# Impact of Cereal Food Structures on Metabolic Effects and Satiety

## The Role of Processing and Product Formulation

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Cover: From crop to food to its effect on the human body. Note: the effects depicted are not necessarily associated with the depicted foods.

(Illustration by Daniel Johansson)

## Impact of Cereal Food Structures on Metabolic Responses and Satiety. The Role of Processing and Product Formulation

### Abstract

Insulin resistance, high blood glucose and raised total blood cholesterol are major risk factors for cardiovascular disease and type 2 diabetes. Partly they are caused by overweight and obesity. Diet is an important modifiable determinant for these risk factors and understanding how food characteristics, *e.g.* composition and structure, influence appetite and metabolic responses might aid in the development of healthy foods.

In this thesis I examined how formulation, processing techniques (baking, cooking, extrusion) and process parameters affect rye, oat and wheat food characteristics (including structure and dietary fibre composition) and the implications of this for appetite and metabolic responses in humans. In vitro digestion methods were used to study structural changes, gastric disintegration, digesta viscosity and glucose release. In human trials, the effect of aeration method (fermentation or whipping) in rye crispbread production on appetite and metabolic responses was evaluated.

When inulin was added to rye porridge, available water was reduced, decreasing starch gelatinisation and viscosity, changes with potentially counteracting effects on glucose response in humans. Smaller oat bran particles in bread increased degradation of β-glucan during fermentation, but also increased their solubility, events with opposing effects on digesta viscosity. Increased viscosity is an underlying mechanism for the ability of β-glucans, and certain other dietary fibres, to lower glucose response and total blood cholesterol. In fermented rye crispbread viscous fibres were more degraded and postprandial insulin response higher when compared with unfermented rye crispbread. However, the effect could also be attributed to slower gastric disintegration for the unfermented rye crispbread. Choice of aeration method had no observable impact on appetite. An amylose layer surrounding starch granules was observed only in sourdough-fermented soft rye bread. This seemed to inhibit glucose release, despite degraded viscous dietary fibres and rapid gastric disintegration.

To conclude, processing and formulation were found to generally affect more than one food characteristic, which might be of relevance for a specific metabolic response. The net effect of characteristics associated with a targeted metabolic response should be considered when designing or selecting foods intended to promote health.

*Keywords:* rye, oats, food microstructure, *in vitro* digestion, appetite, glucose, insulin

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*Sluta aldrig lägga av att låta bli.*
    Emil Jensen

# Contents

# List of Publications

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

I   Daniel P Johansson, José L Vázquez Gutiérrez, Rikard Landberg, Marie Alminger, Maud Langton. Impact of food processing on rye product properties and their in vitro digestion. Submitted.

II  José L.Vázquez-Gutiérrez, Daniel Johansson, Maud Langton (2016). Effects of added inulin and wheat gluten on structure of rye porridge. *LWT-Food Science and Technology* 66, 211-216.

III Daniel P Johansson, Isabella Lee, Ulf Risérus, Maud Langton, Rikard Landberg (2015). Effects of Unfermented and Fermented Whole Grain Rye Crisp Breads Served as Part of a Standardized Breakfast, on Appetite and Postprandial Glucose and Insulin Responses: A Randomized Cross-over Trial. *PLoS ONE* 10(3).

IV  Galia Zamaratskaia, Daniel P Johansson, Matheus Antunes Junqueira, Linda Deissler, Maud Langton, Per M Hellström, Rikard Landberg. The role of sourdough fermentation for appetite and postprandial metabolic responses - A randomised cross-over trial with crispbread. Submitted.

V   Daniel P Johansson, Roger Andersson, Marie Alminger, Rikard Landberg, Maud Langton. Increased particle size of oat bran inhibits degradation and lowers extractability of β-glucan in sourdough bread (manuscript)

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The contribution of Daniel Johansson to the papers included in this thesis was as follows:

I  Designed the study together with supervisors. Performed experiments, statistical evaluation of the results and wrote the manuscript.

II  Designed the study together with co-authors. Contributed with textural and microstructural characterisation and statistical evaluation. Participated in writing and revising manuscript with co-authors.

III  Performed microstructural characterisation, evaluated the results and wrote the manuscript.

IV  Participated in conducting the human trial. Responsible for *in vitro* experiments and microstructural characterisation. Participated in writing of manuscript with co-authors.

V  Designed the study together with supervisors. Performed experiments, statistical evaluation of the results and wrote the manuscript.

# Abbreviations

| | |
|---|---|
| ANOVA | Analysis of variance |
| AUC | Area under the curve |
| CLSM | Confocal laser scanning microscopy |
| extR | Extruded whole grain rye |
| FAO | Food and Agriculture Organization |
| FITC | Fluorescein isothiocyanate |
| GIP | Gastric inhibitory polypeptide |
| GLP-1 | Glucagon-like peptide 1 |
| RCB | Yeast-fermented whole grain rye crispbread |
| RFP | Whole grain rye flake porridge |
| RP | Whole grain rye flour porridge |
| RVA | Rapid Visco Analyzer |
| sRB | Sourdough fermented whole grain rye bread |
| sRCB | Sourdough fermented whole grain rye crispbread |
| TIM | TNO Gastro-Intestinal Model |
| uRCB | Unfermented whole grain rye crispbread |
| WB | Refined wheat bread |
| WCB | Refined wheatcrisp bread |
| WHO | World Health Organization |

# 1 Introduction

Cardiovascular disease and type 2 diabetes are non-communicable diseases and major contributors to global deaths. In 2012 an estimated 17.5 million deaths were attributable to cardiovascular disease and 1.5 million to diabetes (WHO, 2015). Furthermore, in 2014 422 million people had diabetes, the majority being type 2 diabetes (WHO, 2016). Nonfatal complications related to cardiovascular disease and diabetes place a great economic burden on societies. Moreover, cardiovascular disease and diabetes are not issues limited to rich countries, instead being great challenges also for low and middle income countries (WHO, 2015, 2016). Major risk factors for development of type 2 diabetes or incidence of cardiovascular events are insulin resistance, elevated blood glucose, raised blood pressure and raised total blood cholesterol (Laakso & Kuusisto, 2014; Peters *et al*., 2016). Important causes of these risk factors are overweight and obesity (Martin-Rodriguez *et al*., 2015).

Diet and lifestyle are important modifiable determinants for non-communicable diseases and their associated risk factors (WHO, 2015). Elevated postprandial glucose and insulin responses, caused by rapidly digestible carbohydrates, have been indicated as factors linked to the development of type 2 diabetes and cardiovascular disease, although this has not been fully proven (Porte, 2001; Blaak *et al*., 2012). The energy imbalance caused by consumption of readily available energy-rich palatable foods and increasingly sedentary lifestyles is considered to be an important factor leading to overweight and obesity (Mitchell *et al*., 2011; Williams *et al*., 2015). One approach to meet these challenges is the development of healthy foods which elicit more beneficial responses and modifies risk factors (*e.g.* reduces postprandial glucose and insulin responses and total cholesterol) or reduces energy intake by increasing feelings of satiety (Van Kleef *et al*., 2012; Peters *et al*., 2016).

Cereal-based foods provide nearly two-thirds of the global dietary energy intake (Jones & Ejeta, 2016). The health effects associated with cereal foods vary depending on type of cereal and processing (Clark & Slavin, 2013; Tosh & Chu, 2015). In epidemiological studies, whole grain intake has been inversely associated with risk of cardiovascular diseases and type 2 diabetes, as well as lower body weight (Mellen *et al.*, 2008; Aune *et al.*, 2011, 2013; Pol *et al.*, 2013). The mechanisms have not been fully elucidated but have been suggested to be related to high content of dietary fibre and the presence of certain bioactive compounds in whole grain (Fardet, 2010). The content and characteristics of these, and other food constituents, vary between different cereal grains and food types, which can affect the postprandial responses, and may have implications for the outcomes observed in epidemiological studies (Clark & Slavin, 2013; Frølich *et al.*, 2013).

For the development of healthier foods, an increased understanding is needed of how different food constituents and processing techniques interact, altering food properties ultimately relevant for physiological responses in humans.

## 1.1   Cereals, food structures and health effects

Rye and oats are cereals that are mainly consumed as whole grain and have been associated with beneficial health outcomes (Clark & Slavin, 2013). Rye-based foods have been demonstrated to induce higher satiety and lower postprandial insulin response, with or without a corresponding decrease in glucose response, compared with refined wheat foods. Such effects have been attributed to structural features and fibre characteristics (Leinonen *et al.*, 1999; Juntunen *et al.*, 2003a, 2003b; Rosén *et al.*, 2009, 2011). Oat-based foods are rich in β-glucans, a fibre which has been shown to have beneficial effects in improving postprandial glycaemia and total cholesterol levels (Tosh, 2013; Whitehead *et al.*, 2014). Processing technique has been demonstrated to influence postprandial responses for both rye and oat foods (Rosén *et al.*, 2011; Tosh & Chu, 2015).

### 1.1.1  Production and consumption of rye, oats and wheat

Common wheat (*Triticum aestivum*) is the third largest cereal crop world-wide, with global production of 729 million tonnes in 2014 (FAOSTAT, 2016). It is also the largest primary commodity and provides approximately 19% of total available calories in humans (FAO, 2014). Although not as widely cultivated, with production of only 15.3 million tonnes in 2014, rye (*Secale cereal* L.) is an important crop in Eastern, Central and Northern Europe  (Åman *et al.*, 2010;
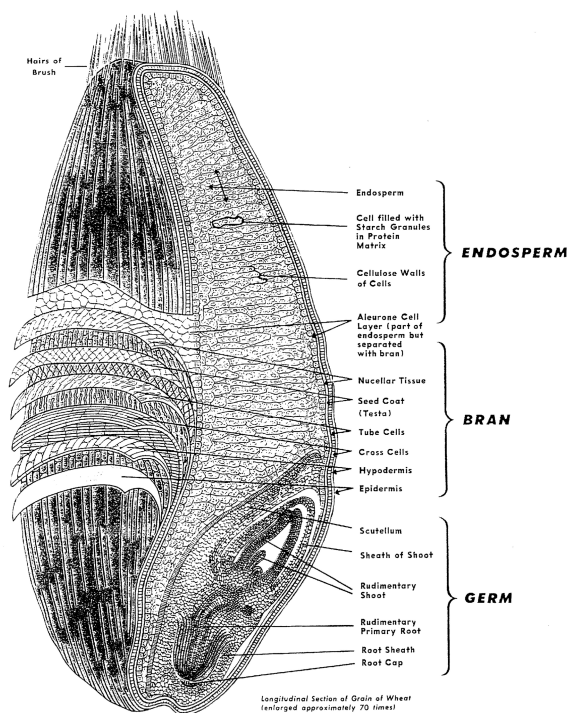
*Figure 1.* Cross section of a wheat kernel. From Slavin *et al* (2000), reproduced with permission from Taylor & Francis.

FAOSTAT, 2016) Production of oats (*Avena sativa* L.) is slightly higher than that of rye, with a total of 23 million tonnes in 2014 (FAOSTAT, 2016). However, as regards consumption of whole grains, rye and oats are more important, *e.g.* they were found to contribute between 20-70% and 6-19%, respectively of total whole grain intake in two large prospective cohort studies in Sweden and Denmark (Kyrø *et al.*, 2012). The definition of whole grain varies slightly in different regions but in Scandinavia it includes all parts of the naked cereal kernel (caryopsis) (Frølich *et al.*, 2013).

### 1.1.2 Kernel characteristics

The kernels (caryopsis) of rye, oats and wheat are all fairly similar in appearance with a pericarp (fruit coat) enclosing a seed consisting of the testa (seed coat), the endosperm and the germ (Figure 1). The pericarp and seed coat consist of several protective layers which are rich in nonstarch polysaccharides. The endosperm consists of the starchy endosperm, mainly starch and protein bodies enclosed in thin cell walls, and the aleurone layer, while germ is the embryo of the seed and is rich in vitamins, enzymes, antioxidants and

nonstarch polysaccharides. During milling, the aleurone is separated from the starchy endosperm together with the pericarp and seed coat, and this fraction is referred to as bran (Delcour & Hoseney, 2010).

*Starch*

Starch is the major component in rye, oats and wheat, constituting approximately 56 to 62 % wet basis, of the grain when consumed as whole grain (*Frølich et al*., 2013; Koehler & Wieser, 2013).

Starch is composed of two polysaccharides, short linear amylose and larger highly branched amylopectin, both based on glucose residues. The ratio of amylose to amylopectin varies between genotypes and species but can roughly be said to be 1:3 in cereals which are not of the waxy type (Eliasson & Larsson, 1993). Starch is synthesised into alternating amorphous and crystalline regions in granular form during the development of the seed (Delcour & Hoseney, 2010). The structure and size distribution of starch granules vary between the different cereal grains. In wheat and rye the size of granules follows a bimodal distribution with larger A-type granules, of average size 25 µm, and smaller B-type granules, of average size 7 µm (Stoddard, 1999). In wheat, a larger fraction of the starch is in the form of B-type granules than in rye. In oats, the granule size distribution is monomodal with diameters between 4 and 12 µm (Mäkelä & Laakso, 1984).

*Nonstarch polysaccharides/dietary fibre*

Cell walls in the starchy endosperm and the bran fraction contain large amounts of nonstarch polysaccharides such as cellulose, arabinoxylan, β-glucan and fructan. Nonstarch polysaccharides, together with resistant starch and resistant oligosaccharides, are currently included in the definition of dietary fibre in Europe (European Commission, 2008).

In wheat and rye, arabinoxylan constitutes a large fraction of the nonstarch polysaccharides, as it is the major component in the cell walls (Frølich *et al*., 2013). For whole grain wheat and rye, the arabinoxylan content has been reported to be around 5.6% and 8.9%, respectively (Andersson *et al*., 2013, 2009). In oats, β-glucan is the major component in the cell walls and the content in whole grain oats is typically around 2 to 6% (Cui & Wang, 2009).

Arabinoxylan consists of a linear backbone of (1→4)-β-D-xylopyranosyl residues substituted to varying degrees with mainly α-L-arabinofuranosyl residues (Knudsen & Laerke, 2010). Arabinoxylan can be divided into a water-extractable and a water water-unextractable fraction, which vary in their substitution degree and pattern. In different rye milling fractions, the weight-

average molecular weight of arabinoxylan is reported to be between 1,070,000 and 1,380,000 g/mol (Rakha *et al.*, 2010).

Cereal β-glucan is a linear polysaccharides consisting of β-D-glucopyranose units. In cereals, the structure is mainly in the form of blocks of three to four (1→4)-linked units connected with a (1→3)-linkage (Cui & Wang, 2009). The average molecular weight of β-glucan in oats is reported to be around 2,200,000 g/mol, while in rye it is reported to be between 860,000 and 1,350,000 g/mol (Rakha *et al.*, 2010; Åman *et al.*, 2004).

*Protein*

Protein content is fairly similar for rye, oats and wheat, with average content between 9 and 13%, although it can vary more, even between different varieties (Frølich *et al.*, 2013; Koehler & Wieser, 2013). Most of the proteins are located in the starchy endosperm although the proportion of protein is higher in the aleurone layer and the germ (Koehler & Wieser, 2013). However, it is the wheat proteins which are best known for their functionality in food processing. In wheat gliadin and glutenins constitute the major fraction of proteins. Together they form the composite protein gluten (Delcour *et al.*, 2012).

*Lipids*

The lipid content of wheat and rye is approximately 1.7 to 2.4% while in oats it is higher, around 7% (Frølich *et al.*, 2013; Koehler & Wieser, 2013). Oat kernels also have high content of lipase, which is set free when the kernels are crushed or milled into flour leading to oxidation of the free fatty acids and rancidity of the product. To extend the shelf-life of oats, they are usually heat-treated to inactivate enzymes (Klensporf & Jeleń, 2008).

### 1.1.3 Processing of cereal grains

Cereal grains are consumed in a wide variety of forms and consequently are subjected to a range of processing techniques after harvest. The initial processing typically includes cleaning, milling, soaking and grading. Subsequent processing, including baking, extrusion and cooking, leads to transformation of the different constituents such as starch, proteins and nonstarch polysaccharides (Delcour & Hoseney, 2010). Baking typically involves formation of a dough or batter by mixing flour and water, usually with inclusion of yeast (and for sourdoughs, lactic acid bacteria) for subsequent fermentation to give a foam structure (Delcour & Hoseney, 2010). Aeration of doughs and batters can also be achieved by including a chemical foaming agent or by incorporation of air through whipping. Extrusion is a continuous process

method where moistened cereals are mixed at high temperature and pressure in stationary barrel with a die at the end. When the cereal mixture is forced through the die at the end of the process, the sudden reduction in pressure and loss of moisture and heat can lead to expansion of the mixture. Extrusion is used to produce a range of food products including breakfast cereals (Singh *et al*., 2007).

*Starch*

In the presence of sufficient water and heat, starch granules undergo a process known as gelatinisation, during which the crystalline structure of the granules changes into a disordered structure. Water absorbed by the granules causes swelling and, if sufficiently swollen, the granules disrupt and starch (mainly amylose) leaks out (Langton & Hermansson, 1989; Copeland *et al*., 2009). In the case of shearing, granules may completely rupture and both amylose and amylopectin leak out (Svegmark & Hermansson, 1991). Gelatinisation of starch can also be achieved at low moisture content by using high temperatures, mechanical shear and pressure, *i.e.* extrusion (Singh *et al*., 2007).

Upon cooling and storage, partial recrystallisation occurs, a process known as retrogradation. For amylose this process occurs within minutes or hours while it is a slower process for amylopectin progressing over hours or days (Copeland *et al*., 2009). Retrogradation of amylopectin is reversible with heating while amylose can form resistant starch which is stable at temperatures up to 150°C (Eerlingen & Delcour, 1995). The formation of resistant starch can also be promoted by organic acids produced during sourdough fermentation (Liljeberg *et al*., 1996a).

*Nonstarch polysaccharides*

Cereal grain contains endogenous enzymes capable of degrading some of its components. These enzymes are mainly located in the bran fraction and the germ (Ballance *et al*., 1976; Vatandoust *et al*., 2012). Degradation of both β-glucan and arabinoxylan by endogenous enzymes takes place during certain phases of cereal processing, *e.g.* fermentation, due to elevated moisture content (Rakha *et al*., 2010, 2011; Isaksson *et al*., 2011). While β-glucan may be substantially degraded, arabinoxylan is more resistant due to its chemical structure (Karppinen *et al*., 2000). Furthermore, the arabinoxylan degrading enzymes are mainly active at elevated temperatures and lower pH (around 4.5) and arabinoxylan is generally not degraded to the same extent as β-glucan (Rasmussen *et al*., 2001). Maintained botanical integrity, *e.g.* whole cell structures, can inhibit the degradation by limiting access for the enzymes (Andersson *et al*., 2004; Åman *et al*., 2004). Furthermore, storage, especially in

frozen conditions, can reduce the solubility of β-glucans (Beer *et al*., 1997; Lan-Pidhainy *et al*., 2007).

*Protein*

Proteins may undergo transitions in their material properties during processing as a result of exposure to temperature and moisture (Roos, 2003). The nature of these transitions and the conditions in which they occur vary depending on protein. For wheat gluten, a transition from a glassy state to a rubbery viscoelastic material occurs when hydrated at room temperature (Delcour *et al*., 2012). This allows the formation of a cohesive viscoelastic network during kneading which stabilises the gas bubbles in wheat dough during fermentation. During baking, the irreversible formation of crosslinks in gluten contributes to setting of the structure (Lagrain *et al*., 2007). Proteins in rye and oats do not form stabilizing networks as wheat gluten does (Lásztity, 1995).

## 1.2   Food digestion

### 1.2.1  Food digestion *in vivo*

During digestion, the ingested food undergoes a number of mechanical, chemical and enzymatic steps of degradation to enable the absorption of nutrients. This process is initiated by mastication, where the food is disintegrated into smaller particles, lubricated by saliva and formed into a bolus to facilitate swallowing (Bornhorst & Singh, 2012). Furthermore, enzymes, mainly α-amylase, which initiates the enzymatic hydrolysis are secreted (Butterworth *et al*., 2011). The point of swallowing appears to be triggered by certain bolus properties, *e.g.* particle size (Peyron *et al*., 2011).

   The food bolus is then transported via the oesophagus to the stomach where peristaltic muscle contractions crush and fragment the solid food bolus. In the stomach, hydrochloric acid (HCl) and pepsinogen, which is converted into pepsin upon contact with the acid, are also secreted (Bornhorst & Singh, 2014). The mechanical grinding of food particles is a rate-limiting step in the emptying of solids into the small intestine and when they reach a size of approximately 1-2 mm or below, food is gradually emptied from the stomach (Hellström *et al*., 2006). Gastric emptying rate may also be affected by viscosity with more viscous digesta being emptied more slowly (Marciani *et al*., 2001).

   The small intestine is divided into three sections: duodenum, jejunum and ileum. In the duodenum, bile, digestive enzymes and bicarbonate, which neutralise the acid digesta from the stomach, are secreted. Bile emulsifies fat and facilitates its enzymatic digestion. Most of the nutrients are absorbed in the

jejunum, while bile and a few other compounds are absorbed in the ileum (Simon, 2014).

Nutrients that have not been digested and absorbed in the small intestine, mainly dietary fibre, can be fermented by the colonic microflora resulting in production of short chain fatty acids which can be absorbed in the colon (Simon, 2014).

### 1.2.2 Simulation of food digestion

Although human studies are naturally the most reliable method for establishing the effect of a food or diet on metabolic outcomes they are expensive and limited by ethical constraints. Moreover, drawing conclusions about mechanistic relationships and the influence of certain food properties can be complicated.

*In vitro* methods are less costly, easily repeatable and can be appropriate for mechanistic studies. They are also widely used to predict the behaviour of food components in the digestive tract by studying structural changes and release of nutrients (Mcclements *et al.*, 2009; Alminger *et al.*, 2012).

*In vitro* methods simulating different stages of digestion can broadly be divided into static and dynamic methods. In static methods, the food of interest is typically digested stepwise in beakers, with a specific retention time for each step. Simulated saliva and gastric and intestinal fluids with suitable digestive enzymes are added sequentially and the pH is adjusted and kept constant for each step of the digestion (Hur *et al.*, 2011). In dynamic models, factors such as flow and transportation of digesta as well as changes in pH and enzyme concentrations with time can also be simulated to more accurately represent the *in vivo* process (Minekus *et al.*, 1995; Guerra *et al.*, 2012; Ménard *et al.*, 2014).

## 1.3 *In vivo* responses

### 1.3.1 Appetite regulation

Appetite is a broad term which concerns all aspects of the drive or inhibition to eat. The regulation of appetite is a complex process which is controlled by both cognitive and physiological responses to environmental cues and food intake (Mela, 2006; Van Kleef *et al.*, 2012). The process can be divided into two different stages, satiation and satiety. Satiation is the intra-meal process which determines the termination of an eating session, while satiety is the process that occurs between meals (in the inter-meal period) and determines the onset of the next meal (Blundell *et al.*, 1988).

Before and during the ingestion of foods, sensory and cognitive signals, such as expectations, sight and the smell of food, as well as the oro-sensory

experience of the food during mastication, induce physiological responses preparing the body for digestion of the food (Smeets *et al*., 2010). These signals influence both the process of satiation and the feelings of satiety after the meal. Once the food reaches the stomach, it increases gastric distension and motility, contributing to satiation and satiety (Feinle-Bisset, 2015). In the small intestine, gut peptides, *e.g.* cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide-YY (PYY), are released in response to the absorption and sensing of nutrients. These peptides regulate gastric and intestinal motility and secretion of enzymes and bile, but are also associated with regulation of appetite (Delzenne *et al*., 2010). Lastly, there is increasing evidence that short-chain fatty acids produced during colonic fermentation may be involved in regulation of appetite and could be beneficial for maintaining energy homeostasis (Byrne *et al*., 2015).

### 1.3.2  Glucose regulation

Blood glucose concentration is controlled via hormonal and neural regulation of uptake in the liver and various tissues and by production of endogenous glucose in the liver (Moore *et al*., 2012; Grayson *et al*., 2013). Insulin, secreted in response to glucose, stimulates glucose uptake in skeletal muscle and adipose tissue and inhibits glucagon production, leading to reduced hepatic glucose production. The incretins GLP-1 and GIP, which are released in response to carbohydrate, fat and protein intake, potentiate insulin secretion and inhibit glucagon (Baggio & Drucker, 2007; Edholm *et al*., 2010).

The blood glucose concentration reflects the net result of absorption rate, endogenous production rate and rate of uptake in different tissues. It has been suggested that, when comparing responses to different foods, a large difference in glucose absorption can be observed as a difference in both postprandial glucose and insulin concentrations, while a smaller difference might only be observed as a difference in insulin concentrations (Eelderink *et al*., 2012a, 2012b).

## 1.4  Food components and characteristics in relation to digestion, metabolic responses and appetite

The digestive process of different foods strongly depends on their characteristics, such as texture, viscosity, structure, botanical integrity and composition, which are partly determined by the processing technique used. These characteristics will influence the subsequent *in vivo* responses in terms of perceived satiety and postprandial glucose and insulin. In this thesis the

focus is mainly on appetite and those responses related to postprandial glycaemia.

### 1.4.1 Viscosity, food form and botanical integrity

High food viscosity has been demonstrated to increase satiety and reduce postprandial glucose and insulin responses, effects attributed to prolonged oral exposure, reduced rate of gastric emptying and reduced diffusion rates in the small intestine (Marciani *et al*., 2000; de Wijk *et al*., 2008; Juvonen *et al*., 2009; Zhu *et al*., 2013b). However, this effect is likely to depend on the type of molecule inducing viscosity, *e.g.* starch or different nonstarch polysaccharides (Zijlstra *et al*., 2009; Wanders *et al*., 2013).

Different effects have been reported with regard to the effect of food form, *e.g.* solid, liquid and volume, on appetite and postprandial glucose and insulin responses. Increased incorporation of water in a food, *e.g.* by mixing solids with added water into a soup, can increase early feelings of satiety by effectively increasing its volume and gastric distension (Rolls *et al*., 1998, 1999; Delzenne *et al.,* 2010; Clegg *et al*., 2013; Zhu *et al*., 2013a). When water is not incorporated but added or served on the side, the effect of gastric distension might be rapidly lost due to liquids preferentially being emptied first (Khoo *et al*., 2009). On the other hand, preferential emptying of liquids can lead to higher glucose response for soups compared with their liquid-solid counterparts, as more nutrients will be present in the liquid phase and enter the small intestine earlier (Clegg *et al*., 2013). With similar volume, solid foods in general induce higher feelings of satiety than their liquid counterparts (Mourao *et al*., 2007; Flood-Obbagy & Rolls, 2009). The effects of different food forms on appetite responses can also be related to increased oro-sensory stimulation due to prolonged oral exposure with solid meals and larger meal volume (Wijk *et al*., 2009; Mattes & Considine, 2013).

For cereal-based foods, botanical integrity can affect appetite and postprandial glucose and insulin responses. Whole kernels compared with milled kernels in wheat bread and rye porridge have been observed to increase feelings of satiety (Hlebowicz *et al*., 2008; Isaksson *et al*., 2011). Lower postprandial glucose and insulin have also been demonstrated for increasing botanical integrity, but with effects observed also for smaller differences in particle size, *e.g.* coarse compared with fine flour (Heaton *et al*., 1988; Liljeberg *et al*., 1994; Behall *et al*., 1999; Edwards *et al*., 2015). These effects are most likely related to encapsulation of starch, resulting in a slower rate of enzymatic hydrolysis and more nutrients reaching the colon, possibly contributing to fermentation and late satiety (Mahasukhonthachat *et al*., 2010; Edwards *et al*., 2015).

### 1.4.2 Starch

In cereals, starch is the most important nutrient contributing to postprandial glucose responses (Blaak *et al*., 2012). One of the main factors determining the rate of digestion of starch is its degree of gelatinisation. More gelatinised starch generally undergoes more rapid enzymatic hydrolysis and induces higher postprandial glucose and insulin responses (Björck *et al*., 1984; Holm *et al*., 1988; Chung *et al*., 2008). Native starch and resistant starch formed by retrogradation of amylose might not be digested in the small intestine but rather by the colonic bacteria (Svihus & Hervik, 2016). Leaked amylose has also been proposed to form a protective layer of resistant starch around starch granules in rye bread (Juntunen *et al*., 2003).

Although solubilised starch can also induce viscosity, the digestion is initiated already during oral processing due to the presence of salivary α-amylase. Consequently, the effect of starch-induced viscosity might be limited in terms of inducing satiety (Zijlstra *et al*., 2009).

### 1.4.3 Dietary fibre

Dietary fibre can regulate appetite and metabolic responses in several ways, de pending on the properties of the fibre. Soluble viscous fibres such as arabinoxylan and β-glucan have been shown to have beneficial effects on appetite, postprandial glucose and insulin responses and total cholesterol levels (Wanders *et al*., 2011; Clark & Slavin, 2013; Whitehead *et al*., 2014).

Soluble fibres may increase the digesta viscosity and influence the diffusion rates of enzymes, nutrients and bile salts (Wood, 2007; Shelat *et al*., 2010, 2011). The cholesterol-lowering effect of soluble fibre is considered to be related to reduced reabsorption of bile salts, leading to increased production of bile salts from circulating cholesterol (Gunness & Gidley, 2010). Viscosity-attributed decreases in postprandial blood glucose concentrations have also been shown for foods supplemented with different types of dietary fibre (Ellis *et al*., 1995; Östman *et al*., 2006; Lightowler & Henry, 2009; Fabek *et al*., 2014). The viscosity induced by soluble dietary fibre is strongly related to its molecular weight and solubility. Maintaining these is therefore beneficial for reducing cholesterol and postprandial glucose responses (Wolever *et al*., 2010; Brummer *et al*., 2012).

Dietary fibre can also act as a barrier in botanically intact foods, thereby limiting the enzymatic availability of encapsulated nutrients which, may aid in reducing postprandial glucose and insulin responses (Grundy *et al*., 2016). Furthermore, gelling fibre, *e.g.* alginate, has been demonstrated to reduce energy intake in subsequent meals possibly due to increased oral exposure time (Wanders *et al*., 2013).

In the colon certain dietary fibres are fermented by the microflora resulting in production of short-chain fatty acids which may be beneficial for appetite regulation (Byrne *et al*., 2015). The susceptibility to fermentation varies between different types of dietary fibres. In general, more soluble fibres with simpler structures, *e.g.* β-glucan and inulin, are more easily fermented (Roberfroid, 1993; Karppinen *et al*., 2000; Slavin, 2013). Inulin, a short fructan with a degree of polymerisation between 2 and 60, is almost completely fermented in the colon (Roberfroid, 1993).

### 1.4.4  Lipids

Lipids were not the main focus in this thesis and are not extensively discussed here. However, they can modulate responses related to the consumption of starch, which might affect the absorption of glucose and postprandial glucose and insulin responses. Lipids have been suggested to efficiently trigger the ileal break, a feedback loop that reduces the gastric emptying rate in response to nutrients reaching the distal small intestine (Maljaars *et al*., 2008). Lipids may also influence postprandial responses due to interaction with starch and formation of complexes with amylose, decreasing its susceptibility to enzymatic hydrolysis (Copeland *et al*., 2009).

### 1.4.5  Protein

Protein is generally considered to be the macronutrient which most potently induces feelings of satiety, potentially by stimulating secretion of gut peptides and through direct stimulation of appetite centres in the brain by circulating amino acids (Veldhorst *et al*., 2008; Belza *et al*., 2013). Proteins may also influence the response to ingestion of digestible carbohydrates although the effect varies depending on protein source. This may be due to differences in the resulting profile of circulating amino acids, as *e.g.* branched chain amino acids are especially efficient in stimulating the secretion of insulin (Gannon & Nuttall, 2010). Protein has also been suggested to affect the digestion of other nutrients by encapsulation (Parada & Aguilera, 2011).

### 1.4.6  Fermentation products

During sourdough fermentation organic acids (lactic and acetic acid) are produced and are suggested to have a range of beneficial effects. These effects include reduced rate of gastric emptying and inhibition of starch hydrolysis, which could affect appetite and postprandial glucose and insulin responses (Liljeberg *et al*., 1995; Liljeberg & Björck, 1998; Östman *et al*., 2006).

# 2   Objectives

The overall aim of this thesis work was to determine the relationships between cereal food processing, composition and structural features and their effects on digestion and subsequent appetite and metabolic responses in humans.

Specific objectives were to investigate:

➢ The effect of processing techniques, *e.g.* fermentation and extrusion, on the structure, fibre characteristics and composition of rye foods (Papers I-IV)
➢ The impact of processing parameters on β-glucan characteristics in breads and the effect on *in vitro* digesta viscosity (Paper V)
➢ The effect of added dietary fibre, in the form of inulin and oat bran, on the structure and texture of rye porridge and wheat breads respectively (Papers II and V)
➢ The role of rye food characteristics in the disintegration of the food matrix, development of viscosity (Papers I, III and IV) and release of glucose (Paper I) during *in vitro* digestion
➢ The effect of different aeration techniques (yeast fermentation, sourdough fermentation and cold whipping) in production of rye crispbreads on satiety and postprandial glucose and insulin responses in humans and how this is related to the characteristics of the foods, *e.g.* structure and fibre composition (Papers III and IV).

# 3   Materials and methods

This section includes a brief description of the materials and methods used in Papers I-V. All the methodology is described in detail in the respective papers.

## 3.1   Food products

The refined wheat bread (WB) used in Paper I was a commercially available bread (Pågen AB, Sweden) and was included as a reference product. Refined wheat crispbread (WCB) was included as a reference in Papers III and IV. The crispbreads used in Papers I, III and IV were produced and supplied by Barilla (Barilla Sweden AB, Sweden).

The sourdough-fermented whole grain rye crispbread (sRCB) studied in Paper IV was made using whole grain rye flour, whole grain rye sourdough, yeast, barley malt, emulsifier, salt and water. The yeast-fermented whole grain rye crispbread (RCB) and unfermented whole grain rye crispbread (uRCB) used in Papers I, III and IV were made from whole grain rye flour, salt and water, with inclusion of yeast in RCB. Both sRCB and RCB were fermented for 120 min at 29 °C, followed by 35 min with an increase from 30 to 38 °C. uRCB was mixed with water at 12 °C and then whipped at 6 °C to incorporate air into the dough. The flour used was of the same origin for sRCB, RCB and uRCB but more coarsely milled for uRCB.

The flour used for RCB and sRCB was also used for production of the sourdough-fermented whole grain rye bread (sRB), extruded whole grain rye (extR) and whole grain rye porridge (RP) studied in Paper I. sRB was prepared from whole grain rye flour, salt, fresh yeast and commercial whole grain rye sourdough starter (Jästbolaget AB, Sweden) and fermented for a total of 70 min. extR was produced from whole grain rye flour and salt in a twin-screw extruder with a pressure of 3.9-4.4 bar and a temperature of 124 °C at the die. The expanded products were cut into breakfast cereal spheres with diameter 5-7 mm. RP was prepared by mixing 64 g whole grain rye flour and 0.58 g NaCl

with 200 g boiling water and stirring with a spoon for 2 min to ensure good mixing.

In Paper II, porridges were made from 50 g whole grain rye flakes or 40 g whole grain rye flakes with addition of a total 12 g of inulin and gluten in varying proportions. The ratios of inulin to gluten studied were 1:3, 1:1 and 3:1. The same amount of boiling water was added to all porridges. The porridges were also used in an intervention trial not included in this thesis. The purpose of the human trial was to investigate the effect of protein and easily fermentable dietary fibres on satiety and postprandial glucose and insulin responses (Lee, 2016).

In Paper V, wheat breads with added oat bran were made according to a $2^3$ factorial design with three centre points; 10, 20 or 30 % of finely milled, coarse, or mixed fine and coarse oat bran and fermentation times of 45, 60 and 75 min were used. Reference products in the form of two breads with β-glucan-rich (14 and 28 %) oat bran fractions and two extruded products (14 % high and low molecular weight β-glucan, respectively) were also included.

## 3.2   Food product characterisation

### 3.2.1  Chemical analysis

For the products used in Papers I, III and IV, fat, protein and amino acids were analysed by external laboratories, dietary fibre was analysed according to the Uppsala method (Theander *et al*., 1995) and β-glucan, fructan and resistant starch content were measured using Megazyme assay kits (Bray, Ireland). Molecular weight of β-glucan ($M_{cf}$) was determined using size exclusion chromatography with fluorescence detection (Rimsten *et al*., 2003). For the breads in Paper V, only β-glucan content and molecular weight were analysed. Dry matter content of the products used in Papers I and III-V was determined according to the AACC method 44-15A.

### 3.2.2  Textural characterisation

In Paper II the development of viscosity during porridge preparation was determined using a Rapid Visco Analyzer (RVA) (Newport Scientific Pvt. Ltd., Australia). Due to the large particles in the rye flake porridge, an RVA, mounted with an impeller, was used.

### 3.2.3  Microstructural characterisation

Microstructural characterisation was performed on the food products in all papers and on masticated and *in vitro* digested, static and dynamic samples in Papers I and IV. Cryosectioning was used for immunolabelled samples in

Papers I, III and IV and for all samples in Paper II. Remaining samples were embedded in plastic. Masticated and digested samples were embedded in agar prior to plastic embedding.

*Staining and labelling methods*

Iodine was used for visualisation of starch and protein, using brightfield microscopy, in all papers. It stains proteins yellow, starch purple or violet, amylose blue and amylopectin beige-brown (Autio *et al*., 2001).

Immunolabelling was used for visualisation of arabinoxylan and β-glucan in Papers I, III and IV and for β-glucan in Paper V. Immunolabelling utilises the specific binding capacity of antibodies to specific targets (antigen). With a suitable label, either on the antibody or on a secondary antibody, the target location can be detected using microscopy (Vázquez-Gutiérrez & Langton, 2015). Confocal laser scanning microscopy (CLSM) was used for visualisation of immunolabelled samples.

For visualisation of inulin in Paper II, inulin labelled with FITC (fluorescein isothiocyanate) was added in small proportions to the porridge. Texas red and Calcofluor white were also used, for visualisation of protein and cell walls respectively. Epifluorescence microscopy was used for imaging.

### 3.3 *In vitro* digestion of food products

Three different *in vitro* methods were used in this thesis work. The products included in Papers I, III and IV were subjected to a static method simulating gastric digestion. The products included in Paper I were also subjected to simulated digestion using the dynamic TNO Gastro Intestinal Model (TIM) (TNO, Zeist, Netherlands). A different static method was used for the oat bran breads in Paper V, for comparison with earlier work on oat β-glucans and viscosity. The *in vitro* methods are more thoroughly described in Papers I and V.

For simulated gastric digestion, we used an adaptation of the standardised static *in vitro* method proposed within the COST Infogest network (Minekus *et al*., 2014). The modifications included *in vivo* mastication by one individual, instead of using simulated saliva and mechanical mixing, and, to follow the development of viscosity during simulated gastric digestion, the use of an RVA with a modified cup. The latter change gave an indication of differences in rate of disintegration due to the interference with measurement caused by larger bolus fragments. Particle size distribution was determined after 2 h using wet sieving. Masticated and digested samples were collected for microstructural characterisation.

The dynamic TIM model developed by Minekus *et al*. (1995) was used for simulating digestion in the stomach and small intestine of the products in Paper I. This model consists of a series of compartments representing the stomach, duodenum, jejunum and ileum, where digestive fluids, enzymes and bile are secreted and pH continuously regulated. Products were masticated *in vivo* by one individual prior to *in vitro* digestion. The oral step was 'standardised' for each product and the same person performed all mastications. Glucose concentration was determined by collecting samples from the duodenal part of the model at 15, 30, 45, 60, 90, 120 and 180 min and using high performance anion exchange chromatography coupled with a pulsed amperometric detector to quantify glucose and maltose concentrations, which were recalculated to glucose. Samples collected at 60, 90 and 120 min from the duodenal compartment were used for microstructural characterisation.

In Paper V, an *in vitro* method simulating gastric and small intestinal digestion was used and combined with enzyme activities according to the protocol described by Tosh *et al*. (2010). The ratio of solid to liquid was increased due to the comparatively low β-glucan content in our study. Viscosity and the molecular weight and concentration of β-glucan in the resulting extract were measured.

## 3.4   Intervention trials

In Papers III and IV, single-blinded, randomised cross-over trials were conducted to investigate the effects of different fermentation methods in production of whole grain rye crispbreads on satiety and postprandial glucose and insulin responses in humans.

### 3.4.1  Study design

Healthy male and female participants between 18 and 70 years of age were recruited in Uppsala, Sweden. After an initial screening to ensure healthy participants, those who met the eligibility criteria (specified in Papers III and IV) were invited to participate in the study. Written informed consent was obtained from all participants and the study was approved by the regional ethics board in Uppsala.

In both studies, the crispbreads were served as part of complete isocaloric breakfasts providing in total 45 g of available carbohydrates. The breakfasts consisted of the test product served with margarine, cheese, a glass of juice and a cup of coffee or tea (Table 1).

Table 1. *Food products and nutrient composition of the breakfasts used in Papers III and IV. For WCB and uRCB, composition is given as the mean of values determined in Papers III and IV. 150 mL coffee or tea was included in all meals. WCB: refined wheat crispbread. RCB: yeast-fermented whole grain rye crispbread. sRCB: sourdough-fermented whole grain rye crispbread. uRCB: unfermented whole grain rye crispbread.*

| | Amount | Nutrient (g)/energy (kJ) per portion | | | | |
|---|---|---|---|---|---|---|
| | (g) | Fat | Protein | CHO | Fibre[1] | Energy[2] |
| *Crispbread* | | | | | | |
| WCB | 52 | 4.1 | 6.4 | 34.9 | 2.9 | 874 |
| RCB | 60 | 1.3 | 5.5 | 38 | 10.2 | 867 |
| sRCB | 59.4 | 1.1 | 5.1 | 36.2 | 9.5 | 821 |
| uRCB | 59 | 1.1 | 5.7 | 35.9 | 11.5 | 837 |
| | | | | | | |
| Margarine[3] | 12/15 | 4.7/5.9 | 0.1 | 0.2 | 0 | 177/222 |
| Cheese | 20 | 5.8 | 4.8 | 0 | 0 | 296 |
| Orange juice | 100 | 0.1 | 0.7 | 9 | 0.7 | 174 |

[1]Fibre content as analysed by the Uppsala method with inclusion of fructans.

[2] Energy content calculated using a conversion factor of 37 kJ/g for fat, 17 kJ/g for proteins and CHO and 8 kJ/g for fibre.

[3]15 g margarine was used in all meals except for WCB in Paper IV, where 12 g was used.

Participants were given instructions regarding physical activity and diet to adhere to on the day before each study visit. On the test days, participants arrived in a fasting state at the clinic, where they were served breakfast. They then remained at the clinic for 4 h after breakfast. During this time, they recorded appetite and blood samples were collected. In Paper III the study visits ended after 4 h, while in Paper IV participants were allowed to leave the clinic but asked to continue recording appetite for a further two hours.

### 3.4.2 Appetite ratings

Subjective appetite ratings were recorded before breakfast, at breakfast and at 30, 60, 90, 120, 150, 180, 210, and 240 min after breakfast in Paper III and further at 270, 300, 330 and 360 min in Paper IV, using an electronic visual analogue scale (VAS). At each time point, participants answered three questions: "How hungry do you feel right now?", "How full do you feel right now?" and "How strong is your desire to eat right now?".

### 3.4.3 Physiological parameters

Venous blood was collected by trained nurses 15 minutes before breakfast and at 15, 35, 65, 95, 125, 185 and 230 minutes after breakfast. Analyses of serum insulin and plasma glucose concentrations were performed by established

routine methods at the certified laboratory of the Department of Clinical Chemistry at Uppsala University Hospital. GLP-1 was analysed by ELISA using the EZFABP3-38K Human FABP3 ELISA Kit from Merck Millipore (Darmstadt, Germany).

## 3.5   Statistical evaluation

SAS version 9.3 or 9.4 (SAS Institute Inc. Cary, NC, USA) was used for statistical analysis in all papers and P<0.05 was considered statistically significant for all tests, except for removal of interaction terms (P>0.10).

Mixed effect models, PROC mixed, suitable for repeated measures and cross-over designs, were used for determining differences between products or treatments in Papers I-IV. In Paper I, the model was used for calculating glucose profile and area under the curve (AUC) for the glucose profile. In Paper II the model was used for evaluating differences in development of viscosity. In Papers III and IV it was used for response variables, *i.e.* profile and AUC of satiety, postprandial glucose and insulin responses, while it was also used for determining GLP-1 in Paper IV. For comparisons at specific time points, where the time x treatment interaction was significant in the model, t-tests with adjustments for multiple testing (Bonferroni or Tukey's honest significance test) were used.

Differences between products with regard to viscosity after and disintegration during simulated gastric digestion in Papers I and IV were evaluated with one-way ANOVA and pair-wise comparisons were made using Tukey's honest significance test.

In Paper V, multiple linear regression was used to estimate the effects of the factors included in the design. Linear regression was also used to model the relationship between logarithmically transformed viscosity and molecular weight x concentration of β-glucan in solution and to estimate the viscosity required for a cholesterol-lowering effect.

# 4 Results and discussion

In this section the results are presented and discussed, starting with the effect of processing on product characteristics. This is followed by the results from the *in vitro* digestion and how they related to the product characteristics. Lastly, the results from the human trials are linked back to the product characteristics and the *in vitro* results.

## 4.1 Processing and effect on product characteristics

In Papers I, III, IV and V, different processing techniques were used to produce a range of food products with varying structural characteristics.

### 4.1.1 Characteristics of the starch/protein matrix

Raw material selection and processing drastically influenced the structural features of the starch protein matrix in cereal products included in Papers I, III, and IV. In Figure 2, an extensive protein network holding the starch granules together can be observed in the wheat products (WB and WCB). The presence of gluten in wheat is well recognised for its ability to form extensive networks in doughs and stabilising bubbles during fermentation and baking of breads (Delcour *et al*., 2012). The aerated rye products (sRB, sRCB, RCB, uRCB and extR), on the other hand, were characterised by a continuous starch phase with different structural appearance (Figure 2, for sRCB see paper IV). The starch phases of the fermented rye crispbreads (RCB, sRCB) appeared fairly similar, while for uRCB starch granules were generally harder to distinguish and leakage of amylose was more prevalent, indicating more gelatinized starch in uRCB. In uRCB, more intact cell structures were visible, due to the coarser flour used compared with the other rye products. In extR, the extrusion process led to complete disruption of the starch granules and formation of a homogeneous starch phase.
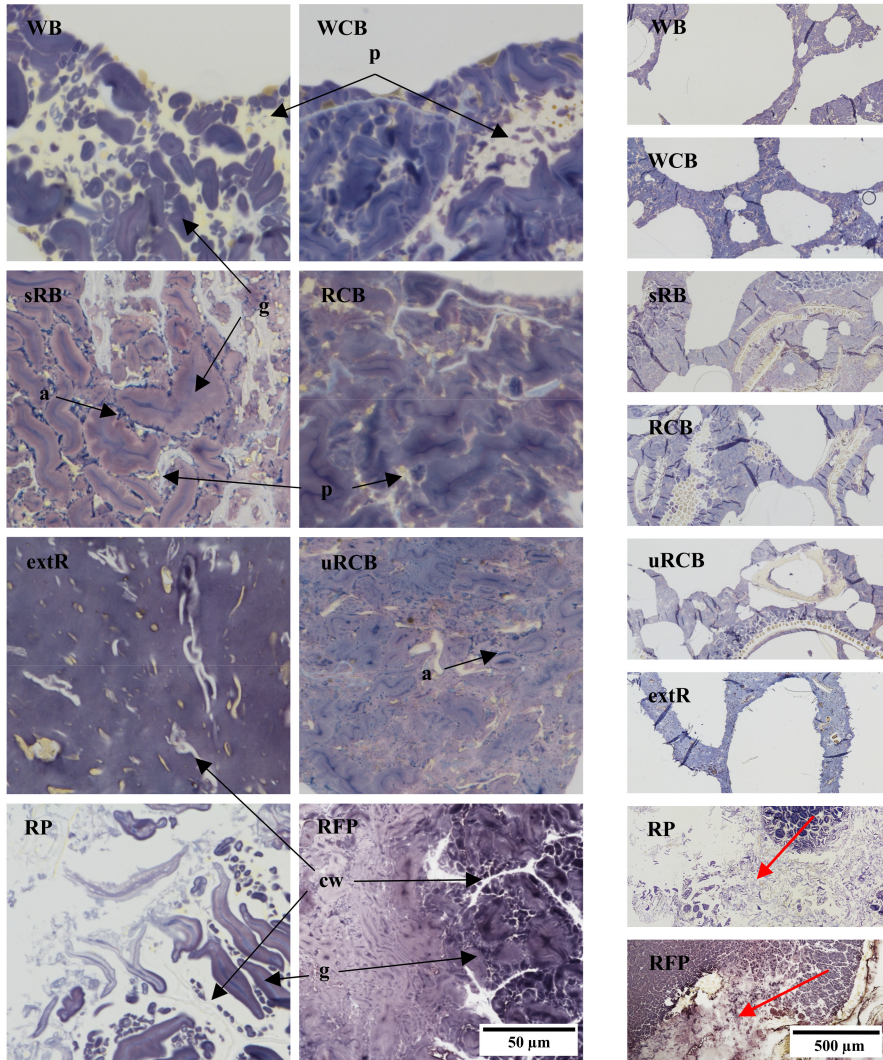
*Figure 2.* Light microscopy micrographs stained with iodine showing the microstructure of products at two different magnifications. Protein (p) is stained yellow, starch (s) purple, amylopectin brown and amylose (a) blue. Cell walls (cw) are unstained, but can be seen in the starch/protein matrix. Arrow (d) indicates transition from intact structure to continuous dilute starch phase in the porridges. WB: refined wheat bread. WCB: refined wheat crispbread. sRB: sourdough-fermented whole grain rye bread. RCB: yeast-fermented whole grain rye crispbread. uRCB: unfermented whole grain rye crispbread. extR: extruded whole grain rye. RP: whole grain rye porridge. RFP: rye flake porridge.

Table 2. *Composition of the products used in Papers I, III and IV, expressed as g/100g dry matter, WB: refined wheat bread. WCB: refined wheat crispbread. sRB: sourdough-fermented whole grain rye bread. RCB: yeast-fermented whole grain rye crispbread. sRCB: sourdough-fermented whole grain rye crispbread. uRCB: unfermented whole grain rye crispbread. extR: extruded whole grain rye. RP: whole grain rye porridge*

|  | WB | WCB | sRB | RCB | sRCB | uRCB | extR | RP |
|---|---|---|---|---|---|---|---|---|
| Protein | 11.8 | 13.1 | 8.7 | 9.6 | 10.2 | 9.6 | 8.3 | 8.3 |
| Fat | 6.7 | 7.5 | 1.6 | 1.7 | 2.2 | 1.6 | 1.6 | 1.7 |
| Starch[a] | 73.4 | 71.6 | 67.2 | 68.0 | 67.4 | 66.0 | 68.9 | 67.1 |
| Resistant starch | 1.8 | 0.5 | 3.3 | 0.8 | 0.7 | 0.7 | 0.1 | 1.2 |
| Ash | 2.2 | 1.8 | 2.3 | 2.4 | 2.7 | 2.3 | 2.3 | 2.2 |
| Dietary fibre |  |  |  |  |  |  |  |  |
| Total[b] | 6.0 | 6.0 | 20.2 | 18.3 | 17.5 | 20.5 | 18.9 | 20.7 |
| *Extractable[c]* | 1.8 | 1.6 | 6.8 | 7.2 | 6.1 | 7.9 | 8.7 | 7.6 |
| *Unextractable* | 4.2 | 4.4 | 13.4 | 11.1 | 11.3 | 12.6 | 10.2 | 13.1 |
| Arabinoxylan[d] |  |  |  |  |  |  |  |  |
| Total | 1.9 | 2.5 | 8.5 | 8.6 | 8.2 | 8.8 | 8.4 | 8.7 |
| *Extractable* | 0.9 | 0.7 | 3.0 | 3.0 | 2.7 | 2.6 | 3.4 | 2.5 |
| *Unextractable* | 1.0 | 1.8 | 5.6 | 5.6 | 5.5 | 6.3 | 5.1 | 6.2 |
| Arabino-galactan | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.2 |
| β-glucan | 0.3 | 0.3 | 2.1 | 2.1 | 2.1 | 2.5 | 2.1 | 2.1 |
| Cellulose and resistant starch[e] | 2.4 | 1.4 | 4.6 | 2.5 | 2.7 | 2.7 | 2.1 | 3.2 |
| Fructan | 0.4 | 0.4 | 2.5 | 2.6 | 1.7 | 4.0 | 3.9 | 4.2 |
| Klason lignin | 0.1 | 0.5 | 1.5 | 1.3 | 1.5 | 1.3 | 1.3 | 1.5 |
| β-glucan $M_{cf}$ ($10^5$ g/mol) | 1.5 | 2.3 | 2.5 | 1.4 | 2.1 | 4.5 | 6.5 | 7.3 |
| Extractability, β-glucan *(%)* | 17 | 36 | 27 | 27 | 37 | 18 | 36 | 16 |

[a]Calculated by difference (total minus fat, protein, fibre and ash).

[b]Calculated as the sum of fructan and total dietary fibre, as analysed by the Uppsala method (Theander *et al*., 1995).

[c]Calculated as the sum of fructan and total extractable dietary fibre, as analysed by the Uppsala method (Theander *et al*., 1995).

[d]Calculated from the sum of arabinose, xylose and galactose, assuming an arabinose to extractable galactose ratio of 0.69 in arabinogalactan (Loosveld *et al*., 1997).

[e]Calculated as the difference between total β-glucan and glucose residues, as analysed by the Uppsala method (Theander *et al*., 1995).

At low magnification (see Figure 2), finer lamellae were visible in the wheat breads, due to the stabilising effect of gluten. However, the lamellae in uRCB and extR also appeared thinner than in the other rye products. For uRCB it may have been related to the method used to incorporate air into the batter (whipping), while for extR it is most likely due to the complete starch gelatinisation followed by rapid expansion and setting of the gelatinised starch during extrusion. The porridges consisted of intact structures (endosperm cells, aleurone layers *etc*.) separated by a phase of highly gelatinised and disrupted starch granules. In sRB, the starch granules were easily distinguishable due to a surrounding amylose layer, which most likely also explains the higher content of resistant starch (Table 2). This is in line with previous observations for sourdough rye breads (Juntunen *et al*., 2003a). The amylose leakage observed in sRB may have been related to the higher water content compared with the crispbreads. An effect of water availability on amylose leakage was also observed in the oat bran breads (Figure 4). High water content promotes retrogradation during storage, which was likely to be limited for the dry products (Liu & Thompson, 1998). Sourdough fermentation and production of organic acids have also been shown to increase the content of resistant starch (Liljeberg *et al*., 1996a).

### 4.1.2 Characteristics of the dietary fibre components

In Papers I and IV, the fermented rye products (sRB, RCB and sRCB) had lower average molecular weight of β-glucan ($M_{cf}$), while the products subjected to heat treatment without prior hydration of the flour (extR and RP) maintained a high molecular weight (Table 2). Fermentation involves hydration of cereal flours, often at elevated temperatures. This results in activation of endogenous enzymes and degradation of dietary fibres such as β-glucan and arabinoxylan (Rakha *et al*., 2011). The hydration of the flour during production of uRCB was also sufficient for degradation to occur, although not to the same extent as for the fermented rye products (Table 2 and Figure 3A). Molecular weight of arabinoxylan was not analysed in any of the papers, but has been shown to be degraded similarly, but not to the same extent, as β-glucan (Rakha *et al*., 2010; Isaksson *et al*., 2011). Arabinoxylan-degrading enzymes are mainly active at higher temperatures and lower pH, around 4.5, than β-glucanases (Rasmussen *et al*., 2001; Rakha *et al*., 2011). While arabinoxylan was most likely less affected, an increase in polydispersity and slight decrease in molecular weight has been reported for fermented rye crispbreads compared with unfermented (Rakha *et al*., 2010). Due to lower dough pH, the use of sourdough (in sRB and sRCB) might also have promoted the degradation of arabinoxylan.
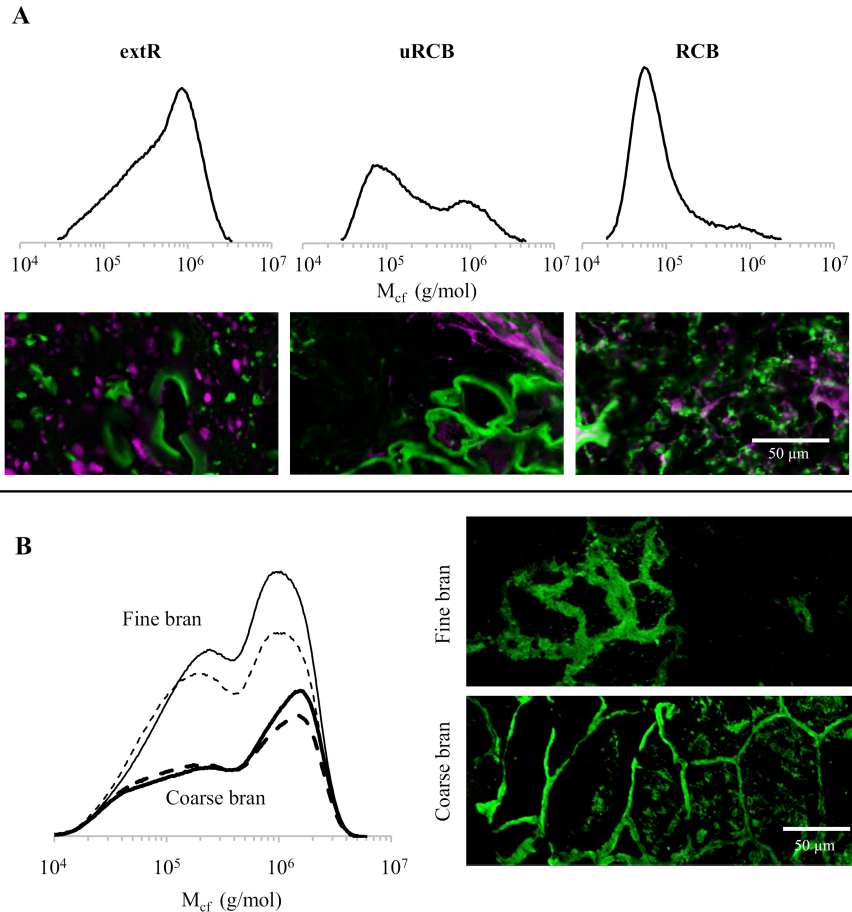
*Figure 3.* Molecular weight distribution of β-glucan (normalised against concentration) and confocal laser scanning microscopy (CLSM) micrographs of immunolabelled β-glucan (green) and arabinoxylan (magenta). A) Extruded rye (extR), unfermented whole grain rye crispbread (uRCB) and yeast-fermented whole grain rye crispbread (RCB). B) Breads with added fine (thin lines) or coarse (fat lines) oat bran. Solid lines: 45 min fermentation. Dashed lines: 75 min fermentation.

While fermentation was detrimental with regard to molecular weight, the extractability of both β-glucan and arabinoxylan appeared higher in the fermented products (sRB and RCB) compared with the unfermented uRCB and RP. Fermentation has previously been associated with increased extractability of arabinoxylan (Lappi *et al.,* 2010). Extrusion was the only process that both maintained molecular weight and yielded high extractability, which is in line with previous findings (Wang & Klopfenstein, 1993). It is possible that the finer particle size of the flour used in sRB and RCB, compared with uRCB,

and the structural disintegration caused by the extrusion process for extR (Figure 3A) contributed to the increased solubility.

The importance of particle size and fermentation time for the molecular weight and solubility of dietary fibres was demonstrated for β-glucan in Paper V. Short fermentation times and large particles are recognised as important factors in maintaining the molecular weight of β-glucan (Andersson *et al.*, 2004; Åman *et al.*, 2004; Rieder *et al.*, 2015). Significant effects on molecular weight depending on fermentation time and particle size were also found in Paper V. However, while smaller particles increased degradation of β-glucan, they also increased its solubility, as can be seen from the chromatograms in Figure 3B (where area under the curve corresponds to concentration in solution).

### 4.1.3  Effect of fibre supplementation on product characteristics

In Papers II and V, different amounts of inulin and oat bran were added to rye flake porridge and wheat bread, respectively. For both the porridges and the breads, increased addition of inulin/oat bran resulted in less gelatinised starch and less leakage of amylose (Figure 4). With addition of the oat bran, smaller bran particles had a similar effect. Although gluten was also added to the porridges, the inulin had a greater impact on starch gelatinisation. This effect
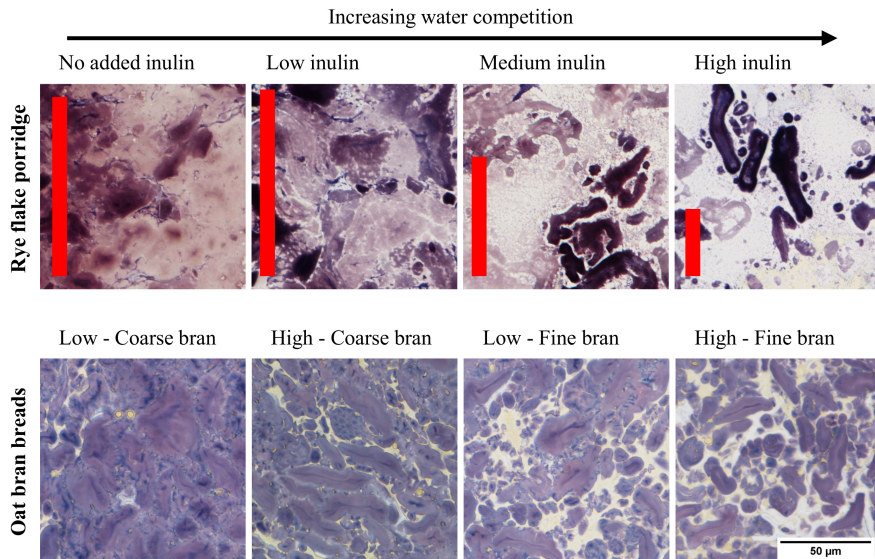


*Figure 4.* Light microscopy micrographs stained with iodine. Protein is stained yellow, starch purple, amylopectin brown and amylose blue Top: microstructure of rye flake porridge without and with increasing addition of inulin. Red bars indicate relative viscosity of the finished porridge at 75°C. Bottom: breads with two levels of coarse and fine oat bran.

on starch gelatinisation was most likely related to increased water competition with the addition of soluble dietary fibre. For the porridges, the limited starch gelatinisation also led to lower porridge viscosity.

As the porridges were not included in any further *in vitro* or *in vivo* studies in this thesis, the potential implications will be discussed shortly. The addition of inulin resulted in two changes that may have opposing effects on postprandial glucose and insulin responses. Less gelatinised starch is more resistant to hydrolysis, while lower viscosity might increase gastric emptying rates and glucose uptake (Holm *et al*., 1988; Phillips *et al*., 2015). Starch-induced viscosity has been demonstrated to be inefficient in modulating responses (Zijlstra *et al*., 2009). However, in this case, the starch would most likely gel at the temperature in the human body, which might have a different effect on the emptying mechanism. In contrast to starch, inulin, at the concentration used here, is not likely to gel at body temperature (Kim *et al*., 2001; Tárrega *et al*., 2011). In the human trial including the porridges, no significant differences were observed between the porridges with regard to glucose and insulin responses during the first 4 h (Lee, 2016). This may be a consequence of two changes with potential to affect postprandial responses counteracting each other.

## 4.2  *In vitro* digestion

In Papers I, IV and V, *in vitro* methods were used to study the digestive process of differently processed wheat, rye and oat products.

### 4.2.1  Food matrix disintegration

In Papers I and IV, two different approaches were used to investigate product disintegration in the gastrointestinal tract. The disintegration of the food matrix of the products was studied using a modified RVA set-up to simulate gastric digestion and the TIM model was used to simulate digestion in the stomach and the small intestine.

*Mastication*

The required masticatory work differed depending on the product, with the rye crispbreads requiring 25-30 masticatory cycles before swallowing, sRB and WCB 20-25 cycles, WB 15-20 cycles, extR 10-15 cycles and the semisolid RP 3-5 cycles. After *in vivo* mastication, bolus water content (% wet basis) was 48-53 % for WB, RCB, sRCB, uRCB and extR, 60-62 % for sRB, and 74-75 % for RP (Papers I and IV). However, only one individual was used for the mastication and differences between individuals could alter the outcomes.

Previous investigations have reported that the inter-individual variation in food bolus particle size is very limited, as is the effect of salivary α-amylase in relation to the action of pancreatic α-amylase (Jalabert-Malbos *et al*., 2007; Woda *et al*., 2010; Woolnough *et al*., 2010). Inter-individual differences may be more important for cereal foods and have been reported to affect the rheological properties of boluses (Loret *et al*., 2011). However, Loret *et al*. (2011) also reported similar characteristics for boluses from different products, despite mastication by different individuals. The use of one individual is a limitation in the current work and the role of food characteristics on individual masticatory parameters and bolus formation should be further studied.

During mastication, the protein/starch matrix of WB, uRCB and extR appeared to be compacted, forming a bolus consisting of aggregates of starch granules held together by protein for WB and of botanically intact fragments connected by a continuous starch phase for uRCB (Figure 5). For extR, continuous starch phase, similar to that in uRCB, was the dominant structure in the bolus. The staining of this starch phase was weaker, possibly due to higher degree of hydration. All other products except for RP, which appeared relatively unaffected by mastication, appeared instead to have fractured, forming fragments retaining structural features of the original food matrix. Refined wheat bread has been reported to form larger particles after mastication than wholegrain and endosperm rye sourdough bread (Pentikäinen *et al*., 2014). This may be related to the hydrated protein network in refined wheat bread giving a more flexible structure and a cohesive bolus compared with the brittle dry structure of WCB, RCB and sRCB and the continuous starch phase in sRB. For uRCB and extR, it is possible that the thinner lamella and more disrupted and gelatinised starch granules compared with the other rye products (Figure 2), resulted in starch being more easily hydrated by saliva during mastication. Hydration of the starch phase could change the mechanical properties, giving a more flexible structure and leading to bolus formation more similar to that of WB.

*Gastric and intestinal digestion*

The appearance of the bolus seemed to affect the behaviour during simulated gastric digestion (Papers I and IV) (Figure 5). For WB compared with WCB and for uRCB and extR compared with sRB, RCB and sRCB, the presence of a cohesive protein or starch network in the bolus appeared to be related to interferences (appearing as peaks) in the viscosity curves occurring over a longer period. This indicates that larger bolus fragments occurred for a longer time in these products, possibly a result of more cohesive boluses. Although this is by no means a true representation of the mechanical forces exerted on
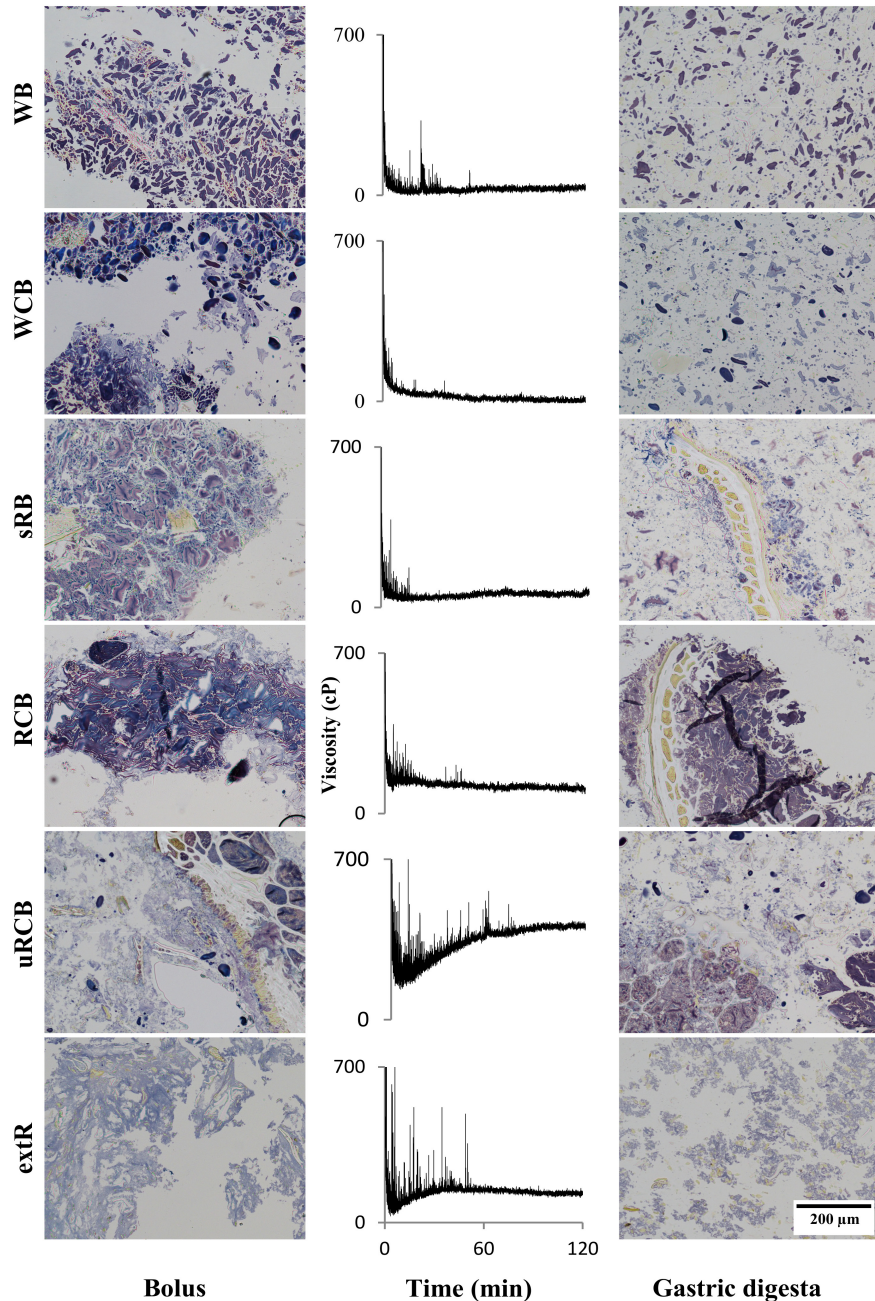
*Figure 5.* Light microscopy micrographs of products after mastication and simulated gastric digestion, and development of viscosity during simulated gastric digestion. WB: refined wheat bread. WCB refined wheat crispbread. sRB: sourdough-fermented whole grain rye bread. RCB: yeast-fermented whole grain rye crispbread. sRCB: sourdough-fermented whole grain rye crispbread. uRCB: unfermented whole grain rye crispbread. extR: extruded whole grain rye.

the boluses during gastric digestion *in vivo*, it can give an indication of differences between foods when the disintegrative process is driven by mechanical forces. As an example, the enzymatic degradation of protein is likely tobe of greater importance for the wheat product than for the rye products during gastric digestion.

After simulated gastric digestion, the particles in the digesta were larger in the rye products, containing intact fragments of the food matrix of millimetre size, whereas the wheat products consisted mainly of starch granules. This is in line with previous observations (Nordlund *et al.*, 2016). It can be attributed to the presence of intact botanical structures and of a continuous starch phase which, unlike the protein network in the wheat products, was not enzymatically hydrolysed during the simulated gastric digestion. In the extR digesta, mainly small (<100 µm) fragments of gelatinised starch could be observed.
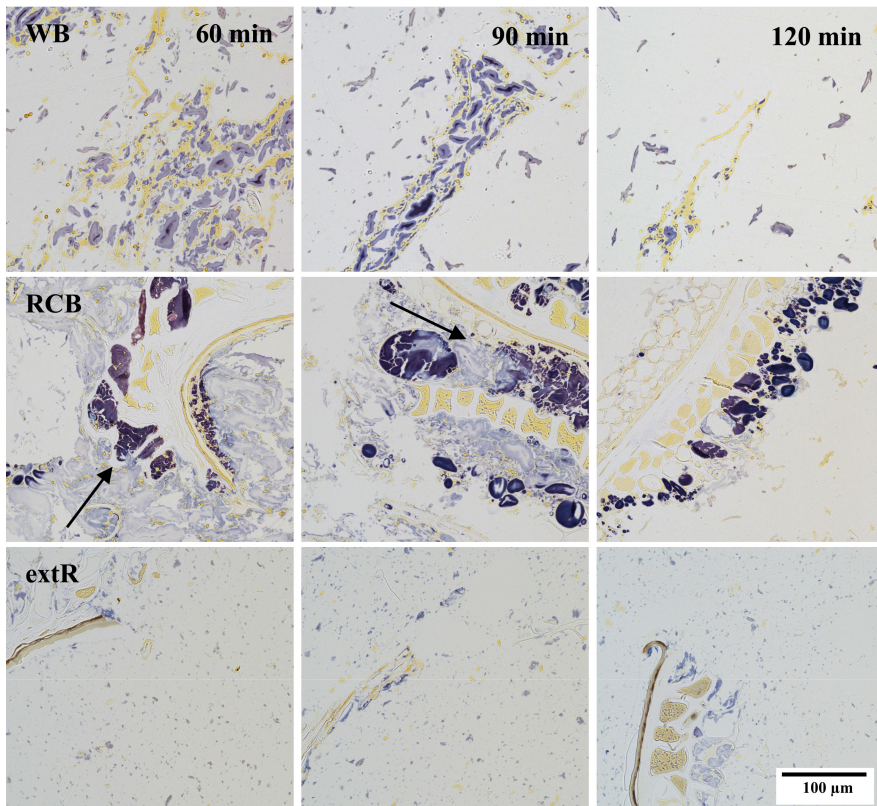


*Figure 6.* Light microscopy micrographs of samples collected from the duodenal section of the TIM model at 60, 90 and 120 min (Paper I). Arrows in the micrographs for RCB indicate the progression of starch digestion. WB: refined wheat bread. RCB: yeast-fermented whole grain rye crispbread. extR: extruded whole grain rye.

Differences in the progression of digestion of rye and wheat products were also apparent in the TIM model (Figure 6). For the refined wheat bread, a transition from larger aggregates consisting of starch granules connected by a protein network to aggregates of decreasing size and increasing proportion of free granules was observed with increasing digestion time. For the rye products, except extR, the starch digestion was instead observed progressing from the periphery towards the centre of the larger fragments. For extR, no larger starch fragments could be observed at any time point, which may have been the result of rapid digestion of starch emptied into the duodenal compartment. It could also have been related to the disintegration process. If a bolus mainly consists of a cohesive starch phase, comparatively small fragments may be detached from the main bolus during gastric digestion.

Due to the sieving effect in which small particles (<1-2 mm) are emptied from the stomach into the duodenum, while the larger solid particles are retained until their size is reduced, the disintegration process is of relevance for the rate at which nutrients become available for digestion and absorption in the small intestine (Hellström *et al*., 2006; Delzenne *et al*., 2010).

### 4.2.2 Fibres, particles and digesta viscosity

In Papers I and IV, changes in viscosity during simulated gastric digestion and in Paper V the viscosity of extracts from *in vitro* digested breads with oat bran and extruded oat bran were measured.

Figure 7A shows the relationship between viscosity and average molecular weight x concentration of β-glucan for *in vitro* extracts from the breads and extruded products in Paper V. Molecular weight and concentration are important factors determining the viscosity of β-glucan and arabinoxylan in solution (Wood *et al*., 2000; Ren *et al*., 2003; Rakha *et al*., 2013). Structural features of arabinoxylan, which may vary between different cultivars or species, can also affect viscosity (Rakha *et al*., 2013). In Paper I, the connection between viscous fibre characteristics and viscosity was not as clear. The viscosity of the unfermented uRCB and RP was significantly higher than that of the fermented sRB and RCB (Figure 7B). This could have been partly related to the less degraded β-glucan and arabinoxylan. However, the viscosity of extR was not significantly higher than for sRB and RCB, despite the higher molecular weight of β-glucan and the higher extractability of β-glucan and arabinoxylan. This was most likely related to the smaller particles in the digesta for extR. Particle characteristics, such as shape and size, are important factors in determining digesta viscosity (Lentle & Janssen, 2008). Furthermore, highly swollen and partly solubilised starch, especially in the case of RP, may contribute to digesta viscosity. For effects on gastric emptying and intestinal

transit rates, as well as mixing of digesta, the viscosity of the whole digesta is relevant (Lentle & Janssen, 2008). When considering diffusion rates, fibre characteristics, such as solubility and molecular weight (and chemical structure) may be more important (Shelat *et al*., 2010, 2011). To get a more complete picture of the role of fibres and viscosity in the digestion of a product, there may be a need for viscosity measurements on both whole digesta and fibre extract, as well as the use of other methods to determine diffusion rates.
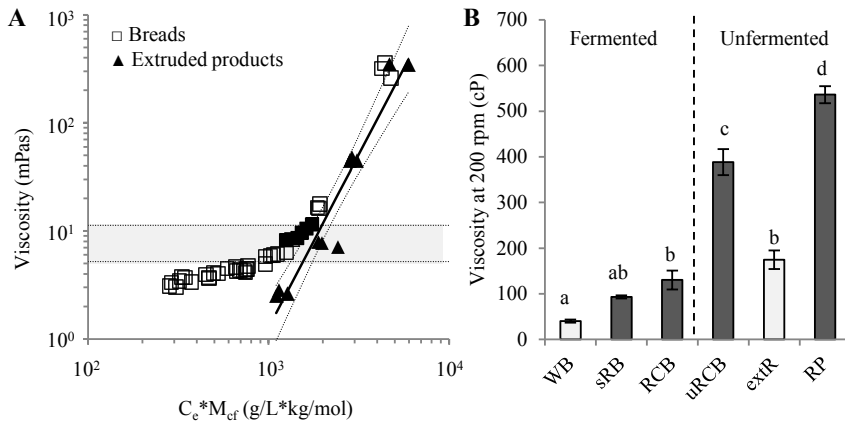


*Figure 7.* A) β-glucan extract viscosity as a function of concentration ($C_e$) x molecular weight ($M_{cf}$) for extruded oat bran and breads with added oat bran. Shaded area indicates range of estimated threshold viscosity needed for a cholesterol-lowering effect *in vivo*. Breads with high content of finely milled oat bran are represented by filled squares. B) Viscosity of simulated gastric digesta of rye products measured with RVA. Bars for products with the smallest particles in the digesta are light grey. WB: refined wheat bread. sRB: sourdough-fermented whole grain rye bread. RCB: yeast-fermented whole grain rye crispbread. uRCB: unfermented whole grain rye crispbread. extR: extruded whole grain rye. RP: whole grain rye porridge.

*Physiological relevance of viscosity measured* in vitro

In Paper V, the possibility of achieving sufficiently high viscosity for a cholesterol-lowering effect from β-glucan by adding oat bran of different particle sizes to breads was investigated. The required viscosity was estimated by comparison to extruded oat bran products previously demonstrated to have an effect by Wolever *et al*. (2010) (Figure 7A). The method used for comparison is described in more detail in Paper V. Interestingly, despite more degraded β-glucan, only breads with 30% finely milled oat bran gave sufficiently high viscosities due to the increased solubility of β-glucan. The aim of studies is often to maintain high molecular weight of β-glucan, thereby ensuring or improving its physiological effect (Åman *et al*., 2004; Rieder *et al*., 2015). Paper V instead demonstrated that sacrificing molecular weight for

increased solubility may be more beneficial with regard to inducing viscosity. Furthermore, as shown in section 4.1.3, the use of fine oat bran also leads to a lower degree of starch gelatinisation, which may have beneficial effects on postprandial glucose and insulin responses.

### 4.2.3 Glucose release in the TIM model

Simulations of digestion in the TIM model (Paper I) showed that in general, the glucose profiles peaked at a later time point (120 min) for the rye products, with the exception of RCB, compared with WB (90 min). Of the rye products with peak glucose concentration at 120 min, RP produced the highest concentration and sRB the lowest. At 180 min, sRB, extR and RP also had higher glucose concentrations than WB. In human trials, intake of rye products has been associated with a low, but prolonged, increase in blood glucose response and a more extended glucose profile, commonly leading to high glucose index (based on the 120 min area) (Rosén *et al.*, 2009). A more extended profile with initially slower release could be beneficial, as it would require lower secretion of insulin for regulation.
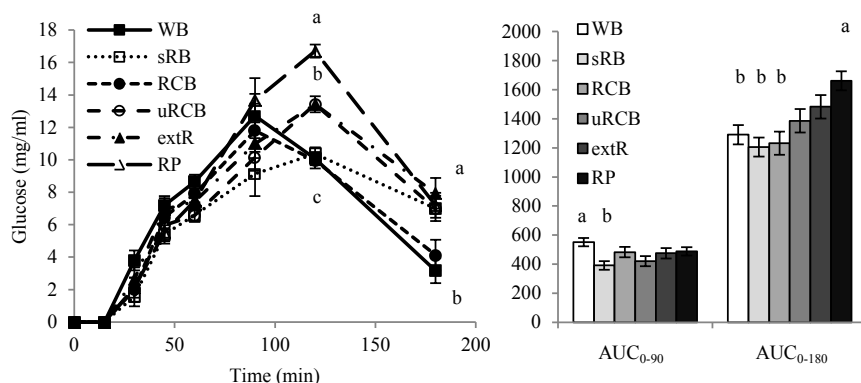


*Figure 8.* Glucose release in the TIM model (Paper 1). Left: glucose profiles for 0-180 min. Right: area under the curve (AUC) for glucose profiles for 0-90 min and 0-180 min. Different letters indicate values that are significantly different from each other. WB: refined wheat bread. sRB: sourdough-fermented whole grain rye bread. RCB: yeast-fermented whole grain rye crispbread. uRCB: unfermented whole grain rye crispbread. extR: extruded whole grain rye. RP: whole grain rye porridge.

From the variation in product characteristics discussed in section 4.1 and the behaviour during simulated digestion with regard to viscosity development and matrix disintegration, it is clear that a number of factors could potentially affect the release of glucose during digestion. Factors which may contribute to faster or delayed/slower glucose release are listed in Table 3 for each product

run in the TIM model (Paper I). The resulting glucose profiles will be the sum of these and possibly additional factors.

Table 3. *Factors which may contribute to faster or delayed/slower glucose release for the products run in the TIM model (Paper I). WB: refined wheat bread. sRB: sourdough-fermented whole grain rye bread. RCB: yeast-fermented whole grain rye crispbread. uRCB: unfermented whole grain rye crispbread. extR: extruded whole grain rye. RP: whole grain rye porridge.*

| Product | Faster glucose release | Delayed/slower glucose release |
|---------|------------------------|-------------------------------|
| WB | Degradation and low content of viscous fibres, fast gastric disintegration and low viscosity, small particles in digesta | Initially large aggregates emptied from gastric compartment |
| sRB | Degradation of viscous fibres, fast gastric disintegration and low viscosity | Protective amylose layer, high solubility of viscous fibres, large particles in digesta, organic acids |
| RCB | Degradation of viscous fibres, fast gastric disintegration and low viscosity | High solubility of viscous fibres, large particles in digesta |
| uRCB | Gelatinised starch, low solubility of viscous fibres | Intact cell structures, limited degradation of viscous fibres, slow gastric disintegration, large particles in digesta, botanical integrity |
| extR | Completely gelatinised starch, no intact cell structures, low viscosity, small particles in digesta | High solubility and very limited degradation of viscous fibres, slow gastric disintegration |
| RP | Highly gelatinised and partially solubilised starch, low solubility of viscous fibres, high product volume (affecting gastric emptying in the TIM model) | Very limited degradation of viscous fibres, gelling of gelatinised starch (affecting gastric emptying), large particles in digesta |

The glucose profiles for WB compared with RCB and for uRCB compared with extR did not differ significantly, despite a number of factors that could have been expected to cause differences with regard to glucose release (Table 3). For WB compared with RCB, the lower fibre content and smaller particles in the digesta of the former would have been expected to lead to more rapid glucose release. It is possible that degradation of soluble fibres in RCB and the emptying of the initially large aggregates from the gastric compartment for WB (Figure 6) were sufficient to compensate for these differences. Similarly for uRCB and extR, the completely gelatinised starch and absence of intact cell structures in the latter (see Figure 2) would have been expected to result in faster glucose release. However, the less degraded and more extractable

viscous fibres in extR (see Figure 3) and the similar disintegration rate of the two (see Figure 5) may have diminished the differences to the point where they were not detectable in the TIM model. The slower disintegration rates and limited degradation of viscous fibres are factors in common for uRCB and extR and may contribute to the later peak compared with RCB and WB.

The sourdough rye bread (sRB) and the rye porridge (RP) were the only rye products with different AUC values than WB (significantly lower and higher, respectively) (Figure 8). For RP, $AUC_{0-180}$ was also significantly higher compared with sRB, and peak glucose concentration was higher compared with all other products. The difference between sRB and RP is in line with findings by Rosén *et al*. (2009) that endosperm and whole grain rye porridges induce higher glucose and insulin responses in humans than corresponding rye breads. For sRB, the lower $AUC_{0-90}$ and more extended glucose release, with a significantly higher concentration at 180 min compared with WB, are also consistent with results from previous studies. Rye breads, both yeast-fermented and sourdough-fermented, have repeatedly been demonstrated to induce lower insulin or lower insulin and glucose responses compared with refined wheat bread (Juntunen *et al*., 2003a; Rosén *et al*., 2011). There are several characteristics of sRB, such as degraded viscous fibres (Table 2), low digesta viscosity and rapid disintegration (Figure 5), which would be expected to lead to rapid digestion and starch release. The presence of an amylose layer around the starch granules may be an important factor contributing to the slow release of glucose in the TIM model (Figure 8). This has previously been suggested to inhibit starch hydrolysis (Juntunen *et al*., 2003a).

The semisolid nature and high volume of RP set it apart from the other products. Although the highly gelatinised and partly solubilised fraction of the starch was most likely rapidly digested, it could also have caused the RP porridge to gel, becoming more solid-like. Gelling is in line with the occurrence of interferences (peaks) in the viscosity measurement during simulated gastric digestion (see Figure 4 in Paper I). This might have had implications for how it emptied from the gastric compartment. In the TIM model, volume and rate of gastric content emptying are constant if the settings are maintained, as was the case in Paper I. This can lead to a faster rate of product emptying for RP compared with the other products due to its large volume. *In vivo* however, the gelling may also lead to reduced gastric emptying rate, partly compensating for this.

## 4.3 Crispbreads and responses in humans

In Papers III and IV, yeast-fermented (RCB) (Paper III), sourdough-fermented (sRCB) (Paper IV) and unfermented (uRCB) (Papers III and IV), whole grain rye crispbreads were compared with yeast-fermented refined wheat crispbread (WCB) in a human trial with regard to appetite and postprandial glucose and insulin responses. In Paper III the average age of the participants was 60.1±12.1 years, while in Paper IV it was 30±11 years. This difference in age between the two study populations may account for some of the difference in results obtained between the papers. Age is known to affect both glucose regulation and appetite ratings (Basu *et al*., 2006; Clarkston *et al*., 1997).

### 4.3.1 Glucose and insulin responses

In Paper III, RCB and uRCB were compared with WCB and found to induce lower insulin responses, but similar glucose responses (Figure 9) as WCB. This is in line with previous observations for soft rye breads compared with wheat breads (Juntunen *et al*., 2003a; Rosén *et al*., 2011). In Paper III, the response also differed between RCB and uRCB, with the former eliciting higher insulin response (Figure 9). Similar results were obtained in Paper IV on comparing uRCB, sRCB and WCB, with uRCB again found to elicit lower insulin responses than both fermented rye crispbread and fermented wheat crispbread, although only for the first 125 min compared with WCB (see Paper IV). The AUC for glucose response was also found to be higher for uRCB compared with sRCB, mainly due to higher values during the latter half of the measurement period, which is in line with the slower, more extended release of glucose observed for some rye products (Rosén *et al*., 2009). No differences in GLP-1 concentrations were observed in Paper IV.

The differences between the unfermented and fermented rye crispbreads were in line with those observed in the TIM model (section 4.2.3), with the fermented product eliciting responses more similar to those of the refined wheat crispbread. However, there was no difference between RCB and WB in the TIM model (Figure 8), but there was between RCB and WCB in the human trials (Paper III). This may have been partly attributable to the differences observed between WB and WCB with regard to disintegrative pattern. As WCB appeared to disintegrate faster (see Figure 5), a greater difference in responses to WCB and RCB could also be expected.

Interestingly, in Paper IV we found no beneficial effect of sourdough-fermentation on glucose or insulin responses, contrary to what has previously been proposed (Lappi *et al*., 2010; Liljeberg *et al*., 1995). Instead, in relation to uRCB, RCB and sRCB were similar with regard to structure and their behaviour in the *in vitro* studies (see Figures 2 and 5 and Paper IV). This

indicates that differences in dietary fibre composition and disintegrative behaviour might play important roles in determining the *in vivo* responses.

In the human studies (Papers III and IV), unlike the *in vitro* studies, the crispbreads were ingested as part of a whole meal and the responses were consequently not solely attributable to the crispbreads. In a mixed meal situation with both solids and liquids, the latter are preferentially emptied first (Khoo *et al.*, 2009). A consequence of this is that in Papers III and IV, the early response to the treatments may have mainly been dependent on the juice provided (contributing approximately 25% of the available carbohydrates), thereby being similar for all treatments. Differences due to type of crispbread that might occur in the early post-ingestive phase would therefore not be observable. This may also explain why no difference in GLP-1 concentrations could be observed.
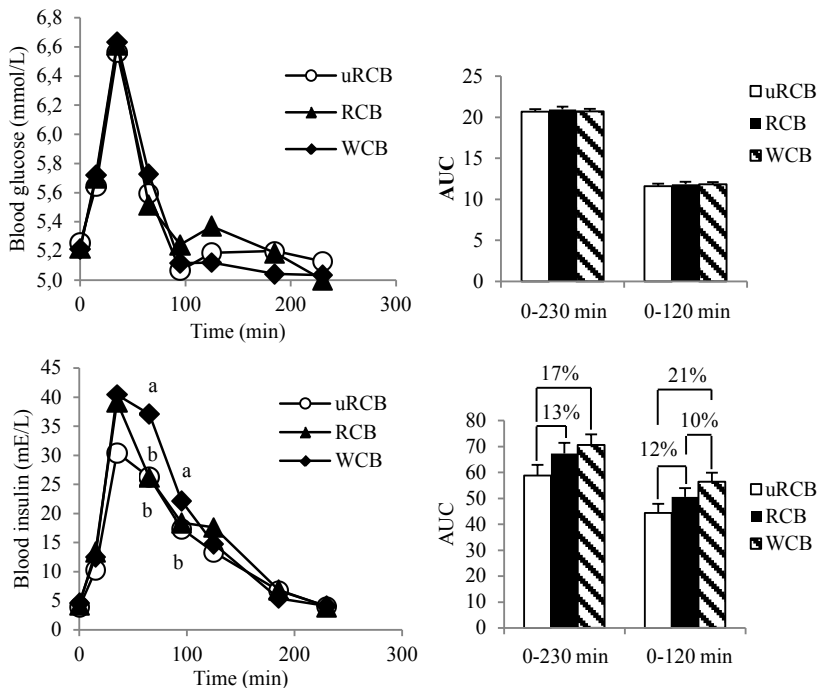


*Figure 9*. Glucose and insulin profiles, and area under the curve (AUC) of each, for unfermented whole grain rye crispbread (uRCB), yeast-fermented whole grain rye crispbread (RCB) and yeast-fermented whole grain rye crispbread. Points marked with different letters and bars given as difference in percent are significantly different from each other.

### 4.3.2 Appetite responses

No differences with regard to appetite ratings were found between the rye crispbreads in either Paper III or IV. In Paper III, both rye crispbreads were found to induce higher fullness and decrease feelings of hunger compared with the wheat crispbread (Figure 10). In Paper IV, however, higher fullness and lower hunger was only observed for the sourdough-fermented rye crispbread (sRCB) compared with WCB, while lower desire to eat was observed for both rye crispbreads compared with the WCB.

The results obtained in Papers III and IV are in line with previous findings that consumption of whole grain rye increases satiety compared with refined wheat products (Isaksson *et al*., 2008, 2009, 2011, 2012; Rosén *et al*., 2009, 2011; Forsberg *et al*., 2014). However, Forsberg *et al*. (2014) was the only previous study to investigate the appetite responses after rye crispbread consumption. They observed a larger effect, but in comparison with soft refined wheat bread. With larger product volume, as for crispbreads compared with soft breads, increased early satiety could be expected (Rolls *et al*., 1999, 1998). Extended oro-sensory exposure can also affect satiation, early satiety and acute energy intake (Wijk *et al*., 2009; Mattes & Considine, 2013). In Papers I and IV, the crispbreads, including the wheat crispbread, required longer mastication times than the soft breads (WB and sRB). The effect of volume might have been smaller than that of oro-sensory stimulation in those cases.
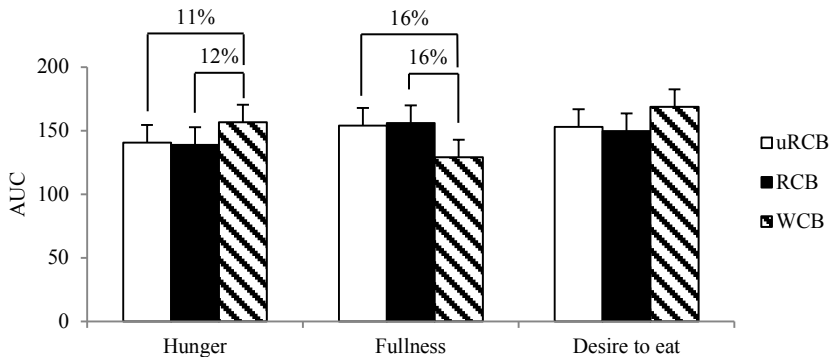


*Figure 10.* Area under the curve (AUC) for appetite ratings for unfermented whole grain rye crispbread (uRCB), yeast-fermented whole grain rye crispbread (RCB) and yeast-fermented refined wheat bread (WCB).

No difference was observed between the rye crispbreads with regard to appetite measures in either Paper III or IV, despite the differences observed in the *in vitro* experiments and the insulin responses *in vivo*. These differences

might not have been sufficient to affect appetite due to the more complex regulation and influence of psychological factors.

The higher feelings of satiety induced by rye foods compared with wheat foods have been attributed to differences in the composition and content of dietary fibre (Clark & Slavin, 2013). In Papers III and IV, the content of dietary fibre in the rye crispbreads was around 20%, while in the refined wheat crispbread it was only 6%. Colonic fermentation has also been suggested as a factor, although this is mainly associated with effects on appetite after a later meal (Ibrügger *et al*., 2014; Joyce & Gahan, 2014). The large effect on hunger and fullness for the sRCB in Paper IV (18% lower and 21% higher, respectively, compared with WCB) could also be a related to production of organic acids during sourdough fermentation. Supplementation of wheat breads with acetic acid has been demonstrated to induce higher feelings of satiety (Liljeberg & Björck, 1998). However, it is unclear whether the acid produced during the sourdough fermentation would be sufficient in this case.

# 5   Conclusions

***Overall conclusion***: A range of food characteristics, including molecular weight and the solubility of certain dietary fibres, botanical integrity and starch gelatinisation, are each relevant for one or more processes occurring during digestion, such as disintegration, viscosity development and starch hydrolysis. This has implications for postprandial responses. Processing generally affects more than one of these characteristics, which may result in effects counteracting each other with regard to postprandial responses. Consequently, the net effect of food characteristics should be considered when aiming to develop healthy foods.

More specific conclusions were that:

➢ Processing technique affects botanical integrity, starch structure and the fibre content and composition of cereal foods based on a particular raw material.
➢ Addition of inulin to rye flake porridge and of oat bran to wheat bread inhibits starch gelatinisation. Less gelatinised starch contributes to slower release of glucose during digestion. However, for rye flake porridge the addition of inulin also results in lower viscosity, which may have the opposite effect.
➢ Fermentation and incubation of hydrated flours at elevated temperatures lead to degradation of β-glucan. While the use of finer particles promotes degradation, the increased solubility can result in overall higher viscosity. Viscosity induced by soluble fibres may be beneficial for glucose regulation and cholesterol reduction.
➢ Inclusion of 30% finely milled oat bran in breads provides enough soluble β-glucan with sufficiently high molecular weight for a potential cholesterol-lowering effect.

- Structural features of foods influence matrix transformations during mastication, which seem to affect the rate of disintegration during gastric digestion. Slower disintegration rate may slow gastric emptying and delay the release of glucose.
- Structural features such as a continuous protein network in wheat bread and intact cell structures in rye foods affect matrix disintegration during gastric and small intestinal digestion.
- Whole grain rye crispbreads elicit lower insulin response and increased feelings of satiety compared with refined wheat crispbread. Unfermented whole grain rye crispbread is more effective than fermented in reducing postprandial insulin response compared with refined wheat crispbread, an effect possibly related to coarser particles, intact fibres and slower gastric disintegration.

# 6 Future perspectives

Much work remains to be done in determining the effects of cereal food processing on digestion, appetite and postprandial metabolic responses. More thorough characterisation of products and more extensive use of both *in vitro* and *in vivo* methodology in combination will likely be helpful in determining factors influencing the outcome with regard to postprandial responses.

For a range of products similar to those used in Paper I, the following tasks would be of interest in future work:

➢ Stepwise static *in vitro* digestion investigating in more detail structural changes, disintegration kinetics, release kinetics and how different parameters such as mechanical work, enzyme activities and pH affect these during oral, gastric and intestinal digestion.

➢ Determination of both bulk and liquid phase viscosity at different stages of digestion (using *in vitro* methods) and relation of this to the molecular weight and solubility of dietary fibre (both β-glucan and arabinoxylan) and particle characteristics such as shape and size (using a combination of *e.g.* laser diffraction and image analysis).

➢ Human intervention trials to verify the results from Paper I and the outcomes of the two research tasks listed above.

For specific food types, *e.g.* rye porridge and rye bread, the importance of specific characteristics could be further investigated:

➢ How starch gelatinisation in porridges affects its gelling in the stomach and gastric emptying in humans.

➢ How specific processing parameters, *e.g.* water content, in baking of rye bread affect the formation of an amylose layer and whether this influences glucose release *in vitro* or postprandial metabolic responses *in vivo*.

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