proteins have a wide range of functions in barley and can be found in the structure of different cell walls, in the aleurone and embryo as enzymes to start metabolic functions, and embedded in the starch granules of the endosperm as a nitrogen source (Finnie & Svensson, 2014). Barley endosperm protein is rich in hordeins (35 to 50% of the total grain nitrogen), which are rich in glutamine (Baik & Ullrich, 2008). Glutamine has been reported to be one of the major energy sources of mucosal epithelial cells and implied in the recovery and preservation of intestinal mucosa (Kaya, et al., 2007). However, for this purpose glutamine has to reach the colon for fermentation and be bound to the dietary fibre to be preserved during digestion in the stomach and small intestine (Kanauchi & Agata, 1997).

Phytate

Phytate is localised primarily in the aleurone and embryo of the barley grains and represents the main source of phosphorous for the seed (Newman & Newman, 2008; Ullrich, 2011). This compound is the salt of phytic acid (*myo*-inositol hexa*kis*phosphoric acid) with strong chelating properties and is often found strongly associated with mineral cations and trace elements such as Fe^{2+}/Fe^{3+}, Zn^{2+} Ca^{2+}, and Mg^{2+} (Ullrich, 2011). The bioavailability of minerals and trace elements is considerably reduced by phytate due to its strong complex forming capacity, and even very low phytate levels have been found to reduce the uptake of trace elements (Hurrell, et al., 2003; Larsson, et al., 1996). Phytate is therefore considered to act as an anti-nutrient, although it also may provide anti-oxidative effects and prevent colon cancer (Jenab & Thompson, 2000; Zielinski, et al., 2007).

Processing of barley and dietary fibre

As with most raw materials, some kind of processing or treatment is often required to make the cereal food more palatable, increase the shelf-life, or to improve the nutritional value and the bioavailability of nutrients (Baik, 2014). Dehulling, pearling, rolling, flaking, grinding, puffing and roasting are some of the most common processes used that affect the fibre content and composition and, consequently, their physiological effects. Dehulling is the removal of the outermost fibre-rich layer by passing the grain between abrasive milling stones, and pearling is the further removal of the bran. After dehulling and/or pearling, the grains can be ground into flour or roller milled into fractionated flours with different fibre contents. Rolling creates cracks in the barley grains, increases the surface area for water uptake and reduces cooking time during boiling for subsequent use as a rice substitute. This treatment may solubilise the insoluble fibre, as observed in a study on wheat by Siljeström, et al. (1986). Similarly, for flaking, pearled grains are soaked, steamed, passed through rollers at high pressure and dried to produce flakes that can be consumed as breakfast cereals (Siljeström, et al., 1986). For puffing, grains are subjected to high pressure and steam in a closed chamber, followed by a sudden release of pressure that results in the expansion of the starchy endosperm. Roasted, dehulled barley increases the flavour and colour due to the formation of Maillard reaction products, and after milling these can be mixed with tea, milk or water for beverages.

Malting

For food purposes, malting increases palatability; the germination softens the grain, modifies the structural properties of some compounds (e.g. protein solubilisation), reduces anti-nutritional compounds (e.g. phytate) (Kaukovirta-Norja, et al., 2004) and may induce changes in dietary fibre that promote beneficial physiological effects (Zhong, et al., 2015b).

Malting is a controlled germination process mainly used for barley and globally it is one of the most common industrial barley processing techniques. Typically used for brewing and distilling, barley malt is also applied in food products as a source of diastatic power (in bread as dough conditioner), flavour and colour (candy, ice cream and other confectionaries) and production of vinegar (Newman & Newman, 2008; Schwarz & Li, 2011).

Malting allows access to the starch granules entrapped in the endosperm cell walls by making use of the endogenous enzymes occurring in the natural development of the seed into the plant. The process consists of three steps: steeping, germination and kilning (Figure 2).
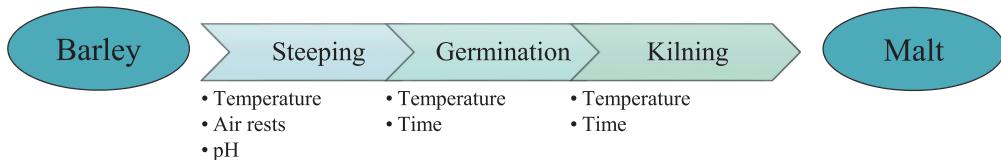
Figure 2
Schematic outline of the malting process and major factors used to control endoperm modification and enzyme activity.

Steeping

The primary purpose of steeping is to increase the moisture content of the grains, from about 12% to above 40%, by soaking them in water. Approximately 35% moisture is enough to induce germination, but steeping is usually complete at a moisture content of 42–44%. These moisture levels are necessary for efficient activation and mobility of the enzymes throughout the endosperm and its uniform modification (Schwarz & Li, 2011).

The steeping temperature is normally maintained at 14–16°C with alternate periods of wetting and draining (Schwarz & Li, 2011). Higher temperatures during steeping can cause problems of irregular germination, but can be used to reduce hydration time (Briggs, 1998) or to control activity of enzymes during germination and thus the modification of barley components (Haraldsson, et al., 2004; Rimsten, et al., 2002).

Changes of steeping water, use of air rests and turning of the grains are needed to supply oxygen, to remove respiration products that inhibit germination and to control the growth of microorganisms (Newman & Newman, 2008; Schwarz & Li, 2011; Swanston, et al., 2014). The reduction of pH through the addition of lactic acid can be used to control growth of microorganisms, but also to regulate enzymatic activity of phytate- and β-glucan- degrading enzymes (Haraldsson, et al., 2004; Rimsten, et al., 2002).

During hydration the grain ends its dormancy state. Water is continuously absorbed into the endosperm and gibberellic hormones are transported from the embryo to the aleurone and starchy endosperm. These hormones activate the release and production of enzymes for the degradation of the cell wall, starch and protein that are mobilised back to the embryo for its growth and start germination. The degradation of cell walls might start in the early stages of steeping since some β-glucan solubilase is present in unmalted barley, unlike other β-glucanases which are synthesised during germination (Chandra, et al., 1999; Swanston, et al., 2014). The main change in the visual appearance of the grain following steeping is the emergence of a rootlet.

Germination

After steeping, barley grains are drained and transferred to germinating beds or tanks. Here, they are kept at a saturated moisture air content, constant temperature (13–21°C) and in contact with a humid stream flow, or turned periodically for 4 to 5 days (Newman

& Newman, 2008; Swanston, et al., 2014). Enzymes activated and produced during steeping work in sequence to degrade the endosperm, starting with xylanases and β-glucanases followed by proteases and amylases (Bamforth, 2010; MacLeod, et al., 1964; MacLeod, 2004).

Depending on the grain composition, the pattern of degradation of the endosperm can vary, and so also the time of the germination phase. For example, if proteins are strongly bound to the cell walls or starch granules, they could limit the degradation of β-glucan and delay the amylases in reaching the site of action (Brennan, et al., 1997; Brennan, et al., 1996). Additionally, the time of germination can be used to tailor the extent of the degradation in order to reduce, for example, the phytate or β-glucan content (Sung, et al., 2005). Germination for brewing is complete with the growth of the rootlets and sprouts (MacLeod, 2004).

Kilning
The germinated barley is kilned to limit starch modification, preserve enzymatic activity, develop colours and flavours, and to achieve a storage-stable product. Once sufficient degradation of the cell wall has occurred, the grains are dried at high temperatures, which depend on the end use of the malt. The process is usually made step-wise, with gradual increase of the temperature, removing moisture from the grain by passing heated air through the grain bed (MacLeod, 2004; Schwarz & Li, 2011). In the first phase at 50–60°C, the moisture drops to about 23–25% of the grain weight, followed by the intermediate phase with a slow increase in temperature over several hours until a moisture content of around 6% is reached. Final kilning temperatures can vary between 80 and 230°C with moisture levels from 4.5 to 1.5%, respectively (Swanston, et al., 2014).

Kilning conditions are one of the largest contributors to the final malt character. At milder temperature profiles the endogenous enzymes are still preserved and malts can be used to add diastatic power for baking or brewing. Higher kilning temperatures create darker malts without diastatic power, which are commonly used as food additives and for brewing dark beers (Newman & Newman, 2008).

The effect of malting on barley grains
During the malting of barley, cultivar differences have been observed to determine the extent and pattern of starch and carbohydrate degradation (Brennan, et al., 1996). The cell-wall matrix is the first to be degraded; the rate and extent of degradation of β-glucan and arabinoxylan in the cell-wall matrix depends on the accessibility and activity of cell-wall degrading enzymes. β-glucan starts by being released from protein and arabinoxylan by activity of e.g. xylanases, arabinofuranosidases, carbopeptidases and feruloyl esterases, making it available for degradation (Bamforth, 2010; Bamforth, et al., 1979; Kanauchi, et al., 2013). Thereafter, rapid depolymerisation of β-glucan occurs with two isoenzymes (I and II) with endo-β-(1,3–1,4)-glucanase activity, which

hydrolyse β-(1,4) linkages adjacent to β-(1,3). Isoenzyme I is synthesised mostly in the scutellum and isoenzyme II in the aleurone layer. Both have the same substrate specificity, are pH sensitive (lose 90% activity at 1.5 units from the optimal pH 4.7) and rapidly lose activity at temperatures above 30°C, but isoenzyme I is more heat-stable (Woodward & Fincher, 1982).These β-glucanases have an optimal temperature activity around 16°C (Bamforth & Martin, 1983), which is the typical temperature during steeping and germination.

Soluble arabinoxylans are degraded before the insoluble ones during the malting process, but it is believed that the insoluble arabinoxylans are not degraded to any great extent (Jamar, et al., 2011; Voragen, et al., 1987b). This may be because most malting processes are performed at temperatures below 35–40°C, which is the optimal temperature range for barley β-(1,4)-endoxylanase activity. However, xylanase activity was found in the early stages of the germination, partially degrading arabinoxylan and exposing β-glucan to degradation (Kuntz & Bamforth, 2007). The pH can also influence the enzymatic products: xylanase activity (producing oligosaccharides) was found predominant at pH > 5, whereas xylosidase activity (producing monosaccharides) was higher at pH < 5 (Kanauchi, et al., 2013).

During germination, α-amylases from the aleurone diffuse into the starchy endosperm and it has therefore been suggested that starch degradation is dependent on cell-wall degradation (Gianinetti, 2009). Starch degradation is relatively limited during controlled malting compared to what occurs in nature. However, as there is extensive degradation of β-glucan and protein during malting, the starch is released from the granules and hydrolysed during the mashing process prior to brewing (Swanston, et al., 2014).

Endogenous phytate-degrading enzymes (phytases) are present in most plants and may be activated e.g. during soaking, fermentation and bread-making. Two phytates have been identified in dry and germinated barley seeds with an optimal pH of 4−5 and an optimal temperature of 45−55°C (Greiner, et al., 2000). Phytase activity was reported to increase after three days of barley germination (Hübner, et al., 2010). Increased germination temperature (Sung, et al., 2005) and low steeping pH have also been shown to increase phytase activity (Haraldsson, et al., 2004).

Brewers' spent grain

Brewers' spent grain (BSG) is a major by-product of the brewing industry. Malt is first milled and mashed with water and then the mixture is separated into a liquid fraction, the wort. The residual solid fraction, the BSG, is removed prior to fermentation (Mussatto, et al., 2006). During mashing, the enzymatic hydrolysis continues and the grain components, mainly starch, are further solubilised into the wort. Mashing temperatures are gradually increased up to 65–70°C, and gelatinisation of starch in barley occurs at 52–68°C, facilitating degradation by different amylases. Amylases in

barley have low activity during malting, but are more active at mashing temperature, thus β-amylase only degrades gelatinised starch within a very narrow temperature span (55–60°C). The most heat-stable of the amylases is α-amylase with some activity remaining at 70°C, and the optimal temperature for limit dextrinase activity is between 40 and 45°C (Swanston, et al., 2014) (brenda-enzymes.org).

Currently, the applications of BSG are limited and it is mostly used as animal feed. BSG is almost depleted of starch but contains high amounts of cell-wall residues rich in hemicellulose, water insoluble proteins, cellulose, and some lipids, lignin, phenolic compounds, minerals and vitamins (Mussatto, et al., 2006). Its fibre-rich composition combined with its high availability at relatively low cost makes this by-product a potential added-value food ingredient. Bakery products and confectionaries are some examples of potential food applications. However, to retain the common organoleptic properties of a certain product (e.g. colour and texture) only a limited amount can be incorporated (5–10%) (Gupta, et al., 2010). Nonetheless, BSG offers a cheap source of dietary fibre with potential beneficial health effects, with composition that can vary according to barley variety and malting process selected. Studies using BSG supplementation demonstrated improvement in the treatment of constipated patients, reduction of total cholesterol and total lipids (Hassona, 1993), possibly due to and increased faecal bulk (Odes, et al., 1986; Åman, et al., 1994).

Colonic health

Formerly thought to be an organ responsible only for absorption of water and electrolytes, the colon is now known to be an important site of metabolic activity with implications in the maintenance of the organ and overall health.

The wall of the colon includes the mucosa, the submucosa, the muscle coat, and the serosa (Figure 3). This structure makes part of a physical barrier between the intestinal lumen and the blood circulation, regulating permeability and influx of different toxic and (pro)-inflammatory components and also of pathogens from the intestinal lumen (Johansson, et al., 2011; Mazzucchelli & Maurer, 2004).

The mucosa comprises a monolayer of tall columnar epithelial cells bound by protein complexes (tight junctions, adherens junctions, desmosomes and gap junctions). This epithelial layer stands on the lamina propria, which contains macrophages, lymphoid cells and blood vessels. Over the epithelial cells lie two mucus layers produced by the goblet cells: the firmly and the loosely adherent mucus layers that serve both as protection from certain colonic bacteria and as a medium for the proliferation of others. A damaged mucus layer might be involved in the genesis of inflammation during colitis (Johansson, et al., 2010).
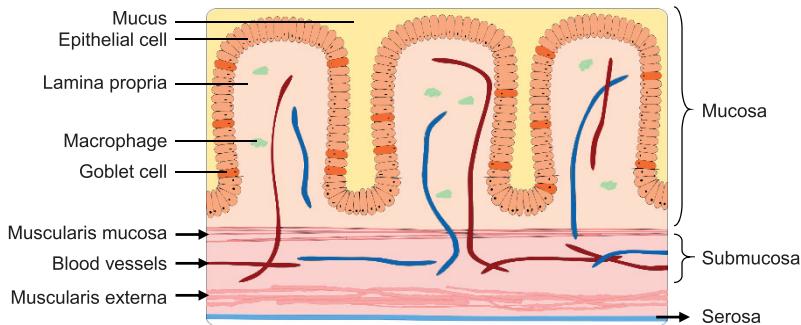
Figure 3
The colonic cell-wall.

Colonic and metabolic diseases

Inflammatory bowel disease (IBD) is a group of gastrointestinal disorders that includes Crohn's disease (CD), ulcerative colitis (UC) and microscopic colitis (MC), differing from each other in behaviour and location. UC is characterised by lesions at the colonic mucosa and submucosa levels, and CD is associated with lesions that pass through the epithelium and that can occur along the whole intestinal tract (Neuman, 2007). MC is an inflammation of the colonic lamina propria, and bile acid malabsorption exists in more than half of the patients.

The origin of IBD is not clear but it is believed to be an interaction between genetic, environmental and immunological factors. The inflammatory flares during IBD are commonly associated with impaired oxidation of the butyric acid, dysfunctional mucosal barrier and changes in microbiota composition. Several studies have reported a shift in colonic microbiota profiles with IBD, with a decrease in butyrogenic bacteria and mucus producers (Martinez, et al., 2006; Matsuoka & Kanai, 2015). The reduced abundance of these bacteria might be related to the usually low SCFA levels in faeces of patients with distal UC. Colonic irrigations of SCFA or butyric acid have shown clinical improvement in UC, highlighting their importance in IBD (Breuer, et al., 1991). One approach would be to provide suitable dietary fibre that could stimulate the growth of microbiota producing butyric acid, and consequently restore the protective mucus layer (Neuman & Nanau, 2012; Pituch-Zdanowska, et al., 2015). Rats fed barley have been shown to increase caecal SCFAs (Zhong, et al., 2015a), and malt was able to modulate rat microbiota to increased butyric acid producers (Zhong, et al., 2015b). Furthermore, IBD patients are at a higher risk of developing colorectal cancer, and malnutrition can occur as frequent inflammatory episodes impair nutrient digestion and absorption (Mijac, et al., 2010).

Colorectal cancer is a consequence of genetic mutations of the epithelial cells into abnormal cells that grow uncontrollably beyond their usual boundaries. A genetic predisposition is one of the causes, but a poor diet and lack of physical activity are risk factors commonly associated with colorectal cancer. Butyric acid and, to some extent, propionic acid have been shown to reduce proliferation of colon cancer cells (Perrin, et al., 2001).

Metabolic syndrome (MS) is a condition consisting of interrelated factors that increase the risk of type-2 diabetes, cardiovascular disease and some types of cancer (Esposito, et al., 2012). The definition of MS varies within health entity groups and over time, but there is a consensus that MS is associated with high triglycerides, low high-density lipoprotein, hypertension, obesity and insulin resistance (Kassi, et al., 2011). The consumption of dietary fibres could help to reduce some MS risk factors: some fibres increase satiety thus helping to control weight; fibres have been linked with decreased blood cholesterol levels and reduced insulin response (Delzenne & Cani, 2005); in recent years a connection has been made between the intake of fermentable fibre in barley and beans and a lower blood glucose response and inflammatory status in healthy people (Johansson, 2014; Johansson, et al., 2013; Nilsson, et al., 2013).

Low-grade inflammation is a chronic condition characterised by elevated blood levels of pro-inflammatory mediators, such as LPS, and considered as a risk factor for MS and type-2 diabetes. The levels of pro-inflammatory mediators are found to be higher in obese than in normal weight people, and adipose tissue is significantly related to the release of pro-inflammatory cytokines (Calder, et al., 2011). Furthermore, high-fat diets have been related to the increase in mucosa permeability allowing contact with microbial material triggering mucosa inflammation (Suzuki & Hara, 2010), downregulation of tight junction proteins genes (e.g. occluding and zonulin) (Cani, et al., 2008), and also affect expression of Toll-like receptors (TLR) (Kim, et al., 2012). One of the causes could be a high content of lipids or bile acids disrupting the cell membranes in the lumen (Suzuki & Hara, 2010). Contrarily, rats fed highly fermentable dietary fibre guar gum have shown an improved bile acid profile in the caecum, resulting in high amounts of propionic acid compared with pectin (acetic acid producer) (Ghaffarzadegan, et al., 2016). Further, intermediary molecular weight had a more pronounced effect than low and high molecular weight guar gum. The bile acid profile was significantly correlated to the SCFAs formed and microbiota composition.

Short-chain fatty acids – formation and function

SCFAs are carboxylic acids of up to 6 carbon atoms and the main anionic products of microbiota fermentation (Wong, et al., 2006). Approximately 90% of SCFAs produced in the colon are acetic, propionic and butyric acid. There are also lower concentrations of valeric and caproic acid (Tan, et al., 2014) and the branched SCFAs, isobutyric and

isovaleric acid, which are products of branched amino acid fermentation (Macfarlane, et al., 1992). Intermediary carboxylic acids such as lactic and succinic acid can be produced and further converted into butyric or propionic acid (Blair, et al., 1995; Bourriaud, et al., 2005). The proximal colon in humans is the dominating site of SCFA production from carbohydrates, which diminishes along the distal colon due to depletion of fibre, thus fermentation of proteins prevails in the distal colon (Hamer, et al., 2008). The fermentation of protein produces ammonia, phenols and hydrogen sulphide and can cause mucosal inflammation when in high concentration (Yao, et al., 2016), whereas dietary fibre increases the formation of acetic, propionic and butyric acid, which are related to various beneficial health effects.

The production of SCFAs is significantly dependent on the fibre source, structure (e.g. molecular weight and branching), amount and mixture of the types of fibres (Kumar, et al., 2012): some types of resistant starch (Englyst, et al., 1987), β-glucan (Berggren, et al., 1993) and possibly arabinoxylan (Hald, et al., 2015) have been reported to increase the butyric acid production, while soluble arabinoxylan could also increase propionic acid formation (Haskå, et al., 2011). The molecular weight is another factor of importance for SCFA production; fructo-oligosaccharides of low molecular weight increased levels of butyric acid in the caecum of rats, whereas those of high molecular weight favoured propionic acid formation (Nilsson & Nyman, 2005). Furthermore, a mixture of arabinoxylan and arabinoxylan-oligosaccharides has been shown to produce higher amounts of butyric acid than each one individually (Damen, et al., 2011). Similarly, rats fed guar gum together with pectin produced higher levels of butyric acid than each one of the fibres alone (Henningsson, et al., 2002). Thus, fibres of different types and physico-chemical properties give different amounts and patterns of SCFAs, which might be controllable by choice of raw materials and processing conditions.

SCFAs are promoters of colonic health and integrity. They serve as an energy source for colonocytes, and 60 to 70% of SCFAs is utilised and/or absorbed into the circulation, whereas less than 10% of the SCFAs is excreted in the faeces in humans (Cummings, et al., 1986; Suzuki, et al., 2008; Wong, et al., 2006). SCFAs contribute to a lower pH in the gut, increasing bio-availability of minerals and inhibiting potentially harmful bacteria (Wong, et al., 2006). Most SCFAs are very efficiently absorbed through diffusion in protonated form, anion exchange or actively by SCFA-transporters (Tan, et al., 2014; Wong, et al., 2006). Therefore, in low colonic pH conditions the SCFA are protonated, which facilitates their diffusion into the circulation (Cook & Sellin, 1998). Most types of dietary fibres are rapidly fermented and the SCFAs formed are rapidly absorbed. However, most colonic diseases occur in the distal part of colon and a supply of fermentable fibres is also needed there. By mixing rapidly fermentable fibres with more slowly fermentable fibres, the formation of SCFAs in the distal part may increase (Henningsson, et al., 2002), which can have implications for the nutritional and possibly inflammatory status of that region.

Butyric acid is the major and preferred source of energy for the colonocytes, for maintenance and functioning of the colon, compared to other SCFAs and to parenteral glucose and glutamine (Wong, et al., 2006). Propionic acid not utilised by the colonocytes is transported to the liver and has been shown to have hypocholesterolemic effects in rats (Chen, et al., 1984). Acetic acid is the main SCFA produced in the colon, readily absorbed and transported to the liver where it is utilised as a precursor of lipogenesis and to stimulate gluconeogenesis, and also in the metabolic organs and tissues of the body (den Besten, et al., 2013).

Butyric acid and, to some degree, propionic acid, have demonstrated anti-inflammatory, anti-tumorigenic and anti-microbial effects (Tan, et al., 2014), with major roles in the regulation of epithelial cell growth and differentiation, stimulation of blood flow and oxygen uptake, and induction of apoptosis in colorectal tumour cell lines (Al-Lahham, et al., 2010; Hamer, et al., 2008; Perrin, et al., 2001). Furthermore, the supply of SCFA enemas, particularly of butyric acid, has shown amelioration of the colonic inflammation, and its depletion linked with the aetiology of inflammatory bowel diseases such as ulcerative colitis (Breuer, et al., 1991; Scheppach, et al., 1992). Its effects, however, seem to be dependent on the dose and degree of inflammation (Hamer, et al., 2008). Therefore, a well-supplied mucosa of butyric acid (followed by propionic acid) could help in the maintenance of the colonic barrier function, thus decreasing permeability and avoiding influx of pathogens and inflammatory compounds that might induce systemic inflammation.

Colonic Microbiota

The majority of the microbiota hosted by humans reside in the gastrointestinal tract. The colon in particular is populated by a great diversity of bacteria functioning in a cooperative network with various substrate and metabolic preferences. Approximately half of the material contained in the lumen of the caecum and colon is microbial biomass, with about 10^{12} colony-forming units per gram of luminal content (Cummings & MacFarlane, 1997; Guarner, 2006). This makes the colon the major site for fermentation of non-digested compounds in the human digestive tract.

The colon of a newborn is essentially sterile but is rapidly colonised through environmental exposure within a few days of birth. Throughout life stages, each individual develops a unique microbiome that can be further modified as a result of diet and health condition (Duncan & Flint, 2013). Despite individual differences, the majority of the species in the colon are anaerobic or facultative aerobic bacteria, and the most abundant phyla are *Bacteroidetes* (mostly gram-negative), *Firmicutes* (mostly gram-positive) and *Actinobacteria* (mostly gram-positive) (den Besten, et al., 2013). Some beneficial effects of a balanced microbiota may include production of vitamins, effective defence against pathogens, proliferation and differentiation of epithelial cells,

and development of immune system (Guarner, 2006). Imbalances in the microbiota composition (dysbiosis) have been linked with inflammatory and metabolic disorders including inflammatory bowel disease, type-2 diabetes and obesity (Ley, et al., 2006). For example, patients with ulcerative colitis have shown reduced microbiota diversity, particularly among *Firmicutes*, with a decrease in the butyric acid producers *Roseburia intestinalis* and *Faecalibacterium prausnitzii* (Matsuoka & Kanai, 2015). *Bacteroides-Prevotella* and *Faecalibacterium prausnitzii* have been found to be lower in subjects suffering from obesity and diabetes, and associated with an increase in inflammatory markers (Furet, et al., 2010; Neyrinck, et al., 2011).

Dietary carbohydrates and, to a lesser extent, dietary and secretion proteins are potential substrates for the microbiota, yielding heat, gases (CO_2, CH_4 and H_2), biomass, and mostly SCFAs. (Cummings, et al., 1986; Cummings & MacFarlane, 1997). Different non-digested components reaching the colon can promote the growth of specific microbiota species, and in this way influence the production pattern of SCFAs. In turn, the products of fermentation (SCFAs or hydrolysates) may promote the growth of other bacteria by, for example, pH changes or bacterial cross-feeding mechanisms (den Besten, et al., 2013; Rios-Covian, et al., 2016). *In vitro* studies have shown that some *Lactobacilli* and *Bifidobacteria* were able to grow at the expense of β-gluco-oligosaccharides produced from partially hydrolysed cereal β-glucan (Snart, et al., 2006), but not when β-glucan polysaccharide was the only carbon source (Crittenden, et al., 2002). Another study showed that some *Bifidobacteria* species could use the arabinose side chains of arabinoxylan more efficiently than the xylose backbone, which could be the prefered substrate for other bacteria (Crittenden, et al., 2002). Resistant starch could change the composition of microbiota towards less *Firmicutes* (Kieffer, et al., 2014; Ordiz, et al., 2015), less *Roseburia* and *Blautia*, and increased *Lactobacillus* (Ordiz, et al., 2015) and *Bacteroidetes* (Kieffer, et al., 2014). However, resistant starch from different sources can be fermented to different extents (Ferguson, et al., 2000).

Although there are many studies on the effect of a single substrate in the microbiota, there are fewer with complex food ingredients, such as barley. Both barley and malt have been associated with an increase in butyric acid in the hindgut of rats, possibly due to the growth of genus such as *Blautia*, *Roseburia*, *Coprococus* and *Lactobacillus*, as a result of high β-glucan content in the diet. However, the combined effect of other fibres, such as arabinoxylan and resistant starch, cannot be dismissed (Zhong, et al., 2015a; Zhong, et al., 2015b).

Digestive and fermentation models

During digestion of a meal in the upper gastrointestinal tract, the properties of food components are modified due to mechanical forces, transport, pH changes and mixing with digestive secretions. Thus, the food reaching the colon has different properties than before consumption. Both gastrointestinal conditions and the food matrix can affect the properties of dietary fibres differently, which can influence the fibre function in the colon. This requires an understanding of what happens to the fibre inside the gastrointestinal tract. *In vitro* systems are therefore important tools to predict *in vivo* behaviour of fibre components and their impact on human colonic health.

In vitro gastrointestinal models

In vitro methods can be useful tools for simulating digestion in the stomach and small intestine, for studying changes in the food structure and composition during digestion, and assessing their functional properties as a substrate for the colonic microbiota. The complexity of the conditions *in vivo* such as passive transport of nutrients and simulation of physiological transit times are not accurately simulated by *in vitro* methods, but they are a reproducible and practical alternative. Static *in vitro* digestion methods allow a large number of samples to be screened as they are usually performed using single additions of enzymes and bile acids, and with simple regulation of pH and temperature conditions at each step of the simulated digestion. Today, a number of more advanced *in vitro* digestion models exist that more closely resemble the *in vivo* situation (Menard, et al., 2014; Minekus, et al., 1995; Wickham, et al., 2009).

The TNO Gastro-Intestinal Model (TIM-1) is a computer-controlled *in vitro* model composed of four compartments (stomach, duodenum, jejunum and ileum) that simulates gastrointestinal transit conditions such as temperature, pH, secretions of enzymes, absorption, and gastric and intestinal mixing and transport by peristaltic movements (Minekus, et al., 1995). The digest volume, and secretions of bicarbonate and hydrochloric acid to control pH levels are monitored in each compartment through sensors connected to the computer, while enzymes, bile and pancreatic secretions are released at a constant rate. The absorption of water and small molecules is achieved with two hollow fibre dialysis devices, connected to the end of the jejunal and ileum compartments for continuous removal of digested nutrients in order to avoid saturation and enzyme inhibition. One of the advantages of the TIM model is the possibility of sampling the digestive fluids at arbitrary time points and sites. Furthermore, as the model is computerised, the variations between runs are reduced, although this can also be a disadvantage when taking biological variations into account. Some of the limitations are related to the difficulties in simulating physiological processes of the gut wall (enterocytes), including active transport and feedback mechanisms (Minekus, et

al., 1995). The mastication process is also absent (unless an oral step with chewing is added in the experimental design of the model), which can be important for studies that rely on food microstructure and particle dimension, either for enzyme accessibility in solid food or shear in oil droplets in liquid foods (McClements & Li, 2010).

In vivo models – rat

The degree of fermentation, formation of SCFAs and microbiota composition in the colon are used to understand the health effects of fibre in humans. However, there are limitations for this assessment in humans as it is difficult to control the experimental conditions and it is impracticable to take samples at the actual site of fermentation (proximal colon). Moreover, faecal samples are usually used for analyses of SCFAs and microbiota, but they are not representative of the metabolic activity and microbiota composition at the actual site of fermentation in the lumen of the colon. Furthermore, it is difficult to work with strictly anaerobic populations. Some of these limitations also apply when using *in vitro* models as most of them use faecal inocula, and it is difficult to simulate absorption of products of fermentation and pH variations.

Animal models can therefore be used, and the rat has become one of the most commonly used animal models for studying fibre fermentation. Some of the limitations relate to the anatomic dissimilarities between the human and the rodent. However, despite the rat being a caecum fermenter, while the human is a colon fermenter, the two species have been shown to correlate well on the degree of fermentation for various fibre sources (Nyman, et al., 1986), and a similar SCFA pattern is formed when using faecal inocula from rats and humans (Barry, et al., 1995). Further, the rat is a full body model and analyses in different organs can be performed, e.g. cholesterol in the liver. It is also easy to follow the products formed from microbial degradation (short-chain fatty acids) at other sites in the body (blood, liver and brain) as well as to measure gene expression in different organs.

Materials and Methods

Study design

This work is based on five studies (Papers I – V) (Figure 4). In the first study, malts of different characteristics were included to study their potential to increase/change SCFA formation, improve barrier function and reduce inflammation in the hindgut of rats, caused by high fat diets (I). In Paper II, barley varieties were malted in different steeping conditions in order to get an insight into the extent to which barley components associated to colonic health could be modified (II). Selected malts or malting conditions from Paper II were used in the subsequent three studies (III, IV and V), together with a malt and BSG produced commercially. To better understand the impact of processing and of barley variety, β-glucan of barley malt products (malts and BSG) was assessed on a broad range of molecular weight distribution (III). In paper IV, different barley malt products were digested *in vitro* to evaluate the effects of gastrointestinal conditions on fibre components before entering the colon and were compared with *in vivo* data from a rat model. In paper V, healthy rats were fed diets containing barley malt products, together with barley extracts of arabinoxylan and β-glucan, to evaluate their influence on the caecal microbiota composition.
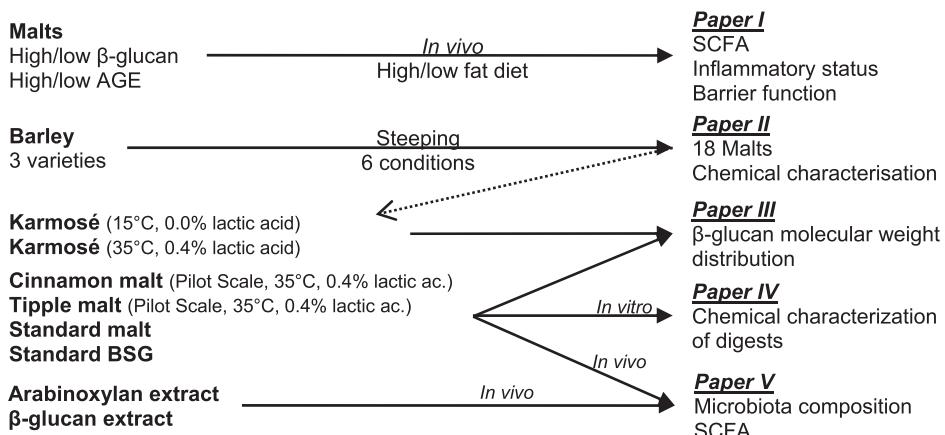
Figure 4
Overview of the analyses and barley materials used in each study.

Paper I – Barley malts with different β-glucan content and AGE levels were evaluated on the basis of their ability to increase SCFA production, reduce inflammatory status, improve barrier function and change microbiota composition in a high-fat setting. Low- and high-fat diets containing microcrystalline cellulose were included as controls. The experiment lasted for 4 weeks with the individual feed intake restricted to 12 g per day for the first two weeks, then increasing to 20 g per day. At the end of the experiment the rats were euthanized and samples were collected for analyses.

Paper II – Three barley varieties were malted on laboratory scale with different conditions of temperature and lactic acid in the steeping water, resulting in eighteen different malted samples. Characterisation of components relevant for colonic health was performed.

Paper III – Five barley malts and one BSG were used to evaluate the differences in molecular weight and size distribution of β-glucan, including those of molecular weight below 10 kDa. The barley products differed with regard to variety, steeping conditions and use of mashing. The protein-carbohydrate aggregation was also evaluated.

Paper IV – Diets containing three barley malts or one BSG were evaluated after passage through the gastrointestinal tract using a dynamic *in vitro* model (TIM-1). The delivery of dietary fibre and protein was analysed to predict the *in vivo* delivery to the colon after digestion. The same diets were given to rats in Paper V, and the faecal excretion of fibres and the caecal proportion of SCFAs were compared and related with the colon delivery from the *in vitro* model.

Paper V – Diets containing malt from four barley varieties and with different fibre composition, BSG, and barley extracts rich in AX or β-glucan were tested on their ability to change the caecal microbiota composition and correlation to SCFAs formed. The experiment lasted 12 days (including 7 days of adaptation to the diet) and the daily feed intake was 12 g per rat. Faeces were collected daily. At the end of the experiment, the rats were euthanized and organs and caecum content and faeces were collected for analyses.

Barley test materials

Table 1.
Barley products used in the different papers.

Papers	Material	Properties
I	Caramelized malt	Caramelised malt used in production of dark beer. Variety unknown
I	Colored malt	Munich malts used in production of dark beer, derived from a mixture of the barley varieties Quench and Breamar
I	50 malt	Pilsner malt produced from the variety Quench with low β-glucan content (0.6 g/100 g)
I	350 malt	Pilsner malt produced from the variety Tipple with high β-glucan content (1.2 g/100 g)
II	Tipple barley	Barley variety used in beer production, with comparatively high β-glucan content (3.9 g/100 g) and low resistant starch and amylose content
II	Karmosé barley	Barley variety with high β-glucan (5.2 g/100 g) and amylose content
II	Cinnamon barley	Barley variety with high β-glucan content (5.1 g/100 g) but no amylose
III – V	Tipple malt	Malt with high β-glucan (2.9 g/100 g), resistant starch and amylose content
III – V	Cinnamon malt	Malt with high β-glucan content (3.9 g/100 g) and low resistant starch and amylose content
III – V	Standard malt	Pilsner malt with low β-glucan content (0.6 g/100 g) from a mixture of the barley varieties Tipple, Quench and Rosalina
III – V	Standard BSG	Barley spent grain of the varieties Tipple, Quench and Rosalina. Low β-glucan content (0.5 g/100 g) and high dietary fibre and protein content
III	Karmosé 1500	Malt with high β-glucan (3.6 g/100 g), resistant starch and amylose content
III	Karmosé 3500	Malt with high β-glucan (4.7 g/100 g), resistant starch and amylose content
V	Arabinoxylan extract	High content of total and soluble arabinoxylan (59 g/100 g)
V	β-glucan extract	High β-glucan content (32 g/100 g) and molecular weight

Analytical methods

The analytical procedures are described or referred to in the Materials and Methods section of each paper.

Table 2.
Methods of analyses used in the characterization of barley products.

Papers	Analyses
I – V	Dietary fibre composition (neutral sugars, AX and glucans)
I – V	β-glucan content
I, II, IV, V	β-glucan molecular weight (HPSEC-FD and Calcofluor)
III	β-glucan size distribution (AF4-FL-MALS-dRI and Calcofluor)
I, III	Amino acids
I	Advanced glycation end-products (AGE) as carboxyl methyl lysine (CML) and carboxyl ethyl lysine (CEL)
II – V	Total and resistant starch
II, V	Amylose content
II, IV, V	Protein content
II	Phytate content
II	Iron content

Table 3.
Methods for analyses of composition of ileum delivery from the TIM-1 *in vitro* model.

Papers	Analyses
IV	Dietary fibre composition (soluble/insoluble, neutral sugars)
IV	β-glucan content
IV	β-glucan molecular weight and size distribution (HPSEC-FD and Calcofluor)
IV	Protein content

Table 4.
Methods for analyses of content of metabolites from colon and blood of rats.

Papers	Analyses
I, V	Short-chain fatty acids in caecum
I	Succinic- and lactic acid in caecum
I	Short-chain fatty acids in portal serum
I	Advanced glycation end-products (AGE), as carboxyl nethyl lysine (CML) and carboxyl ethyl lysine (CEL) in caecum and/or portal plasma
I	Gene expression of occludin, zonucla occludens-1 (ZO-1), toll-like receptor (TLR-3 and TLR-4) in small intestine and distal colon
I	Quantitative PCR for *Akkermansia* in caecum
I	Mucus layer thickness in the transverse colon
V	Composition of dietary fibre in faeces (neutral sugars)
V	Microbiome profiling (next-generation sequencing) in caecum

Malting

Malts in Paper I were produced by Viking Malt at the same steeping and germination conditions, whereas kilning temperatures varied: Caramelized malt was kilned up to 170°C, Colored malt up to 100°C, and 50- and 350-malt up to 85°C. These malts were selected due to their different amounts of β-glucan (50-malt vs 350-malt) and AGE (Colored and Caramelized malt vs 50- and 350-malt) to evaluate the relevance of malt composition for anti-inflammatory effects.

To test the ability to modulate the composition of barley malt, three barley varieties with different chemical composition were malted on a laboratory scale (80 g), at different temperatures (15 or 35°C) and concentrations of lactic acid (0, 0.2 or 0.4% (v/v)) in the steeping water (Paper II). The steeping conditions were based on previous studies in which increased lactic acid concentration (0.8%) and temperature (48°C) during steeping decreased β-glucanase and increased phytase activity (Haraldsson, et al., 2004; Rimsten, et al., 2002). With the ambition to produce malts with a broad β-glucan molecular weight distribution, milder steeping conditions were used in this study to not completely reduce β-glucanase activity. Steeping times were dependent on the variety (32–37 h) to reach a grain moisture of approximately 42%. The steeped grains were transferred into a micro-malting machine where the germination temperature was kept at 15°C for 71 h. The germination time was shorter than traditional malting processes (over three days) in order to limit dietary fibre degradation. Kilning was performed at 55, 70 and 82°C for 10, 8 and 8 h, respectively; higher temperature profiles were not used, as this would increase the content of AGE, which were revealed to attenuate the beneficial effects of malt on the maintenance of intestinal integrity in Paper I.

Tipple and Cinnamon malts (Papers III, IV and V) were produced at steeping conditions of 35°C and 0.4% lactic acid, with germination and kilning conditions as described in Paper II, but performed on a pilot scale (40 kg). These malts were chosen as these steeping conditions better preserved β-glucan content. In contrast, Standard malt with low β-glucan content, and BSG from the same varieties (mixture of Tipple, Quench and Rosalina) were selected. Standard malt was produced at the same conditions as for pilsner beer malt (steeping: 14.5°C and 0% lactic acid, germination: 15°C, kilning: 55–86°C for a total of 20 h), and BSG was prepared by mashing Standard malt (20–70°C), filtering and drying the solids (1°C/min at 48–55°C for a total of 20 h).

Barley extracts

The extracts for β-glucan analysis in Paper III were obtained by incubating malts or BSG in a boiling water-bath for 90 minutes with 0.3 mg/ml $CaCl_2$, as previously described (Rimsten, et al., 2003), and as performed in Paper I, II, IV and V. As starch is gelatinised at these conditions, a thermostable α-amylase (Termamyl) was added to degrade starch and reduce viscosity, which would otherwise hinder the extraction of polysaccharides into the water. In Paper III, the subsequent step of extraction using 50% (v/v) ethanol, as described by Rimsten, et al. (2003), was not performed in order to keep β-glucan of low molecular weight, which was of interest for the analysis. Glucose units were removed from the extracts by dialysing the supernatant for 24 h in deionised water (water change after 2, 4 and 8 h) with a membrane cut-off of 3.5–5 kDa (Spectra/Pore Biotech CE). Samples were freeze-dried and stored in a desiccator until analysis.

Extracts rich in β-glucan and arabinoxylan were obtained from barley and used in the diets in Paper V. Arabinoxylan extract was isolated by Xylophane AB (Gothenburg, Sweden) from barley husks using a process that involved total deacetylation at high pH conditions. β-Glucan extract was obtained by mechanical fractionation of the barley kernels, mostly originating from the aleurone layer cell walls, produced by Lyckeby Starch AB (Kristianstad, Sweden).

Total protein hydrolysis of barley extracts

During analysis of β-glucan molecular weight distribution analysis (Paper III), it was hypothesised that contaminating proteins in the extracts were interfering with the analysis. The residual proteins were removed with a mixture of proteolytic enzymes (protease, leucine aminopeptidase and prolidase), totally hydrolysing proteins to the amino acid level, according to the method described by Baxter, et al. (2004). The samples were incubated with the mixture of enzymes for 20 h at 37°C in sodium phosphate buffer. Each enzyme was tested and resulted negative for β-glucanase activity (assay of endo-β-glucanases using beta glucazyme tablets, Megazyme International, Ireland).

Asymmetric flow field-flow fractionation (AF4)

The broad range of β-glucan molecular weight was analysed with AF4 in combination with different detectors. This method, unlike size-exclusion chromatography, can separate particles over a wider size range (2 nm to 1×10^7 g/mol) across a channel without an additional stationary phase that may potentially interact with the particles and affect the elution.

In brief, the separation with AF4 is based on the diffusion coefficients of the different sized molecules in the sample. First, the sample is loaded into the channel and focused/relaxed where a crossflow is pushing the molecules down towards a semi-permeable accumulation wall, inducing a concentration gradient over the channel height. Due to the diffusion coefficient, larger particles are closer to the accumulation wall and smaller ones higher up in the channel. Then, the particles are eluted along the channel in a laminar flow, separating the particles that migrate at different velocities depending on their size (small particles are faster), whereas the cross-flow ensures that particles are more concentrated close to the ultrafiltration membrane (Figure 5) (Wahlund & Nilsson, 2012). This method has been previously used to separate food macromolecules and aggregates (Nilsson, 2013), including cereal β-glucan (Ulmius, et al., 2012a; Ulmius, et al., 2012b).
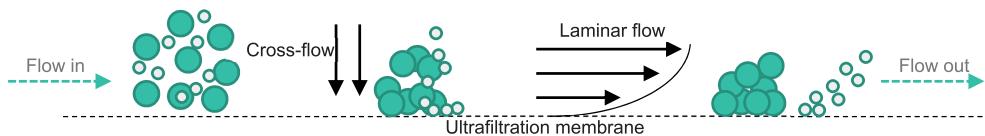
Figure 5
Principle of separation in the AF4 channel. Based on Wahlund and Nilsson (2012).

This methodology aimed to analyse β-glucan molecular weight from barley malt and BSG with the aid of multiple detectors: multi-angle light scattering (MALS), differential refractive index (dRI) and fluorescence (FL) and UV (Paper III). Calcofluor was used to label β-glucan as they form a non-covalently linked complex and emit a fluorescence signal at λ_{ex}: 415 nm λ_{em}: 445 nm. Despite this complex not quantitatively forming with β-glucan of molecular weight < 10 kDa (Jorgensen, et al., 1987; Kim & Inglett, 2006), it was still possible to detect differences between the samples analysed. As the extracts of the barley products contain other components in addition to β-glucan, the fluorescence signal was tested for interference. Neither non-labelled samples nor starch (potato starch and 20% amylose were tested) showed a signal with Calcofluor, indicating that only β-glucan was being labelled and detected by FL.

Test diets and meal preparation

All diets/meals (Paper I, IV and V) were composed of 120 g/kg casein (protein), 1.2 g/kg DL-methionine, 50 g/kg maize oil, 2 g/kg choline chloride, 8 g/kg vitamin mixture and 48 g/kg mineral mixture. The meals/diets in Paper IV and V also contained 100 g/kg of sucrose, whereas the sucrose content in Paper I was reduced to 50 g/kg in order to achieve the required fibre and fat levels. The high-fat diets in Paper I also contained 180 g/kg of butter and 10 g/kg of cholesterol.

The weights of the different barley materials were adjusted to 70 g/kg fibre (Paper I) or 80 g/kg fibre in the diet (Paper IV and V). Microcrystalline cellulose was used in the control groups in Paper I, and it is almost completely resistant to fermentation. Wheat starch was added to adjust for the dry matter content and does not contribute to any SCFA formation, as it is completely digested in the upper part of the gastrointestinal tract of the rats (Björck, et al., 1987).

Male Wistar rats were used in the *in vivo* model, with seven rats per diet. These were kept either in groups of three or four in polycarbonate cages (Paper I), or individually (Paper IV and V). Temperature and light were kept constant (21–22°C) with a 12 h light/dark cycle. Water was given *ad libitum*. Simulated digestion experiments were performed with an *in vitro* model (TIM-1). Duplicate experiments were performed for each meal and an oral step in which the meals were mixed with simulated saliva fluid and amylase before the start of the digestion in the *in vitro* model.

Calculations and statistical analyses

Faecal excretion (during 5 days) and degree of fermentation of specific dietary fibre components for each test diet were calculated as:

Faecal excretion (%) = (g fibre in faeces / g fibre ingested) × 100

Fermentation (%) = 100 – Faecal excretion (%)

SCFAs in caecum were determined either as the proportion of a specific SCFA in relation to the total content (%) or as total amount found in caecum (µmol):

SCFA (µmol) = SCFA (µmol/g) × g caecal content

Data are presented as average and standard error of the mean (SEM) on dry weight basis, unless otherwise stated. SPSS was used for analyses of variance, post-hoc tests (Tukey or Games-Howell), and calculation of the two-tailed Pearson correlation coefficients and Goodman and Kruskal's γ association test. SIMCA (Umetrics) was used for principal component analyses (PCA).

Results and Discussion

Effect of processing on barley malt composition

In Paper I, malts with a wider β-glucan molecular weight distribution slightly increased the formation of SCFAs in the caecum of rats. Thus, one of the primary objectives in Paper II was to modulate the β-glucan molecular weight and reduce the degradation of β-glucan and soluble fibre by changing the steeping conditions of temperature and lactic acid concentration. The final step in malting, i.e. kilning, can be used for the development of colour and flavour, to preserve or reduce diastatic power in the malt. However, if too high temperature is used, one must be aware of the formation of undesired compounds, advanced glycations end-products (AGE), which might affect the intestinal integrity. During mashing, further degradation of starch and β-glucan occurs, the soluble part of the malt is removed, and the remaining part is BSG.

Malting

For the three barley varieties investigated in Paper II, the contents of total arabinoxylan, protein and iron were maintained or were only slightly higher after malting. The increase was most likely due to losses of soluble components, such as glucose, into the steeping water or loss of carbon dioxide formed during germination (Briggs, 1998). The apparent preservation of iron after malting was in accordance with previous studies, where iron and other mineral elements were redistributed between the aleurone, rootlets and shoots (Liu, et al., 1975; Shewry, 2014). Protein, on the other hand, is known to be degraded during malting and some amino acids can be lost through leaching. However, the majority of them are mobilised to the embryo for plant growth (Briggs, 1998). In the present study, the rootlets and sprouts were not removed, resulting in an overall higher content of protein and iron than is usually found in malt. Insoluble arabinoxylan is mostly found in the outer layers of the barley grain and can be expected to be rather resistant to degradation during malting, as found in previous studies (Burton & Fincher, 2014; Hübner, et al., 2010).

Steeping

For beer production it is desirable that β-glucan is highly degraded in order to facilitate filtration of the wort and avoid beer haze at increasing ethanol concentration during fermentation. However, for food uses, a malt in which the dietary fibre is more preserved would be of benefit. To increase intestinal health, a wide range of molecular weight would be preferred.

Soluble fibre content was affected significantly by the different steeping conditions, but to differing degrees depending on the barley variety. The varieties Tipple and Karmosé were more susceptible to the malting process *per se* and to the steeping conditions than the variety Cinnamon. Structural differences, i.e. three-dimensional structure, between these varieties may explain the dissimilarities, as suggested by Brennan, et al. (1997). A loosely packed endosperm may allow higher and more distributed hydration of the grain, leading to a better mobility of the enzymes, which allows them to degrade the substrate more extensively.

β-glucan is partially solubilised and significantly degraded during malting as endogenous enzymes reduce the β-glucan molecular weight. In this study it was possible to regulate β-glucan content through changes of temperature and lactic acid concentration in the steeping water. A higher temperature (35°C) and higher lactic acid concentration (0.4%) were found to produce malts with a higher content of soluble fibre and β-glucan than samples steeped in traditional malting conditions (15°C and without lactic acid) (Figure 6, left). Other studies have shown similar results (Haraldsson, et al., 2004) with most of the loss of soluble fibre being β-glucan (Hübner, et al., 2010).
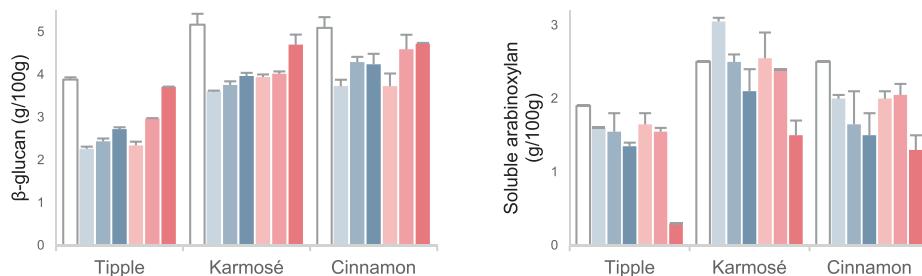
Figure 6
β-glucan and soluble arabinoxylan (mean and SEM) in barley (white) and malts steeped under different conditions (15°C blue, 35°C red, from left to right for each variety and temperature: 0%, 0.2% and 0.4% lactic acid).

The different β-glucan content steeped at different conditions was most likely due to changes in the activity of β-glucanases. This enzyme has been found to be very sensitive to pH changes (Woodward & Fincher, 1982), and a small decrease in pH due to the addition of lactic acid may reduce β-glucanase activity and limit β-glucan degradation (MacWilliam, 1975). The steeping temperature also seems to have an impact on β-

glucan content, at least for the varieties Tipple and Karmosé. The optimal temperature for the activity of endo-β-(1,3-1,4)-glucanase isoenzymes has been reported to be around 16°C (Bamforth & Martin, 1983), but the enzyme rapidly loses activity above 30°C (McCleary, 1986; Woodward & Fincher, 1982). It could thus be expected that malts steeped at 35°C with 0.4% lactic acid had a higher β-glucan content, due to lower β-glucanase activity, than those steeped at 15°C without lactic acid. This was in accordance with previous studies where high temperature (48°C) and a high content of lactic acid (0.8%) in the steeping water reduced β-glucanase activity (Haraldsson, et al., 2004; Rimsten, et al., 2002).

β-glucan molecular weight distribution was analysed with two methodologies: HPSEC-FD (Paper I, II, IV and V) and AF4-MALS-dRI-FD (Paper III). With HPSEC, only β-glucan with a molecular weight higher than 10 kDa was assessed. At this size range the steeping conditions did not seem to affect the β-glucan average molecular weight to any great extent, despite variations in the β-glucan content. Due to these limitations and the availability of equipment, AF4 was used, which enabled the analysis of β-glucan over a wide range of molecular weights, including those lower than 10 kDa. Even though β-glucan-Calcofluor complexation imposes a lower limit of the quantitative detection around 10 kDa, fluoresce detection was still possible at a range of less than 2 kDa. FD signal at a very low molecular weight most probably resulted from a weaker complexation with Calcofluor. A fluorescence signal due to contaminants was discarded (see Material and Methods section), thus only β-glucan was being detected. Taking these limitations into consideration, differences in the β-glucan molecular weight profile, even at the low range, were still taken into account and compared.

Karmosé steeped at 15°C without any lactic acid (K1500) had a lower β-glucan average molecular weight ($3.4×10^5$ g/mol) than the same variety steeped at 35°C with 0.4% lactic acid (K3504) ($6.6×10^5$ g/mol) (Paper III). Furthermore, β-glucan of lower molecular weight ($< 1×10^5$ g/mol) was more predominant in K1500 (73%) than in K3504 (60%). This confirms that steeping at a lower temperature and without lactic acid resulted in a more degraded β-glucan. This is likely explained by a higher activity of β-glucanase during these conditions, which was also seen in the lower β-glucan content in this malt (3.6 g/100g) compared with K3504 (4.7 g/100g) (Paper II).

The impact of variety was shown when the two barley varieties, Tipple and Cinnamon, were malted at the same steeping conditions (35°C and 0.4% lactic acid). These varieties had a similar β-glucan molecular weight before malting ($~1.6×10^6$ g/mol, Paper II), but the β-glucan molecular weight distribution was wider in Tipple malt than in Cinnamon malt. Furthermore, Cinnamon malt contained slightly more β-glucan of high molecular weight (28%, $> 1×10^5$ g/mol) than Tipple malt (20%, $> 1×10^5$ g/mol). Yet again, these differences were probably due to variations in structure and three-dimensional arrangement, resulting in different accessibility of the enzymes to the substrate (Brennan, et al., 1997).

It must be kept in mind that both Cinnamon and Tipple had a high β-glucan molecular weight compared with a traditional malt used when beer is produced (e.g. Standard malt). An increased germination time would possibly increase the time of action of the β-glucanases and further reduce the β-glucans of high molecular weight. For example, for beer production the germination is usually performed over three days (Briggs, 1998; Jadhav, et al., 1998; Mussatto, et al., 2006), as germination time increases dietary fibre degradation (Hübner, et al., 2010). However, one of the goals of this study was to limit the fibre degradation, and germination was therefore restricted to three days. Nevertheless, as the methodology used for measuring molecular weight has limited capacity to detect the low molecular weight compounds, this might have resulted in underestimation of some of these compounds.

The variances in the values of molecular weight between the two methods of molecular weight analysis used could be due to the limit of detection but also to the extraction method used. With regard to the limit of detection, different ranges of molecular weight were taken into account for the analyses in Papers II and III. Thus, β-glucans with molecular weight inferior to 10 kDa were not taken into account with HPSEC-FD (Paper II), whereas the whole range (> 2 kDa) was considered for the calculation of average molecular weight with AF4 (Paper III). The extraction method also differed between Papers II and III. Thus, since the analysis in Paper III was aimed to include also the small-sized β-glucan, the inactivation step of endogenous enzymes with ethanol was not performed resulting in a larger proportion of small-sized β-glucans. Furthermore, it cannot be ruled out that some β-glucan degradation might have occurred during this extraction (Rimsten, et al., 2003) although, due to the short time (90 min) and high temperature (~100°C) of extraction that might have deactivated β-glucanases, it was most probably negligible.

With regard to the *soluble arabinoxylan,* the steeping conditions that preserve β-glucan (35°C and 0.4% lactic acid) seems to degrade soluble arabinoxylan to a greater extent, judging from the lower contents resulting from these conditions (Figure 6, right). Arabinoxylan degradation appears to be caused by the temperature during steeping, being closer to the optimal temperature for barley β-(1-4)-endo-xylanase activity (35–40°C) (Benjavongkulchai & Spencer, 1986). It was expected that the lactic acid concentration had lower effect, as pH has only a slight influence on the xylanase activity (brenda-enzymes.org). However, it cannot be ruled out that the mode of degradation might have been affected, thus affecting arabinoxylan content. In a previous study, optimal xylanase activity was at pH > 5 (release of xylans), whereas xylosidase activity was predominant at pH < 5 (release of xyloses) (Kanauchi, et al., 2013). Therefore, the xylosidase activity could have been predominant in malts steeped with 0.4% lactic acid, releasing xylose units that could leach into the steeping water more easily, resulting in a loss of soluble arabinoxylan. Another possible reason for the lower content could be an underestimation of small-sized xylans during fibre analysis. In most currently used dietary fibre methods, as well as in the gravimetric enzymatic method in this study, the

precipitation of soluble fibre is performed with ethanol 80% (v/v), which fails to precipitate smaller molecules of arabinoxylan.

The degradation of arabinoxylan during malting is usually less than that of β-glucan. These two polymers are non-covalently interlinked in the structure of the cell wall, and it is believed that arabinoxylan, if not degraded, can inhibit the accessibility of β-glucanases to the substrate (Bamforth & Kanauchi, 2001). However, for two of the varieties (Tipple and Karmosé) the contents of β-glucan and soluble arabinoxylan were negatively correlated (r < −0.6, p < 0.05), suggesting that at conditions when arabinoxylan is preserved (e.g. 15°C and without lactic acid), the degradation of β-glucan is possible. This is in contrast with a previous suggested model of cell-wall degradation, where xylanase activity is developed in the early stages of the germination to allow β-glucan degradation (Bamforth & Kanauchi, 2001; Kuntz & Bamforth, 2007). However, the same authors also proposed that a low degradation of arabinoxylan is enough to enable β-glucanase access to β-glucan. Furthermore, some xylanases are involved in the solubilisation of β-glucan, possibly contributing to a higher degradation of β-glucan but not of arabinoxylan (Bamforth, 2010).

Resistant starch was highly correlated with amylose content in malted and unmalted barley samples (r = 0.8, p < 0.01), suggesting that the majority of the resistant starch originated from amylose. Besides the leakage of some solutes into the steeping water, the small increase of amylose content in some malts could also be due to the increase of amylodextrins. Thus, α-amylases and some dextrinases that cleave the α-(1-4)- and α-(1-6)-glycosidic linkages in amylose and amylopectin could yield dextrins of various lengths, including amylodextrins (dextins with 20–30 units), which are assumed to be amylose according to the method of analysis. This could partially explain the increase of amylose in Tipple and Karmosé malts steeped at 35°C, as amylase is activated only at higher temperatures (40–60°C) (Swanston, et al., 2014) (brenda-enzymes.org).

Phytate content decreased after malting, but was little affected by the steeping conditions, particularly by the temperature as reported previously (Rimsten, et al., 2002). However, the lack of effect of the steeping conditions is in contrast to a previous study (Haraldsson, et al., 2004), in which there was significant degradation of phytate at 48°C and 0.8% (v/v) lactic acid in the steeping water. This suggests that the combination of temperature and lactic acid is more important than each factor alone. One possibility for the differences in effects compared with the present study could be the lower steeping temperatures (15 and 35°C) compared with 48°C in the study by Haraldsson, et al. (2004), which was also closer to the optimal temperature for phytase activity (50–57°C) (brenda-enzymes.org) (Bergman, et al., 2000; Bergman, et al., 1999). Another possible reason for the lower degradation of phytate in this study is the more limited time of germination (71 h) and, consequently, less time for phytate degradation. According to some studies, phytase activity has been reported to peak after three days of germination (Hübner, et al., 2010; Sung, et al., 2005).

A malt product for improved intestinal health should thus contain a high content of soluble fibre, especially β-glucan and arabinoxylan such as Cinnamon malt steeped at 35°C and 0.4% lactic acid. Such a product could possibly increase butyric acid in the colon, and a reduced content of phytate would increase iron bioavailability. However, due to the positive correlation between soluble fibre, β-glucan and phytate ($r > 0.6$, $p < 0.05$), and a negative correlation between β-glucan and soluble arabinoxylan in some barley varieties ($r < -0.6$, $p < 0.05$), it becomes difficult to tailor barley into a malt with all the desired characteristics. For such an outcome, other strategies would have to be applied such as the use of external phytates, or increased germination time, at the same time inhibiting β-glucan degradation through the steeping conditions.

Kilning

Colored and Caramelized malts were processed at conditions for production of dark beer, kilned at temperatures up to 100 and 170°C respectively, while 50-malt and 350-malt were only kilned up to 85°C (Paper I). Malts kilned at the higher temperatures contained more carboxylmethyl lysine (CML) and carboxylethyl lysine (CEL), which are markers for advanced glycation end-products (AGE) (Tareke, et al., 2013). AGE are a group of compounds of modified amino acids involving reducing sugars or their degradation products, with pro-oxidant and inflammatory effects (Vistoli, et al., 2013). Their accumulation in the tissues increases naturally with age, but their early presence has been linked with the development of cardiovascular diseases, Alzheimer's disease and diabetes (Luevano-Contreras & Chapman-Novakofski, 2010). Caramelized malt had one of the highest content of AGE markers, which is the probable reason for rats fed this malt in a high-fat setting not showing a significant improvement in the small intestine integrity, in contrast to rats fed the other malts kilned at lower temperatures.

It is therefore necessary to take the kilning temperature into account and avoid the formation of AGE compounds when producing malts for specific health effects. As kilning at high temperatures has been shown to counteract the beneficial effects of malt on the maintenance of intestinal integrity and increase the gut permeability, the temperature profile used in Papers II–V did not exceed 86°C.

Mashing

Standard malt had lower β-glucan average molecular weights than the other malts (Paper III), as it was processed at malting conditions for beer production that focused on the degradation of β-glucan. During mashing of malt, further reduction of starch content and β-glucan molecular weight occur.

BSG used in the present study contained higher amounts of total fibre, arabinoxylan and protein than the Standard malt from which BSG was produced (Table 5), but most of these components were insoluble. Only small proportions of soluble fibre and

arabinoxylan as well as starch were found in this fraction, and similar as previously reported (Jadhav, et al., 1998; Mussatto, et al., 2006).

	Standard Malt	Standard BSG
Total Fibre	15 (10)	55 (3)
Arabinoxylan	5.8 (5)	20 (1)
β-glucan	0.6	0.5
Average ($\times 10^4$)	5.1	3.8
Min – Max	$1700 - 2.0 \times 10^6$	$2000 - 1.0 \times 10^6$
Total starch	53 (27)	12 (80)
Protein	9.1	19

Malts kilned at relatively low temperatures, such as Standard malt (55–86°C), retain diastatic power which enables further starch degradation during mashing. Throughout mashing, amylase activity increases and degradation of starch into oligosaccharides occurs, with these being extracted into the mashing water that are used for brewing. Gelatinised starch is more easily degraded and most amylases in barley (α-amylase, β-amylase and limit dextrinase) are still active at these temperatures (55–70°C) (Swanston, et al., 2014) (brenda-enzymes.org). The gelatinisation of starch in barley has been found to be between 52 and 68°C (Swanston, et al., 2014). Thus, the increasing mashing temperature during production of BSG (20–70°C, 1°C/min), probably enabled starch gelatinisation and degradation.

The β-glucan content was similar in BSG and in Standard malt (0.5 and 0.6 g/100 g), but the β-glucan average molecular weight and the maximum molecular weight were lower in BSG (3.8 vs 5.1×10^4 g/mol in Standard malt) (Table 5). Although the high temperature might denature β-glucanase, its activity is preserved at the low hydration levels during kilning (Swanston, et al., 2014). Therefore, during mashing β-glucanase is still active during the early stages of mashing, thus up to 50% of activity can still remain after 10 min at 45°C (brenda-enzymes.org). The differences in β-glucan distribution after mashing (BSG and Standard malt) were not as obvious as between varieties (Tipple malt and Cinnamon malt) or between steeping conditions (K1500 and K3504) (Paper III). Thus, it seems that the degradation was more predominant in β-glucan of higher molecular weight.

In vitro digestion of barley malt products

Dietary fibres that reach the colon constitute a potential substrate for the microbiota. Many functions of dietary fibre depend on their physico-chemical properties (e.g. solubility and molecular weight), which influence the microbial degradation of the fibres and their implications for colon health. The fibre's properties may already undergo modifications in the upper gastro-intestinal tract. Proteolytic enzymes have been shown to increase β-glucan extractability, thus increasing the solubility and probably also the colonic fermentation (Robertson, et al., 1997). Furthermore, β-glucans of low molecular weight might be degraded to a greater extent than those of high molecular weight by the acidic pH in the stomach, as reported for inulin (Björck & Nilsson, 1991).

Composition of digest and transit time in the TIM-1 model

The *in vitro* model TIM-1 was used in Paper IV to estimate the fate of dietary fibre compounds after ingestion of barley malt and BSG samples. The experiments revealed that after 240 min *in vitro* digestion through the duodenum, jejunum and ileum, greater amounts of soluble fibre exited the ileum with meals containing the malts (Tipple, Cinnamon and Standard) than those containing BSG. This was expected due to the meals' higher proportions of soluble fibre. However, the transit time was also different and the passage along the gastrointestinal tract was slower with Tipple and Cinnamon malts than with BSG (Figure 7, left). As reported in previous studies, soluble fibres can create viscous solutions, significantly related to the concentration and molecular weight (e.g. β-glucan and arabinoxylan), which is in line with our findings (Blackwood, et al., 2000) (Irakli, et al., 2004). The ileal delivery of β-glucan was also slower during the digestion of malts than with BSG (Figure 7, centre), possibly due to the malts' higher β-glucan content and β-glucan average molecular weight than BSG.
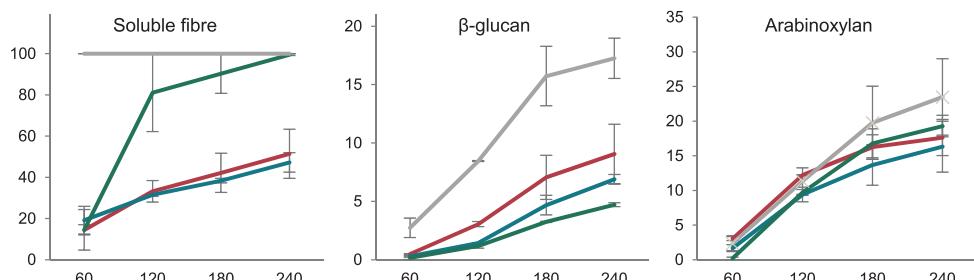
Figure 7
Ileal delivery of soluble fibre, β-glucan and total arabinoxylan in barley malt products (% of intake) at different times of digestion (0-60, 60-120, 120-180 and 180-240 min) in the TIM-1 model. Tipple malt (red), Cinnamon malt (blue), Standard malt (green) and BSG (grey) (mean ± SEM, n = 2) (Paper IV).

The results suggest that, due to the properties and the slow delivery of soluble fibre and β-glucan, Tipple and Cinnamon malt may promote different health effects. For example, viscous fibres have been found to slow down transit time in the small intestine, leading to a postprandial decrease of glucose and cholesterol levels, while satiety is increased. In the long-term this could aid in the control of diabetes, cardiovascular diseases and obesity (Ray, et al., 1983; Rose, et al., 2007). Additionally, it may be speculated whether a slower transit time in the upper part of the gastrointestinal tract may slow the substrate delivery to the colon, resulting in a more complete fermentation of the fibres (Eswaran, et al., 2013). If so, the possibility that the colonic barrier function is maintained increases, while the risk of influx of inflammatory substances is reduced.

Total arabinoxylan transit time was slightly faster in the BSG meal than in meals containing malt, possibly due to a lower proportion of soluble arabinoxylan in BSG (Figure 7, right). Unfortunately, it was not possible to perform analysis of soluble arabinoxylan content in the digests due to the small quantity of sample.

Protein content in the malt diets was slightly higher than in the meals containing BSG (mean 4.1 vs 2.8 g/100 g) (Paper IV), but this did not have any impact on the ileal protein content, which was similar in the digests from all meals.

β-glucan molecular weight during *in vitro* digestion

The β-glucan molecular weight in malts (Tipple, Cinnamon and Standard) decreased with increasing time of digestion. Thus, a longer digestion time shifted the molecular weight from a main peak around 1×10^6 g/mol in favour of those $< 1 \times 10^5$ g/mol (Figures 8, A, B and C). This shift was more noticeable at earlier times of digestion in Tipple and Standard malt than in Cinnamon malt. Differences in physico-chemical structure (dependent on both variety and processing conditions) between these barley malts, such as a more compact cell-wall structure of Cinnamon malt, may be one explanation for the observed differences, as this might influence the action of enzymes or acid, two factors that may be involved in the reduction of β-glucan molecular weight.

The human digestive enzymes (also the enzymes used in the *in vitro* model) do not contain any β-glucanases. The reason for the reduction of β-glucan molecular weight could be a consequence of a decrease in pH during digestion, or by activation of the barley's endogenous β-glucanases and/or other enzyme activities during the *in vitro* digestion. However, a previous study demonstrated that at low pH, as in the stomach, aggregates of pure β-glucan could be disrupted, but they tended to re-aggregate at conditions prevailing in the small intestine (increased pH) (Ulmius, et al., 2012b). Correspondingly, in another study, no degradation of β-glucan in oats was detected at low pH conditions mimicking the human stomach (0.1 M HCl, pH 1, 37°C) (Johansson, et al., 2006). Therefore, the pH changes during *in vitro* digestion would not be expected to change β-glucan molecular weight to any greater extent.

A possible explanation for the decrease in β-glucan molecular weight would be an increased activity of endogenous or microbial enzymes in the malt. Before the meals are introduced into the *in vitro* model, a mixture with artificial saliva and amylase is prepared to simulate the oral phase of digestion. At the temperature used for the oral phase (37°C), the activity of the endogenous enzymes in the barley products may increase, and consequently partly degrade β-glucan before reaching the acidic conditions in the stomach. This has been reported for barley malt prepared as porridge (Haraldsson, et al. (2005).
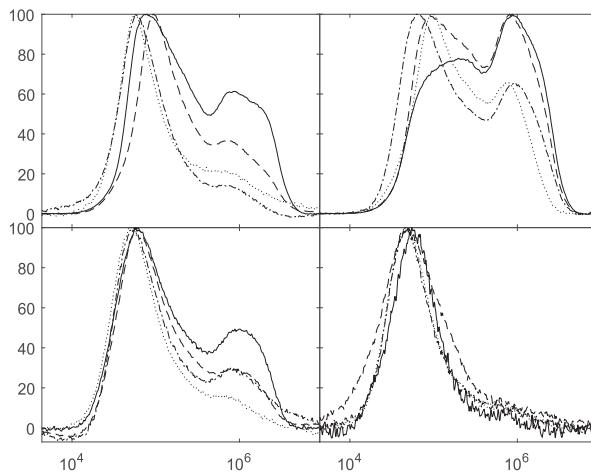
Figure 8
Molecular weight distribution (HPSEC-FD) of extractable β-glucan in samples collected from the ileal delivery during digestion in the TIM-1 model (deliveries were collected during: 0-60 (solid line), 60-120 (dashed line), 120-180 (dash-dot line) and 180-240 min (dotted line)) in diets containing barley products (A – Tipple malt, B – Cinnamon malt, C-Standard malt, D – Standard BSG) (Paper IV).

Another possible explanation for the lower β-glucan molecular weight in the *in vitro* digestion model could be due to the disaggregation with protein, as previously found in rye (Härkönen, et al., 1995). According to old cell-wall structural models, proteins are part of the cell-wall matrix together with β-glucan, arabinoxylan and some celluloses (Forrest & Wainwright, 1977). As a result of the proteolytic activity, there may be a disaggregation between β-glucan and protein in the cell walls, leading to an apparent decrease of β-glucan molecular weight. Similarly, in Paper III, when the extracts of these malts were treated enzymatically with proteases with no β-glucanase activity (Figure 9, with Tipple malt as an example), the β-glucan molecular weight also shifted to a lower range of molecular weight. This fits well with the cell wall structural model, and suggests that if β-glucans are associated with protein they would display an apparently higher molecular weight.
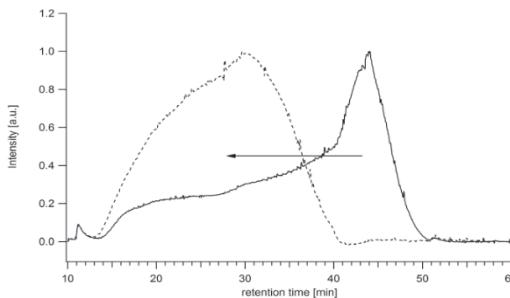
Figure 9
AF4-FL fractogram of extracts from Tipple malt β-glucan labelled with Calcofluor before (solid) and after (dashed lines) proteolitic enzymatic digestion (Paper III).

In BSG, β-glucan molecular weight did not significantly change during *in vitro* digestion. This was possibly due to the mashing process that allowed extensive degradation of the remaining β-glucan, in addition to the rupture of protein-β-glucan bonds.

Small-sized β-glucans would be rapidly fermented into SCFAs in the proximal part of colon, but mixing them with those of high molecular weight would extend the fermentation through the whole colon, including the distal part. After *in vitro* digestion, the high molecular weight β-glucan was better preserved in Cinnamon malt than in the other malts and BSG. This suggests that β-glucan in Cinnamon malt would be more slowly fermented and potentially have a higher capacity to reach the distal colon, there increasing SCFA production.

Effects of barley products in a rat model

The physico-chemical characteristics of fibre (such as monomeric composition, solubility and degree of polymerisation) are of interest as they may determine the utilisation of fibres by the microbiota, which in turn can affect the microbiota composition and the formation of SCFAs.

In the first *in vivo* study, the SCFA formation and gene expression were analysed in conventional rats fed high-fat diets containing commercial barley malts with different β-glucan and AGE content (Paper I). In the second study, the microbiota composition, degree of fermentation and formation of SCFAs were evaluated in rats fed barley malt products (malts and BSG) (Paper V).

Fermentation of dietary fibre
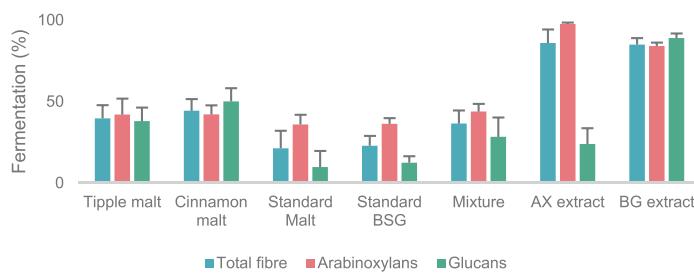
Figure 10
Fermentation of total fibre, arabinoxylan and glucans in the caecum of rats fed barley products (% of intake).

Soluble fibre

As expected, and in accordance with previous studies (Bränning & Nyman, 2011; Zhong & Nyman, 2014), total fibre in Tipple and Cinnamon malt, both containing high amounts of soluble fibre, was fermented to a higher degree in the caecum of rats (40–44%) than Standard malt and BSG (21–23%, Figure 10) (Paper V). In this respect, it is interesting to note that the soluble fibre transit time was slower during the *in vitro* digestion of Tipple and Cinnamon malt than Standard malt and BSG (Figure 7, left). In the *in vivo* situation this would mean that the delivery of soluble fibre to the caecum of rats would be prolonged, with lower amounts of undigested components being fermented at each time point, possibly resulting in a more complete degradation of the fibre (Eswaran, et al., 2013).

The barley extracts (AX and BG) were both highly utilised by the microbiota; AX extract containing 76% soluble arabinoxylan was almost totally fermented (98%), and

BE extract containing ~60% β-glucan of high molecular weight was fermented to 89% (Figure 10). This could be due to their high solubility, but also to the lack of a complex cell structure as in the case of barley malts making them more easily fermented. The diet consisting of a combination of Tipple malt and BSG (70:30) (Mixture diet) showed, as expected, an intermediate degree of fermentation in comparison with the diets containing the single substrates.

β-glucan

Glucose-containing polymers in Tipple and Cinnamon malt were more fermented than BSG (Figure 10, Glucans). As for soluble fibre, a slower delivery of β-glucan to the colon would allow a more complete fermentation, which was observed with both these malts, particularly Cinnamon malt. It may be speculated that slower delivery would also increase the fermentation of more resistant fibres (e.g. cellulose). The slightly higher fermentation in Cinnamon malt may be related to the comparatively higher molecular weight of the β-glucan arriving to the colon, as seen in the ileal delivery of the *in vitro* model (Figure 8B). Standard malt was an exception to the relation between degree of fermentation and molecular weight of β-glucan; although the β-glucan molecular weight was relatively high (1.0×10^6 g/mol) and had a slow transit time *in vitro*, the glucose-containing polymers were little fermented (9%). The reason for that is unclear, but may be explained by an underestimation of the fibre content of polymers of low molecular weight.

Arabinoxylan

Arabinoxylan was similarly fermented in all barley malt products (36–44%), despite the different solubility of the arabinoxylan (2.8–33%). The fermentability could be more related to the transit time, as it was also similar between the meals in the *in vitro* model (Figure 7). The identical fermentation of the arabinoxylan could also be due to the influence of arabinose substitutions or molecular weight, but this was not measured (Pastell, et al., 2009; Pollet, et al., 2012).

Formation of carboxylic acids

In Paper I, the contents of total and specific SCFAs (acetic, propionic and butyric acid) were higher in the caecal pool, distal colon and portal serum of rats fed malts (Caramelized, Colored, 50- and 350-malt) than those in control groups without malt, both in a low or high-fat setting.

Considerably lower amounts of SCFAs were found in the caecum of rats in Paper V than in Paper I (< 78 vs 83–343 µmol) and compared to data from similar studies (Zhong, et al., 2015b). The reason might be due to, for practical reasons, the diets having been removed from the rats more than 6 h before collection of the caecum content. The

SCFAs formed might therefore have been metabolised and regressed to fasting levels as they are rapidly absorbed by the mucosa (Schmitt, et al., 1976). Nevertheless, some differences in SCFA levels between groups were still observed. Thus, total SCFAs in the caecum of rats fed the highly fermentable AX and BG extracts were slightly higher than in those fed the barley malt products. Rats fed Cinnamon malt tended to have higher amounts of total SCFAs than rats fed the other barley malt products. As a result, the degree of fermentation of total fibre was higher, particularly glucose-containing polymers (Paper V).

Butyric acid

Despite that Caramelized, Colored, 50-malt and 350-malt had different content of β-glucan (0.6–1.2g/100g), the contribution of β-glucan to the diet was similar, which may explain the similar formation of SCFA in the caecum of rats (Paper I). Interestingly, some tendencies could be observed due to differences in molecular weight. Malts with lower average and more widely distributed β-glucan molecular weight (Colored and 50-malt) had a higher amount of butyric acid along the hindgut of rats. A high β-glucan content has been associated with a high formation of butyric acid (Berggren, et al., 1993), but the increase of butyric acid formation along the whole colon due to a wider distribution of the molecular weight has not previously been reported. However, due to the limitation in quantifying dietary fibre of low molecular weight, it is not known whether the diets also contained undetected dietary fibre. Thus, in this case, the higher concentration of butyric acid might simply be due to a higher intake of dietary fibre, instead of an effect of the malting on the β-glucan molecular weight. Furthermore, despite the belief that arabinoxylans are quite stable during malting (Voragen, et al., 1987a), it has to be pointed out that some arabinoxylo-oligosaccharides not taken into account in the fibre analysis could have contributed to the butyric acid production (Damen, et al., 2011).

Cinnamon malt and BSG diets resulted in higher amounts of butyric acid in the caecum of rats (7.3 and 6.7 µmol) than those fed control diets (3.4 µmol), but no strong relation was found between butyric acid and any specific fibre component (Paper V). Cinnamon malt contributed to a diet with a high β-glucan content and high molecular weight as well as soluble fibre and soluble arabinoxylan, whereas BSG diet was low in these compounds but high in insoluble arabinoxylan. A slight increase in butyric acid with both Cinnamon malt and BSG indicates that the contribution of butyric acid could be derived from different types of fibre and through different pathways of fermentation. Accordingly, previous studies have shown that both β-glucan (Henningsson, 2002) and water un-extractable arabinoxylan (Damen, et al., 2011) increased the formation of butyric acid. Additionally, a mixture of fibre sources could have a synergetic effect on the formation of SCFAs (Henningsson, et al., 2002).

Propionic acid

Caramelized malt resulted in a lower concentration of propionic acid in the distal colon and portal serum of rats than 50- and 350-malt (Paper I). This was attributed to the higher amounts of CML and CEL in Caramelized malt, which may affect the microbiota producing propionic acid (Mills, et al., 2008), but could also be related to the slightly lower daily feed intake in rats fed this malt.

The formation of propionic acid seemed to be associated with the intake of soluble arabinoxylan. Thus, the proportion of propionic acid was somewhat higher in the caecum of rats fed Cinnamon malt than BSG (17 vs 14% of total SCFA) (Paper IV), and the AX extract also resulted in higher amounts of caecal propionic acid than the other diets (53 vs 25-44 µmol) (Paper V). On the other hand, propionic acid was negatively associated with the intake of total arabinoxylan (Paper IV). An increase of propionic acid was also found in previous studies with wheat fractions rich in water-extractable arabinoxylan (Haskå, et al., 2011) and soluble corn bran arabinoxylan (Lopez, et al., 1999).

Succinic acid

Succinic acid was higher in rats fed 50-malt and 350-malt than in those fed low-fat control diets (4.5–6.4 µmol vs 0.3 µmol, $p < 0.05$) (Paper I). Succinic acid is a precursor of butyric and propionic acid, and its presence could be an indication of low microbial activity, as high amounts have been found after antibiotic treatment (Berggren, 1996). A low microbial activity might also be caused by a high-fat diet, as the succinic acid formation increases considerably with these types of diets (Jakobsdottir, et al., 2013; Mallett, et al., 1984). This carboxylic acid has been found to inhibit colonic mucosa proliferation (Inagaki, et al., 2007) and would therefore not be suitable in the case of IBS patients as their mucosa is compromised. Interestingly, succinic acid was lower with barley malt products compared with diets based on whole-grain barley (1–7 µmol vs 61–94 µmol) (Zhong, et al., 2015a).

Gene expression

Toll-like receptors (TLR) are receptor proteins in leukocytes involved in initiation of pro- and anti-inflammatory genes regulating the adaptive immune responses (Cario, 2010). Their expression increases during inflammatory responses, but overexpression may impair the mucosa homeostasis and trigger or perpetuate inflammatory bowel disease in the intestine (Kim, et al., 2012). Occludin and zonula occludens-1 (ZO-1) are tight junction proteins involved in the control of the passive diffusion of molecules passing through the epithelial cell layers (Furuse, et al., 1993; Van Itallie, et al., 2009). The barrier function can be improved by the expression of these proteins (McCarthy, et al., 1996), but their overexpression can also increase permeability (Balda, et al., 1996).

In Paper I, rats fed high-fat diets had an upregulated expression of occludin, ZO-1 and TLR-3 in the small intestine and distal colon, and of TLR-4 in the distal colon (Paper I). The intake of malts decreased or tended to decrease their expression to values similar to those in rats fed low-fat diets. This was possibly due to an increased formation of butyric acid, as this SCFA was negatively associated with the expression of TLR in the small intestine and distal colon, and with tight junction proteins in the distal colon ($r < -0.4$), which in turn has been shown to improve gut permeability (Peng, et al., 2009). One exception was the increased occludin levels in the small intestine of rats fed Caramelized malt (Paper I). This was perhaps a consequence of a high content of AGE (CML and CEL) in this malt, as AGE products have been known to bind with cell surface receptors or proteins, affecting their structure and function (Eble, et al., 1983; Ott, et al., 2014).

Microbiota

Diversity

Alpha diversity is a measure to determine the number of species or the relative abundance of various bacteria groups within the same subject. A rich microbiota is usually positively associated with the overall health status of the host, whereas a low alpha diversity has been linked to obesity, Crohn's disease and ulcerative colitis (Ley, et al., 2005; Ley, et al., 2006; Qin, et al., 2010; Turnbaugh, et al., 2009). Rats fed barley malt products (malts and BSG) had a higher alpha diversity than those fed the two barley extracts (AX and BG) or the control diet (Paper V). This was probably due to a higher complexity of the substrate properties in the malt products promoting the growth of a higher diversity of microbiota species, compared with the substrates in each extract, which were composed mostly of arabinoxylan or β-glucan. In this respect, the efficacy of some commercial pure fibre extracts advertised for weight loss (e.g. glucomannan, guar gum and chitosan) might also be questioned in case they result in low alpha-diversity (Pittler & Ernst, 2004).

Beta-diversity compares microbiota dissimilarities between subjects, taking into account abundancy and/or phylogeny. Notably, beta-diversity was more similar among rats fed the malt products compared with those fed the two extracts, among which considerable differences were also exhibited. Among the malt products BSG, Standard malt and Cinnamon malt all had different beta-diversity, probably dependent on the content of soluble arabinoxylan. Thus, the higher the content of soluble arabinoxylan in the products, the more similar was the beta-diversity to the AX extract.

The microbiota of rats fed Tipple malt, BSG and Mixture (Tipple malt and BSG, 70:30) was similar at the phylum level, but not within the phylum *Firmicutes*. Rats fed Tipple malt had more *Clostridium*, *Blautia*, *rc4-4* and *Coprobacillus* than those fed BSG, which had a greater abundance of *Ruminococcus* (f. *Lachnospiraceae*). Mixture diet

containing both Tipple malt and BSG, caused an intermediary abundance of most taxa, which was also reflected in the formation of SCFAs, whereas the alpha and beta diversity was more similar to the Tipple malt. This shows the possibility of using BSG together with malt as a food ingredient, for modulation of the microbiota.

Substrate preference

The microbiota composition was more influenced by the different substrates than the caecal formation of SCFAs, most probably due to the effects of the diet on the microbiota remaining for a longer time. As the barley malt products with a high β-glucan content also had a high β-glucan molecular weight, which in some cases coincided with a high content of soluble arabinoxylan, it was difficult to differentiate individual effects of the different polymers on the growth of specific taxa. The inclusion of the AX and BG extracts from barley was useful for reaching a better understanding of the substrate preferences for some bacteria. However, it has to be taken into account that the arabinoxylan and β-glucan present in the extracts might have different physico-chemical properties and do therefore not reflect exactly the function of these fibre components in the barley malt products. For example, the degree of polymerisation and substitution on the xylose chain may differ and can largely affect the production of SCFAs, and thus probably the bacterial growth (Pastell, et al., 2009; Pollet, et al., 2012; Van Craeyveld, et al., 2008; Wilczak, et al., 2015). Furthermore, the β-glucans in BG extract had a higher molecular weight and were more narrowly distributed than those in the barley malt products (1.6 vs $<1.4 \times 10^6$ g/mol). The arabinoxylans in AX extract, according to the supplier, had a high molecular weight (7×10^4 g/mol) and they were also highly soluble (76%), while the molecular weight of arabinoxylans in the malt products may be of different size and the soluble proportion was considerably lower (<10%). The molecular weight of the arabinoxylans was not analysed, therefore it is not possible to make any clear inference about this effect. Some taxa connected to health (for example, *Akkermansia* and *Blautia)* were not as abundant in the caecum of rats fed barley malt products as with the extracts, most probably due to the lower amount of the specific substrate.

Arabinoxylan. *Rikenellaceae* family, *S24-7* family, *Clostridiales* order, *Ruminococcus*, *Odoribacter* and *Oscillospira* appeared to grow better in the presence of insoluble arabinoxylan (Figure 11), and were more abundant in the caecum of rats fed malt products high in total arabinoxylan (Tipple malt, Standard malt, BSG and Mixture) than in Cinnamon malt. This is notable since most of these bacteria have been related with the formation of butyric acid (Evans, et al., 2014). Some taxa positively related with both the content of soluble arabinoxylan in barley malt products and the propionic acid formed were *Clostridium*, *Lachnospiraceae* family and *Blautia*. These taxa have also been associated with butyric acid formation (Evans, et al., 2014), with *Blautia* also having been associated with acetic acid (Lecomte, et al., 2015). However, it should be observed that *Blautia* was more abundant in rats fed the BG extract than AX extract,

indicating that this genus might also grow well in the presence of β-glucan. On the other hand, the *Lachnospiraceae* family was more abundant in rats fed AX extract than those fed BG extract.
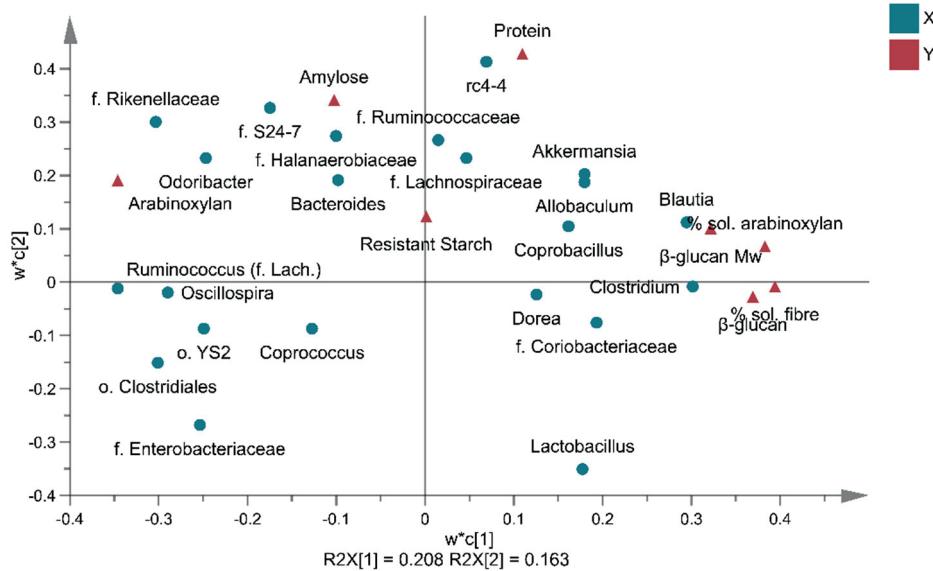

Figure 11
Loading plot of partial least squares (PLS) analysis. Relation between the components in the diet (X) and the taxonomic groups in the caecum (Y) of rats fed Tipple malt, Cinnamon malt, Standard malt, BSG and Mixture diets.

Another bacterium of interest is *Akkermansia*. Low abundance of *Akkermansia muciniphila*, the only known species of the *Akkermansia* genus, has previously been associated with ulcerative colitis and obesity (Rajilić-Stojanović & de Vos, 2014; Schneeberger, et al., 2015). However, *Akkermansia* abundancy was very similar in rats fed the four commercial malts in a high-fat diet (compared to the control diets (Paper I). Similar findings were found in rats fed malt products in low-fat diets (Paper V). However, Tipple, Cinnamon and Standard malts, which contained higher amounts of soluble arabinoxylan than BSG and Mixture, had slightly higher caecal abundance of *Akkermansia* (> 0.66% vs < 0.26%) (Paper V). Furthermore, high abundance of these bacteria was also found in rats fed AX extract (7%), and it therefore seems that the growth of *Akkermansia* is associated with the amount of soluble arabinoxylan in the diet. Interestingly, propionic acid was also higher in the caecum of rats fed AX extract, which is in agreement with previous studies with water-extractable arabinoxylan from both wheat and corn (Haskå, et al., 2011; Lopez, et al., 1999).

A higher ratio of *Firmicutes*/*Bacteroidetes* has been found in obese mice (Ley, et al., 2005) and in humans (Ley, et al., 2006; Mariat, et al., 2009), compared with lean

subjects. At the phylum level, the intake of total arabinoxylan, but not soluble arabinoxylan, was correlated with the increase of *Bacteroidetes,* whereas the intake of soluble fibre was correlated with *Firmicutes*. This finding may explain why rats fed Cinnamon malt had more *Firmicutes* and less *Bacteroidetes* than those fed Standard malt.

β-glucan*.* The growth of some taxa, such as *Blautia*, *Clostridium, Lactobacillus* and *Akkermansia,* was related with the intake of soluble fibres, soluble arabinoxylan, β-glucan or β-glucan molecular weight. The potentially butyrogenic *Blautia* and *Allobaculum*, and *Lactobacillus* seemed to prefer β-glucan as a substrate (Figure 11). Different types of *Lactobacillus* are often added as probiotic bacteria to different food products and therefore substrates that increase the abundance of these types of bacteria in the colon would be considered beneficial. *Lactobacillus* ferment carbohydrates into lactic acid, which is one precursor of butyric acid formation. This genus was more abundant in rats fed Cinnamon malt than Standard malt, and also in those fed BG extract compared to AX extract, suggesting a positive relation between the intake of β-glucan and *Lactobacillus* (Paper V). However, *Lactobacillus* was also abundant in rats fed BSG diet, which had the lowest content of extractable β-glucan and of low molecular weight. These results suggest that β-glucan oligosaccharides and/or other substrates might explain the high *Lactobacillus* abundance. Previous results showed that some *Lactobacillus* grow in the presence of β-glucan oligosaccharides, but are unable to be cultivated on the polymeric form alone (Crittenden, et al., 2002). Taking this into account, in rats fed Cinnamon malt, the *Lactobacillus* could utilise β-glucans depolymerised by other bacteria, through a cross-feeding mechanism. Small-sized β-glucans, which are predominant in the soluble fraction of BSG although in rather low amounts, could have been utilised by *Lactobacillus*. Another reason for the high level of *Lactobacillus* may be their capability to grow at the expense of other bacteria products or substrates. Some strains utilise not only glucose, but also mannose, xylose and galactose (Iyer, et al., 2000). Interestingly, the growth of *Lactobacillus* was correlated with the degree of fermentation of mannose-containing polymers (r>0.3), but not with any other fibre polymer. The higher contribution of *Lactobacillus* in rats fed Cinnamon malt and BSG diets might explain the slightly higher formation of butyric acid.

Conclusions

The malting process can be adapted to modulate fibre functional properties in barley, and in this way increase the potential of malt to improve intestinal health. The amount of soluble fibre and arabinoxylan as well as the β-glucan content and molecular weight affected the SCFA formation, mucosal barrier function and microbiota composition. The specific conclusions from the findings of the present work are summarised below.

- Barley malt increased formation of butyric and propionic acid in caecum and portal blood of rats compared with a control diet (cellulose). A broader molecular weight seemed to increase the caecal SCFA formation.

- Barley malts improved mucosal barrier function and inflammatory state in rats by reducing overly expressed tight junction proteins and toll-like receptors. However, malt with a high content of advanced glycation end-products attenuated the effects on occludin in the small intestine.

- By steeping barley at 35°C with lactic acid (0.4%) rather than steeping at traditional conditions (15°C without lactic acid), a malt with a higher content of β-glucan and soluble fibre was obtained. However, the content of soluble arabinoxylan decreased at these conditions, and total arabinoxylan, protein and iron were unaffected.

- The intrinsic structure of the cell wall is related to the barley variety, and was an important factor for modulation of the fibre components.

- Barley variety and steeping conditions had more pronounced effects on the β-glucan molecular weight distribution than mashing (production of BSG from malt).

- β-glucan molecular weight decreased after *in vitro* digestion of barley malt products and after addition of proteolytic enzymes. This suggested that proteins and β-glucan are closely related in the cell-wall matrix, which might result in an apparently higher molecular weight of β-glucan.

- The *in vitro* transit time of soluble fibre and β-glucan was slower with malts containing higher amounts of soluble fibre and β-glucan of high molecular weight (steeped at 35°C and 0.4% lactic acid) than with malt products lower in those components (Standard malt and BSG). The slower transit time was also associated with a higher degree of fermentation *in vivo*.

- Rats fed barley malt products had a more diverse microbiota composition compared with those fed barley extracts rich in arabinoxylan or β-glucan. This could be due to the higher complexity of the fibre, both concerning composition and the structural arrangement in the malt products compared with the extracts.

- Soluble arabinoxylan in the malt seemed to favour the growth of the propiogenic *Akkermansia*, whereas β-glucan increased the abundance of *Lactobacillus* and *Blautia*. Some butyrogenic bacteria were also associated with insoluble arabinoxylan. By mixing BSG with malt it was possible to modulate the SCFA formation, microbiota composition and diversity into an intermediary composition.

Future perspectives

A number of questions have arisen and in future research it could be interesting to explore other malting parameters, with the purpose of modulating β-glucan molecular weight or reducing phytate content, by using increased germination or by adding β-glucanases or phytases.

Analysis of arabinoxylan molecular weight and content of arabinoxylan-oligosaccharides in the barley products would enable further understanding of the effect of arabinoxylan on the formation of SCFAs and microbiota composition.

To investigate whether the formation of gut metabolites is affected by the phytochemicals in barley products. For example, ferulic acid is covalently bound to arabinoxylan and has been reported to have anti-inflammatory effects.

To analyse other gut metabolites present in the colon, such as secondary bile acids and ammonia that may have negative effects on the barrier function.

To use methodologies for fibre quantification that include fibres of low molecular weight. This would clarify the effect of low molecular weight fibres (β-glucans and arabinoxylans) and whether some dietary fibres are undetected.

To analyse SCFAs along the colon and their ability to reduce inflammation.

To further process BSG in order to increase formation of SCFAs (e.g. increase solubility of arabinoxylan content).

To develop a quantitative methodology of glutamine in order to understand the contribution of this amino acid in the gut barrier function. Barley protein is rich in glutamine, which is one of the major energy sources for the colonic epithelial cells. However, adequate methodologies are missing.

To explore the effect of barley malts in order to prevent or reduce inflammation (intestinal bowel diseases) *in vivo*.

To verify the effect of barley malt products in humans.

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