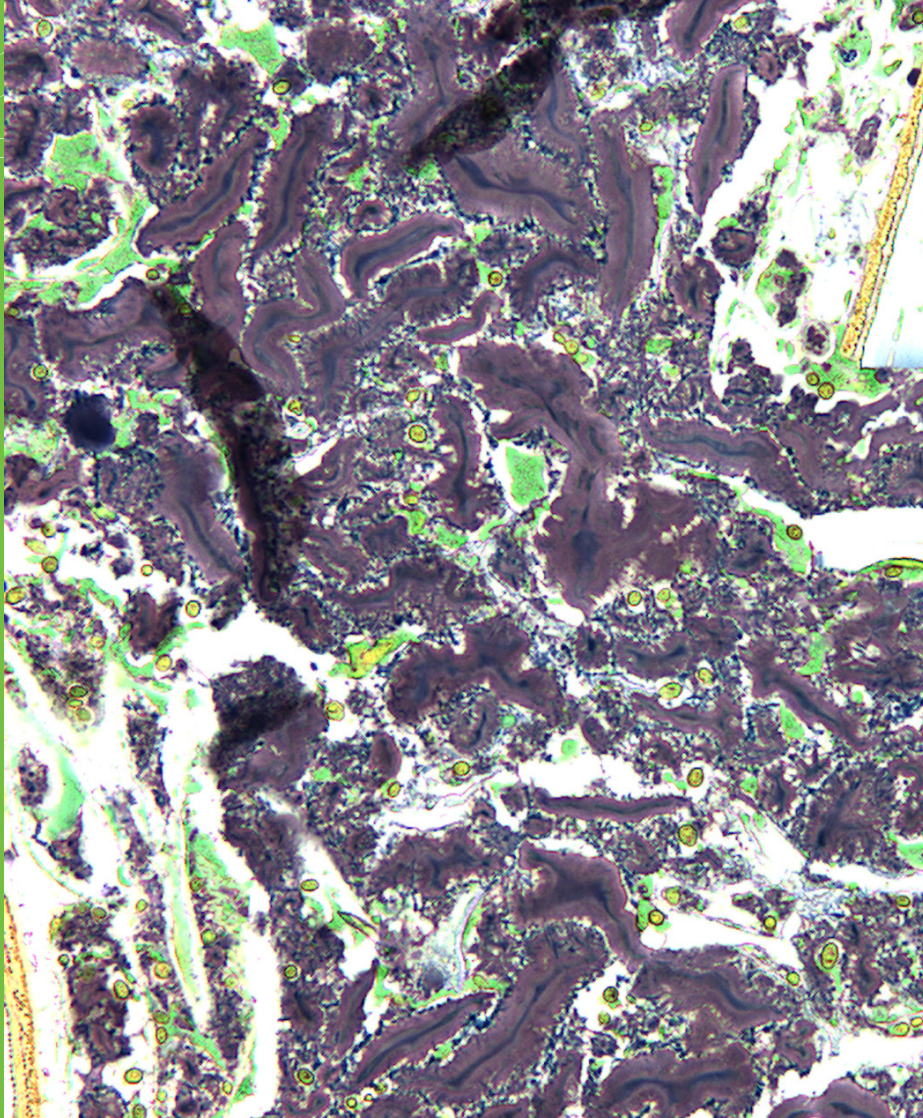


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Dissertation
178

How does cereal food structure influence digestion and satiety

- *In vitro* and *in vivo* approaches

Saara Pentikäinen



How does cereal food structure influence digestion and satiety - *In vitro* and *in vivo* approaches

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Doctoral Programme in Health Sciences, Faculty of Health Sciences,
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VTT Technical Research Centre of Finland Ltd

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Preface

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Espoo, May 2018,
Saara Pentikäinen

Academic dissertation

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List of publications

This thesis is based on the following original publications, which are referred to in the text as I–IV. The publications are reproduced with kind permission from the publishers.

- I **Pentikäinen S, Sozer N, Närväinen J, Ylätaalo S, Teppola P, Jurvelin J, Holopainen-Mantila U, Törrönen R, Aura A-M, Poutanen K.** 2014. Effects of wheat and rye bread structure on mastication process and bolus properties. *Food Research International* 66: 356-364.
- II **Pentikäinen S, Koistinen V, Kolehmainen M, Poutanen K, Hanhineva K, Aura A-M.** Mastication-induced release of compounds from rye and wheat breads to saliva. Submitted.
- III **Pentikäinen S, Sozer N, Närväinen J, Sipilä K, Alam SA, Heiniö R-L, Paananen J, Poutanen K, Kolehmainen M.** 2017. Do rye product structure, product perceptions and oral processing modulate satiety? *Food Quality and Preference* 60: 178-187.
- IV **Pentikäinen S, Karhunen L, Flander L, Katina K, Meynier A, Aymard P, Vinoy S, Poutanen K.** 2014. Enrichment of biscuits and juice with oat β -glucan enhances postprandial satiety. *Appetite* 75: 150-156.

Author's contributions

- I Saara Pentikäinen was responsible for planning the human trial with NS, JN, A-MA, and KP. She was responsible for running the human trial with JN and RT, and for *in vitro* starch hydrolysis analysis, statistical analyses and writing of the publication together with all co-authors.
- II Saara Pentikäinen was responsible for designing the study with MK, KP, KH and A-MA, and responsible for LC-MS analytics with KH. She was responsible for the data analyses and compound identification with KH and VK. She had the main responsibility for writing the publication in collaboration with all co-authors.
- III Saara Pentikäinen was responsible for planning the human trial with NS, JN, KP and MK. She had the main responsibility for running the human trial. She and R-LH were responsible for the sensory analyses. She had the main responsibility for writing the publication in collaboration with all co-authors.
- IV Saara Pentikäinen was responsible for planning the human trial with LK, KK, AM, SV and KP. She was responsible for running the study and analysing the data. She had the main responsibility for writing the publication in collaboration with all co-authors.

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Abstract

Tiivistelmä

List of abbreviations

AACC	American Association for Clinical Chemistry
ANOVA	Analysis of variance
AOAC	Association of Analytical Communities
AUC	Area under the curve
BMI	Body Mass Index
DF	Dietary fibre
EDDS	Eating disorder diagnostic scale
EMG	Electromyography
FC	Fold change
GIP	Gastric inhibitory peptide
GLP-1	Glucagon-like peptide-1
HILIC	Hydrophilic interaction
LC-MS	Liquid chromatography–mass spectrometry
SD	Standard deviation
SEM	Standard error of mean
T2D	Type 2 diabetes
TPA	Texture profile analysis
VAS	Visual analogue scale
XMT	X-ray microtomography

1 Introduction

1.1 Cereal foods in nutrition and health

Cereal foods constitute an important part of diets worldwide. Cereal food supply in the world, in Northern America, in Europe and in Finland was 147, 107, 132 and 115 kg/capita/year, respectively in year 2013 (FAOSTAT, 2013). Cereals provided 1300, 800, 1000 and 950 kcal/capita/day in these areas, respectively. Wheat is globally the most widely used cereal, followed by rice and maize. In Finland, wheat (71.2 %), rye (14.0 %), barley (4.8 %) and oats (5.3 %) are the most consumed cereal grains. By weight basis, breads (92 g), porridge (64 g), savoury pastries (24 g) and pasta (17 g) are the most commonly consumed cereal foods among 25-64 year old Finnish people (Helldan et al., 2013). Cereal foods are a significant source of carbohydrates, protein, vitamins and minerals and they provide over half of all the dietary fibre (DF) depending on the population.

The benefits of consuming a diet with plenty of whole grain and DF are evident and cereal DF complex is considered to provide most of the beneficial effects. Epidemiological studies have shown that higher DF intake is associated with a reduced risk of cardiovascular disease, type 2 diabetes and cancer (Dahl & Stewart, 2015). Cereal DF is considered to be particularly protective against type 2 diabetes compared to DF derived from fruits (Davison & Temple, 2018). Recent meta-analyses show the consumption of whole grains to be associated with reduced type 2 diabetes risk (Aune, Norat, Pål, & Vatten, 2013), reduced risk of coronary heart disease, cardiovascular disease and cancer (Aune et al., 2016). Moreover, there is an inverse relationship between whole grain intake and mortality (G.-C. Chen et al., 2016). A meta-analysis concluded that the risk of mortality decreases by 25% with increasing intake of whole grains up to 100 g/d (Schwingshackl, Schwedhelm, Hoffmann, Lampousi, & Knu, 2017).

Considering the health benefits of a diet rich in wholegrain and specifically cereal DF, it is unfortunate that energy-dense endosperm fraction is commonly separated for food production while the fractions rich in DF, vitamins, minerals and phytochemicals are used for feed or for bioenergy (Poutanen, Sozer, & Della Valle, 2014). While wholegrain wheat contains 10-13 g/100 g DF, the refined wheat flour contains only 3 g/100g DF (Liukkonen et al., 2007). The current whole grain intake is far from optimal in the majority of countries globally (Global Nutrition and Policy Consortium,

2017) (Figure 1). The consumption is low especially among young consumers (Sandvik et al 2014). As consumer appeal for ready-to-eat products is growing (Brennan, Derbyshire, Tiwari, & Brennan, 2013), the variety of snack products could be enriched with high DF options. Challenges related to processing and sensory characteristics have been recognized to be factors limiting the production and consumption of wholegrain foods (Heiniö et al., 2016).

DF rich cereal foods are beneficial for health. However, not only the composition but also the molecular structure, interactions between compounds and food structures are important for the health effects (Poutanen et al., 2014). For example, the structure of starch polymers, their interactions with other components as well as food structure at the macroscopic level define the glycaemic responses. On the other hand, the bioavailability of cereal bran proteins depends partly on structural changes due to food processing. For example bioprocessing with enzymes and yeast has been shown to increase the degradation of the cell wall structure of rye bran increasing the bioavailability of bran proteins (Nordlund, Katina, Aura, & Poutanen, 2013).

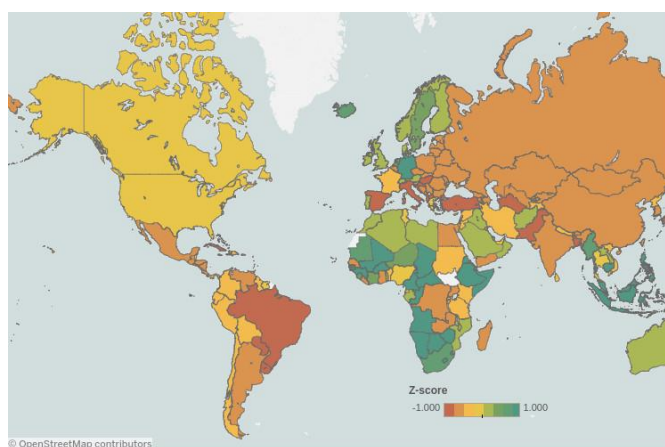


Figure 1. Intake of whole grains (g/d) by country. The colour scheme is based on Z-scores. Red: a detrimental consumption pattern 1+ SD from the global mean, yellow: consumption patterns near the global mean and green: a beneficial consumption pattern 1+ SD from the global mean (Global Nutrition and Policy Consortium, 2017).

1.2 Formation of cereal food structure

Food structure comprises of different length scales from molecular level to microscopic and macroscopic levels (Figure 2). The ingredients and processing methods determine the microstructure of foods that in turn is the basis of the macrostructure and perceived texture (Aguilera, 2006).

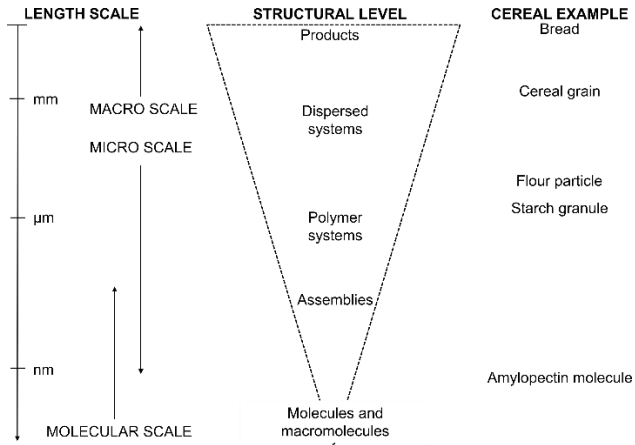


Figure 2. Length scales and structural levels of food materials with cereal examples. Adapted from Aguilera 2006.

Cereal grains as well as other nature made foods have hierarchical structures (Aguilera, 2006) (Figure 3). The natural hierarchical structures serve either some functional purpose (e.g. muscle tissue) or energy storage needs (e.g. starch granules). In food manufacturing, these structures are either preserved and consumed as such, or broken down to produce food ingredients and further transformed to food products by different processing methods. For example, the structure of cereal grain can be broken down by milling to produce flour. The flour is further processed for example by baking to produce bread.

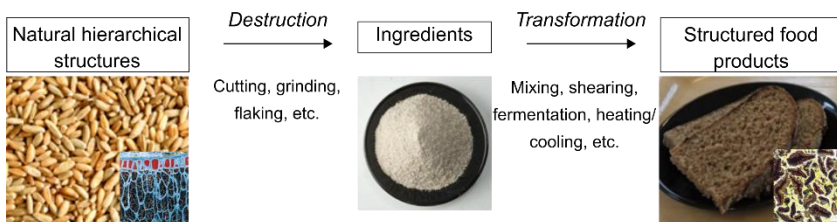


Figure 3. Schematic figure of food processing steps where hierarchical structures occurring in nature are first broken down to produce food ingredients and the ingredients are further transformed into structured food products.

1.2.1 Cereal grain structure and composition

Cereal grains have a well-organized microstructure (Autio & Salmenkallio-Marttila, 2001). All cereal grains contain four morphologically different tissues: embryo, starchy endosperm, aleurone and pericarp (Figure 4). The embryo has the highest concentration of lipids, lipid-soluble vitamins and the highest water content (Evers & Millar, 2002). The starchy endosperm is the largest morphological component occupying the centre of the grain. Starch granules are embedded in a matrix of storage proteins. Cereal bran consists of aleurone and pericarp. Aleurone cells are block-like cells with thick walls and they form a continuous layer surrounding the starchy endosperm. Aleurone cells have relatively high concentrations of protein, lipids, vitamins and minerals. The pericarp consists of dry empty cells.

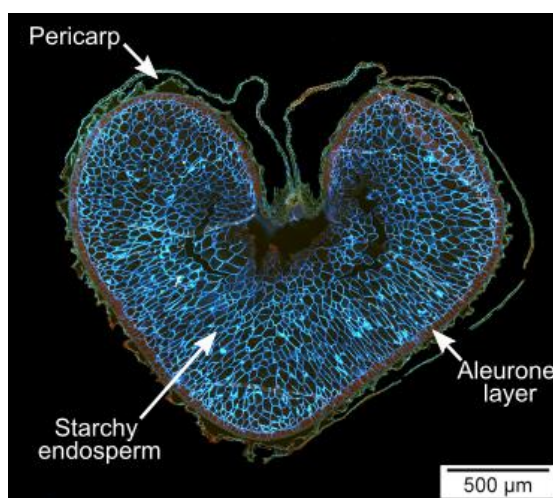


Figure 4. Microstructure of rye grain (VTT).

Although the basic structures of different cereal grains are similar, there are some grain specific structural characteristics such as thick cell walls in rye grain (also in starchy endosperm) and aggregated starch granules in oat grain, which give the different grains specific features (Autio & Salmenkallio-Marttila, 2001). Nutritional composition of rye and wheat grain closely resemble each other but rye contains less starch and more DF (Table 1). Wheat and oats contain more protein compared to rye and oats is high in fat (Frølich, Åman, & Tetens, 2013). Arabinoxylans dominate the cell walls of starchy endosperm of wheat and rye while β -glucans dominate in cell walls of oats.

DFs are important for nutritional and technological functionality of cereal grains. Codex Alimentarius defines DF as “carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by endogenous enzymes in the small intestine of humans and belong to the following categories:

- Edible carbohydrate polymers naturally occurring in the food as consumed
- Carbohydrate polymers which have been obtained from food raw materials by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities,
- Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.”(FAO/WHO, 2011).

Table 1. Nutritional composition of rye, wheat and oat grains (g/100 g), dry-matter basis (Liukkonen et al., 2007; Welch, 2011).

Component	Rye	Wheat	Oat
Protein	10-15	12-14	15
Starch	55-65	67-70	64
Dietary fibre	15-17	10-13	10
Arabinoxylans	8-10	6	-
β -glucan	2-3	0.8	4.4
Cellulose	1-3	2.5	0.6
Lignin	1-2	0.8	-
Fructan	4-6	1.4-2.6	0.7-1.1
Fat	2-3	3	9
Ash	2	2	2

1.2.2 Baking and extrusion cooking in transforming cereal flour to foods

Bread and biscuit baking and extrusion cooking are common processes in transforming cereal flour to foods. The processes are briefly introduced in this chapter. Bread is a staple cereal food worldwide. Bread is baked by mixing flour and other ingredients with water, leavening the dough to produce carbon dioxide and baking to stabilize the foam structure. Breads with different properties are obtained by using varying ingredients and baking methods. In most Western countries, white wheat bread is the standard bread. Mixing wheat flour, salt and water creates a viscoelastic dough (Goesaert et al., 2005). Gluten protein is essential for wheat bread baking since it possess the ability to form a network upon mixing. During dough mixing, the discrete masses of gluten protein disintegrate and form a continuous cohesive gluten protein network. During leavening, the gluten network retains carbon dioxide and heat-induced changes during baking create the solid foam structure characteristic of breads.

Wholemeal rye bread is the traditional bread in Eastern and Northern parts of Europe. Rye bread is traditionally baked using whole grain rye flour and by applying a sourdough process (Katina, Hartikainen, & Poutanen, 2014). In sourdough baking,

whole grain rye flour, water and starter culture are mixed and the mixture is fermented for about 8-18 hours. During fermentation, lactic acid bacteria and yeast grow and produce lactic acid and acetic acid. Acidity increases swelling and degradation of cell walls, arabinoxylans and proteins. After fermentation, more flour and water is added and dough is mixed to incorporate air. Rye dough is left to rise, bread is shaped and left to rise again and finally baked. During baking, starch is gelatinized and gas cells are expanded, creating a solid foam structure.

Biscuits are an important category of baked cereal foods. Short dough used in biscuit baking consists mainly of flour, sugar and fat and a low amount of water (Chevallier, Della Valle, Colonna, Broyart, & Trystram, 2002). During baking, proteins are denatured, starch loses the granular structure and fat melts. Evaporating water causes dough expansion.

Extrusion-cooking is a widely used method to produce cereal foods (e.g. puffed snacks or breakfast cereals), allowing the creation of different shapes and textures (Chanvrier et al., 2013). The process combines mechanical shear and heat treatment (Robin et al., 2012). In extrusion, flour is first mixed and water is added (Sozer & Poutanen, 2011). In the second phase of the process, pressure is created and the mixture is transformed into a homogenous viscoelastic mass that is finally forced through a die. Due to drop in temperature and pressure, the food mass expands. Ingredients, screw configuration, die design and process variables influence the quality of the obtained products.

1.2.3 Structural features of breads, extruded cereal snacks and biscuits

Molecular organization of wheat bread, sourdough fermented rye bread, extruded rye products and biscuits differs in terms of continuous phase, state of starch, protein as well as polymer interactions (Table 2). The gluten network serves as a basis of wheat bread structure and starch granules are embedded in the network (Goesaert et al., 2005). Starch is gelatinized but starch granules remain compact (Autio, Parkkonen, & Fabritius, 1997). Unlike in wheat bread, rye proteins (secalins) do not have the ability to form a network but gelatinized starch granules form the continuous matrix. Starch granules are swollen and some amylose has leached out from the granules and recrystallized. Wholemeal rye bread matrix contains big cell-wall containing particles. Rye bread has typically closed pores and thick cell walls and therefore a denser structure and harder texture than refined wheat bread has.

Short dough biscuit matrix is formed by starch, fat and sugar where gas cell are embedded (Baltsavias, Jurgens, & Van Vliet, 1999). Due to the low water content and low baking temperature, the rate of starch gelatinization is low (Sozer, Cicerelli, Heiniö, & Poutanen, 2014).

In extruded cereal products, the continuous phase is formed of starch while protein phase is discontinuous (Sozer & Poutanen, 2011). During extrusion, proteins denature and complexes form between starch, lipids and proteins. Some part of the insoluble DF might depolymerise and resistant starch might form under harsh conditions (shear and heat) during extrusion process. The macrostructure of extruded cereals depends on ingredients and processing conditions. For example, DF lowers

the expansion capability, which leads to small pores and high density and therefore hard, not crispy texture.

Table 2. Microstructural features of wheat and rye breads, extruded rye snacks and biscuits (Autio et al., 1997; Baltsavias et al., 1999; Goesaert et al., 2005; Katina et al., 2014; Sozer et al., 2014; Sozer & Poutanen, 2011).

	Continuous phase	State of starch	State of protein
Yeast leavened wheat bread	Gluten network where starch granules are embedded	Compact granules, gelatinized starch surrounded by protein network	Networked
Sourdough fermented rye bread	Starch network where protein is embedded	Swollen granules, gelatinized and partly leaked and crystallized amylose	Solubilized, scattered
Extruded rye snacks	Homogenous starch phase	Disrupted starch granules, partly gelatinized starch, some part might transform into resistant starch	Scattered
Short dough biscuits	Starch, fat, sugar	Partly gelatinized	Scattered

1.3 Cereal food structure and digestion

1.3.1 Food digestion process

The gastrointestinal tract consists of the mouth, esophagus, stomach, small intestine, large intestine, rectum and anus (Figure 5). In digestion, food structure is broken down and the nutrients are released from the food matrix, digested and absorbed into the circulation. Food digestion can be divided to three phases. The cephalic phase is the first stage that begins when the food is thought, seen, smelled and continuing with mastication (Smeets, Erkner, & De Graaf, 2010). The cephalic phase is followed by gastric phase that takes place in stomach. Thirdly, the intestinal phase refers to the digestion in small and large intestines. In the mouth, mastication degrades the physical structure of food and saliva lubricates food mass into a bolus that can be swallowed (J. Chen, 2015). Foods should generally achieve a particle size of less than 2 mm for optimal swallowing (Jalabert-Malbos, Mishellany-Dutour,

Woda, & Peyron, 2007). However, foods with soft textures can be safely swallowed in larger particles and therefore the critical particle size varies between 0.8 - 3 mm depending on food texture (Le Bleis, Chaunier, Montigaud, & Della Valle, 2016). Salivary secretion rate (mL/min) during mastication is rather stable and does not depend on the eaten food (Gaviao, Engelen, & Van der Bilt, 2004). However, since foods with different compositions and structures require different chewing times the amount of saliva that mixes with the food in mastication varies. Saliva contains, in addition to water, electrolytes and mucin, salivary α -amylase that hydrolyses α - 1,4 glycosidic bonds of starch into maltose, maltotriose, and α -limit dextrins (Butterworth, Warren, & Ellis, 2011). Sensory input about desirable consistency (particle size and lubrication) of food the bolus triggers swallowing (Steele & Miller, 2010). Muscular contractions of the esophagus transport the swallowed bolus to stomach (Bornhorst, Gouseti, Wickham, & Bakalis, 2016).

The stomach stores and disintegrates food and regulates the delivery of digesta to the duodenum (Minekus et al., 2014). Food digesta consists of a suspension of particulate matter in a fluid phase (Capuano, 2017). Peristaltic contractions mix the bolus with gastric secretions consisting of electrolytes, enzymes, mucus, intrinsic factor and hydrochloric acid (Bornhorst & Singh, 2014). The physical breakdown of food that was initiated by mastication continues in stomach by peristaltic contractions. The gradual decrease in pH inactivates salivary α -amylase and activates pepsin and other gastric enzymes. Acids and enzymes hydrolyse the bolus forming a slurry called chyme, which enters the duodenum (Kong & Singh, 2008). Food particles have to be reduced to a small enough particle size to pass through the pylorus to duodenum, which is the proximal part of small intestine (Meyer, Ohashi, Jehn, Thomson, & Ohashi, 1981).

Gastric emptying of solid foods follows a biphasic pattern consisting of a lag phase, during which solids are broken down to smaller particles and, a linear emptying phase during which the particles pass through the pylorus (Hellström, Grybäck, & Jacobsson, 2006). In addition to particle size, other properties of the ingested meal that regulate gastric emptying are volume, fluid viscosity, caloric content and acidity (Kong & Singh, 2008). In addition, duodenal feedback about the food flow (distention, macronutrients etc.) regulates gastric emptying. The bolus particles that remain larger than 1-2 mm after gastric digestion are emptied by the migrating motor complex during the fasting phase (Hellström et al., 2006).

Peristaltic contractions of the intestinal wall mix the chyme with intestinal secretions and pH of the chyme gradually increases (Bornhorst et al., 2016). Pancreatic proteases (endopeptidases: trypsin, chymotrypsin and elastase; and exopeptidases: carboxypeptidases A and B) degrade proteins into smaller subunits and amylase hydrolyses starch into linear oligosaccharides. Bile salts and phospholipids secreted by the gallbladder stabilize emulsion particles including dietary lipids. Pancreatic lipase hydrolyse triglycerides to monoglycerides and fatty acids (Sullivan, Alpers, & Klein, 2012). Most of the digested nutrients are absorbed in small intestine (duodenum, jejunum and ileum) by active or passive transport mechanisms.

After the small intestinal phase, the remaining chyme enters large intestine where water and some vitamins are absorbed. The microbial population of large intestine

ferment the remaining unabsorbed food material (H. J. Flint, Scott, Duncan, Louis, & Forano, 2012). Microbial fermentation is of great importance for immune homeostasis (Capuano, 2017). Fermentation of DF produces short chain fatty acids (SCFA) that provide supplemental energy and reduce colonic pH. The reduction in pH has several advantages: for example, it inhibits the growth of some pathogens and decreases the production of toxic protein metabolites. SCFAs have also anti-inflammatory and anti-carcinogenic effects.

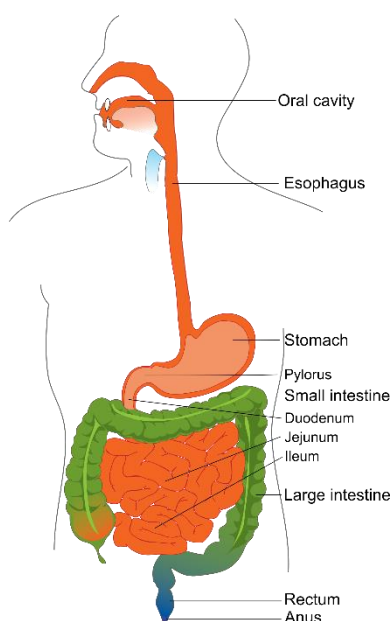


Figure 5. Gastrointestinal tract. Adapted from <https://pixabay.com/en/digestive-system-human-digestion-41529/> Creative Commons (CC0)

1.3.2 Structure disintegration of cereal foods and changes in bolus rheology in mastication and gastric digestion

Food structure is an important determinant of bolus consistency as well as properties during further digestion (Bornhorst & Singh, 2012). Food undergoes various physical changes during mastication: hardness and median particle size decrease and adhesiveness, springiness and cohesiveness increase (Peyron et al., 2011). Chewing time of a food is related to its moisture content and hardness (Engelen,

Fontijn-Tekamp, & Van Der Bilt, 2005). There is also an inverse relationship between food hardness and number of chews required (Hiiemae et al., 1996). For example, mouthfuls of egg white (4 g), emmental (3-4 g) and carrots (4 g) have been found to require approximately 14, 24 and 34 chewing cycles, respectively (Jalabert-Malbos et al., 2007). The durations for mastication processes were 8 s for egg white, 15 s for emmental and 19 s for carrots. Salivary secretion rate is rather constant and therefore those products requiring longer chewing time will be mixed with more saliva (Gaviao et al., 2004). In cereals having a considerable amount of starch, the saliva incorporated in the food mass during mastication may be important for the breakdown of starch (Bornhorst et al 2012). For example, approximately half of wheat bread starch and 25 % of pasta starch have been found to be hydrolysed during mastication (Hoebler et al., 1998). Action of salivary α -amylase during mastication reduces viscosity of starchy bolus (Kong & Singh, 2008).

The bolus particle size distribution after mastication is dependent on food type rather than between individuals (Peyron, Mishellany, & Woda, 2004). Elastic behaviour (Young's modulus) and toughness (fracture stress) influence fracturing of food material (Bornhorst & Singh, 2012). Median particle size (d_{50}) of cereal food varies based on the initial structure being for example 1.5 mm for wheat-flake cereals (Peyron et al., 2011) and 1.9 mm for wheat bread (Le Bleis et al., 2016), whereas masticated pasta remains in particles with length of 2.5 - 30 mm (Hoebler et al., 1998).

Structure disintegration in the stomach is an important factor in digestion since it controls the gastric emptying which in turn influences the rate of further digestion (Bornhorst & Singh, 2012). Different structures show different resistance to physical and chemical (enzymatic, acidic) breakdown in stomach (Bornhorst & Singh, 2014). For example, extruded rye snacks disintegrated to smaller particles after gastric digestion than sourdough fermented rye bread or rye porridge, while rye crispbread contained the highest fraction of large particles (Johansson, Gutiérrez, Landberg, Alminger, & Langton, 2017). Structure disintegration is not dependent only on product category but also on variations within categories. For example, bran-containing brown rice remained in larger particles than white rice, and rye bread particle size reduced less than particle size of wheat bread during gastric digestion (Bornhorst, Kostlan, & Singh, 2013; Johansson et al., 2017; Nordlund, Katina, Mykkänen, & Poutanen, 2016).

Ingestion of a high viscosity meal increases the apparent viscosity of the gastric contents but the viscosity is rapidly reduced with intragastric dilution (Kong & Singh, 2008). Viscosity of the liquid phase of food digesta is generally low and Newtonian, whereas viscosity of the whole digesta is higher and non-Newtonian (Lentle & Janssen, 2010). Dissolved molecules such as non-digestible polysaccharides may increase the apparent viscosity of the liquid phase and decrease the gastric emptying of the liquid phase (Lentle & Janssen, 2010). Disintegration of the food matrix, and hydration, swelling and solubilisation of the food components define the development of digesta viscosity (Lentle & Janssen, 2008). For example sources of oat bran, guar gum or psyllium have the ability to increase viscosity during digestion (Dikeman, Murphy, & Fahey, 2006). However, the solubilisation of DF determines

the concentration in the liquid phase and viscosity (Capuano, 2017). Solubilisation and viscosity formation of DF also depend on whether DF is ingested as an extract or as part of food or meal. Higher viscosity of the liquid phase not only slows down gastric emptying rate but also slows down the permeation of gastric juices into food bolus. The slower permeation slows down the degradation of bolus retaining it longer in the gastric lumen.

1.3.3 Digestibility of starch

Starch is a storage carbohydrate of plants consisting of amylose that is a linear glucose polymer and amylopectin that is a branched glucose polymer (Singh, Dartois, & Kaur, 2010). Starch accounts for 20 % - 50 % of the total energy intake in human nutrition (Bohn et al., 2017). Salivary α -amylase initiates starch digestion in the mouth. The pH optimum for salivary α -amylase is at 6.8 (Pedersen, Bardow, Beier Jensen, & Nauntofte, 2002). The activity has been suggested to continue in the stomach for some time since glucose polymers may stabilize the enzyme. Finally, pH lowering inactivates α -amylase. The last step of starch digestion takes place in small intestine where pancreatic α -amylase hydrolyse it to maltose, maltotriose and α -dextrins. Brush border enzymes (amylglucosidases) further break down maltose, maltotriose and α -dextrins to glucose that is then efficiently absorbed into the bloodstream. Starch digestion *in vivo* can be observed by monitoring changes in blood glucose concentrations. Diets that elicit low postprandial glycaemia have been suggested as being important for the prevention of obesity, type 2 diabetes and cardiovascular diseases (Blaak et al., 2012).

Glycaemic Index (GI) was developed in the 1980's and has become the most popular tool to compare postprandial glycaemic responses of different carbohydrate sources (Bohn et al., 2017; Jenkins et al., 1981). GI is defined as the incremental area under the blood glucose response curve (IAUC) after consumption of a portion of food that contains 50 g of available carbohydrates expressed as a percentage of the IAUC elicited by a portion of a reference food containing equivalent amount of available carbohydrate (ISO, 2010). Available carbohydrate means the part of the carbohydrates that is absorbed into the bloodstream as carbohydrate and capable of increasing blood glucose levels when consumed. Refined wheat bread and glucose solutions are used as reference foods. There exist internationally accepted protocols to measure GI (World Health Organization & Nations, 1998). However, various methodological aspects such as physiology of the subjects, method of blood sampling and other macronutrients than carbohydrates in the test foods cause variation in the measured GI (Hätönen, 2014).

In vitro methods have been developed to complement the laborious *in vivo* glycaemic response tests (Bohn et al., 2017). They are used to predict the *in vivo* responses. *In vitro* tests aim to mimic different phases of human digestion starting with grinding or mastication to represent oral phase, mixing enzymes and adjusting pH according to different phases. Pioneering work of Englyst and colleagues presented an *in vitro* method where starch in food could be classified to three categories according to the *in vitro* hydrolysis rate: rapidly digestible starch (RDS), slowly

digestible starch (SDS) and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). A method to measure the hydrolysis index, which is another fundamental concept to predict metabolic responses, was published the same year (Granfeldt, Bjorck, Drews, & Tovar, 1992). Various *in vitro* methodologies have been suggested since to predict the *in vivo* responses.

Despite the generally good correlation between *in vitro* and *in vivo* measurements of starch digestion, the complexity of food structures and their interactions in the digestive environment may hinder the prediction of *in vivo* responses. For example, gastric emptying rate is in addition to starch hydrolysis, a factor determining glycaemic response and therefore factors influencing gastric emptying should be taken into account when predicting *in vivo* responses (Fardet, Leenhardt, Lioger, Scalbert, & Rémésy, 2006).

The β -cells in the pancreas respond to the nutrients in blood circulation releasing insulin which promotes the absorption of nutrients to tissues. Insulin secretion is primarily triggered by levels of circulating glucose (Fu, Gilbert, & Liu, 2013). Insulin responses have repeatedly observed to be lower for rye breads than for wheat bread even though the glucose responses do not differ (Bondia-Pons, Nordlund, Mattila, Katina, Aura, Kolehmainen, et al., 2011; Johansson, Lee, Risérus, Langton, & Landberg, 2015; Juntunen et al., 2003; Kallio et al., 2008; Leinonen, Liukkonen, Poutanen, Uusitupa, & Mykkänen, 1999; Moazzami, Shrestha, Morrison, Poutanen, & Mykkänen, 2014; Törrönen et al., 2013). The discrepancy in the glucose and insulin responses of rye, which is unusual among carbohydrate-rich foods, has been called a “rye factor” (Moazzami et al., 2012). The mechanism of the “rye factor” is not fully understood.

1.3.4 Structural features of cereal foods influencing digestibility of starch

Microscopic and macroscopic structural features that are relevant for starch digestion are summarized in Table 3. At the microscopic level, features of starch molecules and starch granules resulting from botanical origin or processing influence the susceptibility of starch to hydrolysis. Generally, amylopectin is more susceptible than amylose to hydrolysis and therefore starch with high content of amylose is more resistant to hydrolysis (Singh et al., 2010). Additionally, large starch granules are digested more slowly than small starch granules, which is suggested to be due to their smaller granule specific surface area. Close contact with other macromolecules such as gluten proteins may cover starch granules restricting their gelatinization and limiting the accessibility of digestive enzymes (Singh et al., 2010). For example in pasta, starch is encapsulated by proteins, which makes starch less susceptible for starch hydrolysis (Petitot et al., 2009). Amylose can form complexes with small hydrophobic molecules such as fatty acids. Formation of these complexes decreases solubility of starch and increases gelatinization temperature and resistance towards digestive enzymes.

Table 3. Microstructural characteristics and food matrix characteristics influencing starch digestion

Microstructural characteristics
Amylose-amylopectin ratio of starch
Size and surface of starch granules
Structural integrity of starch granules
DF encapsulation
Starch-protein interactions
Amylose-lipid complexes
Gelatinization and re-crystallization of starch
Food matrix characteristics
Open porosity
Density
Viscosity
Intact grain structures

Native starch is extremely resistant to hydrolysis and processing may drastically influence starch digestibility (Mishra, Hardacre, & Monro, 2012). Processes that disrupt the integrity of starch granules and increase the surface area, such as grinding, make starch more susceptible for digestion while intact grain structures form a physical barrier protecting starch from enzyme action (Singh et al., 2010). Gelatinization, which is an irreversible transition that starch undergoes when heated in water, makes starch more easily available for enzymatic action (Schirmer, Jekle, & Becker, 2015). The starch to water ratio of food determines the extent of gelatinization: starch in pudding is fully gelatinized whereas in biscuits it is only partly gelatinized. Upon cooling, starch chains of gelatinized starch start to re-crystallize (retrogradation) (Singh et al., 2010). The semi-crystalline structures formed in retrogradation resist amylase digestion (form of resistant starch).

The food matrix forming during processing influences the accessibility of digestive fluids to starch in the food and the effectiveness of α -amylase to starch hydrolysis (Mishra et al., 2012). Foods that have an open porous structure have a high internal surface area that is easily available for amylase action. Leavened breads and cakes as well as puffed products have open porous structures. Pasta and other food matrices with dense configurations are not easily available for digestive fluids. In such food matrices, digestive enzymes will be exposed to starch only by eroding superficial layers of food. Therefore, the surface area of bolus particles is relevant for the starch digestion in such dense foods.

In addition to direct effects of food structure on digestibility of starch, the changes occurring in digesta viscosity also influence digestibility of starch due to solubilized substances etc. When viscosity of the bolus in the stomach increases, gastric emptying rate decreases, which will impact the rate of nutrient flow to duodenum as well as rate of hydrolysis (Lentle & Janssen, 2008). Viscosity of chyme also hinders the access of digestive juices to macromolecules in the bolus.

1.4 Cereal foods and satiety

Satiety is a concept referring to a 'process that leads to inhibition of further eating, decline in hunger, increase in fullness after a meal has finished' (Blundell et al., 2010). It is also called 'inter-meal satiety'. *Satiation* means a 'process that leads to the termination of eating' and is also called 'intra-meal satiety'. Satiety enhancing foods are considered to help consumers to control their eating and to reduce negative psychological aspects of dieting (Halford & Harrold, 2012).

Cereal foods are an interesting food group from the weight maintenance and satiety perspectives since they are rich in DF and DF intake is associated with lower body weight and less weight gain as well as enhanced postprandial satiety (Du et al., 2010; Liu et al., 2003; Wanders et al., 2011). However, DF content of cereal foods varies and DF comprises of a wide variety of compounds with distinct physicochemical properties and thus varying impacts on appetite (Poutanen et al., 2017). Additionally, food structure influences the effectiveness of DF rich food to control appetite.

Rye products have repetitively been shown to have good appetite-reducing properties (Isaksson et al., 2011, 2012; Isaksson, Fredriksson, Andersson, Olsson, & Aman, 2009; Isaksson, Sundberg, Åman, Fredriksson, & Olsson, 2008; Johansson, Lee, Riserus, Langton, & Landberg, 2015; Rosén et al., 2009; Rosén, Östman, Shewry, et al., 2011; Rosén, Östman, & Björck, 2011). Typically the studies have compared rye products with refined wheat bread but some studies also compare satiety responses to rye products with different structures (Isaksson et al., 2011; Rosén, Östman, & Björck, 2011). Rye porridge with kernels was more effective than rye porridge with milled kernels to enhance satiety but there was no difference between rye bread with whole kernels and rye bread with milled kernels (Isaksson et al., 2011). Breakfast with boiled rye kernels was more effective in maintaining satiety than wholegrain rye bread (Rosén, Östman, & Björck, 2011).

Viscous DF such as oat β -glucan is generally considered to be beneficial for satiety (Wanders et al., 2011). However, in a recent systematic review, the viscosity forming ability of DF was not found to be consistently related to satiety (Poutanen et al., 2017). Regarding oat β -glucan and satiety, the results have also varied considerably with some showing beneficial effects (Beck, Tosh, Batterham, Tapsell, & Huang, 2009; Lyly et al., 2009, 2010) and others showing no difference compared to control (Hlebowicz, Darwiche, Björge, & Almér, 2008; Juvonen et al., 2011). Differences in food matrices where oat bran/ β -glucan has been incorporated is one potential source of variation in these studies.

1.4.1 Satiety signals originating from food ingestion and digestion

Food properties influence the satiety response through different signalling routes before and during food ingestion, after ingestion and after absorption (Blundell et al., 2010). The current study focuses on the cephalic and gastric phases of digestion and their links to satiety in an acute postprandial setting (3.5 h). The cephalic phase

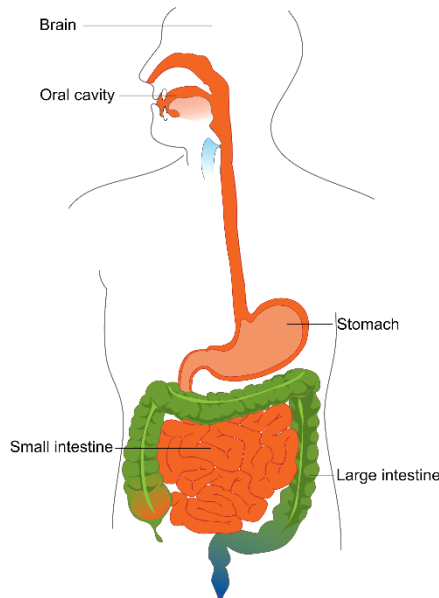
is the first stage of digestion beginning when the food is thought, seen, smelled and continues with mastication (Smeets et al., 2010). Already prior to ingestion, the appearance of food raises expectations of the satiating capacity of food and these expectations influence the actual satiety response (Brunstrom, Brown, Hinton, Rogers, & Fay, 2011) (Figure 6). Palatability of food increases food intake (Sørensen, Møller, Flint, Martens, & Raben, 2003). Palatability has been suggested to also influence postprandial appetite sensations, but the results have been mixed. Increased oro-sensory exposure has been concluded to improve satiety but potentially also postprandial satiety response (Hogenkamp & Schiöth, 2013). The gastric phase and specifically gastric distention and emptying are considered as key physiological functions related to satiety (Delzenne et al., 2010). Gastric emptying is naturally related to small intestinal phase of digestion determining the rate of nutrient flow. The exposure of the upper small intestine to nutrient stimuli is one of the determinants of satiety. Finally, some microbial fermentation products have been associated with satiety (Nilsson, Ostman, Holst, & Björck, 2008).

1. CEPHALIC PHASE

- Expectations (familiarity, sensory properties of food)
- Palatability (taste, texture of food)
- Oral residence time (viscosity, moisture content, hardness, fracturability and adhesion of food)

3. SMALL INTESTINAL PHASE

- Nutrient delivery (viscosity and macronutrients of chyme)



2. GASTRIC PHASE

- Gastric distention (volume of meal)
- Gastric emptying rate (bolus characteristics: viscosity, particle size distribution, fracturability of particles, macronutrients (fat), interactions between meal components, acidity)

4. LARGE INTESTINAL PHASE

- Microbial fermentation (fermentable non-digestible carbohydrates of chyme)

Figure 6. Satiety cues originating from different stages of digestion and properties of food, bolus and chyme influencing the cues. Adapted from <https://pixabay.com/en/digestive-system-human-digestion-41529/> Creative Commons (CC0)

1.4.2 Structural properties of food, bolus and chyme determining acute postprandial satiety

There are structural properties of food and digesta that evoke satiety signals at each stage of food digestion. During oral processing, food texture is sensed and textural features, such as creaminess, provide cues about the satiating capacity of food, based on earlier experiences about the associations between certain sensory properties and satiety (Yeomans & Chambers, 2011). Food structure determines satiety via oral processing. The importance of structure is especially evident when comparing liquid and solid structures. Regarding liquids, the oral phase is fast and the satiety providing signals are weak (Hogenkamp & Schiöth, 2013). Different solid foods require different oral processing depending on hardness and moisture content of the product (J. Chen, Khandelwal, Liu, & Funami, 2013; Jalabert-Malbos et al., 2007). However, it is not clear if the differences in oral processing are relevant for satiety response when comparing solid and semisolid foods (Hogenkamp & Schiöth, 2013).

Increased gastric distention and reduced gastric emptying rate have been recognised to contribute to satiety (Delzenne et al., 2010). Increasing gastric distention by increasing the volume of the food has been shown to enhance satiety response (Rolls & Roe, 2002). Particularly, increasing gastric volume by incorporating liquid into food instead of consuming it separately, has been found to be effective in increasing fullness in the early postprandial phase (Clegg, Ranawana, Shafat, & Henry, 2013; Rolls, Bell, & Thorwart, 1999; Zhu, Hsu, & Hollis, 2013). Gastric emptying rate is influenced by numerous factors related to food structure. Firstly, viscous food mass is emptied at a slower rate than less viscous food mass. Therefore, foods that result in a high viscosity food bolus due to viscous soluble fibres induce greater satiety (Marciani et al., 2001). Alterations in viscosity are dependent not only on DF type and content but also on other food properties such as particle size, and structure (Dikeman & Fahey, 2006). For example, solubility of β -glucan from fine barley flour is higher than that in coarse barley flour (Capuano, 2017). Secondly, the stomach regulates the flow of digesta through the pylorus, allowing only small enough particles to pass into the duodenum. Food particles that are highly resistant for gastric size reduction, such as meat or pasta, empty slower. Gastric emptying is selective meaning that generally, liquids are emptied first and solids remain in stomach longer. The interactions of liquid and solid phase could also influence emptying (Marciani et al., 2013). Solid substances that retain liquid could slower gastric emptying.

Chyme viscosity determines the flow rate in small intestine (Lentle & Janssen, 2008). Prolonged exposure of the small intestine to nutrients leads to increased release of gut peptides that mediate satiety response (Delzenne et al., 2010). Metabolites resulting from colonic fermentation of non-digestible carbohydrates are hypothesised to improve satiety (Nilsson et al., 2008).

2 Aims of the study

The aim of this work was to investigate how cereal food structure influences digestion and postprandial satiety

The specific aims were:

1. to study the effect of bread structure on mastication-induced structure disintegration and starch hydrolysis (I),
2. to investigate the dissolution of compounds from rye and wheat bread matrices to saliva in mastication (II),
3. to study the role of cereal food structure in postprandial satiety (III)
4. to focus on the role of food matrix in satiety responses of oat bran enriched meals (IV)

The main hypotheses were:

1. Rye breads require more mastication effort than wheat bread and rye breads disintegrate into larger particles from which starch hydrolyses at a slower rate (I)
2. Rye and wheat breads differ regarding the compounds that are dissolved from the bread matrix to saliva in mastication (II)
3. Cereal food structures that require intensive mastication result in stronger feeling of satiety than those requiring less intensive mastication (III)
4. Oat bran included in juice is more effective to maintain satiety than oat bran included in biscuit matrix (IV)

3 Materials and methods

The work is based on three trials of which *trial 1* is the basis for publications I and II, *trial 2* for publication III and *trial 3* for publication IV. The publications are hereinafter referred with Roman numerals (I - IV). Materials and methods used in the *trials 1-3* are summarized below and presented in detail in publications I-IV.

3.1 Participants and study designs

The trials were carried out at the Kuopio Campus of the University of Eastern Finland (*trial 1*) and at VTT Technical Research Centre of Finland Ltd (Otaniemi, Espoo) (*trials 2 and 3*) (Table 4). The study participants were recruited through advertising and email lists in the campus areas near the study locations. Normal-weight (Body Mass Index (BMI) 18.5–25 kg/m²) young (20–40 years in *trials 1 and 2* and 20–45 years in *trial 3*) females were recruited to the studies (Table 4). Food aversions or allergies to the study products and regular smoking were among exclusion criteria in each trial. Volunteers with missing teeth (except 3rd molars) and those with diagnosed functional mastication problems were excluded from mastication trials (*trials 1 and 2*). Pregnant or lactating volunteers and those with unstable body weight (± 4 kg) during the previous year or abnormal eating behaviour (evaluated with Eating Disorder Diagnostic Scale (EDDS) in *trial 2* and with Three-factor Eating Questionnaire (TFEQ) in *trial 3*) were excluded from the satiety trials (*trials 2 and 3*) (Stice, Telch, & Rizvi, 2000; Stunkard & Messick, 1985). Before the initiation of the studies, the study procedures were explained to the participants in detail and the volunteers were asked to sign an informed consent.

The study visits were organized during morning hours to limit the influence of circadian variation occurring in physiological processes (e. g. saliva secretion rate) (Panda, 2016). The participants of the mastication trials (*trials 1 and 2*) were instructed to eat breakfast 1 to 1.5 hours before the study visit and the satiety trial participants (*trials 2 and 3*) were instructed to fast at least 10 hours before the study visit. Each trial followed a crossover design where all participants tested each test product or portion. In the mastication trials, all products were tested during one visit, whereas in the satiety trials, one test portion was evaluated during one visit and there were at least two washout days between the visits. The study products were presented to the participants as ‘cereal products’ but no further details were explained.

The study protocols were approved by The Research Ethics Committee of Hospital District of Northern Savo (*trial 1*) and the Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (*trials 2 and 3*). The studies were conducted according to the ethical principles of good research and clinical practice described in the declaration of Helsinki.

Table 4. Types of trials, study products, number of participants and study participants' age and BMI

	<i>Trial 1</i>	<i>Trial 2</i>	<i>Trial 3</i>
Trials	Mastication trial	Mastication trial and 3.5-hour postprandial satiety trial	3.5-hour postprandial satiety trial
Study products	Rye and wheat breads	Rye and wheat breads, extruded rye flakes and puffs, rye smoothie	Wheat and oat bran biscuits, orange juice with or without added oat bran
Number of participants	n=15	n=26 in mastication trial, n=16 in satiety trial	n=30
Age (y), mean \pm SD	24.6 \pm 4.4	31.7 \pm 7.5	24.3 \pm 3.8
BMI (kg/m ²), mean \pm SD	22.0 \pm 1.4	22.2 \pm 1.9	21.7 \pm 1.9

3.1.1 Mastication trials

The coded food samples were served to the participants in random order, each sample in three portions in the mastication trials (*trial 1* and *2*). The order of food samples for each participant was randomized using list randomizer (www.random.org). One portion represented a mouthful of food (Table 5). The research group determined the portion sizes in pre-tests. The weights of bread cubes differed between *trials 1* and *2* since the breads that were used had different densities. However, the size of the cube (2 \times 2 \times 2 cm) was kept constant. The participants were instructed to masticate each portion until subjective swallowing point. At that point, the bolus was expectorated and placed in a plastic container, which was kept on ice. There was a short break between the food sample types during which the mouth was rinsed with water. Mastication process (number of chews, mastication time, work etc.) was measured with electromyography (EMG). The collected boluses were divided into aliquots and stored in -70 °C. Later the thawed aliquots were used to measure moisture content of the boluses, analyse particle size distribution, define salivary α -amylase induced starch hydrolysis and analyse dissolved compounds (Figure 7).

Table 5. Portion sizes of food samples in mastication trials

	Measure of a portion	Average weight of a portion (g)	Trial
Refined wheat bread ¹	2 × 2 × 2 cm	9.1	1
Wholemeal rye bread ¹	2 × 2 × 2 cm	15.1	1
Endosperm rye bread ¹	2 × 2 × 2 cm	9.6	1
Endosperm rye bread with gluten ¹	2 × 2 × 2 cm	10.9	1
Wholemeal rye bread ²	2 × 2 × 2 cm	7.7	2
Refined wheat bread ²	2 × 2 × 2 cm	4.0	2
Extruded wholemeal rye flakes	1 table spoon	3.5	2
Extruded wholemeal rye puffs	two 2 cm pieces	1	2
Rye smoothie with grinded rye flakes	1 table spoon	16.8	2

¹ No crust included

² Crust included on one side

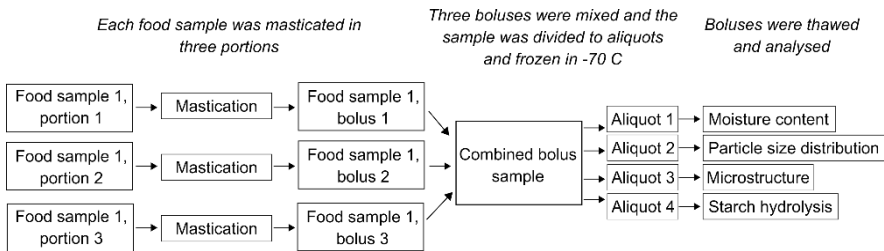


Figure 7 Flow of the food and bolus samples in *trial 1*. Each food sample (n=4) was masticated in three portions by each participant (n=15).

Electrical activity of masticatory muscles was measured by EMG equipment (Mega Electronics, Kuopio, Finland) using disposable dermal Ag/AgCl electrodes. Masseter and temporal muscles were identified by palpation while the participant clenched one's teeth. Ethanol (70 %) was used to clean the skin and bipolar electrodes were placed on the masticatory muscles on both sides of the face. EMG activity was measured throughout the mastication trial. Parameters describing the mastication process (number of chews, mastication time, work etc.) were extracted from the EMG data for each product. Matlab® (The MathWorks Inc., Natick, MA, USA) was used for EMG data analysis.

3.1.2 Satiety trials

Expected satiety

The participants in *trial 2* were asked to anticipate the satiating capacity of the food products before and after masticating a sample of the product (Table 6). The evaluation before the mastication was based on a visual cue (photograph) and the evaluation after mastication was based on both visual cue (photograph) and sensory cues obtained during mastication (Figure 8). The photograph showed a food portion with a fixed amount of the food sample and a glass of juice. The portions were similar to those served in the satiety trial. The participants anticipated their satiety using 10 cm visual analogue scale (VAS).

Table 6. Scales used in satiety trials adapted from Blundell et al 2010.

Scale	Questions	Anchors
Expected satiety before mastication	<i>Imagine that you would eat the whole portion of food shown in the photograph. Evaluate how satiated you would feel after one hour.</i>	0=not at all satiated, 10=extremely satiated
Expected satiety after mastication	<i>You have just masticated the product shown in the photograph. Imagine that you would eat the whole portion of food shown in the photograph. Evaluate how satiated you would feel after one hour</i>	0=not at all satiated, 10=extremely satiated
Hunger	<i>How hungry are you?</i>	0=not at all hungry, 10=extremely hungry
Fullness	<i>How full are you?</i>	0=not at all full, 10=extremely full
Satiety	<i>How satiated are you?</i>	0=not at all satiated, 10=extremely satiated
Desire to eat	<i>How strong is your desire to eat?</i>	0=not at strong, 10=extremely strong
Prospective food consumption	<i>How much do you think you could eat right now?</i>	0=nothing at all, 10=a very large amount

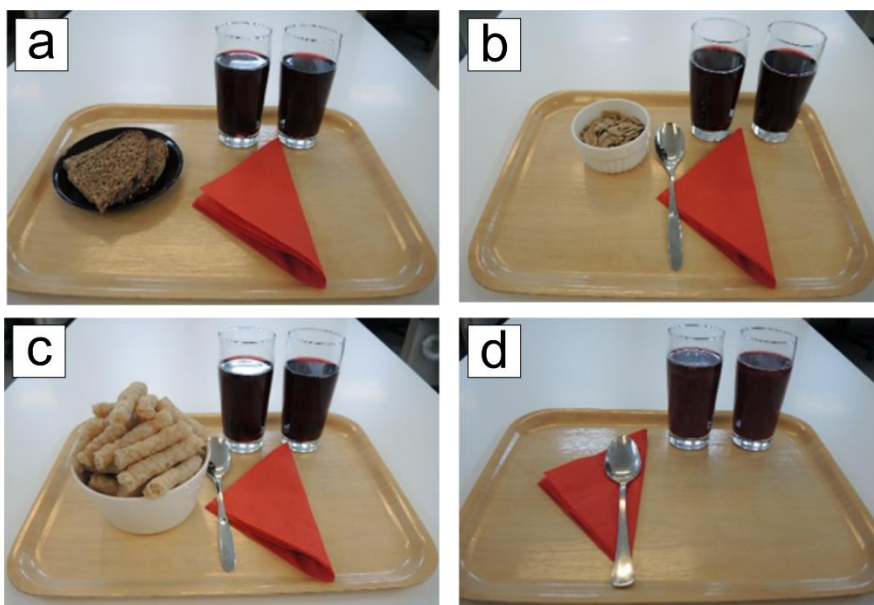


Figure 8 Photographs of the food portions a) wholemeal rye bread and juice, b) extruded wholemeal rye flakes and juice, c) extruded wholemeal rye puffs and juice, d) wholemeal rye smoothie.

Postprandial satiety

In *trials 2* and *3* the participants were served food portions consisting of cereal products and juice, one of the portions in each study visit, in a random order (Table 7). The order of the portions was randomized with the computerised data-collecting system (Compusense five 5.2) that was used for the evaluations. In *trial 2* the portion sizes were matched by energy contents, whereas in *trial 3* the portion sizes were matched by sample weights. The participants were instructed to eat and drink the food portions at their own pace but not to spend more than 20 min on eating in *trial 2* or not more than 10 minutes in *trial 3*. Satiety and related sensations were evaluated prior and after consuming the food portion and repetitively every 30 minutes until 210 min after starting point of the consumption using visual analogue scales (VAS) anchored with extremes (Table 6). The evaluated sensations were hunger, fullness, satiety, desire to eat and prospective food consumption. Additionally, *pleasantness* was evaluated right after consuming the food portion. Average appetite score was afterwards calculated as $[\text{desire to eat} + \text{hunger} + (10 - \text{fullness}) + \text{prospective food consumption}] / 4$. Computerised data-collecting system (CSA, Computerised Sensory Analysis System, Compusense, Guelph, Canada, Compusense five 5.2) was used to collect the evaluations.

Table 7. Portion sizes of food samples in postprandial satiety trials

	Weight of cereal component (g)	Weight of juice (g)	Energy in portion (kcal)	Trial
Wholemeal rye bread and juice	95	500	382	2
Refined wheat bread and juice	75	500	382	2
Extruded wholemeal rye flakes and juice	59	500	382	2
Extruded wholemeal rye puffs and juice	58	500	382	2
Wholemeal rye smoothie	58	500	382	2
Wheat biscuits and juice	78	400	467	3
Oat bran biscuits and juice	78	400	429	3
Wheat biscuits and oat bran juice	78	400 ¹	526	3
Oat bran biscuits and oat bran juice	78	400 ¹	488	3

¹Includes oat bran concentrate

3.2 Study products

3.2.1 Ingredients and preparation

Rye and wheat breads

The breads for *trial 1* were baked at VTT Technical Research Centre of Finland Ltd. The aim was to include breads with different structures for the trial. To reach this aim, refined wheat flour, wholemeal rye flour and endosperm rye flour were used as basic ingredients and either yeast leavening or sourdough fermentation were applied. Yeast leavened wheat bread and sourdough fermented wholemeal rye bread represented structural extremes and sourdough fermented endosperm rye bread was in the between those structures. Moreover, wheat gluten was added to endosperm rye bread to achieve a rye bread with some characteristics of refined wheat bread.

Refined wheat bread consisted of wheat flour, water, sugar, salt, vegetable margarine and emulsifier (PANDOAN®), and it was leavened with fresh yeast. Wholemeal rye bread comprised wholemeal rye flour, wholemeal rye sourdough (wholemeal rye flour, *Lactobacillus brevis*, *Lactobacillus plantarum*, fresh yeast and water),

water, fresh yeast and salt). Endosperm rye bread consisted of refined rye flour, refined rye sourdough (refined rye flour, *Lactobacillus brevis*, *Lactobacillus plantarum*, fresh yeast and water), water, fresh yeast and salt. Endosperm rye bread with gluten had otherwise similar ingredients but the refined rye flour was partly replaced with wheat gluten to change the structure of the bread to resemble that of wheat bread.

Wholemeal rye products and refined wheat bread

Extruded wholemeal rye puffs and flakes for *trial 2* were prepared at VTT using whole grain rye flour and salt as ingredients. A twin screw extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, UK) was used to produce the extrudates with a constant feed rate of 60 g/min and temperature profile of 80-95-110-120 °C with the screw speed of 350 and 250 rpm for puffs and flakes, respectively.

Wholemeal rye smoothie was prepared mixing ground wholemeal rye flakes with blackcurrant juice and letting the mixture stand for 15 min resulting in a thick smoothie-like heterogeneous texture. Blackcurrant juice was a commercial product (Marli).

Sourdough fermented wholemeal rye bread and refined wheat bread in *trial 2* were commercially available products (Emil Halme, Espoo, Finland). Wholemeal rye bread consisted of wholemeal rye flour, water and salt and refined wheat bread consisted of wheat flour, water, yeast, sugar, rapeseed oil and salt.

Biscuits and oat bran juice

The biscuits for *trial 3* were baked at VTT. The recipe for wheat biscuits comprised of wheat flour, water, sugar, rapeseed oil, emulsifier (PANDOAN®), ammonium bicarbonate, sodium bicarbonate, disodium pyrophosphate and salt. Oat bran biscuits included also oat flakes and oat bran concentrate (Oatwell 22, CreaNutrition).

Oat bran juice was prepared by mixing oat bran concentrate (Oatwell 22, CreaNutrition) with orange juice. Oat bran concentrate was mixed with juice individually for each portion just before the consumption to avoid viscosity formation prior to consumption.

3.2.2 Nutrient composition

AOAC methods 2009.01 and 2011.25 were used to determine the DF content, AOAC method 996.11 and AACC method 76.13 for starch content and Kjeldahl method (nitrogen × 6.25, according to 90/496/EEC) for protein content (I-IV). AACCI method 32-23-01 was used to determine the β-glucan content of biscuits (IV).

3.2.3 Structure and texture

Analyses of macrostructure

Specific volumes of fresh breads were determined by a Pregelbauer infrared device (Bread Vol Scan, Pregelbauer, Germany) (I). Bread structure was characterised by X-ray microtomography (Model 1172, SkyScan, Aartselaar, Belgium). 1 × 1 × 1 cm pieces of breadcrumb were scanned. The X-ray tube was operated at a voltage of 40 kV/250 µA and a 12-bit cooled CCD camera (2000 × 2000 pixels) was used to collect the X-ray data. Radiographs were loaded into NRecon reconstruction software (v. 1.6.6). Cell walls of the solid matrix appear grey and air cells appear black. The reconstructed 2-D slices were then loaded into CTAn software (v. 1.12, SkyScan, Belgium) to obtain the parameters of porosity, cell wall thickness and cell diameter.

Textural properties of breads (I and III) were extracted by TA-XT plus texture analyser (texture profile analysis) (Stable Micro System, Godalming, Surrey, UK) with a 25 mm diameter probe, 30 kg load cell and 40 % (I) / 60 % (III) strain on 25 mm thick cylindrical pieces of breads. The measurement was carried out only for pieces of breadcrumb (I) or including the upper crust on top side of the bread piece (III). The acquisition rate was 200 points/s and the test speed was 1.7 mm/s. TPA software (Exponent v.6, Stable Micro System, Godalming, Surrey, UK) was used to extract force-deformation curve, which was the basis of the parameter calculations (hardness, stickiness, cohesiveness, chewiness, adhesiveness). Textural properties of extruded wholemeal rye products were extracted by using a Texture Analyser TA-HDi, HD371 (Stable Micro Systems, United Kingdom) with a 36 mm probe and 250 kg load cell (III). The parameters were defined based on pre-tests. Puffed extrudates were cut to 10 mm height and flakes were analysed as is. The acquisition rate was 200 points/s and the test speed was 1 mm/s. Texture Exponent software v.5.1.2.0 (Stable Micro Systems, UK) was used for parameter calculations (hardness, crispiness).

Microscopy

The bread samples were prepared, stained and imaged according to Andersson and colleagues (Andersson et al., 2011) (I). Cereal cell wall protein was stained using Acid Fuchsin and β-glucan using Calcofluor. Protein and starch were stained with Light Green and Lugol's iodine, respectively. When examined in exciting light (excitation, 400–410 nm; emission, 445 nm) protein stained by Acid Fuchsin appears red and cell walls rich in β-glucan stained by Calcofluor appear blue. In bright-field, protein stained by Light Green appears green or yellow. Lugol's iodine stains native starch purple, while the amylose component of starch appears blue and amylopectin brown.

Analysis of sensory texture

The sensory profile of the breads, extruded products and smoothie was analysed by descriptive analysis (Lawless & Heymann, 2010) (III). The vocabulary of the sensory attributes was developed by describing the differences between the samples.

The selected textural attributes included moisture, hardness, work needed for mastication, porosity, crumbliness, crunchiness, crispiness and adhesion to teeth. Reference samples were used to define the extremes for most of the attributes, and all descriptors were also verbally anchored. All sensory intensities were evaluated using a 10 cm scale anchored from “not at all” to “extremely”. All samples were evaluated by sensory profiling by a trained sensory panel in duplicate sessions in two consecutive days. The scores were recorded and collected using computerized software (Compusense Five, Ver 5.4.15, CSA, Computerized Sensory Analysis System, Compusense Inc., Guelph, ON, Canada).

3.3 Food bolus characteristics, *in vitro* digestion and rheology

3.3.1 Bolus characterization

Saliva impregnation

The amount of saliva absorbed by masticated breads was determined based on the moisture content of bread crumbs and moisture content of bolus samples (I). Wet bolus (WB) samples were weighed and placed in an oven at 105 °C overnight and the dried bolus (DB) was weighed again. The water content of boluses was determined by the following formula: $WB-DB/WB \times 100$. The amount of saliva absorbed by different breads was determined by the difference between the water content of bolus and the water content of breadcrumb.

Particle size distribution

Particle size distribution of masticated breads was analysed with a method that was developed at VTT for this study. The bolus samples representing approximately one piece of masticated bread were diluted into 100 ml of water, mixed with magnetic stirring at room temperature for 25 min and let stand for 5 min in order to get bigger particles settled in the bottom (I). Then the turbid liquid containing the smallest particles that could not be imaged was removed and the sample volume was increased with water up to 100 ml. The diluted samples were poured on 9 cm petri dishes and adjusted so that they were as little as possible in contact with each other. Digital images were taken of each petri dish. Images were calibrated utilising a precision stage micrometre slide and particle areas were determined using Cell[^]P imaging software (Olympus, BX50).

Microscopy

The microscopy analyses of the bolus samples were carried out as those for food samples (described above) (I).

Metabolite profiling

Bolus samples of 200 mg by dry weight basis were diluted with 610 μ l of water, centrifuged and 100 μ l of the supernatant was collected (II). Methanol (200 μ l) was

used to extract metabolites and to precipitate proteins in the sample. The samples were filtered (0.2 μm PTFE membrane; PALL corporation) prior to liquid chromatography quadrupole time-of-flight mass spectrometry (LC-qTOF-MS) using hydrophilic interaction (HILIC) chromatography. The liquid chromatography was performed on a 1290 Infinity Binary UPLC system (Agilent Technologies, Santa Clara, CA, USA) and the mass spectrometric analysis was performed on a 6540 UHD Accurate-Mass Q-TOF (Agilent Technologies).

The data were collected by using the vendor software (MassHunter Qualitative Analysis B.05.00; Agilent Technologies), and the output was transferred in compound exchange format (.cef) into the Mass Profiler Professional software (MPP 2.2; Agilent Technologies) for data pre-processing.

The features were normalized row-wise and clustered, based on peak areas, into 15 clusters by k-means clustering by Multiple Experiment Viewer software (version 4.9). Features in specific clusters were identified. Exact masses of the positive ions and MS/MS fragmentation data were compared to entries in METLIN Metabolomics Database, other publicly available spectral databases, and in in-house standard library. MS-DIAL software version 2.64 was used in the identification process.

3.3.2 Salivary α -amylase induced starch hydrolysis rate *in vitro*

The method for measuring the rate of *in vitro* starch digestion of Granfeldt and colleagues was modified and used to determine salivary α -amylase induced starch hydrolysis of the boluses (Granfeldt et al., 1992) (I). Bolus samples obtained from the *in vivo* mastication trial were used in this *in vitro* analysis. An adequate weight for each individual bolus sample was determined based on the moisture content of the sample and starch content of the bread. The bolus samples were thawed and a sample with standard starch content (0.5 g) was transferred to dialysis tubing (Spectra/Por No. 2, flat width 45 mm, molecular weight cut off 12–14 kD) with cold phosphate buffer (pH 6.9). The tubing was incubated in a beaker with 0.05 M phosphate buffer. Incubation time was shortened to 30 min to reflect the time when salivary α -amylase could be active before being inactivated by low pH in stomach *in vivo*. Aliquots were removed from the buffer outside of the dialysis tubing at time points 0, 1.5, 3, 6, 9, 12, 15 and 30 min, and frozen ($-20\text{ }^{\circ}\text{C}$). Removed samples were incubated with amyloglucosidase (Megazyme) to hydrolyse the solubilised starch to glucose. Free glucose was determined by treating the samples with glucose oxidase peroxidase reagent (Megazyme) and the absorbance was read at 510 nm. Glucose solution (1 g/1 l) was used as a standard. The amount of released glucose was converted to starch multiplying with 0.9 and the degree of starch hydrolysis was calculated as the proportion of the solubilized starch of the original starch of the bolus.

3.3.3 Viscosity formation *in vitro*

An *in vitro* digestion tool with a rheometer (MCR 300, Anton Paar, Physica) and a titration station was used to measure *in vitro* viscosities of the test breakfasts with biscuits and juice (IV) (Aymard & Wahl, 2007). Biscuits were ground and blended with juice in the rheometer, pH was adjusted to 2 and pepsin was added to mimic gastric conditions. Evolution of viscosity was monitored for 70 min at low shear rate (10 s^{-1}). Next, pH was adjusted to 6.3 and pancreatin and bile salts were added to mimic small intestinal conditions. Evolution of viscosity was monitored for 90 min at low shear rate (10 s^{-1}). Viscosity values were taken at the end of the gastric and small intestinal phase.

3.4 Statistical analyses

SPSS software (IBM SPSS Statistics; versions 20, 22 and 14) was used for statistical analyses. Values of $p < 0.05$ were considered to be statistically significant with the exception of t-tests comparing fold changes in publication II where $p < 0.01$ was considered as statistically significant.

Product characteristics

Kruskal-Wallis test was used to compare textural properties of breads (I). Sensory differences between study products were compared using one-way ANOVA and Tukey's test for pair-wise comparison and satiety expectations and pleasantness evaluations were compared using repeated measures ANOVA (publication III).

Mastication

Friedman's non-parametric test for related samples was used to compare the parameters describing mastication (I and III). A non-parametric test was chosen since the variables were not normally distributed. Simple linear regression was conducted to evaluate to what extent the bread properties explain total work required to masticate bread (I).

Postprandial satiety

Linear mixed-effects models were used to compare the effects of the test portions on the profiles of postprandial satiety responses (III and IV). The models included participant as a random factor, and product, time, and product \times time interaction as fixed factors. When a significant main effect of a product or product \times time interaction was observed, post hoc analyses were performed using the Sidak correction for multiple comparisons in order to identify the statistically significant differences between the test portions. The contribution of cephalic phase factors was evaluated by adding parameters of oral processing, evaluated pleasantness and satiety expectations to the model as fixed factors one at a time and Schwarz's Bayesian Criterion (BIC) was then used to compare goodness of fit between the models (III).

Bolus properties

Repeated measures ANOVA was used to compare the starch hydrolysis rate of boluses (I). T-tests with Benjamini-Hochberg FDR correction were conducted to examine whether the fold changes were statistically significant (II).

4 Results

Results of the current work are summarized below and presented in detail in publications I-IV.

4.1 Characteristics of the study products

4.1.1 Nutrient content

Table 8 presents the nutrient contents of the studied cereal products (I-IV). Wholemeal rye products (I-III) were characterized by high DF and protein content, and low fat content. Endosperm rye breads had similarly low fat content but somewhat less DF and protein than the wholemeal rye products. Refined wheat breads (I-III) were low in DF, high in protein and relatively high in fat. Wheat biscuits were characterized by high fat content; relatively high protein content and low DF content (IV). Similarly, biscuits with oat bran addition were high in fat but also rich in DF and protein. Specifically β -glucan content of oat bran biscuits was high (5.1 g / 100 g).

Table 8. Nutrient contents of the cereal food samples in the articles I-IV.

Nutrients (g/100 g)	Articles I and II				Article III				Article IV	
	Re- fined wheat bread	Whole- meal rye bread	Endo- sperm rye bread	Endo- sperm rye bread with glu- ten	Refined wheat bread	Whole- meal rye bread	Ex- truded whole- meal rye flakes	Ex- truded whole- meal rye puffs	Wheat biscuits	Oat bran biscuits
Starch	40	34.8	46.0	43.9	46.4	35.4	57.7	59.8	59.7	40.6
Protein	7.4	6.1	4.1	5.4	9.1	6.5	9.7	9.8	8.2	12.4
Fat	5.4	0.9	0.3	0.4	2.4	0.6	1.2	1.3	14.6	13.2
Total DF	2.8	11.2	5.7	5.8	4.7	13.3	20.7	19.8	2.3	13.2
Soluble DF	0.8	3.6	2.1	1.9	2.3	7.5	9.5	10.7	N.a.	N.a.
Insoluble DF	2.0	7.7	3.6	3.9	1.5	3.6	3.7	4.0	N.a.	N.a.
Soluble arabinoxylan	0.3	0.7	0.5	0.5	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.
β -glucan	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	0.16	5.1
Oligosaccharides	N.a.	N.a.	N.a.	N.a.	1.0	2.2	7.6	5.2	N.a.	N.a.

N.a.) not analyzed

4.1.2 Structure and texture

Breads

The breads varied in structure and texture as designed. Wholemeal rye bread had the lowest and refined wheat bread the highest specific volume and total porosity (Figure 9, Table 9). Specific volume and total porosity of the two endosperm rye breads were in between those of wholemeal rye bread and refined wheat bread. Rye breads had higher closed porosity and thicker cell walls than refined wheat bread. Cell diameter was the largest in endosperm rye bread with gluten and the smallest in wholemeal rye bread. Rye breads were harder, less springy and less cohesive than refined wheat bread. Endosperm rye bread was the most chewy bread and wholemeal rye bread the least chewy.

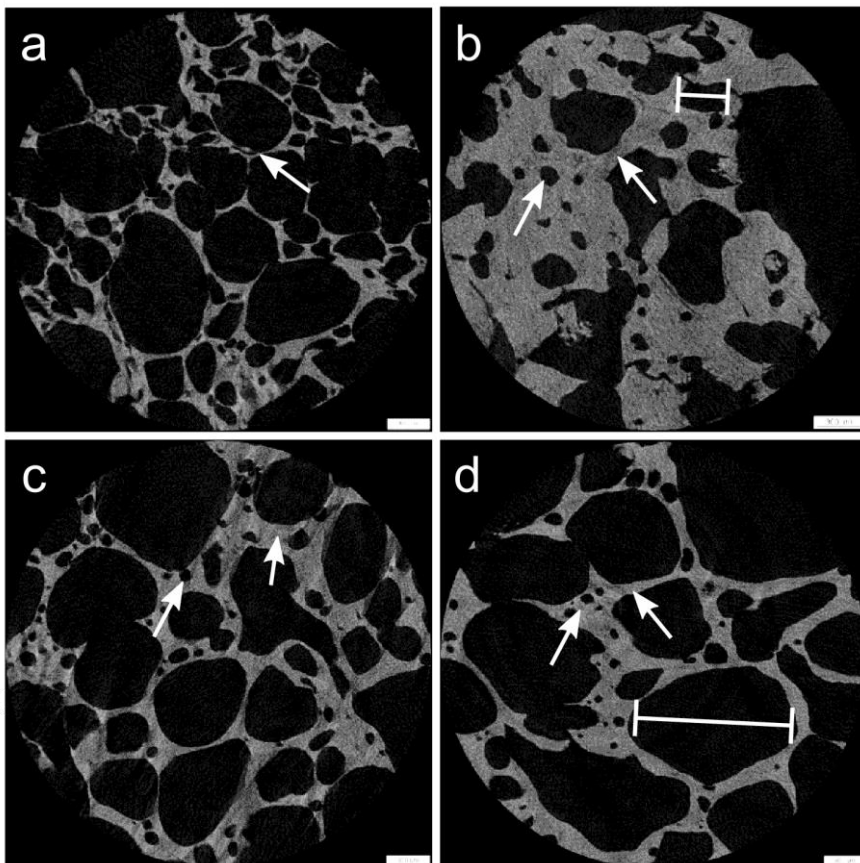


Figure 9. 2D XRT images of breads a) refined wheat bread, b) wholemeal rye bread, c) endosperm rye bread, d) endosperm rye bread with gluten. The arrows point out

cell walls, closed and open pores and line segments point out pore diameter. White bar is 900 μm . Figure has been adapted from publication I.

Table 9. Structural and instrumental textural characteristics of the crumbs ($n=10$) (I). Values are means \pm standard deviations. Different superscript letters indicate pairwise differences between breads ($p < 0.05$).

	Refined wheat bread	Whole-meal rye bread	Endosperm rye bread	Endosperm rye bread with gluten	p
Specific volume (ml/g)	5.1 \pm 0.1 ^c	2.0 \pm 0.1 ^a	3.2 \pm 0.2 ^b	3.1 \pm 0.0 ^b	<0.001
Total porosity (%)	83 \pm 3 ^c	55 \pm 2 ^a	73 \pm 7 ^b	76 \pm 2 ^{bc}	<0.001
Closed porosity (%)	0.4 \pm 0.1 ^a	1.8 \pm 0.5 ^b	1.3 \pm 0.5 ^b	1.2 \pm 0.5 ^{ab}	0.002
Cell wall thickness (μm)	111 \pm 8 ^a	146 \pm 30 ^{abc}	152 \pm 15 ^{bc}	176 \pm 31 ^c	0.003
Cell diameter (μm)	993 \pm 126 ^{ab}	865 \pm 105 ^a	1339 \pm 300 ^{bc}	1560 \pm 206 ^c	<0.001
Hardness (g)	170 \pm 28 ^a	757 \pm 99 ^d	648 \pm 133 ^c	601 \pm 98 ^b	<0.001
Cohesiveness	0.5 \pm 0.05 ^c	0.11 \pm 0.01 ^a	0.22 \pm 0.03 ^b	0.21 \pm 0.03 ^b	<0.001
Chewiness (g)	76 \pm 7 ^b	28 \pm 6 ^a	98 \pm 26 ^c	88 \pm 19 ^{bc}	<0.001
Springiness (%)	0.9 \pm 0.02 ^c	0.3 \pm 0.04 ^a	0.7 \pm 0.06 ^b	0.7 \pm 0.06 ^b	<0.001

The continuous phase in rye bread matrices was formed by starch, whereas in refined wheat bread protein formed the continuous network (Figure 10). Unlike in refined wheat bread where protein formed a continuous network, in endosperm rye bread with gluten, protein was aggregated as distinguishable chunks. Starch granules in refined wheat bread were compact, whereas in rye breads they were swollen and partly degraded. Especially in wholemeal rye bread, starch granules were swollen and some amylose had leaked out and formed crystals. Bran particles were present in wholemeal rye bread.

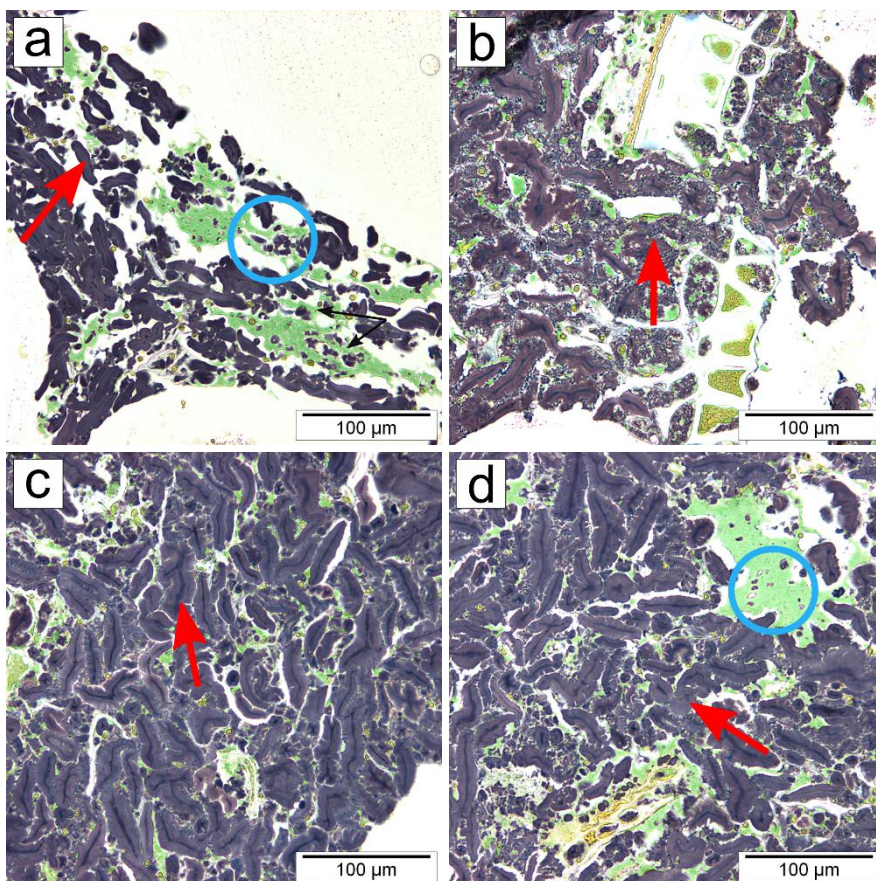


Figure 10. Light micrographs of breads (I) a) refined wheat bread, b) wholemeal rye bread, c) endosperm rye bread, d) endosperm rye bread with gluten. Protein appears green (stained with Light Green) and starch granules purple (stained with Lugol's iodine). White bar is 100 μm . Blue circles point out continuous protein network in refined wheat bread (a) and chunks of protein in endosperm rye bread with wheat gluten. Red arrows point out starch granules.

Breads, extruded products and smoothie

Rye flakes had the hardest and wheat bread the least hard texture (Table 10) (III). Hardness of wholemeal rye puffs was similar to that of wholemeal rye bread. Wholemeal rye bread was harder, less cohesive, more chewy and adhesive than refined wheat bread. Extruded wholemeal puffs were crispier than extruded wholemeal flakes.

Table 10. Instrumental textural characteristics of breads (n=10) and extruded products (n=20) (III). Values are means \pm standard deviations. Crumbs with crust on top side were used to measure textural properties.

	Refined wheat bread	Wholemeal rye bread	Extruded wholemeal rye flakes	Extruded wholemeal rye puffs
Hardness (g)	4 \pm 1	24 \pm 8	1530 \pm 390	27 \pm 3
Cohesiveness	0.7 \pm 0.0	0.4 \pm 0.1	-	-
Chewiness (g)	2.0 \pm 0.5	5.1 \pm 1.8	-	-
Adhesiveness	-0.13 \pm 0.33	-0.01 \pm 0.01	-	-
Crispiness work			98.3 \pm 37.3	0.6 \pm 0.1
Crispiness index ($\times 10^{-3}$)			0.004 \pm 0.002	21 \pm 5

The study products (III) varied significantly in all sensory attributes describing texture (ANOVA, $p < 0.001$) (Figure 11). The extruded wholemeal rye flakes and puffs were the driest products and smoothie the moistest. Breads were intermediate regarding moisture and rye bread was perceived moister compared to refined wheat bread. Wholemeal rye flakes were harder and required more work for mastication than the other products. Regarding the breads, wholemeal rye bread was harder and required more work for mastication than wheat bread. The breads and wholemeal rye puffs were porous. Rye puffs had the most porous structure. The both extruded products were crumblier, crunchier and crispier than the breads or smoothie. Wholemeal rye bread was crumblier than refined wheat bread. Wholemeal rye puffs adhered to teeth more than the flakes, the breads or wholemeal rye smoothie.

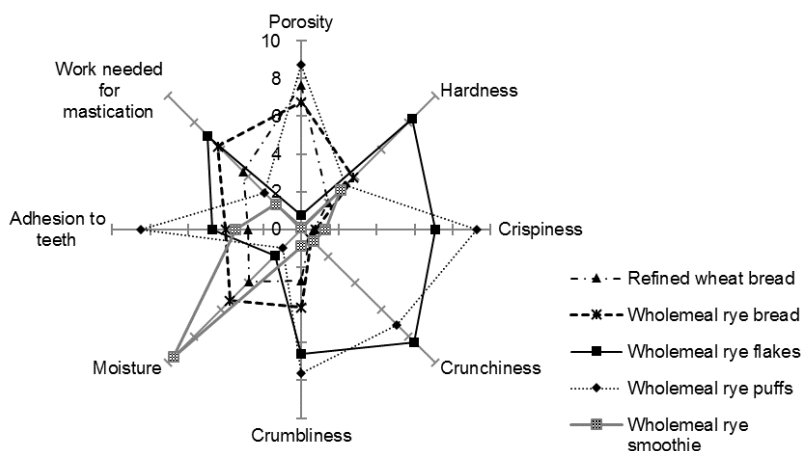


Figure 11. Perceived textural differences between study products (III). The study products differed statistically significantly in each attribute (ANOVA, $p < 0.001$).

4.2 Relevance of cereal food structure to digestion

4.2.1 Impact of food structure on mastication

Breads with different structures

Mastication processes of a piece of refined wheat bread, wholemeal rye bread, endosperm rye bread and endosperm rye bread with gluten did not differ regarding number of chews ($p = 0.244$) or chewing time ($p = 0.232$) (Figure 12) (I). However, the breads differed in total work required to masticate bread ($p = 0.004$) and regarding work/bite ($p = 0.026$). Wholemeal rye bread required more total work than refined wheat bread and endosperm rye bread with gluten required more work per bite than refined wheat bread.

There was a strong positive correlation between closed porosity and mastication work ($R^2 = 0.96$) and strong negative correlation between specific volume and mastication work ($R^2 = -0.98$) as well as between cohesiveness and mastication work ($R^2 = -0.94$). Hardness and cell wall thickness correlated weakly with mastication work ($R^2 = 0.71$, $R^2 = 0.41$, respectively).

Wholemeal rye products with different structures

Mastication processes of mouthfuls of wholemeal rye products with different structures (bread, extruded flakes and puffs, smoothie) and refined wheat bread differed regarding the number of chews, chewing time and relative work ($p < 0.001$ for all) (III) (Figure 12). Wholemeal rye bread and flakes and refined wheat bread required

more chews, longer chewing time and more work compared to wholemeal rye puffs or smoothie ($p < 0.05$ for all).

The mouthfuls of food samples had different weights and therefore the measured mastication process attributes were extrapolated to represent the mastication process of a fixed portion of the product. Similarly as for mouthfuls, there were statistically significant differences in the extrapolated values regarding number of chews, chewing time and relative work between portions of products ($p < 0.001$ for all). Number of chews per portion was higher for flakes, puffs and wheat bread than for rye bread or rye smoothie ($p < 0.05$ for all). Masticating a portion of flakes and puffs required the longest time and the most work, whereas processing a portion of smoothie required the shortest time and the least work ($p < 0.05$ for all).

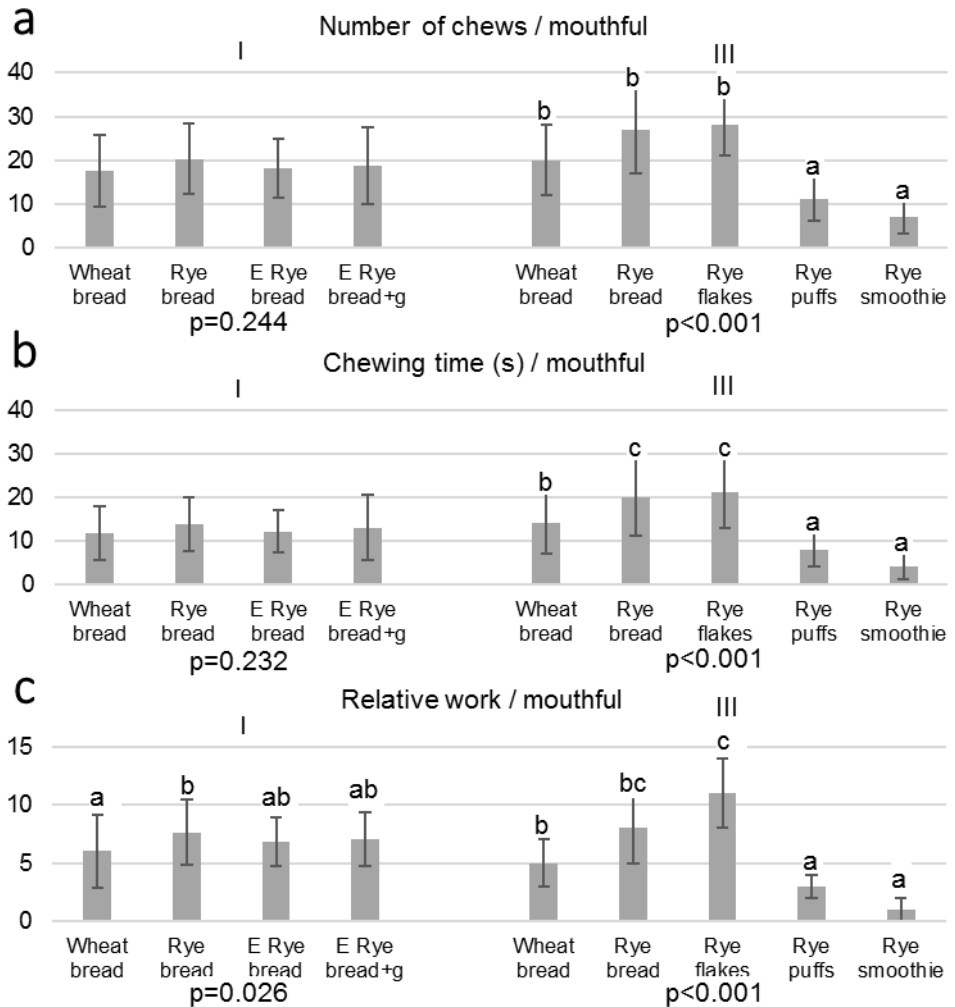


Figure 12. a) Number of chews, b) chewing time and c) relative work for mouthful of products (mean ± SD) in I and III. Work was related to a mastication process of a control product (I) or mastication process of chewing gum (III). E = endosperm, g = gluten.

4.2.2 Impact of food structure on bolus characteristics and *in vitro* digestion

The breads absorbed on average 0.3 g of saliva per 1 g of bread during mastication with no differences between bread types (I). Wholemeal rye bread, endosperm rye

bread and endosperm rye bread with gluten were degraded into smaller and visually more compact particles than wheat bread. In rye breads, particles smaller than 1 mm² covered 50-60% of the surface area while particles smaller than 1 mm² covered less than 40% of the surface area of wheat bread particles. Mastication had no major impact on microstructure of the breads. While starch granules were swollen and some amylose had leaked out from the granules already in rye breads as such, in masticated refined wheat bread the starch granules also appeared swollen and some amylose had leaked out.

Dissolution of compounds from masticated breads to saliva

Altogether 1807 features occurred in mere saliva samples and saliva samples isolated from boluses of four breads (wholemeal rye bread, endosperm rye bread, endosperm rye bread with gluten and refined wheat bread) (II). More features were dissolved to saliva from masticated rye breads than from masticated wheat breads. Features were divided to 15 clusters of which four were specific to rye bread boluses and one to wheat bread boluses. These specific clusters of features were examined in detail. Rye bread boluses were characterized by a greater dissolution of peptides and amino acids, whereas sugars and nucleosides were characteristic for wheat bread boluses. Twenty-four different peptides and 10 amino acids (asparagine, leucine, phenylalanine, isoleucine, saccharopine, methyllysine, citrulline, aspartic acid, lysine, pipercolic acid) were more pronounced in the rye bread boluses than in the wheat bread boluses. Nucleosides; 2'-deoxyadenosine, cytidine and 1-methyladenosine were more pronounced in the wheat bread boluses than in the rye bread boluses. Sugar compounds were more pronounced in wheat bread boluses than in rye bread boluses.

There was a trend for slower starch hydrolysis rate in rye bread boluses compared to refined wheat bread boluses during 30 min incubation ($p = 0.098$) (Table 11) (I). At 30 min, the amount of solubilised starch of the original starch content was 20.6 ± 2.3 % in wholemeal rye bread bolus, 19.0 ± 1.8 % in endosperm rye bread bolus and 18.7 ± 1.5 % in bolus of endosperm rye bread with gluten, whereas the amount of solubilized starch was 24.3 ± 2.3 % in refined wheat bread bolus.

Table 11. Salivary α -amylase induced starch hydrolysis rates (%/min) in bread boluses

	Refined wheat bread	Wholemeal rye bread	Endosperm rye bread	Endo-sperm rye bread with gluten	<i>p</i>
Starch hydrolysis rate (%/min)	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	.098

***In vitro* viscosity development of portions of biscuits and juice with oat bran additions**

In vitro viscosity of the portions with biscuits and juice was studied after gastric and small intestinal conditions. Viscosity was the highest in the portion with oat bran addition both in biscuits and in juice (21.3 Pa s after gastric conditions and 22.7 Pa s after small intestinal conditions) (IV). The food portions with oat bran addition either in biscuits or in juice gave rise to similar viscosities (7.8 Pa s and 4.8 Pa s after gastric conditions (respectively), 8.2 Pa s and 6.3 Pa s after intestinal conditions (respectively)). The control portion with no oat bran addition in biscuits or juice had very low viscosity during *in vitro* digestion (< 0.06 Pa s). The results indicate that the total amount of oat bran in food portion governs viscosity in gastric and intestinal conditions rather than the food matrix where oat bran has been added.

4.3 Relevance of cereal food structure to satiety

4.3.1 Impact of food structure on expected satiety

There were statistically significant differences in satiety expectations between the food portions with matching ingredients but different structures (ANOVA, $p < 0.001$ both before and after mastication) (III). The food portion with wholemeal rye bread and juice was anticipated to be the most satiating food portion, whereas wholemeal rye smoothie was evaluated as the least satiating food portion. Evaluation of expected satiety was higher after masticating rye bread, rye flakes and rye smoothie than before masticating those sample foods ($p = 0.001$, $p < 0.001$, and $p < 0.001$, respectively).

4.3.2 Impact of food structure on postprandial satiety

Wholemeal rye products with different structures

The food portions with matching ingredients and energy contents but different structures influenced some aspects of postprandial satiety responses differently in the early postprandial period (30 min and 60 min) (III). Thirty minutes after the initiation of eating a portion of extruded wholemeal rye flakes and juice, the feeling of fullness was weaker and the feeling of hunger was stronger than at the same time point after consuming a portion of extruded wholemeal rye puffs and juice ($p = 0.012$ and $p = 0.028$, respectively). Desire to eat was significantly stronger at 60 min after consumption of a portion of extruded wholemeal rye flakes and juice than after consuming wholemeal rye bread and juice ($p = 0.038$). The amount of food that the participants evaluated being able to eat ("prospective food consumption") was larger after consuming flakes and juice portion than after consuming puffs and juice portion at 30 min and 60 min ($p = 0.002$ and $p = 0.028$, respectively) or rye bread and juice portion at 30 min ($p = 0.018$).

Average appetite is a parameter derived from the evaluations of fullness, prospective food consumption, hunger and desire to eat. Average appetite was significantly higher 30 min and 60 min after consuming a portion of extruded wholemeal rye flakes and juice than after consuming a portion of extruded wholemeal rye puffs and juice ($p = 0.011$, $p = 0.045$). At time point 30 min average appetite was also significantly higher after consuming the rye flake portion compared to rye bread portion ($p = 0.034$). Except for the differences reported above there were no other statistically significant differences at any time points between any of the food portions.

Goodness of fit (BIC value) of the mixed model where product and time were fixed factors and subject a random factor was 2195. Adding the number of chews in the model did not improve model fit (BIC value 2165, p -value for product 0.051) but adding a parameter of relative work did improve it (BIC value 1911, p -value for product 0.001) as well as adding evaluated pleasantness (BIC 1965, p -value for product 0.001).

Oat bran addition to biscuit and juice matrices

The food portions with matching volumes of biscuits and juice but differing oat bran additions (to biscuits, juice or both) influenced some aspects of postprandial satiety responses differently (IV). The feelings of satiety and fullness were stronger and the amount of food that the participants evaluated being able to eat (“prospective food consumption”) was smaller after consuming bran-added biscuits and bran-added juice, compared to the control portion without oat bran addition ($p < 0.001$ for satiety and fullness, $p = 0.01$ for prospective food consumption). The evaluated satiety and fullness were also stronger after consuming the portion with oat bran addition in both biscuits and juice compared to the portion with oat bran addition only in biscuits ($p = 0.007$, $p < 0.001$, respectively). Desire to eat and evaluated ability to eat were lower after consuming a food portion with oat bran addition both in biscuits and in juice compared to the portion with oat bran addition only in juice ($p = 0.003$, $p = 0.002$, respectively).

The feelings of satiety and fullness were stronger after consuming the food portion where oat bran was added to juice compared to the control portion ($p < 0.001$ for both). The feeling of fullness was also stronger after consuming the breakfast with oat bran addition in juice compared to that with oat bran addition in biscuits ($p < 0.001$). There were no significant differences between the food portions regarding either the feeling of hunger or the average appetite score.

5 Discussion

In the bread mastication study, only small differences were observed between rye and wheat breads in mastication processes but rye breads disintegrated to smaller particles than wheat bread and starch in rye bread boluses tended to hydrolyse at a slower rate. A diverse array of compounds was dissolved from masticated breads and mixed with saliva. Specifically, peptides and amino acids were dissolved from rye breads and sugar compounds from wheat bread.

Comparison of wholemeal rye products with different structures (bread, extruded flakes, extruded puffs or smoothie) showed that portions of bread or puffs and juice were more effective than portion of flakes and juice to maintain some aspects of satiety. Intensity of oral processing did not relate to satiety response but perceived pleasantness and satiety expectations did. Oat bran that was added to juice was more effective in maintaining the feelings of satiety and fullness than oat bran incorporated in biscuit matrix.

5.1 Bread structures and first steps of digestion

5.1.1 Bread structures

Microscopic analysis showed that the continuous phase of refined wheat bread was based on gluten network, whereas swollen starch granules formed the continuous phase embedding protein in rye breads (I). Wholemeal rye bread matrix contained large particles with cell wall. The results regarding the microstructures are in line with previous studies (Autio et al., 1997; Autio & Salmenkallio-Marttila, 2001; Goesaert et al., 2005). Wheat gluten that was added in endosperm rye bread did not form a network but protein remained as distinct chunks. The reason might be that the rye bread baking process did not include intense mixing that is required for formation of strong gluten network (Goesaert et al., 2005).

The distinct microstructures were reflected in the macroscopic structures and instrumental textures. Wheat bread was more porous and cohesive compared to the rye breads resulting from cohesive and elastic gluten network with the ability to retain carbon dioxide during baking. There were no cell wall containing particles that could have hardened the texture. Rye proteins do not possess a similar ability as wheat proteins to form a network that would efficiently hold expanding gases during baking (Autio et al., 1997). The three rye breads had more closed pores, thicker cell walls and harder and denser structure than wheat bread had. Endosperm rye breads were denser and less porous than wheat bread but less dense and more porous than wholemeal rye bread. Cell wall containing particles in wholemeal rye bread have likely increased its hardness compared to the other breads that were baked from refined flour (Autio & Salmenkallio-Marttila, 2001).

5.1.2 Transformation of bread to bolus in mastication

Despite the above-described structural differences in the four breads, there were no major differences in the mastication processes. Of the observed parameters, there were differences only regarding work: more total work was required to masticate wholemeal rye bread and more work/bite to masticate endosperm rye bread with gluten when comparing to wheat bread. Similar amounts of saliva were incorporated to different breads during mastication process. Rye breads disintegrated to smaller particles than wheat bread that had bigger, ragged particles remaining in the bolus.

Bread crust is a major factor for overall bread structure and mastication properties (Gao, Wong, Lim, Henry, & Zhou, 2015). It was seen also in the current study: wheat bread without crust had more chewy texture than rye bread (I) whereas rye bread with crust (III) had more chewy texture compared to wheat bread with crust. In study I, the breads were offered in similar size cubes with no crust. In addition, regardless of the differences in bread structures, they all belong to the same food category of solid cereal foams. These factors probably explain the small differences that were found in mastication processes. Hardness of wholemeal rye bread as well as the high content of arabinoxylan that might cause part of bolus to adhere to the oral cavity could explain the difference in total work needed to masticate wholemeal rye bread compared to wheat bread that was less hard and contained less arabinoxylan. On the other hand, wholemeal rye bread was the moistest bread and since adequate moisture content is one determinant of mastication process, the high initial moisture content probably limited the need for mastication.

There were no differences between breads regarding saliva uptake in mastication. Porosity has been shown to be an important factor determining hydration of wheat breads and firmness has been found to be inversely related to water holding capacity of breads with different flours (Bornhorst & Singh, 2013; Mathieu et al., 2016). In addition, bread moisture content has been found to influence the amount of liquid absorbed by the bolus during *in vitro* mastication (Bornhorst & Singh, 2013). When considering the observed differences between breads regarding porosity, hardness and moisture content in the current study, it is somewhat surprising that there were no significant differences between the breads in saliva uptake. However, saliva secretion rate has been found to be rather stable during mastication of different food types (Gaviao et al., 2004). Therefore, mastication time is an important factor determining the incorporation of saliva to food bolus. Since the length of mastication did not differ in different breads, it is natural that similar amounts of saliva were incorporated to the breads. In addition, there is large inter-individual variation in saliva secretion and that might have overrode the small differences between bread types (Pedersen et al., 2002).

Rye breads disintegrated to smaller particles than wheat bread during mastication. Previously, wheat bread with hard crust has been shown to disintegrate to smaller particles in mastication than wheat bread with soft crust (Gao et al., 2015). However, as the current study observed the mastication process of bread crumbs (with no crust) this is not a possible explanation for the found differences (I). Differ-

ences in bread densities do not appear to explain the differences either, since density of bread has been concluded to be an unimportant factor for particle size distribution of bolus (Le Bleis, Chaunier, Della Valle, Panouillé, & Réguerre, 2013; Le Bleis et al., 2016). Different structural bases offer one potential explanation for the smaller particles in masticated rye breads: wheat bread had a strong gluten network that was reflected as cohesive bread texture. On the other hand, rye breads did not have similar network and they had less cohesive textures. Therefore, rye breads might fragment to small particles more easily than wheat breads in mastication.

Even though wheat bread remained in larger particles in mastication compared to rye breads, the situation appears to change when proceeding further in digestion. Earlier, a long lag phase was observed in rye bread disintegration in *in vitro* gastric conditions (Bornhorst & Singh, 2013). Similarly, rye breads were found to remain as larger particles compared to wheat breads after mastication and *in vitro* gastric digestion (Nordlund et al., 2016). While rye bread digesta particles remained to have the continuous network of starch granules after mastication and *in vitro* gastric digestion, wheat bread residue was extensively hydrolysed. When reflecting those results with the results of the current work, it seems that even though larger particles remain after mastication of wheat breads compared to rye breads, the wheat bread particles are more susceptible to pepsin hydrolysis in gastric conditions, possibly due to easily accessible gluten network, resulting in drastic reduction in particle size. On the contrary, rye bread particle size did not reduce much after *in vitro* gastric digestion compared to the situation after mastication. This resistance to enzyme action in gastric conditions could be attributable to the closed porosity that hinders the penetration of gastric secretions inside the bread structure and swollen starch granules and viscosity forming DF in rye bread particles, which likely protect protein. Additionally, other properties of the digesta, such as increased viscosity of liquid phase due to dissolved compounds, could slow down the action of gastric enzymes.

Food particle size after mastication and gastric digestion is among determinants of postprandial responses. For example, high proportion of larger particles after *in vitro* digestion was negatively correlated with *in vivo* insulin response and the percentage of small particles correlated positively with *in vivo* glucose responses (Nordlund et al., 2016; Ranawana, Monro, Mishra, & Henry, 2010). Therefore, studies on particle size reduction after mastication and *in vitro* gastric digestion offer potential explanations for differences in postprandial metabolism, for example those that have been observed in insulin metabolism. Mastication-induced dissolution of compounds from bread matrices

The rate of salivary α -amylase induced starch hydrolysis tended to be slower in rye breads than in wheat bread (I). In line with this observation, non-targeted LC-MS metabolic profiling showed that less di- and tri-saccharides were dissolved from masticated rye breads than from masticated wheat bread (II). In addition to sugar compounds, metabolic profiling of boluses showed that a diverse array of compounds was dissolved from masticated breads to saliva. This study was the first to demonstrate the wealth of compounds dissolving from the food matrix already after mastication using a non-targeted metabolomics approach. Especially, peptides and amino acids were dissolved from rye bread matrices to higher extent than from

wheat bread matrix after mastication (II). Other interesting compounds that were dissolved from the bread matrices included ribitol, betaines, vitamins and amines.

One might expect that smaller particles with more surface area compared to mass, such as rye bread particles in the current study, would be more susceptible to enzyme action than larger particles. However, there was a trend for slower starch hydrolysis rate in all the rye bread boluses. Therefore, rye bread must have specific properties resisting the impact of salivary α -amylase. Firstly, the cell walls in rye breads were thicker and there were more closed pores compared to wheat bread making the penetration of saliva inside rye bread more difficult. Accordingly, the structure of the rye bread bolus particles was compact probably making them less easily accessible for salivary α -amylase. As opposite to rye bread particles, the wheat bread particles were airy, likely making them easily accessible for salivary α -amylase. Similarly, starch in highly porous industrial wheat bread was found to hydrolyse faster during mastication than starch in artisan wheat bread or whole wheat bread, which both had a less porous, denser structure (Joubert et al., 2017). Secondly, we found the structures of the studied products to be different even at the level of starch granules, most probably attributable to sourdough fermentation (Poutanen, Flander, & Katina, 2009). The starch granules of rye breads were swollen and some amylose had leached out from the granules and recrystallized. This form of starch resists amylase action and thus, might partly explain the slower starch hydrolysis (Singh et al., 2010). Thirdly, all the rye breads, including the endosperm rye breads contained more DF, especially arabinoxylan, compared to wheat bread. DF, which was scattered in the bread matrix may have formed a physical barrier to the enzyme action and on the other hand, the soluble DF together with saliva may have formed a viscous layer slowing down the penetration of salivary enzymes to particles. In line with the current study, Joubert et al found that starch in wholemeal wheat bread with more DF was hydrolysed slower than starch in refined wheat bread (Joubert et al., 2017). Lastly, pH of sourdough fermented rye breads is around 4.2-4.3 (Katina et al., 2014), which is below the optimal pH for salivary α -amylase.

More peptides and amino acids were dissolved from masticated rye breads than from masticated wheat bread indicating that the first pool of protein hydrolysis products was released more easily from various rye breads compared to refined wheat bread. In the case of rye breads, this phenomenon likely results from the sourdough fermentation process during which the proteins in the dough are already partly hydrolysed (Poutanen et al., 2009; Tuukkanen, Lojonen, Mikola, Sontag-strohm, & Salovaara, 2005). However, more water-soluble proteins were dissolved also from wholemeal wheat bread after mastication compared to refined wheat bread after mastication (Joubert et al., 2017). Sourdough fermentation was not applied in baking and thus cannot explain the observed differences in released proteins in that study. One possible explanation for the slower dissolution of protein from refined wheat bread compared to wholemeal wheat bread or to various rye breads could be that the protein pool in refined wheat bread is well-networked and remains stable during mastication, whereas protein in the other bread types is not as networked and thus could be dissolved more easily.

5.1.3 Potential nutritional relevance of compounds dissolved to saliva in mastication

The compounds that are released from food matrix during digestion may act as signal molecules in the digestive tract for example stimulating the secretion of hormones or activating vagal nerve receptors (Delzenne et al., 2010; Raybould, 2008). After mastication, the food bolus enters the stomach, where it is mixed with gastric secretions. The liquid phase with solute compounds generally passes through the stomach faster than the solid phase and may reach the gut in front (Siegel et al., 1988). Therefore, it is interesting to observe the compounds that are released from food matrix already because of mastication process and dissolved in saliva and other digestive fluids.

Rye and wheat typically breads induce similar postprandial glucose responses but the insulin response to rye bread is attenuated compared to wheat bread (Bondia-Pons, Nordlund, Mattila, Katina, Aura, Kolehmainen, et al., 2011; Johansson, Lee, Risérus, et al., 2015; Juntunen et al., 2003; Kallio et al., 2008; Leinonen et al., 1999; Moazzami et al., 2014; Törrönen et al., 2013). Differences in the starch hydrolysis rate that we found after mastication and others have found after *in vitro* digestion (Juntunen et al., 2003) might explain the difference knowing that the glucose entrance rate to duodenum is among the factors regulating insulin response (Pilichiewicz et al., 2007). Supporting the hypothesis, the concentration of gastric inhibitory peptide (GIP), which is stimulated by glucose flow to the duodenum and which in turn stimulates the insulin secretion from pancreatic β cells (Yabe & Seino, 2011) has been shown to be slower for rye breads compared to wheat bread (Juntunen et al., 2003). Furthermore, an array of peptides and amino acids was dissolved from rye breads to saliva during mastication and may proceed from stomach to duodenum in front with the liquid phase. Nutrients entering to the trigger negative feedback on gastric emptying (Hellström et al., 2006). Slower gastric emptying could also be a factor slowing down the glucose entrance rate to duodenum.

Many studies have shown the beneficial effects of rye foods on satiety which occur most probably due to high DF content of rye products (Isaksson et al., 2009, 2008; Johansson, Lee, Riserus, et al., 2015; Rosén, Östman, Shewry, et al., 2011). However, the current study raises interesting questions regarding the role of compounds, namely protein hydrolysis products and ribitol, dissolved in mastication to saliva and their potential role in the regulation of satiety. Protein hydrolysis products, peptides and amino acids as well as ribitol were more pronounced in rye bread boluses. Protein hydrolysates in digestive tract increase cholecystokinin release, which is an appetite suppressing hormone released shortly after beginning of eating episode (Delzenne et al., 2010; Raybould, 2008). The rapid release of protein hydrolysates could offer one additional explanation for satiety-promoting effects of rye bread. Rye bread intake has been observed to increase ribitol concentration in plasma and it has been suggested to mediate the enhanced satiety response to rye (Bondia-Pons, Nordlund, Mattila, Katina, Aura, & Kolehmainen, 2011; Lankinen et al., 2011). The current study found that ribitol was dissolved from whole-meal rye

bread and endosperm rye bread and it was mixed with saliva supporting the potential role of ribitol for enhanced satiety responses.

The current study was pioneering work in the field of digestion studying the dissolved compounds from masticated breads by non-targeted metabolomics. The results raise interesting hypothesis about the role of these compounds in signalling during digestion. However, further studies are needed to understand the relevance of the compounds released in early steps of digestion for the overall digestion and postprandial responses.

5.2 Cereal food structure and satiety

5.2.1 Effects on cephalic phase factors

Rye bread or extruded rye puffs with juice were more effective to maintain some aspects of satiety than extruded wholemeal rye flakes with juice (III). Rye smoothie did not differ statistically significantly from the other portions. The differences in satiety responses (average appetite score) occurred in the early postprandial phase (30 min and 60 min). The second satiety trial showed that the amount of added oat bran but also the matrix where oat bran was added were relevant for feelings of satiety and fullness: incorporation of oat bran in a liquid matrix (juice) was more effective than incorporating it in a solid matrix (biscuits) (IV). Structure of wholemeal rye products influenced cephalic phase factors: oral processing, perceived pleasantness, and expected satiety (III). Mastication of portions of the driest products, extruded flakes and puffs, required the longest time and the most work, whereas processing a portion of smoothie, that was clearly the moistest product, required the shortest time and the least work. Rye bread was evaluated to be the most pleasant product whereas extruded rye puffs were evaluated as the least pleasant product. Rye smoothie was anticipated to induce the poorest feeling of satiety while rye bread, which was assumedly the most familiar rye product, was expected to induce the most intense feeling of satiety. Cephalic phase signals prepare gastrointestinal tract for food processing and this phase has also been suggested to influence satiety response (Smeets et al., 2010).

Unlike we expected, the intensity of oral processing did not relate to satiety response. In fact, rye flakes that required the most intense mastication (high orosensory exposure) resulted in the poorest feeling of satiety. A recent review suggested that orosensory exposure, referring to number of chews, chewing time etc., would influence at least to satiation but possibly also to postprandial satiety (Hogenkamp & Schiöth, 2013). This means that foods with high orosensory exposure might lead to enhanced postprandial satiety when comparing to those with low orosensory exposure. The results of the current study did not support this. The rye smoothie, which results in mastication process that could be defined as low orosensory exposure did not differ from the other portions with high orosensory exposure (bread,

extrudates). Differences in oral processing could indicate that the foods have distinct structural features for example related to density that might be relevant for bolus properties and later stages of digestion and satiety. Those will be discussed in the next chapter.

Perceived pleasantness influenced the feelings of satiety to some extent regarding rye products with different structures. Specifically the stronger feeling of satiety after eating rye puffs may have partly occurred because the product was perceived unpleasant. Similarly, in study IV, where the influence of biscuits and juice portions to satiety were studied, the consumption of the least pleasant combination resulted in the strongest feelings of satiety. The importance of palatability of foods on postprandial satiety feelings has been concluded to remain unclear (Sørensen et al., 2003). The results of the two satiety trials in the current work imply that portions that are disliked result in enhanced satiety response. However, decreasing palatability of food is not of course a feasible strategy when aiming to develop satiety enhancing foods.

Satiety expectations did influence the actual postprandial satiety in the current study. In fact, the differences in satiety responses vanished when the evaluations of expected satiety were taken into account. The result is in line with an earlier study that showed a relationship between expected satiety and actual satiety (Brunstrom et al., 2011). The results regarding the importance of perceptions (pleasantness and expected satiety) highlight the significance of other than purely physiological determinants (stomach distention, nutrient absorption etc.) of satiety response. The differences between study products in evaluated pleasantness as well as anticipated satiety should therefore be taken into account when planning satiety studies and interpreting the results. In some other culture where rye products are not as familiar as in Finland, the results could have been different. In the light of the results from the rye product study, studying the satiety expectations also in the biscuit study might have helped to interpret the results. Hypothetically, the juice with oat bran may have been perceived thicker than normal juice evoking expectations on its satiating capacity, which via expected satiety influenced the actual feeling of satiety.

5.2.2 Interactions in gastric digestion

In addition to the cephalic factors discussed above, inevitably also the changes in food structure and interactions between the solid and liquid components during gastric digestion were relevant for satiety. Especially regarding the rye products, the differences in satiety responses occurred already during the first postprandial hour indicating that gastric phase factors were more important than small intestinal phase factors. The gastric phase of digestion usually takes 3-4 hours (Minekus et al., 2014). Gastric distention and gastric emptying rate are key physiological factors associated with appetite regulation (Delzenne et al., 2010).

The both satiety trials in the current study tested meals with cereal products and juice. During gastric digestion, liquids leave the stomach first and after that, there is a lag phase, during which solids are processed into small enough particles to pass the pylorus (Hellström et al., 2006). The ability of the food to hydrate and to retain

water might therefore partly influence gastric emptying and distention. Hydration properties of DF (including the swelling capacity and water retention capacity) depend on size, shape and elasticity of particles and total surface area of particles as well as the form the DF is ingested (Capuano, 2017). In both current satiety trials, the products were equal in DF type and content within the studies but the food structures were different. Rye flakes that maintained some aspects of satiety poorer than bread or puffs, were less porous and harder than those products, and therefore, probably the ability of flakes to hydrate and the capacity to hold water in gastric phase were relatively poor (Bornhorst & Singh, 2013; Mathieu et al., 2016). Additionally, wholemeal rye bread fragmented to very small particles in mastication, particles with area under 1 mm² covering 50 % of the surface area (I). Alam et al measured particle size distribution of masticated extruded rye products with a method similar to the current study. They found out that the area covered with particles smaller than 1 mm² was only 10 - 35 % in masticated extruded rye products, which indicates that extruded rye products remained as larger particles after mastication than breads in the current study (Alam et al., 2016). Especially rye flake boluses contained large particles with smaller surface area in relation to mass, probably resisting hydration. To summarize, rye flake particles were hydrated slower and retained less water due to low porosity and high hardness as well as fragmenting to large particles. For these reasons, solubilisation of DF was probably poorer and the liquid phase might have emptied more rapidly than regarding the portions where hydration was better. However, we did not study gastric emptying rate, which prevents us from making firm conclusions about hydration and its impact on gastric emptying.

The solid and liquid matrices were in interaction already before ingestion in two of the studied food portions: the rye smoothie in the rye product study and the oat bran added to juice in the biscuit study. In those portions, the soluble DF (in rye smoothie, mainly arabinoxylan and in oat bran juice, mainly β -glucan) has been probably already partly dissolved in the moment of consumption, increasing the viscosity of the liquid phase and slowing down the gastric emptying of the liquid (Marciani et al., 2001). On the contrary, the corresponding combinations with liquid and solid matrices ingested separately (rye flakes and juice in the rye product study and juice and biscuits with oat bran in the biscuit study) the interaction started only at the moment of consumption and the liquid phase was probably emptied from stomach at a faster rate. Satiety response of those meals with separately ingested cereal and juice parts was poorer than of the corresponding mixed meals. Our observation is in line with the suggestion of Wanders et al (2011): DF provided in liquid form might induce stronger appetite reduction compared to those provided as part of solid foods (Wanders et al., 2011).

Taken together, stomach distention and emptying rate are important factors for satiety (Delzenne et al., 2010). Water holding capacity of solid phase as well as viscosity of the liquid phase (Marciani et al., 2001) are determining gastric emptying. Therefore, the interactions between the meal components are important, specifically in the case of cereal products that contain substances (e.g. soluble DF) that dissolve

from food matrix increasing the viscosity of liquid phase and on the other hand that typically have porous structures with ability to hydrate and retain water.

5.3 Methodological considerations

5.3.1 Modification and characterization of food structure

Different food structures were produced to explore the relevance of cereal food structure to digestion and satiety in the current study. The different structures were created by using different raw materials (wholemeal rye, endosperm rye, refined wheat, wheat gluten, oat bran) and processing methods (sourdough baking, straight dough baking, extrusion). Cereal food structure as other food structures can be observed in different length scales. At microscopic level, type (e.g. amylose-amylopectin ratio) and state of molecules (e.g. gelatinized starch), interactions between molecules (e.g. starch-protein interactions) and polymer systems are forming the food structure. Microstructure of foods determines macrostructure and perceived texture (Aguilera, 2006). The factors that form the structure are interrelated and therefore modifying some structural feature influences inevitably to other features as well. Thus, the interpretation of the obtained results is not always straightforward but different aspects of the structure need to be considered. For example, density of bread has been found to influence starch digestibility but density is related to many other structural characteristics making the understanding about the principal component behind the effect hard to identify. Therefore, when studying realistic foods instead of model foods it is important to characterize the structure thoroughly.

Light microscopy, X-ray microtomography (XMT), texture profiling and descriptive sensory profiling were used in the current study to define food structures. Light microscopy with specific staining of chemical components visualizes the microstructure showing the arrangement of compounds (e.g. starch and protein) in food and therefore offer explanations on the observed differences in macroscopic structure and texture (Autio & Salmenkallio-Marttila, 2001). For example, light microscopy added understanding about the state of starch and the location of proteins in different bread matrices in the current study. XMT combined with image analysis provides quantitative information of cell diameter, cell wall thickness, and porosity, which are important attributes in cellular solid foods such as breads (Alam & Sozer, 2016). Texture profile analysis quantifies the mechanical characteristics of foods such as hardness, cohesiveness and chewiness, which relate to the sensory texture of the product. Descriptive sensory analyses provide objective descriptions of products in terms of the perceived sensory attributes (Lawless & Heymann, 2010).

Each of the methods is valuable in adding understanding about the food structure. Optimally, the different methods should have been used more consistently in the three trials. The most comprehensive structure analysis was done for breads in *trial 1* (XMT, texture profiling, light microscopy), whereas the analyses were more limited in *trial 2* (texture profiling and descriptive sensory profiling) and in *trial 3* food structure was not quantified but only food bolus rheology *in vitro* was observed. By

applying more uniform structural analysis and uniform analysis of the structure disintegration too, the comparability between the studies would have been more straightforward. Due to the variety of structures (breads being springy solid foams, extruded being brittle solid foams, smoothie being liquid with solid particles) not all the methods would have been similarly applicable for all products. However, for example descriptive sensory profiling and light microscopy are methods that can cover very different structures.

5.3.2 *In vivo* studies

Mastication trials

Human trials were conducted to study mastication process of rye and wheat breads with different structures and rye products with different structures. Young healthy females were chosen as participants to outline some of the inter-individual variation in mastication. The number of participants was 15 in the first trial. Even though the study population was limited to young healthy females, we found large variation in mastication and possibly, there was not enough statistical power to show the differences that various bread structures brought about in mastication. Therefore, more participants (n=26) were recruited to the next mastication trial.

Mastication process in the current study was characterized with electromyography (I and III). Other possible methods for characterizing mastication process include for example, timing the chewing sequences and counting the chewing cycles (Jalabert-Malbos et al., 2007; Peyron et al., 2004), recording mandibular movement (Hedjazi, Guessasma, Yven, Della Valle, & Salles, 2013) and recording intraoral forces with sensors placed inside oral cavity (Shimada et al., 2012). Electromyography proved to be a useful method providing information not only about the time needed to masticate the sample but also information about the relative work needed to masticate different foods. In addition, it was a comfortable method from the participants' point of view. In *trial 1*, one food product outside the study set was used as a reference for force and work parameters and in *trial 2* chewing gum was used as a reference for those parameters. Chewing gum being a familiar product for all the participants was found to be a more suitable option. Mastication of chewing gum provided clear and consistent data about individual's EMG signal that could be used as reference data.

The study foods were served as "mouthfuls". The volume of a mouthful was based on pre-tests. Due to the anatomical differences, the portion sizes were not fit for all the participants and that possibly distracted the natural mastication process. In further mastication studies, some options will be to offer larger portions and let the participants consume the whole portion habitually or to conduct individual pre-tests to define a suitable portion for each individual. In *trial 1*, bread crust was removed from the samples, which most likely narrowed the structural differences between breads. Therefore, crust was included in one side of the bread cubes in the next mastication trial (*trial 2*) to gain more realistic understanding about the mastication processes of wheat and rye breads.

Satiety trials

Measuring appetite has, based on our studies and studies of other groups, turned out to be a complex task. Satiety is a subjective feeling, influenced not only by quality and quantity of the consumed food but influenced also for instance, by expectations, beliefs, and liking (Blundell et al., 2010). For example, liking appeared to influence satiety response in the both satiety trials. In addition, familiarity of the studied products can have marked influence on expected satiety and actual satiety (Brunstrom, Shakeshaft, & Scott-Samuel, 2008; Irvine, Brunstrom, Gee, & Rogers, 2013). For example, in *trial 2* the study products were not equally familiar to the participants. Therefore, the unintentional differences between products that may influence the results should be recognized and, if possible, eliminated. The current study setting would have been benefitted from familiarizing the participants with the study products beforehand. Unfortunately, that was not foreseen when planning the trial.

The principle of defining the portion size in postprandial satiety studies is a key consideration. Amount of energy, amount of fibre or volume are examples of different bases to define the portion size. However, keeping one attribute constant inevitably leads to differences in the other attributes. Energy content was chosen as a basis to define portion sizes in *trial 2*, and weight of biscuits and volume of juice were kept constant in *trial 3*. These choices led to a situation where the amount of food as grams slightly varied between portions (*trial 2*) or to a situation where energy contents of the portions varied (*trial 3*). With hindsight, in both the studies, the energy content could have been the basis for defining portion sizes and weight of the portion could have been adjusted by adding more water to the juice.

'Preload - test meal paradigm' is a methodology routinely used to evaluate post-meal satiety response to food portions (Blundell, 2017). Fixed amount of food (preload) is followed by test meal (amount eaten *ad libitum* is measured) or subjective evaluations of satiety sensations at regular time intervals. The five scales recommended by Blundell et al (2010) were used in the current study to define satiety responses. All the parameters have been concluded to explain subsequent energy intake (A. Flint, Raben, Blundell, & Astrup, 2000). However, the different attributes do not necessarily provide consistent results, which has been seen in earlier studies and in the current study. However, assumedly a diet consisting of foods with good satiating capacity is one means to alleviate weight control.

In the satiety trial where wholemeal rye product portions were compared, differences in the satiety related responses were seen already during the early postprandial period. The mastication processes of the portions also differed. Regarding those products, satiation (intra-meal satiety) would have been interesting study topic in addition to post-meal satiety that was studied. It could be assumed that there would have been differences in the total amount consumed *ad libitum* regarding different products. For example, the portion of extruded wholemeal rye puffs was considerably larger in volume than portion of extruded wholemeal rye flakes although they were similar in weight and energy content. Probably larger volume and extensive need for mastication would have been reflected as a smaller amount eaten. This remains as an interesting topic for future studies.

5.3.3 *In vitro* studies

Human nutrition trials are considered as “gold standard” providing information about health effects of foods (Minekus et al., 2014). However, *in vitro* methods that simulate digestion process offer complementing information about gastro-intestinal functions of foods. Additionally they are rapid and cost-effective. The current study applied both *in vivo* and *in vitro* methods.

Starch hydrolysis rate has been recognised as a key parameter determining postprandial glycaemic response. The duodenum is the main site for starch hydrolysis in the digestive tract. However, saliva contains salivary α -amylase that initiates starch digestion. In starchy and porous foods, such as breads, already mastication that softens the structure and the starch hydrolysis by salivary α -amylase might be relevant for the overall digestion (Bornhorst & Singh, 2013). Salivary α -amylase induced starch hydrolysis rate was evaluated by measuring the release of soluble starch from food matrix during incubation in buffer. The method was based on a method to measure starch hydrolysis rate (Granfeldt et al., 1992). Instead of using alimentary enzymes, only enzymes originating from *in vivo* mastication were present in the samples. To improve the method for the future purposes, references with ground breads mixed with buffer could be added to the data set to be able to differentiate the part of starch, which is dissolved from matrix to water from the part of solubilized starch that has been actually released by mastication process and action of salivary α -amylase.

In vitro viscosity evolution of portions of biscuits and juice were measured in *trial* 3. In the applied method, biscuits were ground and mixed with juice and the viscosity of the whole mixture after *in vitro* gastric and small intestinal phases was observed. This approach did not take into account the selective nature of gastric emptying that might influence gastric distention. Taking into account the interactions between meal components and the impact of those interactions on gastric emptying in *in vitro* methods would in the future studies provide valuable information of gastric digestion process of heterogeneous meals.

5.4 Limitations of the study

The trials were conducted in various projects with varying main goals. This basis resulted in some heterogeneity in the applied methods. More uniform analyses of food structure and bolus properties would have improved the possibilities to interpret the results more thoroughly and to draw firmer conclusions. Specifically, understanding of the viscosity evolution of food boluses and interactions between meal components (hydration of solid components, compounds dissolved in liquid phase) in trials 2 and 3 and about the particle size distribution of food boluses in trials 2 and 3 would have been useful. In addition, measurements of gastric emptying would have been useful for making conclusions about gastric emptying rate in relation to satiety.

The study participants were young healthy females in each of the trials. Therefore, the results cannot be directly generalized to general population. However, the study results provide useful indications about the mastication process of cereal foods and their satiety effects.

5.5 Evaluation of the main hypotheses

1. *Rye breads require more mastication effort than wheat bread and rye breads disintegrate into larger particles from which starch hydrolyses at a slower rate (I)*
 - There were only minor differences in the mastication processes of rye breads and wheat bread. As opposed to the hypothesis, rye breads disintegrated into smaller particles than wheat breads in mastication. According to the hypothesis, starch tended to hydrolyse at a slower rate from rye breads than from wheat bread.
2. *Rye and wheat breads differ regarding the compounds that are dissolved from the bread matrix to saliva in mastication (II)*
 - As hypothesised, the compounds that were dissolved from rye and wheat bread matrices in mastication differed largely.
3. *Cereal food structures that require intensive mastication result in stronger feeling of satiety than those requiring less intensive mastication (III)*
 - As opposed to the hypothesis, structure that required the most intensive mastication did not result in stronger feeling of satiety than those requiring less intensive mastication.
4. *Oat bran included in juice is more effective to maintain satiety than oat bran included in biscuit matrix (IV)*
 - As hypothesised, oat bran included in juice was more effective to maintain some aspects of satiety than oat bran included in biscuit matrix.

5.6 Combining food and nutrition sciences in food development

The current study explored the mastication process and postprandial satiety responses of DF rich cereal products with different structures. Mastication is an interesting interface between food and nutrition sciences being the endpoint for food

technology and the starting point for nutrition. Food scientists should be able to develop foods that have among other important attributes a good nutritional profile and appealing sensory properties. As it has been realised that unrefined foods high in DF are important for health, it is important to combine forces for their increased availability and use. High DF foods are demanded especially in the snack food category.

Profound understanding about food structure is required in order to create foods with appealing textures. However, food structure is not important only for the perceived texture but also for digestion. Disintegration of food structure in mastication and further digestion is determining the physiological functionality of food. Structure disintegration, for example, defines gastric emptying rate, which is related to postprandial glycaemia and insulinaemia. Wholegrain and DF rich foods have exceptionally beneficial impact on health, but their consumption is far from adequate. Attractive wholegrain foods and DF rich foods with proven functionality in GI tract should be developed to increase the intake. This goal can be achieved with close collaboration between food scientists, biochemists, nutritionists and sensory scientists.

6 Conclusions

The work showed that food ingredients, processing and the resulting form of food are important contributors for digestion and satiety. Intriguingly, a vast array of protein hydrolysis products was dissolved to saliva from masticated rye breads. The rapid dissolution was most probably a consequence of partial hydrolysis of protein in the bread baking process. Starch, in turn, tended to hydrolyse more slowly in masticated rye breads than wheat bread, even though rye breads were disintegrated to smaller particles. Therefore, particle size was less important determinant of starch digestibility than other properties of bread particles. Most likely, factors related to macro and microstructure of rye breads (thick cell walls, closed pores, recrystallized starch) resisted the influence of salivary α -amylase.

Structural differences between foods prepared using similar ingredients influenced the expectations towards satiating capacity of food, palatability and mastication process. Surprisingly, the food structure that required the most intense mastication was less able to maintain some aspects of satiety than products requiring less intense mastication. However, other cephalic phase factors, expectations and liking did contribute to satiety. Expectations about the satiating capacity of food seemed to be self-fulfilling prophecies and less pleasant food portions resulted in enhanced satiety. These results highlight the importance of considering not only the direct physiological satiety targets (e.g. stomach emptying rate) but also subjective perceptions about the studied foods when designing satiety trials and interpreting the results.

The results suggest that hydration of the food matrix and its DF component is important for satiety. Foods with potentially good hydration capacities (porous products, bread and extruded puffs) or those, which were ingested as partly hydrated (rye smoothie, juice with oat bran) enhanced some aspects of satiety compared to those products likely hydrating at a slower rate (dense flakes, oat bran biscuit).

The current research raises some interesting questions for future studies. Firstly, what is the significance of the compounds that are released from food matrix already in mastication? These compounds may precede through the alimentary tract potentially acting as the first signal molecules. Could they be, for example, regulating postprandial metabolism or satiety? Secondly, how do meal components with different structures interact in stomach and how do the interactions influence gastric emptying, postprandial metabolism and satiety? Thirdly, how do the wholemeal rye portions with different structures contribute to intra-meal satiety?

The results provided more understanding on the significance of food structure to the first steps of digestion and to postprandial satiety: breads baked with different raw materials and methods had distinct structures and were digested differently already in mouth, and meals with similar ingredients but different structures resulted in distinct responses of some aspects of satiety. Therefore, understanding is needed about not only the chemical composition of foods but also the formation of

structure in food processing and the disintegration of structure in digestion. Mastication is an interesting study topic being the interface between food science and nutrition contributing not only to sensory perception, but also to the form in which food mass proceeds to further digestion. Holistic understanding on structuring and destruction of food calls for increased collaboration of food scientists, biochemists and nutrition scientists.

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PUBLICATION I

**Effects of wheat and rye bread
structure on mastication process and
bolus properties**

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Effects of wheat and rye bread structure on mastication process and bolus properties



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ABSTRACT

Chemical composition, baking process and structure of breads influence their degradation in digestion leading to different postprandial responses. Rye bread has a very different structure as compared to wheat bread, and rye breads are known to induce lower postprandial insulin responses than wheat bread. The aim of this study was to find out potential differences in mastication and initial starch hydrolysis rate of rye and wheat breads. Three rye breads (wholemeal rye, endosperm rye and endosperm rye with gluten) and wheat bread were masticated by fifteen participants and the process was monitored using electromyography. The particle size distribution and initial *in vitro* starch hydrolysis of the bread boluses were analysed. Specific volume correlated negatively and closed porosity of breads correlated positively with work required for mastication. When compared to wheat bread, wholemeal rye bread required more work for mastication process ($p = 0.004$). Rye breads were degraded to smaller particles than wheat bread during mastication. There was a trend ($p = 0.098$) towards slower *in vitro* starch hydrolysis rate in rye bread boluses than in wheat bread boluses. The results indicate that the digestion process of rye breads differs from that of wheat bread already in the early phase of digestion. This may be one reason behind the unique postprandial responses reported for rye breads.

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1. Introduction

Breads are an elementary part of diets worldwide. Due to varying chemical compositions of flours and applied baking processes, breads form a food group with heterogeneous structures. White wheat bread is a commodity usually baked of starchy endosperm flour. During dough mixing, wheat gluten proteins are transformed into network in which carbon dioxide generated by yeast fermentation is retained leading to expansion during fermentation and baking (Goesaert et al., 2005). Rye bread is usually baked of whole grain flour using lactic acid fermentation (Autio, Parkkonen, & Fabritius, 1997). Rye proteins do not form a continuous network (Lorenz, 2003). The continuous phase in rye dough is composed of protein–starch matrix (Autio et al., 1997). Gas retention properties of rye dough, attributed to arabinoxylans, are weaker than those of wheat dough (Vinkx & Delcour, 1996). Due to smaller number of pores and greater number of large particles the structure of rye bread is harder than that of wheat bread (Autio et al., 1997).

Abbreviations: WHEAT, Refined wheat bread; RYE-WHOLE, Wholemeal rye bread; RYE-ENDO, Endosperm (refined) rye bread; RYE-ENDO-GLUT, Endosperm (refined) rye bread with gluten. * Corresponding author at: VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Finland.

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Depending on chemical composition, baking process and the resulting structure, breads cause different postprandial glucose (Scazzina, Siebenhandl-Ehn, & Pellegrini, 2013), insulin (Juntunen et al., 2003), (Rizkalla et al., 2007) and satiety responses (Keogh, Atkinson, Eisenhauer, Inamdar, & Brand-Miller, 2011), (Forsberg, Aman, & Landberg, 2014). Food digestion process leading to different postprandial responses begins already at the cephalic phase when food is seen, smelled, tasted and masticated (Smeets, Erkner, & De Graaf, 2010). Mastication disintegrates food to smaller particles and saliva lubricates food mass into a bolus, which can be swallowed (Bornhorst & Singh, 2012). Salivary α -amylase initiates the degradation of starch (Butterworth, Warren, & Ellis, 2011). Studies regarding this stage of bread digestion and its role in the overall digestion are scarce. Mastication process and bolus formation of breads have been studied by Hoebler et al. (Hoebler et al., 1998) who found that food structure had a great impact on mastication process and starch hydrolysis of food bolus. Tournier et al. (Tournier, Grass, Zope, Salles, & Bertrand, 2012) found out that baguettes with lower water content and higher crust/crumbs weight ratio required longer mastication than toast bread and rye bread. Le Bleis et al. (Le Bleis, Chaunier, Della Valle, Panouillé, & Réguerre, 2013) observed that country type wheat bread with higher bulk density required longer mastication time than wheat bread with a considerably lower bulk density.

We have previously studied the postprandial glucose and insulin responses to various rye breads including traditional wholemeal rye bread and endosperm rye bread, compared to white wheat bread and found that postprandial insulin responses have repeatedly and constantly been lower for rye bread (Leinonen, Liukkonen, Poutanen, Uusitupa, & Mykkänen, 1999), (Juntunen et al., 2003), (Törrönen et al., 2013). The current study aimed at exploring differences in mastication process and initial starch hydrolysis rate of rye and white wheat breads. In addition to the traditional wholemeal sourdough rye bread, we also used endosperm rye flour in baking and used wheat gluten addition to achieve a wider range of textural and structural properties of rye breads.

2. Materials and methods

2.1. Test breads

2.1.1. Baking

Test breads were refined wheat bread (WHEAT), wholemeal rye bread (RYE-WHOLE), endosperm (refined) rye bread (RYE-ENDO) and endosperm (refined) rye bread with wheat gluten (RYE-ENDO-GLUT). WHEAT comprised medium-coarse wheat flour (Sunnuntai medium-coarse wheat flour, Raisio, Finland) (3824 g), water (2485 g), fresh yeast (172 g), sugar (76 g), salt (57 g), vegetable fat margarine (459 g) and emulsifier, PANODAM® (18 g). RYE-WHOLE formula comprised commercial wholemeal rye flour (Sunnuntai wholemeal rye flour, Raisio, Finland) (2036 g), wholemeal rye sourdough (2949 g), water (772 g), fresh yeast (88 g) and salt (47 g). Wholemeal rye sourdough was prepared from wholemeal rye flour (1153 g), L62 (1.4 g *Lactobacillus brevis*), L73 (1.4 g *Lactobacillus plantarum*), fresh yeast (11.4 g) and water (1920 g). RYE-ENDO formula comprised refined rye flour (Mylly-Matti endosperm rye flour, Helsinki Mills, Finland) (2633 g), refined rye sourdough (2139 g), water (1258 g), fresh yeast (55 g) and salt (37 g). Refined rye sourdough was prepared from refined rye flour (1366 g), L62 (1.2 g *L. brevis*), L73 (1.2 g *L. plantarum*), fresh yeast (13.4 g) and water (2278 g). The formula of RYE-ENDO-GLUT was otherwise similar to that of endosperm rye bread but the refined rye flour was partly (103 g) replaced with gluten (Vital Wheat Gluten, Amilina, Lithuania). Baking temperatures and times for WHEAT, RYE-WHOLE, and both endosperm rye breads were 225 °C/20 min, 240 °C/10 min + 220 °C/40 min, and 240 °C/10 min + 220 °C/30 min, respectively. Test breads were stored frozen at –20 °C and defrosted at +4 °C overnight before textural measurements and mastication trial.

2.1.2. Bread characteristics

The dietary fibre (DF) content of the breads was determined according to AOAC Method 2009.01 and AOAC Method 2011.25, starch content according to AOAC Method 996.11 and AACC Method 76.13. The protein content was determined by Kjeldahl method (nitrogen \times 6.25, according to 90/496/EEC). Moisture content of bread crumbs was analysed by first drying the samples at room temperature until moisture content of bread and air were similar (approximately for 20 h). The samples were then ground and dried in oven at 130 °C for 1 h.

Bread samples for X-ray microtomography (XMT) were made by cutting $1 \times 1 \times 1$ cm cube pieces from 5 different locations of each bread crumb. After cutting, each sample was gently sealed in airtight plastic bags to avoid moisture loss during analysis. Samples were scanned using a desktop XMT system (Model 1172, SkyScan, Aartselaar, Belgium) consisting of an X-ray tube, an X-ray detector and a charge-coupled devices (CCD) camera. The X-ray tube was operated at a voltage of 40 kV/250 μ A to obtain optimum contrast between air cells and cell walls according to a modified method (Sozer, Bruins, Dietzel, Franke, & Kokini, 2011; Sozer, Dogan, & Kokini, 2011). A 12-bit cooled CCD camera (2000 \times 2000 pixels) was used to collect the X-ray data. Samples were rotated by a total of 180° during the scanning process with a pixel size of 12.85 μ m to obtain optimum resolution, resulting in a

total scanning time of 24 min. The initial X-ray radiographs or raw images were obtained at every 0.7° of rotation. Samples were scanned in five replicates. After scanning, radiographs were loaded into NRecon reconstruction software (v. 1.6.6). The software combines the images graphically into a 3-D object from which 2-D cross-sectional images can be taken. Ring artefact correction was set to 12 and beam hardening correction was set to 40% in order to reduce the number of artefacts. Cell walls of the solid matrix appear grey, whereas air cells appear black. The reconstructed 2-D slices were then loaded into CTAn software (v. 1.12, Skyscan, Belgium) to obtain the parameters of porosity, cell wall thickness (t) and cell diameter (D).

The samples were prepared for microscopy, stained and imaged according to Andersson et al. (Andersson et al., 2011). Protein and β -glucan in cereal cell walls as well as protein and starch were stained using Acid Fuchsin/Calcofluor and Light Green/Lugol's iodine, respectively. Protein stained by Acid Fuchsin appears red and cell walls rich in β -glucan stained by Calcofluor appear blue when examined in exciting light (excitation, 400–410 nm; emission, >455 nm; Fulcher & Wong 1980, Wood et al. 1983). In brightfield, protein stained by Light Green appears green or yellow. Lugol's iodine stains native starch purple, while the amylose component of starch appears blue and amylopectin brown.

Specific volumes of fresh breads were determined by Pregesbauer infrared device (Bread Vol Scan, Pregesbauer, Germany) from six parallel breads. Texture profile analysis was used to extract the primary and secondary mechanical characteristics by using TA-XT plus Texture Analyser (Stable Micro System, Godalming, Surrey, UK) with a 25-mm diameter probe SMS P/36, 30-kg load cell, 40% strain on 25-mm thick slices from six parallel slices of breads which were cut by the help of a mould from the centre of two breads. Pre-test and test speed were 1.7 mm/s and post-test speed was 10 mm/s. TPA software (Exponent v.6, Stable Micro System, Godalming, Surrey, UK) was used to extract parameters such as hardness, stickiness, cohesiveness, chewiness and resilience from the resulting force-deformation curve.

2.2. Mastication trial

2.2.1. Participants

Fifteen young (20–40 years) females were recruited to the study through email lists and bulletin boards from the University of Eastern Finland. Inclusion criteria were normal weight, no smoking, no missing teeth except 3rd molars and no diagnosed functional mastication problems. The mean age of participants (\pm SD) was 24.6 (\pm 4.4) years and mean Body Mass Index (BMI) was 22.0 (\pm 1.4) kg/m². The study was conducted according to the ethical principles of good research and clinical practice described in the declaration of Helsinki. Ethical approval was obtained from the Research Ethics Committee, Hospital District of Northern Savo, Finland. The participants gave written informed consent to their participation in the study.

2.2.2. Procedure

The participants attended one study visit. The experiments took place between 8–11 a.m., and the participants were instructed to eat breakfast 1 to 1.5 h before that. They were familiarised with the study procedure before the actual mastication trial. Four bread samples were offered to each participant in random order. The samples were blind-coded by using 3-digit numbers. Breads were served in three portions of $2 \times 2 \times 2$ cm-size cube. Average weight (\pm SD) of all three portions of WHEAT, RYE-WHOLE, RYE-ENDO, RYE-ENDO-GLUT was 9.1 ± 2.4 g, 15.1 ± 1.0 g, 9.6 ± 0.8 g, and 10.9 ± 1.9 g, respectively. The participant masticated the bread portion until it was considered to be ready for swallowing. Instead of swallowing the bolus was expectorated to a plastic container which was kept on ice. The three portions of each bread were masticated in a row and between different breads there was a break of two minutes during which the mouth was rinsed with water.

Table 1
Macronutrient composition of the test breads, g/100 g per wet weight.

	WHEAT	RYE-WHOLE	RYE-ENDO	RYE-ENDO-GLUT
Starch	40.0 ± 0.1	34.8 ± 0.0	46.0 ± 0.1	43.9 ± 0.1
Total dietary fibre ^a	2.8	11.2	5.7	5.8
Insoluble dietary fibre	2.0 ± 0.0	7.7 ± 0.1	3.6 ± 0.0	3.9 ± 0.0
Soluble dietary fibre	0.8 ± 0.1	3.6 ± 0.2	2.1 ± 0.1	1.9 ± 0.0
Water extractable arabinoxylan	0.3 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Protein	7.4 ± 0.0	6.1 ± 0.0	4.1 ± 0.0	5.4 ± 0.0
Fat	5.4 ± 0.0	0.9 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
Moisture ^b	43.0 ± 0.0	54.5 ± 0.0	47.9 ± 0.0	47.0 ± 0.0

^a Sum of Insoluble dietary fibre and soluble dietary fibre.

^b Bread crumb.

2.2.3. Electromyography (EMG) measurements

The mastication process was characterised by measuring the electrical activity of facial muscles by EMG. EMG was measured with NeurOne system (Mega Electronics, Kuopio, Finland) using disposable dermal Ag/AgCl electrodes. The skin was cleaned with alcohol, and bipolar electrodes were placed on the masseter and temporal muscles on both sides of the face. The muscles were identified by touch when the participant gritted her teeth. EMG activity was measured continuously throughout the whole mastication trial and the data blocks for each chewing period were isolated for analysis by visual inspection and double-checked against experiment minutes records. From the EMG time series, the onset, duration and amplitude of each bite event were extracted by applying chemometric techniques for the elimination of high frequencies and background fluctuations. As a result of data

processing and analyses, the duration of chewing, number and frequency of bites were calculated for each food product tested. The bite force used was estimated from the amplitude of smoothed EMG root-mean-square signal. Correlations between bread properties (specific volume, hardness, cohesiveness, cell wall thickness, and closed porosity) and total mastication work were studied.

2.3. Bolus analyses

2.3.1. Saliva impregnation

Food bolus saliva impregnation was determined based on moisture content of bread crumbs and bolus samples. Wet bolus (WB) samples were weighed and placed in an oven at 105 °C overnight and the dried bolus (DB) was weighed again. The water content of boluses was determined by following formula: $WB-DB/WB \times 100$. The saliva impregnation of boluses was determined by the difference between the water content of boluses and the water content of bread crumb.

2.3.2. Particle size distribution

The bolus samples were diluted into 100 ml of water, mixed with magnetic stirring for 25 min and let stand for 5 min in order to get bigger particles settled in the bottom. Then the turbid liquid containing the smallest particles that could not be imaged was removed and the sample volume was increased with water up to 100 ml. The liquids containing the bigger particles were poured on petri dishes for imaging. Around 8 to 12 petri dishes were needed depending on the sample. The particles were adjusted on petri dishes so that they were as little as possible in contact with each other. Digital images were taken of each petri dish. Images were calibrated and particle areas were determined using Cell^P imaging software (Olympus, BX50).

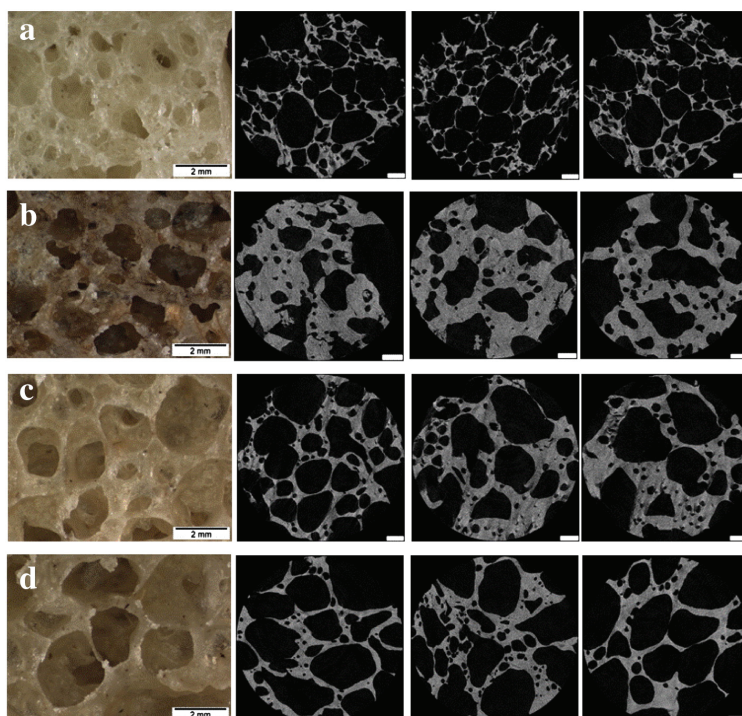


Fig. 1. Stereomicroscopy images (first column) and representative 2D XRT images (3 replicates, columns 2–4) of test breads a) WHEAT, b) RYE-WHOLE, c) RYE-ENDO, d) RYE-ENDO-GLUT. White bar in stereomicroscopy images is 2 mm and in XRT images 900 μ m.

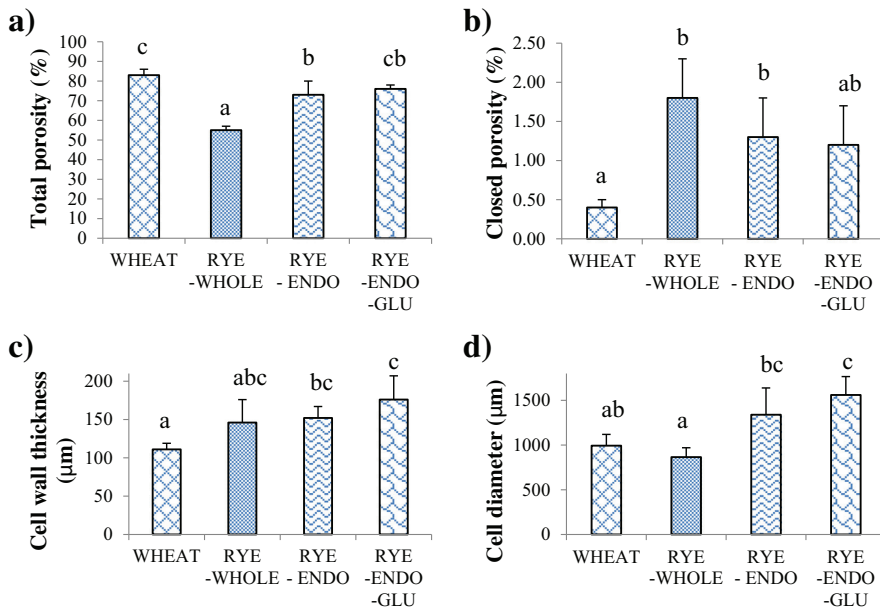


Fig. 2. a) Total porosity, b) Closed porosity, c) Cell wall thickness and d) Cell diameter of the test breads. Data represent the mean \pm standard deviation from 5 replicates. Bars with different letters differ statistically significantly ($p < 0.05$).

2.3.3. Initial starch hydrolysis rate

The rate of *in vitro* initial starch hydrolysis of boluses was determined by a method modified from “Rate of in-vitro starch digestion in products ‘as eaten’” (Granfeldt, Björck, Drews, & Tovar, 1992). An adequate weight of bolus sample was determined for each sample based on moisture content of the sample and starch content of the bread. A bolus sample containing 0.5 g starch was transferred to dialysis tubing (Spectra/Por No. 2, flat width 45 mm, molecular weight cut off 12–14 kD) with 15 ml cold phosphate buffer (pH 6.9). The tubing was incubated in a beaker with 0.05 M phosphate buffer (400 ml) at 37 °C for 30 min, with magnetic stirring. The salivary α -amylase present in the bolus sample initiated the starch hydrolysis. Two millilitre aliquots were removed at time points 0, 1.5, 3, 6, 9, 12, 15 and 30 min, and frozen (-20 °C). Removed samples were incubated with amyloglucosidase (Megazyme) at 40 °C for 15 min to hydrolyse the solubilised starch to glucose. Free glucose was determined by treating the samples with glucose oxidase peroxidase reagent (Megazyme) for 20 min, and the absorbance was read at 510 nm. Glucose solution (100 μ g/0.1 ml) was used as a standard. The amount of released glucose was converted to starch multiplying with 0.9. The degree of starch hydrolysis was calculated as the proportion of the released starch from the original starch content of the bolus.

2.3.4. Microscopy

The microscopy analyses of food boluses were conducted as described in Section 2.1.2.

2.4. Statistical analyses

The results are presented as means \pm SDs or \pm SEMs, as indicated. SPSS software (IBM SPSS statistics 20) was used for statistical analyses.

Kruskal–Wallis Test was used to compare textural properties of breads. Friedman’s non-parametric test for related samples was used to compare the parameters describing mastication. Simple linear regression was conducted to evaluate the correlation between bread properties and total work required to masticate bread. Repeated measures ANOVA was used to compare the starch hydrolysis rate of boluses. Values of $p < 0.05$ were considered to be statistically significant.

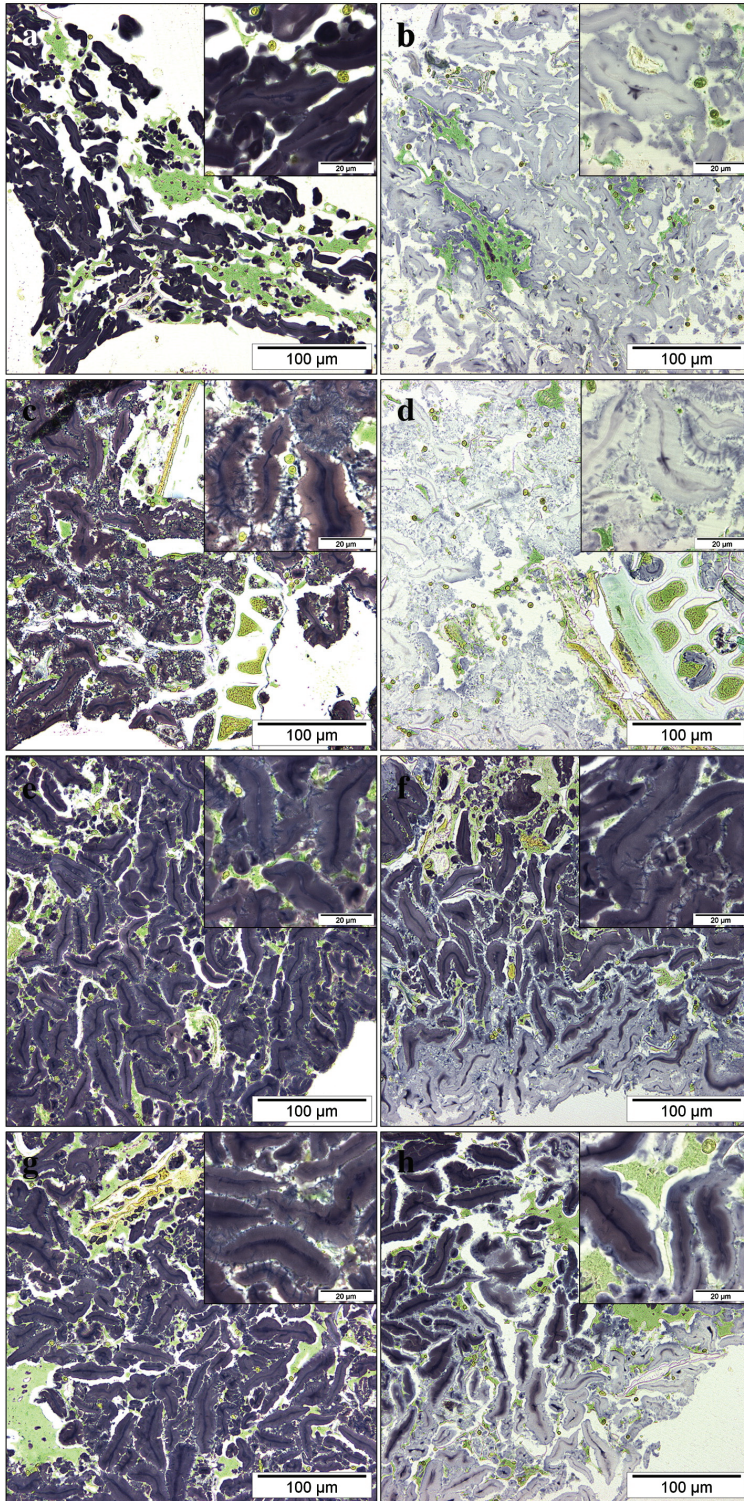
3. Results

3.1. Bread characteristics

Rye breads were high in DF and moister than wheat bread, RYE-WHOLE having the highest DF and moisture content (Table 1). Rye breads had higher closed porosity and thicker cell walls than wheat bread (Figs. 1, 2). RYE-WHOLE had lower total porosity than other breads, and RYE-ENDO and RYE-ENDO-GLUT had wider cell diameter than RYE-WHOLE and WHEAT. Starch formed the continuous phase and protein was scattered all over the matrix in rye breads while protein formed a continuous network in WHEAT (Fig. 3). In RYE-ENDO-GLUT protein was aggregated as clearly distinguishable areas. Starch granules in RYE-WHOLE were swollen and amylose had leaked out from granules forming crystals while starch granules in WHEAT had a less swollen, compact structure. Starch granules in RYE-ENDO and RYE-ENDO-GLUT (Fig. 3e and g, respectively) were also degraded but to a lesser extent than starch granules in RYE-WHOLE. Large bran pieces that also occasionally contain cutin could be observed in RYE-WHOLE (Fig. 4c).

RYE-WHOLE had the lowest specific volume (2.0 ± 0.1 ml/g) and WHEAT the highest (5.1 ± 0.1 ml/g), the two endosperm rye breads being in between (RYE-ENDO 3.2 ± 0.2 ml/g, and RYE-ENDO-GLUT 3.1 ± 0.0 ml/g) (Fig. 4). There were statistically significant differences

Fig. 3. Light micrographs of breads and corresponding boluses a) WHEAT, b) WHEAT bolus, c) RYE-WHOLE, d) RYE-WHOLE bolus, e) RYE-ENDO, f) RYE-ENDO bolus, g) RYE-ENDO-GLUT and h) RYE-ENDO-GLUT bolus. Protein appears green (stained with Light Green) and starch granules purple (stained with Lugol’s iodine). White bar is 100 μ m in the main figures and 20 μ m in the subfigures.



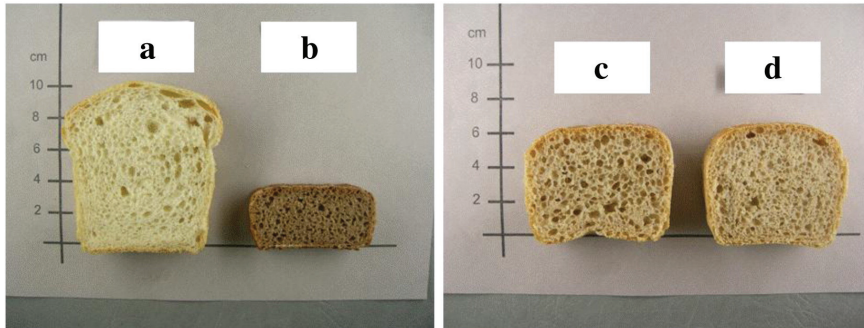


Fig. 4. Photographs of the test breads: a) WHEAT, b) RYE-WHOLE, c) RYE-ENDO, d) RYE-ENDO-GLUT.

in specific volumes between breads ($p < 0.001$) except between RYE-ENDO and RYE-ENDO-GLUT ($p = 0.4$). RYE-WHOLE had a harder, less cohesive and less springy texture than the wheat bread (Table 2). Whole meal rye bread was less springy and less chewy than the other breads.

3.2. Mastication

Mastication of RYE-WHOLE required more work than that of WHEAT ($p = 0.004$), and mastication of RYE-ENDO-GLUT required more work per bite than mastication of WHEAT ($p = 0.026$) (Table 3). There were no statistically significant differences between the test breads in the number of bites, chewing time, EMG activity time or duty cycle. There was a high positive correlation between closed porosity and total work ($R^2 = 0.96$) and high negative correlations between specific volume and total work ($R^2 = 0.98$) as well as between cohesiveness and total work ($R^2 = 0.94$). Hardness and total work and cell wall thickness and total work correlated weakly ($R^2 = 0.71$, $R^2 = 0.41$, respectively).

3.3. Bolus samples

Saliva impregnation in masticated breads was on average 0.3 g saliva per 1 g of bread for all breads (Table 3). The average saliva impregnation varied statistically significantly from person to person ($p < 0.001$) the average for all four breads being between 0.24 ± 0.02 and 0.78 ± 0.06 g saliva per 1 g of bread. Photographs of masticated bread particles and the distribution of particles with different particle areas are presented in granulometric curves in Fig. 5. The curves represent cumulative percentage of the total area occupied by particles. Particles of the rye bread boluses were compact while particles of the white wheat bread bolus were fluffy (Fig. 5, a–d). The rye breads were degraded into small particles while there was a greater amount of bigger particles left in the masticated wheat bread.

Micrographs of bread boluses showed that protein and pieces of grain were not affected by mastication. Pieces of grain and also some intact cells were seen in the RYE-WHOLE bolus. Iodine stain was not

properly attached to starch granules, especially in the WHEAT and RYE-WHOLE boluses. Starch granules in RYE-WHOLE (bread) were more degraded than those in WHEAT (bread), and that was reflected also in the WHEAT bolus (Fig. 3c, a, and b, respectively). Some amylose from the WHEAT bolus had leaked out from the starch granules but had not formed crystals as in the RYE-WHOLE bread. The starch granules of RYE-ENDO bolus and RYE-ENDO-GLUT bolus were less swollen than the starch granules of the RYE-WHOLE bolus (Fig. 3f, h and d, respectively).

The release of glucose from the starch in the bolus samples showed a 9-min-lag for all breads, after which the initial rate of glucose release was steady for all breads (WHEAT 0.9 ± 0.1 ; RYE-WHOLE 0.7 ± 0.1 ; RYE-ENDO 0.7 ± 0.1 ; RYE-ENDO-GLUT 0.7 ± 0.0 /min) and was not significantly different between the breads ($p = 0.098$) (Fig. 6). There was, however, a trend that glucose was released at slightly lower rate from all the RYE breads than from the WHEAT bread. The extent of starch solubilisation at 30 min was 20.6 ± 2.3 ; 19.0 ± 1.8 ; and $18.7 \pm 1.5\%$ for RYE-WHOLE, RYE-ENDO, and RYE-ENDO-GLUT, respectively whereas it was 24.3 ± 2.3 for WHEAT.

4. Discussion

Whole meal rye bread required more work for mastication than wheat bread and endosperm rye bread with gluten required more work per bite than wheat bread. The studied correlations between structural properties typical to rye breads and total work required for mastication showed that the denser the bread, the larger closed porosity, and the less cohesive structure, the more work required to break the bread down in the mouth. The result was similar to that of Le Bleis et al. (Le Bleis et al., 2013) who observed that wheat bread with higher bulk density required longer mastication time than bread with considerably lower bulk density. There were notable differences in structures of rye and wheat breads. Rye breads had thicker cell walls, higher closed porosity, lower specific volume and harder and less cohesive texture. Wheat bread was baked from refined flour by yeast fermentation which gave a soft and elastic texture due to formation of gluten network. The rye breads were baked with sourdough fermentation, and had harder and denser structure. Wheat bread and wholemeal rye bread were the two extremes in terms of total porosity closed porosity, hardness, springiness and cohesiveness. Endosperm rye breads were included in the study to obtain breads with textural and microstructural properties in between wheat bread and traditional rye bread (Fig. 2, Table 2).

The initial starch hydrolysis rate evoked by salivary α -amylase tended to be slower for rye breads than for wheat bread, the amount of solubilised starch after 30 min incubation being around 20% for rye breads and 24% for wheat bread. To the best of our knowledge the starch hydrolysis rate of rye and wheat breads by solely salivary α -amylase has not reported before. Starch hydrolysis rate of rye and wheat breads

Table 2
Texture profile of the test breads.

	WHEAT	RYE-WHOLE	RYE-ENDO	RYE-ENDO-GLUT	p
Hardness (g)	170 \pm 29 ^a	757 \pm 100 ^d	648 \pm 133 ^c	602 \pm 99 ^b	.000
Springiness (%)	0.90 \pm 0.03 ^c	0.34 \pm 0.04 ^a	0.72 \pm 0.07 ^b	0.72 \pm 0.06 ^b	.000
Cohesiveness	0.50 \pm 0.05 ^c	0.11 \pm 0.01 ^a	0.22 \pm 0.04 ^b	0.21 \pm 0.03 ^b	.000
Chewiness (g)	76 \pm 7 ^b	28 \pm 6 ^a	98 \pm 26 ^c	88 \pm 19 ^{bc}	.000

Values with different letters in a row differ statistically significantly ($p < 0.05$).

Table 3Mastication parameters. Each parameter is presented as mean of 15 subjects \pm SD.

	WHEAT	RYE-WHOLE	RYE-ENDO	RYE-ENDO-GLUT	χ^2	p
Number of bites	17.5 \pm 8.3	20.3 \pm 8.1	18.1 \pm 6.8	18.6 \pm 8.8	4.168	.244
Chewing time (s)	11.6 \pm 6.2	13.8 \pm 6.2	12.1 \pm 4.9	13.0 \pm 7.4	5.094	.232
EMG activity time (s)	2.8 \pm 1.2	3.5 \pm 1.5	3.0 \pm 1.2	3.2 \pm 1.7	4.289	.165
Duty cycle ¹	0.26 \pm 0.04	0.26 \pm 0.03	0.25 \pm 0.03	0.25 \pm 0.02	4.611	.203
Total work (%) ²	0.60 \pm 0.31 ^a	0.76 \pm 0.28 ^b	0.68 \pm 0.21 ^{ab}	0.70 \pm 0.23 ^{ab}	13.349	.004
Work/bite (%) ²	0.59 \pm 0.22 ^a	0.65 \pm 0.15 ^{ab}	0.67 \pm 0.19 ^{ab}	0.68 \pm 0.19 ^b	9.242	.026
Saliva uptake (g per 1 g of bread)	0.3 \pm 0.2	0.3 \pm 0.2	0.3 \pm 0.2	0.3 \pm 0.2	6.440	.092

Values with different letters in a row differ significantly ($P < 0.05$).¹ EMG activity time to total time ratio.² Normalized to corresponding values of a reference product.

including *in vitro* stomach phase and duodenal phase has been studied by Juntunen et al. (2003) and Rosen et al. (2009). Juntunen et al. (2003) found hydrolysis of starch to be slower for rye breads than for wheat bread. The amount of hydrolysed starch after pepsin treatment and 30 min of incubation with pancreatic α -amylase reflected the results obtained in this study after 30 min incubation with salivary α -amylase. Rosen et al. (2009) observed hydrolysis index (HI) of only endosperm rye bread to be lower than that of wheat bread, while HI for whole grain rye bread, whole grain rye bread with lactic acid or rye bran bread did not differ from wheat bread. Differences in starch accessibility in bread may result from differences both in processing methods and ingredients. Rye breads were baked using sourdough fermentation in the current study and in the study of Juntunen et al. (2003) whereas sourdough fermentation was not applied in the study of Rosen et al. (2009). During sourdough fermentation acids are produced resulting in lower pH and activation of endogenous enzymes (Poutanen, Flander, & Katina, 2009). The sourdough baking process changes the structure of starch granules. This can be observed in microscopy images of the test breads in the current study: the starch granules in rye breads were swollen and amylose leached out from the granules had re-crystallised. Re-crystallised amylose has been proposed to resist

α -amylase-induced hydrolysis (Singh, Dartois, & Kaur, 2010). Microscopy images of the bread boluses showed no remarkable changes in bread microstructure by mastication. However, iodine stain did not attach properly to WHEAT, RYE-WHOLE and the peripheral areas of RYE-ENDO and RYE-ENDO-GLUT samples. This might be an indication of influence of salivary α -amylase on the surface of the sample, reflected as reduced affinity of iodine on starch.

Physical structure is the most important factor determining the postprandial glycaemic response of bread (Fardet, Leenhardt, Lioger, Scalbert, & Remesy, 2006). Porous bread is easily disintegrated in digestion and starch granules are released from food matrix being easily accessible to α -amylases. Rye breads were disintegrated to smaller but more compact particles than wheat bread during mastication. Wheat bread particles were more airy, which may result from preserved gluten network that was observed in light microscopy images. The open porous structure of wheat bolus particles may facilitate the access of salivary α -amylase to starch granules and thus affect the faster starch hydrolysis that was observed in this study.

Postprandial insulin responses have been shown to be lower for rye breads than for wheat bread in earlier studies (Leinonen et al., 1999; Juntunen et al., 2003; Rosen et al., 2009; Torronen et al., 2013) but the

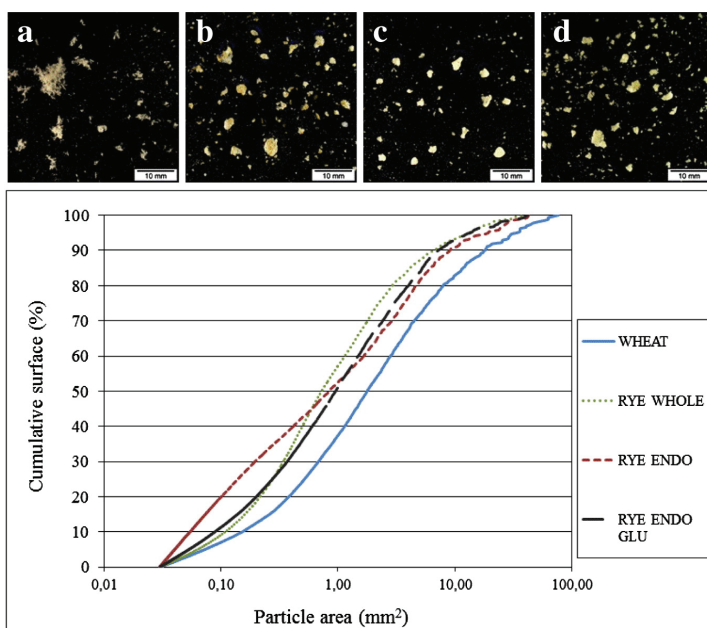


Fig. 5. Photographs of masticated breads a) WHEAT, b) RYE-WHOLE, c) RYE-ENDO, d) RYE-ENDO-GLUT (white bar is 10 mm) and particle area distribution of masticated bread samples (averages of 8 subjects). The curves represent a cumulated percentage of the total area occupied by particles. Values for particle area are logarithmic.

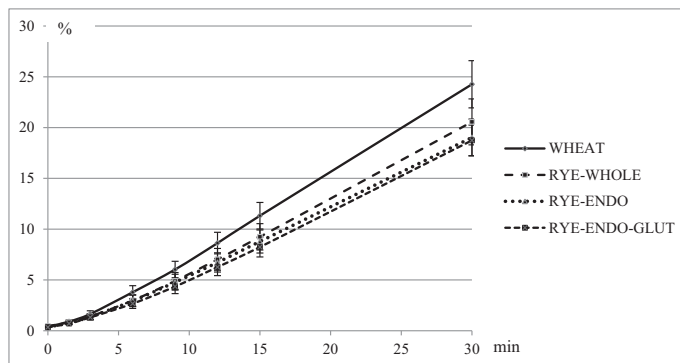


Fig. 6. Initial (0–30 min) starch hydrolysis rate measured *in vitro* (averages of 15 subjects). Values are average percentages (%) of hydrolysed starch from initial starch content with SEM.

underlying reason for the difference is unclear. Cephalic phase contributes to insulin response to foods (Teff, 2010). Cephalic phase insulin response (CPIR) is activated via the vagal nerve by food stimuli in mouth making differences in mastication processes between rye and wheat breads an interesting subject to study. Number of bites, chewing time, EMG activity time, duty cycle, total work, and work/bite were parameters extracted from EMG data to characterise the mastication process. The amplitude of the smoothed EMG root-mean-square signal is relative to the muscle power, but the actual bite force depends on the individual bone geometry as well as impedance of the electrode connection to the skin. EMG amplitude was normalized to one sample for each person to provide a reasonably good estimate of a relative bite force. The results of this study show that structural properties that are characteristic to rye breads are typical for larger mastication work. Since cephalic phase insulin response is, among other properties such as taste, stimulated by sensed texture, it would be interesting to study CPIR to rye breads and wheat bread.

The glucose entrance rate to duodenum is among the factors regulating insulin response (Pilichiewicz et al., 2007). The initial rate of starch hydrolysis by salivary α -amylase was studied with an *in vitro* method modified from the method of Granfeldt et al. (1992). The original method includes, in addition to mastication, incubation of samples in low pH with pepsin and subsequent incubation with pancreatic α -amylase mimicking later phases of digestion. Since it has been shown by Juntunen et al. (2003) that the concentration of gastric inhibitory peptide (GIP), which is stimulated by glucose flow to the duodenum and which in turn stimulates the insulin secretion from pancreatic β cells (Yabe & Seino, 2011), was already higher 30 min after ingestion of wheat bread than rye bread, we were interested in observing the very early rate of glucose release. The salivary α -amylase which is active in the mouth and early gastric digestion, initiates starch hydrolysis (Minekus et al., 2014). Thus, the bolus samples were incubated for 30 min to mimic the initial phase of starch digestion. The results show that there is a trend towards slower initial starch hydrolysis for rye breads than for wheat and this may lead to differences in glucose entrance rate to the duodenum and resulting insulin response.

Large inter-individual variations were found in mastication process, impregnation of saliva to bolus samples and starch hydrolysis rate. We assume that regardless of differences in bread structure all the breads belong to same food category which may accentuate the role of habitual mastication. Large individual variation may conceal some differences due to bread type. Higher number of participants may have been needed to obtain more statistically significant results.

To conclude, we characterised textural and microstructural properties of breads and studied their mastication process and initial starch hydrolysis rate. Properties that are characteristic to rye breads correlated with mastication work but there were no large differences in

mastication processes of the studied breads. The *in vitro* starch hydrolysis measurement showed a trend towards slower starch hydrolysis rate of rye breads compared to wheat bread.

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PUBLICATION II

**Mastication-induced release of
compounds from rye and wheat
breads to saliva**

Submitted manuscript

Mastication-induced release of compounds from rye and wheat breads to saliva

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1 **Abstract**

2 Mastication initiates digestion, disintegrating food structure and mixing it with saliva. This study
3 aimed to provide understanding about the first step of bread digestion by exploring releasing
4 compounds from bread matrix in mastication. Further, the aim was to identify compound groups that
5 differentiate rye and wheat breads.

6 Fifteen participants masticated whole-meal rye bread, endosperm rye bread, endosperm rye bread
7 with added gluten, and wheat bread. The masticated samples were studied with non-targeted LC-MS
8 metabolic profiling.

9 A great number of compounds was released from bread matrices in mastication, and the identified
10 compounds differed largely between bread types. Specifically, rye bread samples were characterized
11 by a greater release of peptides and amino acids, whereas sugars and nucleosides were characteristic
12 for wheat bread. These compounds could potentially act as signal molecules in the alimentary tract
13 and may explain, at least partly, the postprandial physiological effects of the breads identified in
14 earlier studies.

15 **Keywords:** bread; mastication; metabolomics; peptides; rye

16

17 **1 Background**

18 Breads are an important part of diets all over the world. Cereal flour is the main ingredient in bread
19 baking, but due to the use of different cereal grains, as well as grinding and baking processes, breads
20 constitute a wide range of food items with distinct nutritional profiles, structures and health effects.
21 Rye bread and refined wheat bread represent very different structures, refined wheat bread having
22 more cohesive and springy but less hard texture than rye bread, which is reflected in the mastication
23 process (Pentikäinen et al., 2014). The differences between rye breads and wheat bread in the
24 nutritional content, *in vitro* digestion of starch and protein, and postprandial metabolism have been
25 studied extensively; however, the first step of digestion, namely mastication, has been thus far largely
26 neglected. Mastication initiates digestion by disintegrating food to smaller particles and by lubricating
27 the food mass with saliva. Ingested food is transformed into food bolus that is swallowed and
28 processed in further digestion (Bornhorst & Singh, 2012).

29 Disintegration of foods and the consequences for sensory perception have been studied using different
30 foods and food models (J. Chen, 2015). However, food structure and mastication process do not
31 determine only the sensory perception and the physical form (particle size, cohesion etc.), in which
32 the bolus proceeds to further digestion, but they also determine, which compounds and to what extent
33 are dissolved from food matrix first to saliva and further to other digestive fluids. After mastication,
34 the food bolus enters the stomach, where it is mixed with gastric juice and where protein digestion is
35 initiated. The liquid phase with solute compounds generally passes through the stomach faster than
36 the solid phase and may reach the gut in front (Siegel et al., 1988). The compounds that are released
37 in mastication may act as flavour agents in the mouth but also act as signal molecules in further
38 digestion for example stimulating hormone excretion or activating vagal nerve receptors (Delzenne
39 et al., 2010; Raybould, 2008).

40 For various types of rye breads, the low acute postprandial insulin response compared to wheat bread
41 is a specific feature (Bondia-Pons, Nordlund, Mattila, Katina, Aura, & Kolehmainen, 2011;

42 Johansson, Lee, Riserus, Langton, & Landberg, 2015; Juntunen et al., 2003; Kallio et al., 2008;
43 Leinonen, Liukkonen, Poutanen, Uusitupa, & Mykkänen, 1999; Moazzami, Shrestha, Morrison,
44 Poutanen, & Mykkänen, 2014; Törrönen et al., 2013). Blood glucose concentration is the main trigger
45 for insulin secretion and insulinogenic amino acids absorbed into the circulation augment insulin
46 secretion (Nilsson, Stenberg, Frid, & Holst, 2004). In addition to insulin-triggering effect of
47 postprandial nutrient concentration in blood, already the digestion of amino acids and glucose in the
48 gut stimulates the secretion of incretin hormones (gastric inhibitory peptide (GIP) and glucagon-like
49 peptide-1 (GLP-1)), which, in turn, augment glucose induced insulin secretion (Fu, Gilbert, & Liu,
50 2013). Thus, also the flow of nutrients to gut lumen is one aspect determining insulin response.

51 Starch in breads begins to hydrolyse already during mastication (Hoebler et al., 1998; Pentikäinen et
52 al., 2014). Little is known about other compounds, which in addition to starch hydrolysis products
53 could be released from bread matrix to saliva during mastication. We hypothesize that the wheat and
54 rye breads, which are known to have distinct postprandial metabolic responses differ already
55 regarding the compounds that are released from the bread matrix and mix with saliva in mastication.
56 We aimed to explore specifically those compounds that differentiate rye breads from wheat bread.

57 **2 Methods**

58 **2.1 Test breads**

59 The test breads were three sourdough-baked rye breads: wholemeal rye bread (WRB), endosperm
60 rye bread (ERB) and endosperm rye bread with added gluten (ERBG) and a refined yeast leavened
61 wheat bread (WB). The breads were designed to represent different structures. The test breads were
62 baked at VTT Technical Research Centre of Finland Ltd. Table 1 shows the macronutrient
63 composition of the test breads by dry weight basis. The recipes, baking processes, and structural
64 properties of the breads are described in detail in our previous paper (Pentikäinen et al., 2014).

65

66 Table 1 Macronutrient composition of the test breads. Values are percentage of dry weight.

	Wholemeal rye bread	Endosperm rye bread	Endosperm rye bread with gluten	Refined wheat bread
Starch	59.1 ± 0.1	76.2 ± 0.1	74.0 ± 0.2	66.0 ± 0.2
Total dietary fibre	19.1	9.4	9.9	4.6
Insoluble dietary fibre	13.0 ± 0.1	5.9 ± 0.0	6.6 ± 0.0	3.3 ± 0.0
Soluble dietary fibre	6.0 ± 0.3	3.5 ± 0.2	3.2 ± 0.0	1.4 ± 0.2
Protein	10.3 ± 0.0	6.8 ± 0.1	9.1 ± 0.1	12.3 ± 0.0
Fat	1.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	8.9 ± 0.0

67

68 2.2 Mastication trial

69 Mastication trial with 15 females aged 20–40 years was conducted in the end of the year 2013. Only
70 female participants were included to outline some of the inter-individual variation in mastication.
71 Exclusion criteria were smoking, missing teeth (except 3rd molars) and diagnosed functional
72 mastication problems. The study was conducted according to the ethical principles of good research
73 and clinical practice described in the declaration of Helsinki. Research Ethics Committee, Hospital
74 District of Northern Savo gave ethical approval to the study (record 87/2013). Written informed
75 consents were collected from the participants prior the study.

76 The participants attended the study visit in the morning between 8–11 a.m. They were instructed to
77 eat their habitual breakfast 1–1.5 hours prior the study visit. The breads were served in random order
78 as three 2 × 2 × 2 cm cubes, which were masticated one after the other. Each bread cube was
79 masticated until the subjective swallowing point. At that point, the bolus was expectorated to a plastic

80 container, which was kept on ice. There was a break of two minutes between the bread types. Mouth
81 was rinsed with water during the break. Bolus samples were stored in -70°C .

82 **2.3 Metabolite profiling**

83 *2.3.1 Bolus sample preparation*

84

85 200 mg bolus sample by dry weight basis was weighed in 2 ml plastic tubes and 610 μl of water was
86 added. The tubes were centrifuged and the supernatant was collected. The metabolites were extracted
87 and proteins precipitated by adding 200 μl methanol to 100 μl of sample. The tubes were mixed, let
88 stand on ice for 30 minutes and centrifuged. The supernatant was collected and stored in -70°C .

89 *2.3.2 Non-targeted LC-MS metabolite profiling analysis*

90

91 The samples were filtered (0.2 μm PTFE membrane; PALL corporation) prior to analysis by the liquid
92 chromatography quadrupole time-of-flight mass spectrometry (LC-qTOF-MS). The samples were
93 analysed in a random order using hydrophilic interaction (HILIC) chromatography. The quality
94 control samples were injected after every nine samples.

95 The liquid chromatography was performed on a 1290 Infinity Binary UPLC system (Agilent
96 Technologies, Santa Clara, CA, USA). For the separation, an Acquity UPLC BEH amide column
97 (Waters, Milford, MA, USA; dimensions 2.1 \times 100 mm, particle size 1.7 μm) was used. The column
98 temperature was $+50^{\circ}\text{C}$, flow rate 0.5 mL/min, injection volume 2 μL , and sample tray temperature
99 $+4^{\circ}\text{C}$. The gradient elution consisted of HPLC grade water (solution A) and HPLC grade methanol
100 (solution B), both containing formic acid (0.1 % v/v). A following gradient was used: 0–10 min: 2 %
101 \rightarrow 100 % of solution B; 10–14.5 min: 100 % of solution B; 14.5–14.51 min: 100 % \rightarrow 2 % of solution
102 B; and 14.51–16.5 min: 2 % of solution B. The mass spectrometric analysis was performed on a 6540
103 UHD Accurate-Mass Q-TOF (Agilent Technologies). The ionization was carried out using jet stream

104 electrospray ionization (ESI) in the positive mode. The collision energies for the MS/MS analysis
105 were chosen as 10, 20 and 40 V, for compatibility with spectral databases.

106 2.3.3 *Metabolomics data analysis*

107

108 The data were collected by using the vendor software (MassHunter Qualitative Analysis B.05.00;
109 Agilent Technologies), and the output was transferred in compound exchange format (.cef) into the
110 Mass Profiler Professional software (MPP 2.2; Agilent Technologies) for data pre-processing
111 (Koistinen, Katina, Nordlund, Poutanen, & Hanhineva, 2016). Only features that were found in at
112 least 80 % of replicates, in at least one of the sample types (four masticated breads) were included in
113 the analyses. The features were normalized row-wise and clustered, based on peak areas, into 15
114 clusters by k-means clustering using Multiple Experiment Viewer software (version 4.9). Clustering
115 was conducted in order to categorize features occurring in a similar manner within certain sample
116 types into distinct groups.

117 Features in specific clusters were identified. Exact masses of the positive ions and MS/MS
118 fragmentation data were compared to entries in METLIN, other publicly available spectral databases,
119 and in our in-house standard library. MS-DIAL software version 2.64 (Tsugawa et al., 2015) was
120 used in the identification process.

121 Fold changes were calculated as the ratio (B/A) of the average peak area of identified compounds in
122 rye bread boluses (B) against the corresponding average peak areas in wheat bread boluses (A). In
123 the cases where fold change was below 1 the negative inverse was calculated. T-tests with Benjamini-
124 Hochberg FDR correction were conducted to examine whether the fold changes were statistically
125 significant. *P*-value 0.01 was set as a limit for statistical significance.

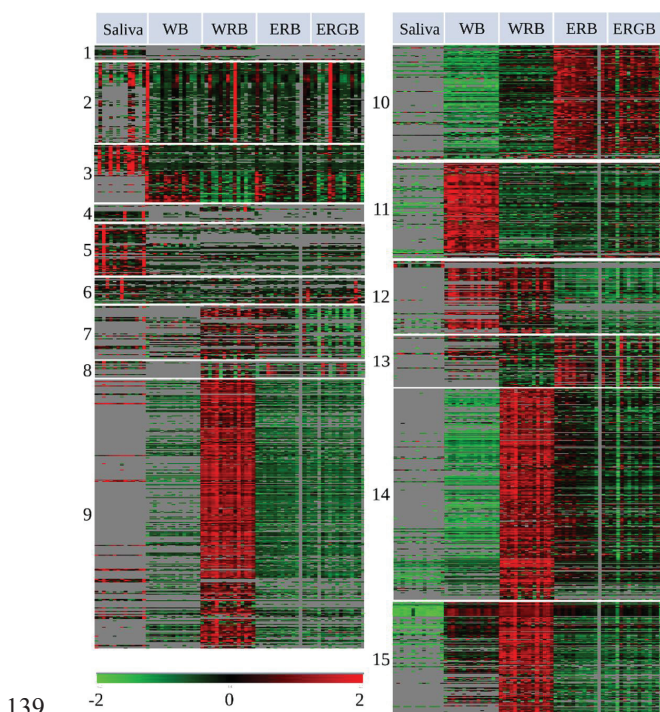
126

127 **3 Results**

128 **3.1 Overview of the features released from breads to saliva in mastication**

129

130 Altogether 1807 features were included in the data matrix collected from the non-targeted metabolite
131 profiling analysis of saliva and bread bolus samples. Figure 1 provides an overview on how the
132 features clustered across different sample types based on their peak areas. Approximately 57 % of the
133 features were located in clusters 9, 10, 14 and 15, which contained those features specifically
134 pronounced in rye bread boluses. Approximately 8 % of the features were located in cluster 11
135 representing compounds more pronounced in wheat bread boluses when compared to rye bread
136 boluses. These five specific clusters, which differentiated most clearly rye bread bolus samples from
137 wheat bread bolus samples, were further examined. Details about the identified compounds are
138 presented in supplementary table.



140 Figure 1 k-Means cluster analysis of metabolic features (n = 1807) in the dataset.

141 **3.2 Differential compounds released from masticated rye versus wheat breads**

142

143 *3.2.1 Clusters of features more pronounced in masticated rye breads compared to wheat bread*

144

145 Cluster 9 included 388 features of which 22 were identified (Table 2). The majority of the identified
146 compounds were peptides. In addition, two amino acids, ribitol and pyridoxine were among the
147 identified compounds. The fold changes were mainly positive, meaning that these compounds were
148 mainly present in rye bread boluses and to a lesser extent in wheat bread boluses. All the identified
149 peptides were more pronounced in wholemeal rye bread boluses than in wheat bread boluses, and
150 generally, this was the case also with endosperm rye bread boluses yet with smaller fold changes and
151 with less consistence. Both amino acids (L-asparagine and L-histidine) were more pronounced in
152 wholemeal rye bread boluses and L-asparagine was more pronounced in endosperm rye bread
153 boluses, when compared to the wheat bread boluses.

154 Cluster 14 contained 305 features, of which 25 were identified. The identified compounds included
155 several amino acids and peptides, betaines, nucleosides, one polyamine and thiamine. Among
156 compound groups, peptides had the most identifications, but this cluster was not as dominated by
157 peptides as cluster 9. All the identified compounds were mainly found in the three types of rye bread
158 boluses but had relatively low levels in wheat bread boluses. Fold changes were generally higher for
159 wholemeal rye bread boluses compared to endosperm rye bread boluses.

160 Twenty-three of the 167 features in cluster 15 were identified. The compound groups included amino
161 acids, peptides, betaines and nucleobases. In addition, phenylethanolamine and glucose 6-phosphate
162 were identified. All the identified compounds were found in particular in wholemeal rye bread
163 boluses; phenylethanolamine and some of the peptides were completely missing from the other
164 boluses. Regarding the endosperm rye bread boluses, the fold changes for some amino acids, betaines,
165 peptides, nucleobase and glucose 6-phosphate were negative, indicating that these compounds were

166 more pronounced in wheat bread boluses than in endosperm rye bread boluses. Thus, the identified
 167 compounds in this cluster seem to be specific to whole-meal rye bread.

168

169 Table 2 Identified compounds in clusters 9, 14 and 15. The fold changes (FC) are listed for the rye
 170 breads with comparison to wheat bread. The statistical significance of the fold change is marked with
 171 asterisks: $p < 0.01$ *, $p < 0.001$ ** and $p < 0.0001$ ***. WB: Wheat bread, WRB: Wholemeal rye bread,
 172 ERB: Endosperm rye bread, ERGB: Endosperm rye bread with gluten.

Compound group	Identification	FC, WRB/WB	p	FC, ERB/WB	p	FC, ERGB/WB	p
<i>Cluster 9</i>							
peptide	Thr-Val-Leu	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Leu-Leu-Ala	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Ile-Val-Lys	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Ile-Ile-Arg	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Leu-Cys-Arg	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Ile-Val-Glu	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Ala-Pro-Leu	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Val-Val-Leu	21.4***	1.2×10^{-26}	3.6***	8.1×10^{-16}	2.5***	1.2×10^{-6}
peptide	Leu-Val-Ile	13.7***	4.1×10^{-24}	2.4***	3.3×10^{-11}	1.7*	6.2×10^{-3}
amino acid	L-asparagine	12.3***	3.7×10^{-30}	3.8***	7.7×10^{-23}	3.4***	1.2×10^{-16}
peptide	Val-Leu	11.7***	1.2×10^{-26}	2.1***	5.7×10^{-12}	1.7**	1.0×10^{-4}
peptide	diprotin B	8.4***	8.6×10^{-27}	1.7***	1.0×10^{-8}	1.6***	9.1×10^{-7}
vitamin	pyridoxine (vitamin B6)	6.7***	2.4×10^{-28}	2.3***	4.7×10^{-12}	2.4***	8.6×10^{-10}
peptide	Ile-Thr-Leu	6.2***	3.0×10^{-27}	2.0***	1.7×10^{-9}	1.6***	7.0×10^{-8}
peptide	Ala-Val-Leu	6.0***	1.9×10^{-18}	-1.0	0.84	-1.2	0.22
peptide	Leu-Thr-Lys	4.8***	1.5×10^{-18}	1.4***	7.9×10^{-5}	1.3**	2.7×10^{-4}
peptide	Val-Arg	4.7***	2.2×10^{-30}	1.1*	7.2×10^{-3}	-1.2	0.21
peptide	Ala-Val-Arg	4.0***	4.8×10^{-16}	1.4***	2.0×10^{-5}	-1.0	0.86
peptide	Ala-Ile-Lys	3.2***	2.2×10^{-20}	-1.1	0.52	-1.1	0.082
peptide	Val-Ser	2.2***	5.5×10^{-08}	-1.5***	2.6×10^{-7}	-1.5***	2.7×10^{-6}
sugar alcohol	ribitol	1.7***	1.2×10^{-13}	1.3***	5.8×10^{-5}	1.1	0.59
amino acid	L-histidine	1.5***	1.1×10^{-22}	-1.6	0.062	-1.2***	3.1×10^{-7}
<i>Cluster 14</i>							
betaine	acetylcholine	204.5***	6.5×10^{-17}	120.4***	1.3×10^{-15}	107.1***	7.8×10^{-15}
peptide	Pro-Leu	31.8***	9.1×10^{-30}	7.4***	1.1×10^{-20}	5.4***	2.2×10^{-11}
peptide	(contains Leu / Ile)	28.5***	3.5×10^{-36}	10.3***	6.1×10^{-28}	8.3***	2.0×10^{-16}

Compound group	Identification	FC, WRB/WB	p	FC, ERB/WB	p	FC, ERGB/WB	p
amino acid	L-leucine	26.9***	2.8×10^{-32}	3.7***	4.0×10^{-20}	3.2***	7.7×10^{-11}
amino acid	L-phenylalanine	25.4***	4.5×10^{-34}	5.0***	3.1×10^{-24}	4.4***	2.2×10^{-17}
peptide	Leu-Leu-Leu	24.8***	2.4×10^{-29}	8.6***	7.0×10^{-22}	6.2***	3.0×10^{-13}
peptide	Leu/Ile-Arg	18.0***	1.4×10^{-19}	2.4***	5.2×10^{-9}	2.1***	2.5×10^{-5}
polyamine	N8-acetylspermidine	15.7***	2.9×10^{-27}	8.7***	1.4×10^{-23}	7.8***	2.1×10^{-17}
peptide	Leu-Leu	13.8***	5.7×10^{-26}	1.8***	7.2×10^{-5}	1.5**	1.9×10^{-4}
nucleobase	adenine	10.9***	3.7×10^{-24}	3.6***	5.6×10^{-16}	3.4***	4.2×10^{-14}
amino acid	L-isoleucine	10.2***	2.7×10^{-29}	2.0***	6.0×10^{-14}	1.8***	5.6×10^{-6}
polyamine	1,2-diamino-2-methylpropane	9.1***	5.7×10^{-29}	2.6***	2.5×10^{-18}	2.2***	3.1×10^{-8}
amino acid	L-saccharopine	5.6***	4.8×10^{-27}	5.3***	2.3×10^{-26}	4.4***	4.1×10^{-16}
amino acid	tyrosine	3.8***	9.5×10^{-20}	1.3*	1.2×10^{-3}	1.2	0.089
betaine	trigonelline	3.7***	4.4×10^{-27}	2.0***	1.7×10^{-18}	1.7***	2.6×10^{-10}
amino acid	N-methyllysine	3.6***	2.2×10^{-25}	3.3***	5.3×10^{-22}	2.7***	8.8×10^{-11}
nucleoside	N6-methyladenine	3.5***	1.9×10^{-26}	1.7***	6.6×10^{-14}	1.5**	2.8×10^{-4}
vitamin	thiamine	3.4***	9.0×10^{-25}	2.0***	9.4×10^{-16}	1.8***	4.6×10^{-6}
amino acid	L-citrulline	3.3***	8.4×10^{-16}	1.7***	1.3×10^{-7}	1.7***	3.1×10^{-5}
peptide	Ile/Leu-Glu-Arg	3.3***	5.9×10^{-13}	$-\infty$ ***	0	$-\infty$ ***	0
amino acid	L-citrulline	3.3***	7.9×10^{-16}	1.7***	1.4×10^{-7}	1.6***	7.3×10^{-5}
amino acid	aspartic acid	3.1***	2.1×10^{-21}	2.8***	1.6×10^{-19}	2.6***	1.1×10^{-12}
betaine	L-carnitine	2.6***	8.5×10^{-18}	1.8***	2.0×10^{-12}	1.5**	6.6×10^{-4}
nucleoside	5'-methylthio-adenosine	2.4***	1.5×10^{-14}	2.1***	4.6×10^{-12}	1.9***	4.4×10^{-7}
nucleoside	4-guanidino-butanoate	2.1***	1.7×10^{-22}	1.8***	4.0×10^{-18}	1.7***	1.1×10^{-12}

Cluster 15

amine	phenylethanolamine	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Met-Leu-Phe	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Ile-Pro-Ile	∞ ***	0	—	—	—	—
peptide	Ala-Ile-Arg	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Leu-Leu-Ala	∞ ***	0	∞ ***	0	∞ ***	0
peptide	Phe-Ile	∞ ***	0	∞ ***	0	∞ ***	0
betaine	valine betaine	7.2***	5.7×10^{-29}	-3.0***	3.2×10^{-21}	-2.7***	2.6×10^{-10}
amino acid	L-threonine	3.9***	3.9×10^{-14}	-1.2	0.25	-1.0	0.80
nucleobase	guanine	3.5***	2.2×10^{-18}	-1.1	0.13	-1.1	0.41
amino acid	L-arginine	3.2***	3.5×10^{-19}	-1.0	0.65	-1.2	0.18
peptide	Leu-Ala-Lys	2.3***	2.4×10^{-10}	-1.5***	1.6×10^{-6}	-1.5***	2.6×10^{-5}
betaine	choline	1.8***	5.2×10^{-13}	-2.3***	8.9×10^{-14}	-2.4***	2.1×10^{-6}
peptide	Ile-Pro	1.7*	3.3×10^{-3}	1.0	0.89	1.0	0.95
amino acid	L-lysine	1.7***	5.4×10^{-18}	-1.8***	4.5×10^{-17}	-1.9***	3.4×10^{-9}
sugar	glucose 6-phosphate	1.7***	2.5×10^{-7}	-1.6	0.058	-1.3	0.013
phosphocholine	glycerophosphocholine	1.6***	2.4×10^{-16}	-1.0	0.92	1.3**	1.4×10^{-4}
nucleobase	5-methylcytosine	1.5*	1.5×10^{-3}	-2.4***	1.0×10^{-12}	-2.1***	1.1×10^{-7}
amino acid	L-arginine	1.5***	3.1×10^{-20}	-1.4	0.39	-1.1	0.11

Compound group	Identification	FC, WRB/WB	<i>p</i>	FC, ERB/WB	<i>p</i>	FC, ERGB/WB	<i>p</i>
amino acid	L-pipecolic acid	1.4***	1.6×10^{-7}	-1.3***	2.1×10^{-5}	-1.7**	1.8×10^{-4}
betaine	glycine betaine	1.3***	5.4×10^{-21}	-1.4	0.49	1.1**	9.9×10^{-4}
amino acid	<i>N,N</i> -dimethyl-L-arginine	1.3*	6.1×10^{-3}	-1.2	0.057	-1.3	0.023
peptide	Arg-Ala	1.2	0.079	-∞***	0	-4.1***	5.1×10^{-8}
betaine	proline betaine	1.1	0.10	-1.0	0.94	-1.2	0.044

173

174 Cluster 10 contained 165 features of which seven were identified including tri- and tetrasaccharides,
175 4-aminobutylguanidine (nucleobase derivative), 5'-*S*-methyl-5'-thioadenosine (nucleoside), and
176 spermidine (polyamine) (Table 3). Sugars were more pronounced in the endosperm rye bread boluses
177 than in wheat bread boluses, whereas they were less pronounced in wholemeal rye vs. wheat bread
178 boluses. This was the case also for spermidine, whereas 4-aminobutylguanidine and 5'-*S*-methyl-5'-
179 thioadenosine were mainly characteristic of all rye bread boluses.

180 Table 3 Identified compounds in cluster 10. The fold changes (FC) are listed for the rye breads with
181 comparison to wheat bread. The statistical significance of the fold change is marked with asterisks: *p*
182 < 0.01*, *p* < 0.001** and *p* < 0.0001***. WB: Wheat bread, WRB: Wholemeal rye bread, ERB:
183 Endosperm rye bread, ERGB: Endosperm rye bread with gluten.

Compound group	Identification	FC, WRB/WB	<i>p</i>	FC, ERB/WB	<i>p</i>	FC, ERGB/WB	<i>p</i>
nucleobase derivative	4-aminobutylguanidine	6.1***	6.3×10^{-30}	6.4***	2.6×10^{-28}	5.8***	9.2×10^{-21}
nucleoside	5'- <i>S</i> -methyl-5'-thioadenosine	1.3***	1.5×10^{-13}	1.4***	7.5×10^{-12}	1.3***	3.4×10^{-6}
sugar	tetrasaccharide	-1.1*	2.3×10^{-3}	1.3***	1.4×10^{-12}	1.2***	1.4×10^{-6}
sugar	trisaccharide	-1.3*	0.011	1.5***	9.8×10^{-9}	1.3***	1.5×10^{-5}
sugar	trisaccharide	-1.3***	5.4×10^{-5}	3.7*	3.5×10^{-3}	3.6*	4.1×10^{-3}
polyamine	spermidine	-1.5***	2.3×10^{-5}	2.4***	1.2×10^{-9}	2.2***	2.6×10^{-11}

sugar trisaccharide -2.0*** 2.7×10^{-13} 3.3*** 2.6×10^{-10} 2.9*** 4.3×10^{-7}

184 3.2.2 Cluster of features more pronounced in masticated wheat bread compared to rye breads
185

186 Cluster 11 contained 138 features, of which 14 were identified (Table 4). The identified compounds
187 included unidentified di-, tri- and tetrasaccharides, phosphocholines, nucleosides, pantothenic acid,
188 and one peptide. The fold changes between the rye and wheat bread boluses were negative for most
189 of the identified compounds, indicating that these compounds were found in particular in wheat bread
190 boluses. All identified nucleosides and pantothenic acid were statistically significantly more
191 pronounced in wheat bread than in rye bread boluses. The majority of the identified sugar compounds
192 was also more pronounced in wheat bread bolus than in the three different rye bread boluses.
193 Phosphocholines were more abundant in wheat bread boluses than in endosperm rye bread boluses,
194 whereas there were no statistically significant differences between wheat bread boluses and
195 wholemeal rye bread boluses.

196 Table 4 Identified compounds in cluster 11. The fold changes (FC) are listed for the rye breads with
197 comparison to wheat bread. The statistical significance of the fold change is marked with asterisks: p
198 < 0.01*, p < 0.001** and p < 0.0001***. WB: Wheat bread, WRB: Wholemeal rye bread, ERB:
199 Endosperm rye bread, ERGB: Endosperm rye bread with gluten.

Compound group	Identification	FC, p		FC, p		FC, p	
		WRB/WB		ERB/WB		ERGB/WB	
nucleoside	2'-deoxyadenosine	-8.3***	2.8×10^{-24}	-3.7***	2.8×10^{-14}	-3.4***	7.7×10^{-15}
nucleoside	cytidine	-6.5***	8.9×10^{-25}	-7.3***	2.0×10^{-22}	-8.2***	8.3×10^{-16}
nucleobase	cytosine	-4.2***	1.7×10^{-24}	-4.7***	4.4×10^{-21}	-5.3***	2.3×10^{-14}
sugar	trisaccharide	-3.2***	8.9×10^{-16}	-1.1	0.071	-1.2	0.017
nucleoside	adenosine	-3.0***	2.2×10^{-29}	-3.0***	1.0×10^{-23}	-2.9	2.9×10^{-27}

nucleoside	1-methyladenosine	-2.9***	2.7×10^{-25}	-2.6***	2.1×10^{-20}	-2.5***	3.6×10^{-11}
sugar	disaccharide	-2.0***	8.3×10^{-7}	-1.5**	2.4×10^{-4}	-1.6**	1.8×10^{-4}
vitamin	pantothenic acid (vitamin B5)	-1.9***	4.1×10^{-11}	-2.6***	7.2×10^{-14}	-2.9***	3.0×10^{-10}
peptide	glutathione	-1.8***	5.4×10^{-6}	-2.1***	6.2×10^{-5}	-3.7***	3.6×10^{-8}
amino acid	glutamine	-1.7***	2.6×10^{-14}	-1.8***	5.6×10^{-14}	-2.1***	1.4×10^{-7}
sugar	trisaccharide	-1.3**	9.4×10^{-4}	-1.7	0.059	-1.5	0.036
sugar	tetrasaccharide	-1.2	0.13	-1.3	0.39	-1.1	0.18
phosphocholine	LysoPC(16:0)	-1.2	0.082	-1.6*	2.1×10^{-3}	-2.2**	3.5×10^{-4}
phosphocholine	unknown phosphocholine	-1.1	0.30	-1.5*	4.0×10^{-3}	-2.2**	5.0×10^{-4}
peptide	Leu-Leu-Arg	∞	0	–	–	–	–

200 4 Discussion

201 This is the first study demonstrating the wealth of compounds released from bread matrices and mixed
202 with saliva in mastication. Intriguingly, identified compound groups and the relative amounts of those
203 compounds differed between wheat bread and three types of rye breads, as well as between the
204 different types of rye breads. The most evident differences between masticated rye and wheat breads
205 was the greater release of peptides and amino acids from rye breads and release of sugar compounds
206 from wheat bread.

207 Postprandial gastric inhibitory peptide (GIP) response has been found to be lower for rye breads than
208 for wheat bread suggesting that there could be differences in the nutrient flow in the gut (Juntunen et
209 al., 2003). We expected that protein hydrolysis products with incretin release stimulating activity
210 could partly explain the higher insulin response after wheat bread vs. rye bread consumption, which
211 has been observed in previous studies. However, as opposed to what we expected, the release of
212 peptides and amino acids was greater from masticated rye than wheat breads. For example, leucine,

213 isoleucine and phenylalanine, the concentration of which in blood has been previously connected
214 with insulin response (Bondia-Pons, Nordlund, Mattila, Katina, Aura, & Kolehmainen, 2011;
215 Moazzami et al., 2014), were more abundant in rye bread boluses than in wheat bread boluses.

216 The greater release of peptides and amino acids from rye breads might be explained by differences in
217 the bread baking processes. Sourdough fermentation is typically applied in rye bread baking, as was
218 the case also in the current study. During fermentation, endogenous rye proteases hydrolyze proteins
219 and produce peptides and amino acids (Poutanen, Flander, & Katina, 2009; Tuukkanen, Loponen,
220 Mikola, Sontag-strohm, & Salovaara, 2005). However, the main sites for protein digestion are in the
221 stomach and small intestine. Thus, even though amino acids and peptides were released from rye
222 breads in mastication to a greater extent than from wheat bread, the following steps of digestion might
223 turn the situation around. The study of Bondia-Pons et al. showed that *in vitro* protein hydrolysis was
224 slower from sourdough endosperm rye bread than from wheat bread (Bondia-Pons, Nordlund,
225 Mattila, Katina, Aura, & Kolehmainen, 2011). However, in the study, the relative content of soluble
226 proteins and smaller molecular weight peptides was higher in rye bread compared to wheat bread
227 both in the beginning and in the end of the *in vitro* hydrolysis. As observed by Nordlund et al.,
228 sourdough rye breads were less disintegrated than wheat breads after chewing and gastric digestion
229 *in vitro* (Nordlund, Katina, Mykkänen, & Poutanen, 2016). It could be interpreted that even if there
230 is a pool of readily available peptides and amino acids in rye breads, the main protein pool remains
231 intact for some time and hydrolyses more slowly than the protein pool of wheat bread. Compounds
232 released from food matrix could also have relevance for postprandial satiety responses, which are
233 enhanced for rye products compared to refined wheat products (Isaksson, Fredriksson, Andersson,
234 Olsson, & Aman, 2009; Rosén, Östman, Shewry, et al., 2011; Rosén, Östman, & Björck, 2011) but
235 differ among rye products with varying structures (Isaksson et al., 2011; Pentikäinen et al., 2017).
236 Protein hydrolysates in digestive tract increase cholecystokinin release (Raybould, 2008).
237 Cholecystokinin is an appetite suppressing hormone that is released shortly after beginning of eating

238 episode (Delzenne et al., 2010). Therefore, protein hydrolysates in digestive tract could offer one
239 explanation for satiety-promoting effects of rye bread. The concentration of ribitol in plasma has been
240 observed to increase after rye bread intake, in acute and 8-week interventions (Bondia-Pons,
241 Nordlund, Mattila, Katina, Aura, Kolehmainen, et al., 2011; Lankinen et al., 2011) and it has been
242 suggested to mediate the satiety response. The current study found that ribitol was released from
243 whole-meal rye bread and endosperm rye bread and it was mixed with saliva supporting the potential
244 role of ribitol for enhanced satiety responses.

245 Tri-, tetra- and monosaccharides were a distinct group of compounds released from masticated wheat
246 bread to greater extent than from masticated rye breads. The result is in line with our earlier study
247 related to glucose release, where we found a trend for faster salivary alpha-amylase induced starch
248 hydrolysis in wheat bread compared to rye breads (Pentikäinen et al., 2014) and with a study where
249 starch of wheat bread was hydrolysed faster than starch of rye breads (Juntunen et al., 2003). On the
250 contrary, Bondia-Pons et al. found the starch hydrolysis rate from endosperm rye bread to be faster
251 than that from wheat bread (Bondia-Pons, Nordlund, Mattila, Katina, Aura, & Kolehmainen, 2011).
252 It seems that some part of the wheat bread starch starts to hydrolyze in the very beginning of digestion
253 process. Faster starch hydrolysis, which stimulates the release of incretin hormones, could explain at
254 least to some extent the higher postprandial insulin response to wheat bread very soon after ingestion.
255 However, the compound identification did not reveal, of which sugar moieties the mono-, tri-, and
256 tetrasaccharides were comprised. It will remain uncertain if these compounds comprised of glucose
257 units or of some other monosaccharides, and if those compounds could influence incretin and insulin
258 secretions. The sugar compounds could be studied in depth in future studies.

259 In addition to peptides, amino acids and sugars, a great variety of other compound groups such as
260 vitamins, amines, and betaines were identified from bread boluses. Betaines were found in particular
261 in masticated rye breads. Previously, betaine concentration in plasma has been linked to whole grain
262 consumption in humans (Ross et al., 2011). In mice urine, concentrations of betaines were increased

263 after rye containing diet (Pekkinen et al., 2015). This is the first study to show that some betaines are
264 released from bread matrix already in mastication and mixed with saliva.

265 Whole grain consumption has been associated with many health benefits (G.-C. Chen et al., 2016;
266 Schwingshackl, Schwedhelm, Hoffmann, Lampousi, & Knu, 2017). About one third of the features
267 in the current data were related to wholemeal rye bread. These compounds will be an interesting field
268 for future studies when aiming to understand the mechanisms for the benefits of whole grain
269 consumption.

270 The clusters of interest contained 1163 features of which we were able to identify 83 compounds (7
271 %) meaning that the majority of the metabolic features remained unidentified. Food bolus samples
272 represent a new sample type and there are no references regarding this specific sample type yet. The
273 small proportion of identifications is a limitation of this study. We suggest that compounds that are
274 released from food bolus in different stages of digestion could act as signal molecules for endocrine
275 and neural responses. However, at this stage it is uncertain which compounds could be relevant and
276 how large fold changes could be expected to deliver some difference in further physiological
277 responses.

278 The current study revealed that, a diverse array of compounds was released from masticated bread
279 samples and mixed with saliva. The study also outlined the magnitude of differences between
280 different bread types. Peptides, amino acids and sugars were the most evident compound groups that
281 differentiated rye and wheat breads. We expect these results and the metabolomics approach to inspire
282 further research inspecting the actions of the released compounds in the gut lumen.

283 **Authors' contributions**

284 SP, MK, KP, KH, and A-MA designed the study. SP conducted the mastication trial. KH and SP were
285 responsible of LC-MS analytics. KH, VK and SP were responsible for the data analyses and

286 compound identification. SP drafted the manuscript and all authors contributed to the interpretation
287 of the data and processing of the manuscript. All authors read and approved the final manuscript.

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407

408 **Figure caption**

409 (title):

410 Figure 1. *k*-Means cluster analysis of metabolic features ($n = 1807$) in the dataset.

411 (description of the illustration):

412 Each row represents one molecular feature. The columns Saliva, WB, WRB, ERB and ERGB contain
413 replicates obtained from bolus samples of 15 individual participants. Red color indicates upregulation
414 (large relative peak area) whereas green color indicates downregulation (small relative peak area).
415 WB: Wheat bread, WRB: Wholemeal rye bread, ERB: Endosperm rye bread, ERGB: Endosperm rye
416 bread with gluten.

417 **Supplementary files**

418 title:

419 Supplementary file 1. Compound identification table

420 (description of the supplementary file):

421 RT: retention time; CID: collision induced dissociation, M: mass.

PUBLICATION III

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Do rye product structure, product perceptions and oral processing modulate satiety?



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ABSTRACT

Food structure and cephalic phase factors are hypothesized to contribute to postprandial satiety in addition to established food properties such as energy content, energy density, and macronutrient and fibre composition of a preload. This study aimed to evaluate if the structure of rye products has an impact on subjective feelings of satiety, and whether cephalic phase factors including oral processing, satiety expectations and perceived pleasantness modulate the interaction. Four wholegrain rye based samples (extruded flakes and puffs, bread and smoothie) were studied in terms of texture characteristics, *in vivo* oral processing, and expected satiety ($n = 26$) and satiety as well as perceived pleasantness ($n = 16$) (ClinicalTrials.gov number: NCT02554162). The vast textural differences between products were reflected in mastication process, perceived pleasantness and satiety expectations. Extruded products required the most intensive mastication. Rye puffs and rye bread which were characterised by a solid and porous structure, and showed better satiety effect in the early postprandial phase compared to other products. Mastication effort interacted with satiety response. However, the products requiring the most intense mastication effort were not the most satiating ones. It seems that there are some food structure related factors that influence both mastication process and postprandial satiety, the mastication process itself not being the mediating factor. Higher palatability seems to weaken postprandial satiety response.

1. Introduction

The feeling of satiety has been proposed to support weight management through various routes such as greater food reward, reduced hunger and better control of energy intake (Hetherington et al., 2013). For instance, the amount and type of dietary fibre in food, macronutrient composition and energy density of food contribute to the modulation of satiety. In addition, cognitive and sensory signals generated before and during eating (cephalic phase) are proposed to influence satiation (intra-meal satiety) and satiety (inter-meal satiety) (Blundell et al., 2010). Cephalic phase responses such as stimulation of hormone and enzyme secretion are hypothesized to enhance nutrient processing and thus to enhance also satiety response (Smeets,

Erkner, & De Graaf, 2010).

Signals that are generated already during oral processing are needed for optimal appetite regulation, in addition to signals originating from later phases of digestion (Smeets et al., 2010). The importance of oral phase for appetite regulation has been well established in studies where appetite suppression has been incomplete after infusing food directly to stomach. Hogenkamp and Schiöth recently reviewed studies on oral processing of food, satiation and satiety, and concluded that viscosity of food had consistent impact on *ad libitum* food intake (satiation) and that orosensory exposure was the mediating factor between viscosity and satiation (Hogenkamp & Schiöth, 2013). Later, Bolhuis et al. showed that hard foods which were eaten in smaller bites than soft foods and processed longer in mouth, reduced the energy intake during the meal,

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Table 1

Nutrient content of the food samples and nutrient content and portion sizes of portions served in the satiety trial.

	Samples (/100 g)					Satiety trial portions (/portion)				
	WG sourdough bread	Extruded rye flakes	Extruded WG rye puffs	Refined wheat bread	Black- currant juice	WG sourdough rye bread + juice	Extruded WG rye flakes + juice	Extruded WG rye puffs + juice	WG rye smoothie	Refined wheat bread + juice
Nutrient content										
Energy (kcal)	200	322	330	253	38	382	382	382	382	382
Starch (g)	35.4	57.7	59.8	46.4	ns	33.7	34.1	34.5	34.1	34.8
Protein (g)	6.5	9.7	9.8	9.1	ns	6.2	5.7	5.6	5.7	6.8
Fat (g)	0.6	1.2	1.3	2.4	ns	0.6	0.7	0.7	0.7	1.8
Total dietary fibre (g)	13.3	20.7	19.8	4.7	ns	12.6	12.2	11.4	12.2	3.6
Soluble dietary fibre (g)	7.5	9.5	10.7	2.3	ns	7.2	5.6	6.2	5.6	1.7
Insoluble dietary fibre (g)	3.6	3.7	4.0	1.5	ns	3.4	2.2	2.3	2.2	1.1
Oligosaccharides (g)	2.2	7.6	5.2	1.0	ns	2.0	4.5	3.0	4.5	0.7
Sugar (g)	–	–	–	–	9.6	48	48	48	48	48
Portion sizes (g)										
Cereal product						95	59	58	58	75
Juice						500	500	500	500	500
Total						595	559	558	559	575

and that the effect was sustained over the following meal (Bolhuis et al., 2014). They also concluded that the differences in oral processing might mediate this effect. Mastication process has also shown to suppress gastric emptying rate (Ohmure et al., 2012).

The effects of preload texture and resulting oral processing on postprandial satiety have been investigated in several studies. Energy intake at next meal context is adjusted only partly after a liquid preload while it is fully adjusted after semi-solid or solid preload (Almiron-Roig et al., 2013). This leads to lower overall caloric intake (preload and *ad libitum* meal) after semi-solid or solid preloads compared to liquid preload. This indicates that food texture, at least when liquids are compared to solids or semi-solids, plays a role not only in satiation but also in satiety response. However, the results concerning food textures other than liquids, resulting in varying orosensory exposure, are somewhat inconsistent (Hogenkamp & Schiöth, 2013). Satiety effect of foods with either solid or heterogeneous texture, assumed to induce high orosensory exposure, or corresponding comminuted texture, assumed to induce low orosensory exposure, have been compared by various groups: Mattes et al. found that there were no differences in satiety responses between solid and semi-solid foods (apple vs. apple soup, peanut vs. peanut soup or chicken vs. chicken soup) (Mattes, 2005) whereas later (Flood-Obbagy & Rolls, 2009) a whole apple was concluded to induce more pronounced satiety than apple sauce and the whole apple also reduced energy intake in the following meal. Martens et al. showed that solid food (steamed chicken breast) resulted in enhanced satiety response compared to liquefied food (blended steamed chicken breast) (Martens, Lemmens, Born, & Westerterp-Plantenga, 2011) whereas Flood and Rolls showed that there was no difference in satiety response whether soup was offered as separate broth and vegetables versus pureed soup (Flood & Rolls, 2007). In addition heterogeneous and homogeneous yoghurts resulted in similar satiety response (Tsuchiya, Almiron-Roig, Lluch, Guyonnet, & Drewnowski, 2006). To summarize, the evidence regarding the importance of food texture and oral processing on satiety is inconsistent. Most of the studies do not report oral processing precisely. The influence of oral processing on appetite has been studied also in experimental settings where the same foods have been eaten varying the number of chews or mastication time as instructed by the researchers. The results of such studies have been inconsistent: some reports indicate that increasing number of chews or mastication time improves satiety but others show no connection (Hogenkamp & Schiöth, 2013).

Sensory characteristics of foods such as chewiness and saltiness (Forde, van Kuijk, Thaler, de Graaf, & Martin, 2013), anticipated creaminess (McCrickerd, Lensing, & Yeomans, 2015) and thickness and creaminess (Yeomans & Chambers, 2011) have been found to influence on expected satiety. Even expectations about the satiating capacity of foods evoked by visual and other sensory perceptible cues have shown to influence the actual satiety response: In the study of Brunstrom et al participants were shown either a large or a small portion of fruits prior to consuming an equal size fruit smoothie (Brunstrom, Brown, Hinton, Rogers, & Fay, 2011). The participants who saw the larger fruit portion reported higher expectations of satiety and in fact also experienced enhanced satiety for three hours. Liking of food has also been repeatedly shown to influence appetite reflected as an increased intake as palatability increases (Sørensen, Møller, Flint, Martens, & Raben, 2003). However, results concerning the importance of palatability on postprandial satiety remain inconclusive. To summarize, cephalic phase factors including oral processing, perception about pleasantness of food as well as expectations about its satiating capacity may all work together to modulate the satiety response.

The current study aimed to evaluate if the structure of rye products influences subjective feelings of satiety, and if cephalic phase factors including oral processing, satiety expectations and evaluated pleasantness are mediating the interaction. The use of rye products as model foods allowed the comparison of extreme food structures with only minor differences in chemical composition.

2. Materials and methods

2.1. Products and their nutrient contents

The test foods were wholegrain rye products representing various structures; wholegrain sourdough rye bread, extruded wholegrain rye flakes, extruded wholegrain rye puffs and wholegrain rye smoothie (Table 1 and Fig. 1). Wheat bread was included as a control product. Wholegrain sourdough rye bread (wholegrain rye flour, water, salt) and refined wheat bread (wheat flour, water, yeast, sugar, rapeseed oil, salt) were commercially available products by local bakery (Emil Halme). Wholegrain rye puffs and flakes were prepared at VTT using whole grain rye flour (Oy Karl Fazer AB/Fazer Mills and Mixes, Lahti, Finland) and salt (0.8%) as ingredients. A twin screw extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, UK) was used to produce the extrudates with a constant feed rate of 60 g/min and temperature

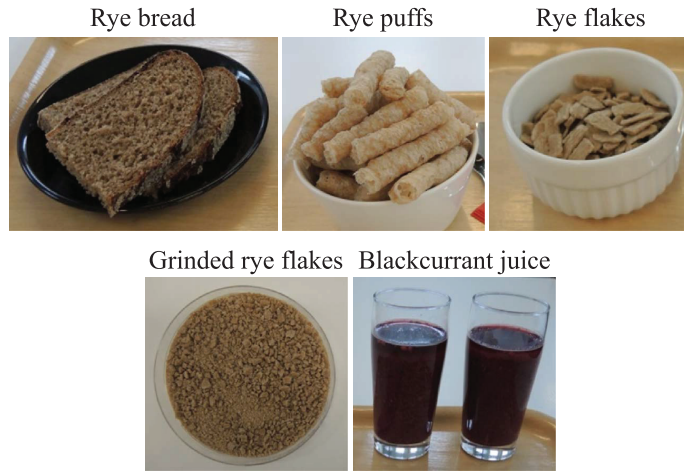


Fig. 1. Photographs of the rye food samples. Rye smoothie was prepared mixing grinded wholegrain rye flakes with blackcurrant juice and letting the mixture stand for 15 min.

profile of 80–95–110–120 °C (Section 1 to die exit) with the screw speed of 350 and 250 rpm for puffs and flakes, respectively. Water was pumped into the extruder barrel in order to obtain desired moisture contents in the extrudates. Extruded products were collected continuously from the exit die (diameter 3 mm) and dried immediately in an oven at 100 °C, 30 min for puffs and 90 min for flakes. Wholegrain rye smoothie was prepared mixing grinded wholegrain rye flakes with blackcurrant juice and letting the mixture stand for 15 min resulting in a thick smoothie-like heterogeneous texture. Blackcurrant juice was a commercial product (Marli).

2.1.1. Instrumental texture

Texture profile analysis was used to extract the primary and secondary mechanical characteristics of breads by using a texture analyser (TA-XT plus Texture Analyser, Stable Micro System, Godalming, Surrey, UK) with a 25-mm diameter cylinder probe (P/25L Lap Perspex), 30-kg load cell, 60% strain on 25-mm thick cylindrical pieces of breads which were cut by the help of a mould. Upper crust was included in the pieces. The acquisition rate was 200 points/s and the test speed was 1.7 mm/s. TPA software (Exponent v.6, Stable Micro System, Godalming, Surrey, UK) was used to extract force-deformation curve. Hardness, cohesiveness, chewiness, and adhesiveness were calculated based on force-deformation curve.

Textural properties of extruded puffs and flakes were analyzed by the uniaxial compression test using a texture analyser (Texture Analyser TA-HDi, HD3071, Stable Micro Systems, United Kingdom) equipped with a 250 kg load cell and a cylindrical 36 mm aluminium probe using a protocol used by Alam et al. (2014). Extruded ribbons (puffs) were cut to 10 mm height for analysis and flakes were analysed as is. The samples (50 replicates for each samples) were deformed at 70% strain with a test speed of 1 mm/s and the acquisition rate 200 points/s. Texture Exponent software v.5.1.2.0 (Stable Micro Systems, UK) was used to obtain values of hardness (F_{max}), crispiness work (C_w) and crispiness index (C_i). High crispiness is accompanied by a high C_i and low C_w value, whereas low crispiness corresponds to a low C_i and high C_w value. The analysis was performed using the algorithms described by Alam et al. (2014).

2.1.2. Perceived characteristics

All assessors of VTT's internal trained sensory panel ($n = 12$) have passed the basic taste test, the odour test and the colour vision test and trained for sensory profiling. The trained sensory panel was first familiarized with the sensory assessment of diverse cereal samples.

The method in sensory profiling was descriptive analysis (Lawless & Heymann, 2010). The vocabulary of the sensory attributes was developed by describing the differences between the samples. The assessors familiarized themselves with the products, discussed and defined the key attributes differentiating the products in a training session aiming to produce the descriptors for the sensory profile. The selected attributes included *colour darkness*, *rye flavour intensity*, *flavour intensity*, *visual porosity*, *hardness*, *crispiness*, *crunchiness*, *crumbliness*, *moisture*, *adhesion to teeth* and *work needed for mastication*. In sensory profiling the latter was evaluated according to the instructions: "Masticate the sample using your back teeth until the sample is ready to be swallowed. After that, please evaluate how much work was needed for mastication". Actual reference samples were used to define the extremes for most of the attributes, and all descriptors were also verbally anchored. All sensory intensities were evaluated using 10 cm scale anchored from "not at all" to "extremely". All samples were evaluated by sensory profiling in duplicate sessions in two consecutive days by all the panellists. The samples were blind-coded by 3-digit numbers, and the presentation order of the samples was randomized within each test day. Water was served to the assessors for cleaning the palate between the different samples. The scores were recorded and collected using computerized software (Compusense Five, Ver 5.4.15, CSA, Computerized Sensory Analysis System, Compusense Inc., Guelph, ON, Canada).

2.2. Participants

Participants ($n = 26$) were recruited through public advertisements and email advertisements in Otaniemi campus area nearby the study location. The eligibility of the volunteers was checked beforehand through screening questionnaire. The criteria were: female gender, age 20–40 years, BMI between 18.5 and 27 kg/m², stable body weight (± 4 kg during the previous year) and a habit of eating breakfast. Smokers, pregnant or lactating women, persons with missing teeth (except 3rd molars) or with diagnosed acute temporomandibular disorders (TMD) (self-reported) and persons with dietary restrictions possibly affecting the study participation (celiac disease, allergies or aversions to cereal foods or high carbohydrate foods) or abnormal eating behaviour according Eating Disorder Diagnostic Scale (EDDS) were excluded. Young healthy females were recruited to diminish the variation in mastication pattern. The interested volunteers fulfilling the inclusion criteria were invited to an info visit. Volunteers deciding to participate signed an informed consent form. The whole study popula-

Table 2

Characteristics of the study participants. Values are means \pm SD, $n = 26$ in the mastication trial and $n = 16$ (subset) in the satiety trial.

	Mastication trial $n = 26$		Mastication trial and satiety trial $n = 16$ (subset)	
	Mean \pm SD	Range	Mean \pm SD	Range
Age	31.7 \pm 7.5	19–50	32.9 \pm 8.2	22–50
BMI	22.2 \pm 1.9	19.1–27.3	22.4 \pm 2.2	19.8–27.3
Eating behaviour ¹				
Cognitive restraint	45.7 \pm 16.6	11–72	51.7 \pm 12.1	17–72
Uncontrolled eating	27.6 \pm 10.3	11–48	27.6 \pm 11.2	11–48
Emotional eating	33.3 \pm 24.7	0–89	41.4 \pm 26.8	0–72

¹ Eating behaviour was measured with 18-item Three-Factor Eating Questionnaire (TFEQ) (Karlsson, Persson, Sjöström, & Sullivan, 2000).

tion ($n = 26$) participated in mastication trial and a subgroup of 20 participants started the satiety trial. The both trials were conducted during October–December 2015. Sixteen of the 20 participants completed all the study visits and four discontinued due to personal reasons. Characteristics of the participants are described in Table 2. Two participants were older than 40 years (48 and 50 years). However, since they fulfilled all the other inclusion criteria they were included in the study, as the number of recruited participants was not as high as desired. The participants were given one movie ticket per study visit to compensate their time and effort. The study protocol was approved by the Coordinating Research Ethics Committee of the Helsinki and Uusimaa Hospital District. The study was conducted according to the ethical principles of good research and clinical practice described in the declaration of Helsinki. The trial was registered in ClinicalTrials.gov (NCT02554162).

2.3. Mastication trial

2.3.1. Procedure

The mastication trial followed a cross-over, single-blind design, in which all participants masticated the five samples in random order. The

participants were instructed to eat a breakfast 1–1.5 h before the visit scheduled between 8–11 a.m. The study procedure was first practiced with a test sample and the coded food samples were served to the participant in random order, each sample in three portions. Portion sizes represented a mouthful of food: $2 \times 2 \times 2$ cm-size cube of bread (including crust in one side) (approx. 7.7 g), one table spoon of flakes (3.5 g), two 2 cm pieces of puffs (1 g) and one table spoon of rye smoothie (16.8 g). The participants were instructed to masticate each portion of sample until subjective swallowing point and then expectorate the bolus. The three portions of each sample were masticated in a row and there was break between different samples during which mouth was rinsed with water and the expected satiety rating for each sample was evaluated. As a final sample, the participant was served three portions (=piece) of chewing gum and she was asked to chew each piece for 20 s. Oral processing was characterised by measuring electrical activity of facial muscles with electromyography. Even if the measured voltage is linearly relative to the force generated by the muscle, the calibration varies between different subjects and even the four muscles monitored. Thus, to get an indication of the relative force needed to masticate each of the samples individual data on oral processing of chewing gum was used as a reference for force parameters. The mastication trial visits were video recorded to support data analysis.

2.3.2. Electromyography (EMG) measurements

The mastication process was characterised by measuring the electrical activity of masticatory muscles by EMG equipment (Mega Electronics, Kuopio, Finland) using disposable dermal Ag/AgCl electrodes. Masseter and temporal muscles were identified by touch when the participant gritted her teeth. Skin was cleaned with 70% ethanol alcohol and bipolar electrodes were placed on the muscles on both sides of the face. A reference electrode was placed on cervical vertebra. EMG activity was measured continuously throughout the whole mastication trial. The data block starts and ends for each chewing period were both marked in the EMG acquisition system (Fig. 2A) and recorded manually. From the EMG time series, the onset, duration and amplitude of each chew were extracted by applying chemometric techniques for the elimination of high frequencies and background fluctuations as in the study of Pentikäinen, Sozer et al. (2014) (Fig. 2B).

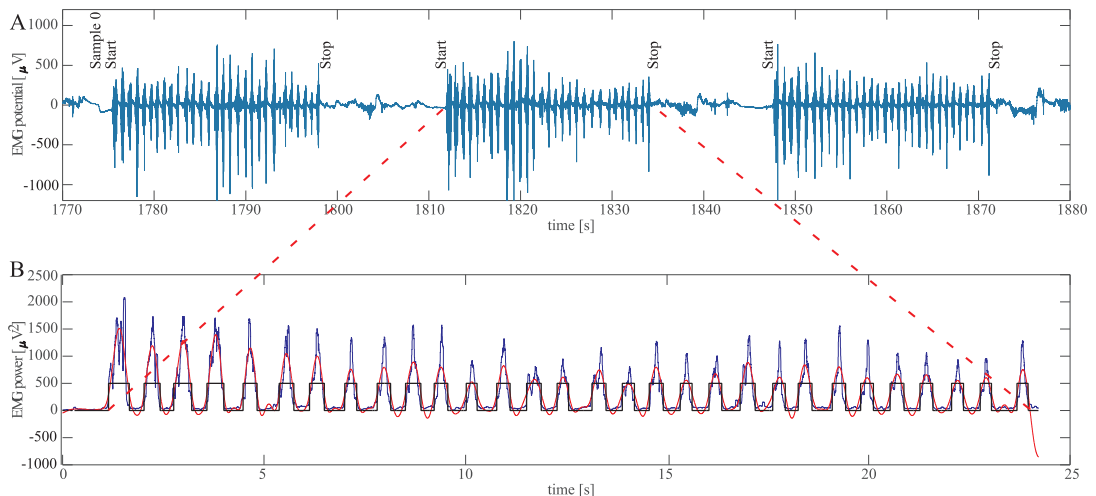


Fig. 2. A: EMG data after 50 Hz notch filtering for a single participant, chewing gum sample. The three mastication sequences are each labeled with 'start' and 'stop'. B: Further analysis of the second mastication sequence of the data above. EMG power was computed, highpass-filtered, squared (blue curve) and smoothed (red curve), after which chews were detected (black block curve). The event data were used for number of chews, total oral processing time, time of EMG activity and duty cycle. The smoothed EMG power was used for relative force and, when multiplied by time of EMG activity, the relative work. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Chewing force and work parameters were normalized to chewing process of chewing gum. As a result of data processing and analyses, the duration of oral processing, duration of EMG activity, duty cycle (duration of EMG activity/duration of chewing), number of chews, relative chewing force (highest EMG amplitude for the product normalized to highest EMG amplitude for chewing gum) and relative work (time of EMG activity x relative chewing force) were calculated for each test food. All analysis of EMG data was done using Matlab® (The MathWorks Inc., Natick, MA, USA). The values for duration of EMG activity, duration of oral processing, number of chews and relative work were extrapolated to represent the amount served later in the satiety trial. The coefficients were determined by dividing the weight of the whole portion served in the satiety trial by the weight of one mouthful of food used in the mastication trial. Coefficients for rye bread, rye smoothie, rye puffs, rye flakes and wheat bread were 12.4; 32.8; 58; 16.9 and 19.2, respectively.

2.3.3. Expected satiety

The participant was asked to anticipate the satiating capacity of the samples before and after mastication of each food sample. This part was included in order to find out whether food structure evaluated based on visual cue (picture) or with both visual and sensory cues (mastication) influences anticipated satiety effect. The evaluation was based on a photograph showing a food portion including a fixed amount of sample and a glass of juice. The portions in photographs were the same size as the portions that were later used in the satiety trial. The questions, as translated from Finnish were: (before mastication) “Imagine that you would eat the whole portion of food shown in the photograph. Evaluate how satiated you would feel after one hour.” and (after mastication) “You have just masticated the product shown in the photograph. Imagine that you would eat the whole portion of food shown in the photograph. Evaluate how satiated you would feel after one hour”. The evaluation was done on 10 cm visual analogue scale (VAS) anchored with 0 = not at all satiated, 10 = extremely satiated.

2.4. Satiety trial

The satiety trial followed a cross-over, single-blind design, in which all participants tested the five study portions in random order, each portion on a separate day. There were at least two washout days between two consecutive study visits. The participants were instructed to follow their usual eating and exercise habits during the day preceding each study visit and to fast at least 10 h before arriving to the study visit.

The study visits started in the morning between 7 and 9 a.m. The test portion sizes were matched by energy content each portion providing 380 kcal of energy (Table 1). The portions consisted of blackcurrant juice (5 dl) and of either 95 g of wholegrain (WG) sourdough rye bread, 59 g of WG rye flakes, 58 g of WG rye puffs or 75 g refined wheat bread. WG rye smoothie was prepared by mixing 59 g of grinded rye flakes in 5 dl blackcurrant juice. The participants were instructed to eat and drink the test products at their own pace but not to spend more than 20 min on eating. Satiety related sensations were evaluated before and right after consuming the test portion and

repetitively every 30 min until 210 min after starting point of the consumption using 10 cm visual analogue scales (VAS) anchored with extremes (0 = not at all, 10 = extremely). The evaluated sensations were *hunger*, *fullness*, *satiety*, *desire to eat* and *prospective food consumption* (“How much would you be able to eat right now?”). In addition, *pleasantness* of the test portion was evaluated after consuming the portion. Average appetite score was afterwards calculated as [desire to eat + hunger + (10-fullness) + prospective food consumption]/4. Computerised data-collecting system (CSA, Computerised Sensory Analysis System, Compusense, Guelph, Canada, Compusense five 5.2) was used to collect the evaluations.

2.5. Statistical analyses

IBM SPSS Statistics 22 was used to analyse the data. Oneway ANOVA was used to study the sensory differences of study products. Pair-wise comparison was conducted by using Tukey’s test. Repeated measures ANOVA was used to study the differences in satiety expectations and pleasantness evaluations. Friedman’s non-parametric test for related samples was used to compare the parameters describing mastication process. P-value < 0.05 was considered as statistically significant.

Regarding the satiety evaluations, baseline value of each visual analogue scale parameter was subtracted from the values of subsequent time points to take into account the possible effect of baseline differences on the analysis. Linear mixed-effects models were used to compare the effects of the test portions on the profiles of postprandial satiety responses. The used models included participant as a random factor, and product, time, and product * time interaction as fixed factors. When a significant main effect of a product or product * time interaction was observed, post hoc analyses were performed using the Sidak correction for multiple comparisons in order to identify the statistically significant differences between the test portions. The contribution of cephalic phase factors was evaluated by adding parameters of oral processing, evaluated pleasantness and satiety expectations to the model as fixed factors one at a time and Schwarz’s Bayesian Criterion (BIC) was then used to compare goodness of fit between the models. The smaller the BIC value is the better the model fit is.

3. Results

3.1. Characteristics of study products

3.1.1. Instrumental texture

Instrumental texture of the solid products was measured using a texture analyser. The extrudates were dry products with hard and fragile texture whereas breads were springy and moist (Table 3). Rye flakes had the hardest texture and wheat bread the least hard. Hardness of rye puffs and rye bread was similar whereas they had otherwise different textural properties rye puffs being crispy and rye bread being springy. Rye bread was less cohesive, more chewy and adhesive than wheat bread. Puffs were crispier than flakes, indicated by higher crispiness index and lower crispiness work.

Table 3
Moisture contents of the samples and textural properties measured with TPA (breads) and TA (extrudates).

	WG sourdough rye bread	Refined wheat bread	Extruded WG rye flakes	Extruded WG rye puffs
Moisture (%)	39.3 ± 0.1	32.3 ± 0.4	7.0 ± 0.0	5.5 ± 0.0
Hardness (N)	24 ± 8	4 ± 1	1530 ± 390	27 ± 3
Cohesiveness	0.4 ± 0.1	0.7 ± 0.0	–	–
Chewiness	5.1 ± 1.8	2.0 ± 0.5	–	–
Adhesiveness	–0.010 ± 0.014	–0.133 ± 0.332	–	–
Crispiness work			98.3 ± 37.3	0.6 ± 0.1
Crispiness index (x ^{10–3})			0.004 ± 0.002	21 ± 5

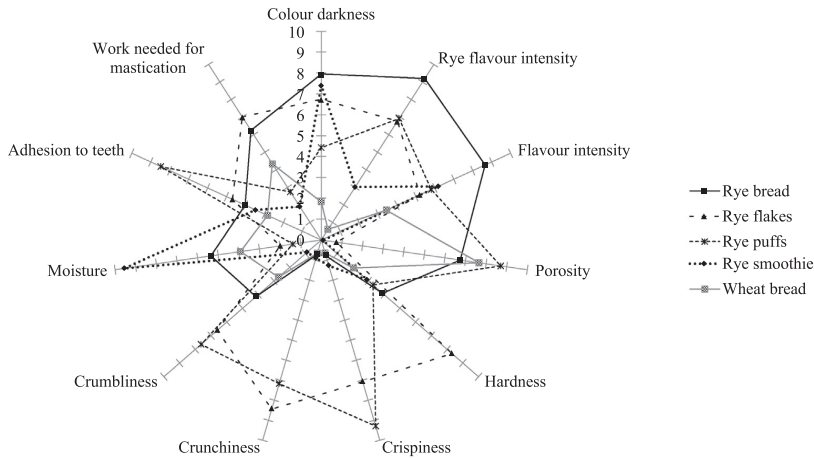


Fig. 3. Perceived characteristics of the food samples evaluated by the trained sensory panel ($n = 2 \times 12$). Sensory intensities were evaluated on an intensity scale 0–10. Values are means. There were statistically significant differences ($p < 0.001$) between the samples in each attribute.

3.1.2. Perceived characteristics

The sensory characteristics of the samples were evaluated by a trained sensory panel. The products varied significantly in all the evaluated sensory attributes ($p < 0.001$ for all) (Fig. 3) as was intended. Rye flakes and rye bread were evaluated to require more work for mastication than the other products (rye flakes vs. rye puffs, smoothie and wheat bread $p < 0.001$; rye bread vs. rye puffs and smoothie $p < 0.001$, rye bread vs. wheat bread $p = 0.004$). Rye puffs adhered to teeth more than the flakes, breads or smoothie ($p < 0.001$ for all). Rye flakes and puffs were crumblier, crunchier and crispier compared to the other products ($p < 0.001$ for all). Rye flakes were crunchier than rye puffs ($p = 0.015$) and rye puffs were crispier than rye flakes ($p < 0.001$). Rye flakes were harder than the other products ($p < 0.001$ for all) and rye bread was harder than wheat bread ($p = 0.009$). Rye puffs and both breads were more porous than rye flakes or smoothie ($p < 0.001$). Both overall flavour and rye flavour were more intense in rye bread than in other products ($p < 0.001$ for all).

3.1.3. Expected satiety and evaluated pleasantness

The participants of the mastication trial ($n = 26$) evaluated the expected satiating capacity of the products before and after masticating them. The evaluation was based on picture representing isocaloric portions of the products. The satiety expectations differed significantly between the products ($p < 0.001$ for both before and after mastication) (Fig. 4A). The portion containing wholegrain sourdough rye bread was evaluated to be more satiating than the other portions both before mastication (rye bread vs. rye flakes, smoothie and wheat bread $p < 0.001$; rye bread vs. rye puffs $p = 0.031$) and after mastication ($p < 0.001$ for all) whereas wholegrain rye smoothie portion was evaluated as less satiating than the other portions before mastication ($p < 0.001$ for all) and less satiating than rye bread and rye flakes ($p < 0.001$ for both) and wheat bread ($p = 0.005$) after mastication. Expected satiety effects of rye bread, rye flakes and rye smoothie were evaluated higher after than before mastication ($p = 0.001$, $p < 0.001$, and $p < 0.001$, respectively). There were no differences in the evaluations before and after mastication of rye puffs or wheat bread. The participants of the satiety trial ($n = 16$) evaluated the pleasantness of the consumed portions. There were significant differences in the ratings of pleasantness between the portions ($p < 0.001$) (Fig. 4B). The rye bread portion was evaluated as more pleasant than the other portions (rye bread vs. smoothie $p = 0.002$; vs. rye puffs $p < 0.001$; vs. wheat bread $p = 0.011$; vs. rye flakes $p = 0.005$) and extruded rye

puff portion was evaluated less pleasant than rye bread ($p < 0.001$), wheat bread ($p = 0.001$) and rye flake portion ($p = 0.006$).

3.2. Mastication properties

Mastication was characterised by monitoring the electrical activity of facial muscles during masticating mouthful of sample. There were significant differences between food samples in all the measured oral processing attributes: number of chews, total oral processing time, total EMG activity time, duty cycle, relative force and relative work ($p < 0.001$ for all). Table 4 shows the values for the parameters and the results of pairwise comparisons. Total oral processing time, total EMG activity time and relative work for mouthful of sample were the highest for rye bread and rye flakes and the lowest for puffs and smoothie. The number of chews was the highest for mouthful of rye flakes and the lowest for puffs and smoothie. It should be noted, however, that for smoothie the events detected as chews are mostly other muscle motions than actual chewing.

When the measured oral processing attributes were extrapolated to represent the process of chewing the whole portion of the product (as amount served in the satiety trial) there were also statistically significant differences between products in all the attributes ($p < 0.001$). Total oral processing time, EMG activity time and relative work per portion were the highest for flakes and puffs and the lowest for smoothie. Number of chews per portion was higher for flakes, puffs and wheat bread than for rye bread or rye smoothie.

3.3. Postprandial satiety responses to food portions

Portions of the test products were served to subgroup of 16 participants in the satiety trial. Each portion was served in separate day. The mean VAS ratings for hunger, fullness, desire to eat, prospective food consumption, satiety and average appetite score for the 210 min period are presented in Fig. 5. Hunger (Fig. 5A) was significantly lower and fullness (Fig. 5B) higher at 30 min after consumption of puff portion compared to flake portion ($p = 0.012$ and $p = 0.028$, respectively) whereas there were no statistically significant differences between other portions. Desire to eat (Fig. 5C) was significantly higher at 60 min after consumption of flake portion than rye bread portion ($p = 0.038$) but there were no differences between other portions. Prospective food consumption (Fig. 5D) was significantly higher after consuming flakes compared to puffs at 30 min and 60 min ($p = 0.002$ and $p = 0.028$, respectively) and compared to

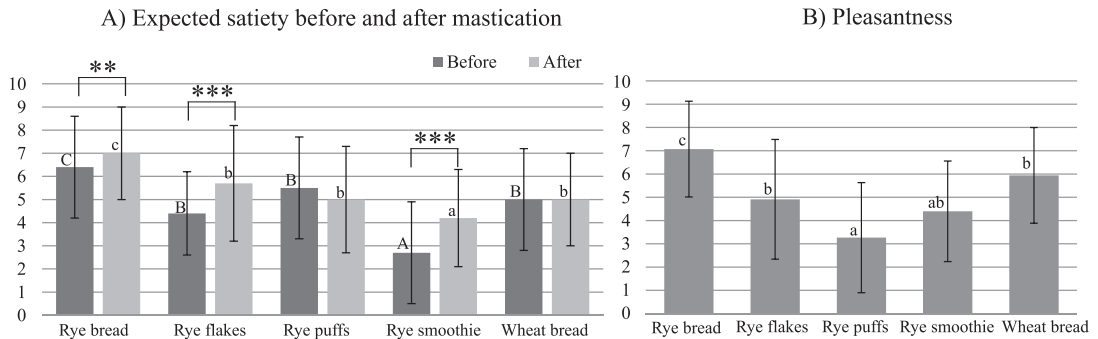


Fig. 4. A) Expected satiety before and after mastication ($n = 26$) and B) pleasantness of the portions after eating the portion ($n = 16$). Expected satiety was evaluated based on photograph representing study portions together with mastication trial. Pleasantness of each study portion was evaluated together with satiety trial right after consuming the portion. The evaluations were done on a VAS scale 0–10. Values are means \pm SD. Different letters above bars indicate statistically significant difference between evaluations (in 2A uppercase letters for values before mastication and lowercase letters for values after mastication). Asterisks in 2A indicate significant difference within product before and after mastication trial ** $p < 0.01$, *** $p < 0.001$.

rye bread at 30 min ($p = 0.018$). However, there were no other differences between products or in other time points. There were no statistically significant differences in satiety ratings (Fig. 5E). Average appetite (a parameter derived from fullness, prospective food consumption, hunger and desire to eat) (Fig. 5F) was significantly higher after consuming flakes compared to puffs at 30 min and 60 min ($p = 0.011$, $p = 0.045$) and compared to rye bread at 30 min ($p = 0.034$). Between other products no differences were seen.

3.4. Postprandial average appetite in relation to oral processing, evaluated pleasantness and satiety expectations

Mixed model including product and time as fixed factors, subject as a random factor and average appetite as dependent factor was taken as starting point to study the contribution of cephalic phase factors on average appetite (a parameter derived from fullness, prospective food consumption, hunger and desire to eat). BIC value describing the goodness of fit for this model was 2195. Parameters of oral processing (number of chews per portion and relative work); evaluated pleasantness and satiety expectations were then added to the model as fixed factors one at a time to see whether they influenced the goodness of model fit. Adding the number of chews in the model did not improve the fit (BIC value 2165, p -value for product 0.051) but adding a parameter for relative work did improve it (BIC value 1911, p -value for product 0.001). Including evaluated pleasantness improved the fit as well (BIC 1965, p -value for product 0.001). The differences between

products were abolished when the evaluations about expected satiety before mastication (BIC 1966, p -value 0.109) and after mastication (BIC 1968, p value for product 0.304) were added in the model.

4. Discussion

The results showed that rye product portions matched by energy content but varying in structure required different type of mastication process and influenced on postprandial satiety measures differently in the early postprandial period. Mastication effort, measured as relative mastication work, and perceived pleasantness seem to interact with satiety response. The portion with rye flakes showed the weakest satiety impact, puffs and rye bread showing the strongest impact and rye smoothie intermediate. Rye puffs and rye bread, having the most beneficial influence on satiety, were both characterised by a solid and porous structure with comparable instrumental and sensory hardness. However, there were many characteristics that differentiate these products: rye bread was soft and springy product and rye puffs crispy, with strong adhesion to teeth, probably attributable of the combination of high content of arabinoxylan and big particle surface area in mastication. Rye flakes, resulting in the weakest satiety response, were hard and crunchy and had a non-porous structure requiring intensive mastication effort. The differences in satiety responses occurred already in the early postprandial phase (30 min and 60 min) indicating that cephalic and gastric phase factors accounted for the differences.

The mastication process was analysed in a mastication trial measur-

Table 4

Oral processing parameters. Values are means \pm SD, $n = 26$. Different superscript letters in a row indicate statistically significant difference ($p < 0.05$) between products. Extrapolated parameters represent oral processing parameters for the portion size served in the satiety trial.

	WG sourdough rye bread	Extruded WG rye flakes	Extruded WG rye puffs	WG rye smoothie	Refined wheat bread	χ^2	Sig.
Parameters for mouthful of food							
Number of chews	27 \pm 10 ^b	28 \pm 7 ^b	11 \pm 5 ^a	7 \pm 4 ^a	20 \pm 8 ^b	85.8	< 0.001
Total oral processing time (s)	20 \pm 9 ^c	21 \pm 8 ^c	8 \pm 4 ^a	4 \pm 3 ^a	14 \pm 6 ^b	84.9	< 0.001
Time of EMG activity (s)	9 \pm 3 ^{bc}	10 \pm 3 ^c	4 \pm 2 ^a	2 \pm 1 ^a	7 \pm 3 ^b	85.6	< 0.001
Duty cycle (%) ¹	46 \pm 3 ^a	48 \pm 4 ^a	53 \pm 6 ^b	61 \pm 13 ^b	48 \pm 3 ^a	46.6	< 0.001
Relative force (%) ²	90 \pm 15 ^b	101 \pm 25 ^b	75 \pm 23 ^{ab}	45 \pm 23 ^a	80 \pm 17 ^b	60.0	< 0.001
Relative work ³	8 \pm 3 ^{bc}	11 \pm 3 ^c	3 \pm 1 ^a	1 \pm 1 ^a	5 \pm 2 ^b	80.7	< 0.001
Extrapolated parameters for food portion							
Number of chews	340 \pm 130 ^a	480 \pm 120 ^b	640 \pm 260 ^b	210 \pm 130 ^a	380 \pm 160 ^b	80.3	< 0.001
Total oral processing time (s)	250 \pm 110 ^{ab}	360 \pm 130 ^c	440 \pm 210 ^c	140 \pm 100 ^a	280 \pm 110 ^b	73.7	< 0.001
Time of EMG activity (s)	110 \pm 40 ^{ab}	170 \pm 50 ^c	220 \pm 90 ^c	70 \pm 40 ^a	130 \pm 50 ^b	82.2	< 0.001
Relative work ³	100 \pm 30 ^b	190 \pm 50 ^c	160 \pm 70 ^c	40 \pm 40 ^a	100 \pm 40 ^b	70.2	< 0.001

¹ Time of EMG activity/Total oral processing time.

² Chewing force of the product related to chewing force of chewing gum.

³ Time of EMG activity \times relative force.

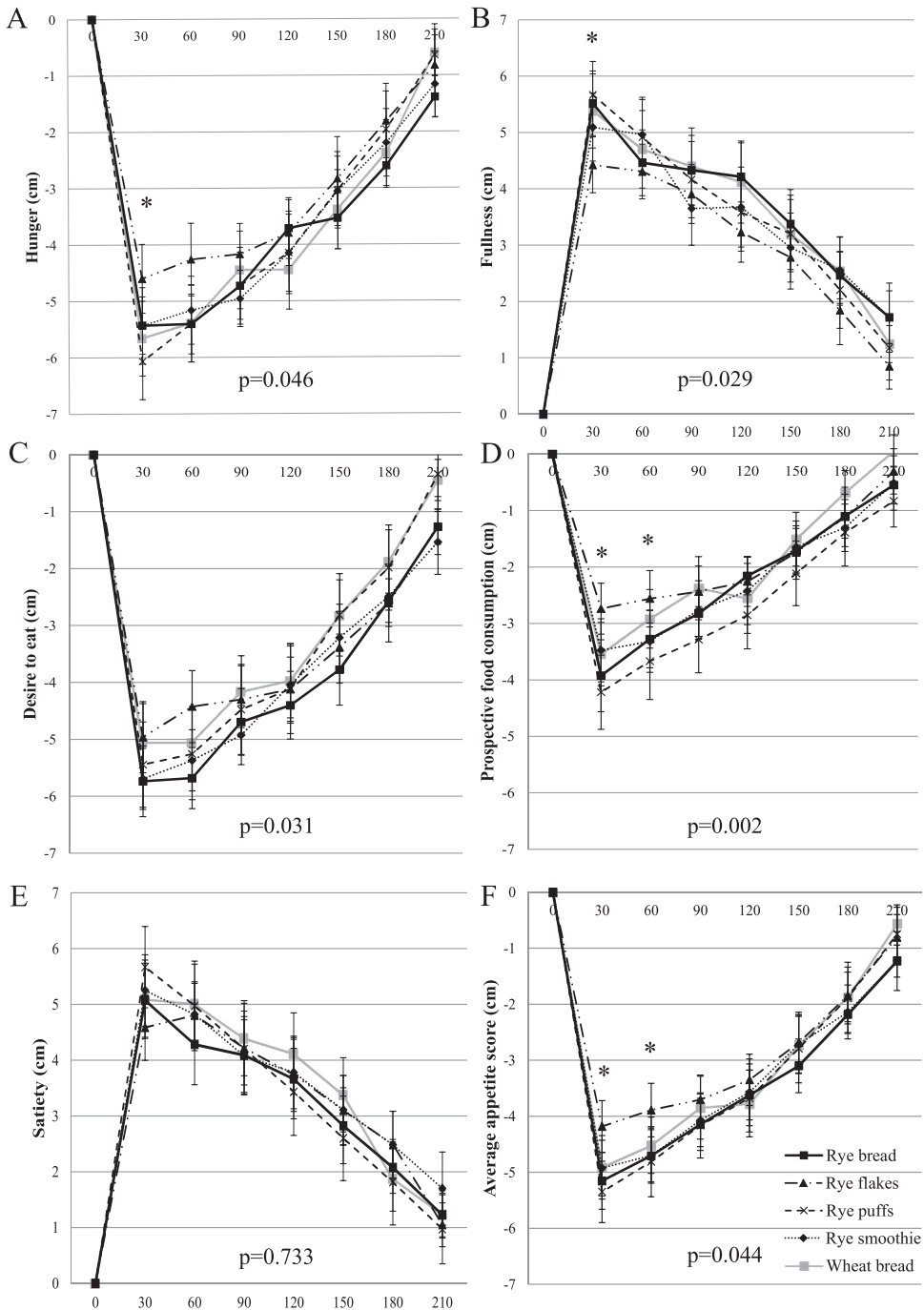


Fig. 5. Changes VAS ratings for A) hunger, B) fullness, C) desire to eat, D) prospective food consumption, E) satiety and F) average appetite score during 210 min postprandial period in healthy women for wholegrain rye bread (—■—), wholegrain rye smoothie (—◆—), wholegrain rye puffs (—×—), wholegrain rye flakes (—▲—) and refined wheat bread (—□—). Values are means with their standard errors represented by vertical bars, n = 16. Significant product effect was found for hunger, fullness, desire to eat, prospective food consumption and average appetite score. The time points with statistically significant differences (p < 0.05) between products are marked with asterisk (*).

ing the process with EMG. The method makes it possible to evaluate not only mastication time or number of chews but also relative chewing force and mastication effort that is needed to disintegrate the sample in the mouth. The results show that the mouthfuls of samples required different mastication patterns, rye bread and flakes needing the highest number of chews and the longest processing time. Since the number of mouthfuls needed to consume a portion of food (with fixed energy amount) varies, the mastication parameters were extrapolated to represent the values for portions served in the satiety trial. The results show that the number of chews, oral processing time and mastication effort were the highest for portions of rye flakes and rye puffs. Thus, the driest products required the most mastication effort among the studied products.

Number of chews and mastication effort (derived as a product of chewing time and force), were used to represent the mastication process in the statistical models to reveal possible contributions to the satiety. These two parameters were chosen because they are reasonably uncorrelated, while e.g. number of chews and chewing time are strongly dependent. Mastication effort was found to improve the model while the number of chews did not influence the goodness of the fit. This indicates that mastication effort would be more relevant oral processing factor than the mere number of chews with respect to the appetite response. However, the obtained result does not support the hypothesis that higher mastication effort would be beneficial for satiety response since the flakes requiring the most intense mastication effort actually resulted in the weakest satiety response. We assume that there are structural properties that are reflected in mastication parameters but actually are relevant for other satiety inducing mechanisms in the body. Differences in stomach distention could offer one plausible explanation: rye bread and rye puffs were porous products which most probably were disintegrated into fairly small particles with good hydration capacities compared to the flakes that have hard and dense structure resulting assumedly bigger particles in mastication. The beverage consumed alongside the flakes is probably emptied rapidly from stomach causing less stomach distention which is among factors influencing satiety. The period of the observed differences supports this hypothesis: the differences in the satiety responses were seen during the first hour after consumption. The rheology of the boluses would be interesting to study *in vitro* to better understand the impact of food structure for stomach digestion phase.

Rye smoothie portion and portion with rye flakes and juice is an interesting pair to compare since these portions include exactly the same ingredients and similarly produced cereal product (extruded flakes), energy content and volume but in different forms. The smoothie was designed to represent the flakes portion without the need for extensive mastication. Despite being structurally very different, both the products possess properties potentially beneficial for satiety: the flakes required more mastication effort which might be a beneficial property for satiety whereas rye smoothie was a soup-like product which is a food type generally considered having good satiating capacity. Some researchers believe that for maximum satiating power, the water should be incorporated in the food, as opposed to being consumed alongside the food as a beverage (Almiron-Roig et al., 2013). Indeed, rye smoothie tended to induce better satiety compared to rye flake portion although the difference was not statistically significant. One possible explanation may be again in hydration: the rye smoothie was let stand for 15 min before the satiety trial thus resulting in thick texture with hydrated rye flake particles. Dry rye flakes, which are characterised with low porosity and which have been shown to remain in bigger particles than extruded puffs in mastication (Alam et al., 2016), assumedly do not absorb water promptly and the beverage consumed alongside the flakes is probably emptied rapidly from stomach causing less stomach distention than the juice that is incorporated in the food product. Dhingra et al. concluded in their review about dietary fibre in foods that hydration properties are relevant in explaining the physiological effects of fibres and that for example

substrate pore volume impacts the hydration capacity (Dhingra, Michael, Rajput, & Patil, 2012). Moreover our earlier study showed that beta-glucan which was added in juice resulted in better satiety response than the same ingredient added in biscuits in study setting having the same basic products (Pentikäinen, Karhunen, et al., 2014).

In addition to mastication process other cephalic phase related factors, such as perceived expectations about the satiating capacity of the food as well as perceived pleasantness may influence the actual satiety response. In the current study the study portions, even though matched with energy, were evaluated differently regarding their satiating capacity: rye bread was evaluated as the most powerful satiety-maintaining product whereas the rye smoothie was evaluated to be poorest to suppress appetite. In addition, the evaluations of the satiating capacities were enhanced after oral processing of the food, especially for rye flakes and rye smoothie which apparently were also unfamiliar foods for the participants. It has been shown that expectations about the satiating capacity of food can influence the actual satiety response and that the effect can last up to three hours (Brunstrom et al., 2011). Adding the evaluated satiety expectations into the mixed model abolished the differences between products. Thus, we assume that the expectations about the satiating capacity of the portions influenced the results.

Rye puff portion was evaluated as the least pleasant, rye bread portion as the most pleasant and other portions intermediate. Regarding the previous studies about the possible influence of pleasantness on satiety these clear differences could not be neglected. Addition of pleasantness ratings into statistical model enhanced the model as well as increased the statistical significance between products ($p = 0.001$ vs. original p -value of 0.044). Thus, the evaluated pleasantness of the products indeed was influencing the result. Lower pleasantness ratings for rye puffs may have resulted from considerably big volume of the portion resulting from airy structure. Additionally strong adhesion to teeth might have influenced the poorer pleasantness ratings.

Differences in oral processing can be achieved either by instructing participants to masticate food during a fixed time or by applying fixed number of chews or by providing textures that result in longer or more intense oral processing. The latter approach is preferable when trying to develop products that would naturally help to control food intake and enhance satiety response. The current study was successful in producing various food structures resulting in different oral processing patterns. They were not only foods as such and with comminuted structure but realistic products with structural differences including ductile and chewy texture (bread), hard and crunchy texture (flakes) and hard, airy, crispy texture (puffs) and a soup-like texture (smoothie).

As a drawback the current study's setting is that the familiarity of the products (even though it was not specifically asked) assumedly was different. Rye bread is a staple food in Finland whereas both extruded rye products and rye smoothie are uncommon food items. It has been seen in earlier studies that earlier experiences about foods help to evaluate their satiety effect (Brunstrom, Shakeshaft, & Scott-Samuel, 2008). Thus, in further study settings it would be good to familiarize the study participants to each study product beforehand to exclude the possible mixing impact of familiarity. Postprandial satiety responses were measured during 210 min following the established practices (3–5 h) (Blundell et al., 2010). However, in the current study or similar studies where differences in satiety responses are hypothesized to occur mainly due to cephalic phase or stomach phase factors it might be more informative to measure the responses more frequently during a shorter period.

To conclude, the vast textural differences between products were reflected in mastication process and also in the satiety responses to food portions with standardised energy contents. The results did not support the hypothesis that mastication process itself would mediate the interaction between food structure and postprandial satiety but there appears to be other mechanisms possibly related to stomach phase

digestion modulating the interaction. Palatability seems to weaken postprandial satiety response.

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PUBLICATION IV

**Enrichment of biscuits and juice with
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Research report

Enrichment of biscuits and juice with oat β -glucan enhances postprandial satiety[☆]



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ABSTRACT

Effects of fibre and β -glucan on satiety have been reported in many studies, but no consensus has been reached. The aim of this study was to examine the effects of breakfasts varying in the dose of oat bran (4 g or 8 g β -glucan). The approach was to study whether the food matrix (solid or liquid) into which the oat bran is incorporated influences postprandial satiety in otherwise similar meal settings. Thirty healthy females were offered four different breakfasts: biscuits + juice (0 g β -glucan), enriched biscuits + juice (4 g β -glucan), biscuits + enriched juice (4 g β -glucan) and enriched biscuits + enriched juice (8 g β -glucan) in a random order on separate test days. The sensations associated with hunger and satiety were evaluated using visual analogue scales (VAS) before and after ingesting the test breakfasts and every 30 min until 210 min. Oat bran addition in breakfasts increased postprandial satiety especially when both juice and biscuits were enriched (8 g of β -glucan). Addition of oat bran to juice enhanced satiety and related feelings more effectively than the addition into biscuits.

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Introduction

Adequate dietary fibre consumption provides extensive health benefits, including beneficial effects on GI function, lipid metabolism and body weight regulation (Ye, Chacko, Chou, Kugizaki, & Liu, 2012; Slavin & Green, 2007; Slavin, 2005). β -Glucan is a major constituent of grain fibres, abundant especially in barley and oats. It consists of glucose molecules bound to each other with β -(1 → 4) and β -(1 → 3) linkages (Barsanti, Passarelli, Evangelista, Frassanito, & Gualtieri, 2011). β -Glucan exhibits high viscosity at relatively low concentrations (1%) (Sadiq Butt, Tahir-Nadeem, Khan, Shabir, & Butt, 2008). Viscosity in the lumen of the gut is suggested to be important for the physiological properties of β -glucan (Wood, 2007). The benefits of β -glucan on health, including improvement of cholesterol and glucose metabolism, are well known (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011; Food and Drug Administration, HHS, 2002). In addition, β -glucan has been proposed to contribute to enhanced satiety. Enhanced satiety offers many potential benefits to consumers with weight management goals (Hetherington et al., 2013). Reductions in perceived

deprivation during energy restriction, improved compliance with healthy eating and mood benefits have been suggested.

In one recent review on the effects of dietary fibre intake on appetite, energy intake and body weight and in another concerning dietary fibre and satiety, it was concluded that different dietary fibres have different effects on appetite and acute energy intake (Wanders et al., 2011; Slavin & Green, 2007). According to these authors, more viscous fibres efficiently reduce appetite. This is suggested to be a consequence of increased exposure time in the oral cavity, greater water holding capacity and subsequently increased stomach distension and gastric vagal signalling due to delayed gastric emptying as well as increased release of appetite-regulating peptides throughout the intestine (Wanders et al., 2011).

The ability of oat bran to enhance subjective postprandial satiety has been studied in different experimental settings (Beck, Tosh, Batterham, Tapsell, & Huang, 2009; Hlebowicz, Darwiche, Bjorgell, & Almer, 2008; Juvonen et al., 2011; Lyly et al., 2009, 2010). The results have been mixed. In the first three studies the results showed positive effects on satiety, whereas in the last two β -glucans had no significant effect on satiety. In a study by Beck et al. (2009), varying doses of β -glucans (2.16; 3.82; 5.45 and 5.65 g per serving) in extruded breakfast cereals increased fullness significantly as compared to a control product with no β -glucans. However, there were only trends but no significant differences in other scores (hunger, satiety, prospective food consumption). Similarly, in a

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study by Lyly et al. (2009), a beverage with 30 g of oat bran (about 5 g of β -glucans) induced a significant effect on fullness compared to control beverage, but there were only insignificant trends in other hunger- and satiety-related scores.

Lyly et al. (2010) also compared beverages at two energy levels (700 kJ and 1400 kJ) and with different fibre contents (0, 5 and 10 g dietary fibre containing 0, 2.5 or 5 g β -glucans, respectively). Among the beverages with 700 kJ, both fibre-containing beverages decreased hunger and increased satiety as compared with the non-fibre control. The fibre content did not make a difference. The significant effect of the highest amount of dietary fibre (10 g) on increased satiety and decreased hunger was similar at the two energy levels. Juvonen et al. (2011) compared postprandial appetite ratings after eating puddings with added oat bran (30 g) or wheat bran (19 g) or with no added fibre. They observed no significant difference in appetite ratings. Hlebowicz et al. (2008) also observed no significant effects of β -glucans on satiety. In their study, β -glucans (4 g) were added to muesli that was eaten with vanilla yoghurt.

It has been observed that the textural properties of foods also affect satiation and satiety (de Graaf, 2012). Solid foods are more satiating than liquids even if the energy and macronutrient contents are the same. It has been suggested that the effect of texture on satiety is mediated by the oral residence time of food in the mouth, meaning that foods that need more oral processing spend more time in the mouth and induce a stronger cephalic phase response, which in turn could contribute to greater satiety (de Graaf, 2012). This interpretation supports the idea of fibre-enriched solid food as a potential enhancer for satiety, as potentially more chewing would be needed. On the other hand, food matrix influences the fibre hydration rate, which may be an important factor determining satiety response (Wanders et al., 2011). High water content favours hydration, suggesting that liquid food matrix would enhance the effect of fibre on satiety.

The current study examined the satiating effect of oat bran-enriched biscuits and/or juice consumed as part of a breakfast. The specific aims of the study were to evaluate: (1) the dose–response effect of oat β -glucans (0, 4, 8 g per breakfast) and (2) the influence of food matrix (solid or liquid) enriched with oat bran on perceived satiety in healthy, lean women. We hypothesised that addition of oat bran in juice or biscuits would enhance satiety compared to the control breakfast, and that addition of oat bran in juice would be more effective than addition of oat bran in biscuits. Furthermore, we hypothesised that addition of a double dose of oat bran would further enhance satiety.

Methods

Participants

30 Females were recruited to the study through advertising and email lists from universities and polytechnics in the Espoo region. To participate, candidates had to be of normal weight and in the habit of eating breakfast. Exclusion criteria were overweight (BMI > 25), underweight (BMI < 18.5), restrictive diet or remarkably restrained eating patterns (cognitive restraint score over 15 in the Three Factor Eating Questionnaire) (Stunkard & Messick, 1985), physical or mental illness or medication likely to interfere with metabolism or dietary habits, food allergies relevant to this study, pregnancy or lactation, participation in another clinical trial, general anaesthesia in the month prior to the study, significant changes in body weight (± 4 kg) during the previous year and regular smoking. The baseline characteristics of the participants are shown in Table 1. The participants gave written informed consent to their participation in the study. Ethical approval was obtained

from the Ethics Committee of the Hospital District of Helsinki and Uusimaa.

Procedure

This was a crossover, single blind study. Each participant tested four different breakfasts on four separate days. The order of the test breakfasts was randomized. There were at least two washout days between two consecutive study visits. The participants were instructed to follow their usual eating and exercise habits and to avoid alcohol consumption during the day preceding each study visit. They were also instructed not to smoke in the morning before and during the visits.

The participants came to the study visits in the morning between 7 and 9 a.m. after a minimum of 10 h fast. They were instructed to drink a glass of water in the morning before the study visit if they were thirsty. The four test breakfasts were presented to each participant in a random order. Participants were instructed to eat the test breakfast at their own pace but not to spend more than 10 min on eating. The participants evaluated their sensations before eating (T0) and repetitively after eating the breakfast.

The participants were familiarised with the study procedure at a visit preceding the beginning of the actual study. During this initial visit the subjects were trained to use the rating scales with a test breakfast containing white wheat bread and juice as the test products. In addition, baseline measurements (weight, height, blood pressure, heart rate, and waist circumference) were made to confirm the suitability of the participants for the study.

Satiety ratings

The satiety-related sensations were evaluated using 10 cm visual analogue scales (VAS), as recommended by Blundell et al. (2010). The evaluated sensations were *hunger*, *fullness*, *satiety*, *desire to eat* and *prospective food consumption* (“How much would you be able to eat right now?”). In addition, the ratings of the *thirstiness* and *pleasantness of the test breakfast* were included in the ratings. Evaluations were made before and after ingesting the breakfast and every 30 min until 210 min after breakfast consumption. Pleasantness of the test breakfast was evaluated only once, immediately after ingestion. The VAS scores were collected by using a computerised data-collecting system (CSA, Computerised Sensory Analysis System, Compusense, Guelph, Canada, Compusense five 5.2). The areas under the curves (AUC, cm \times min) were calculated for describing the overall changes in the sensations during the 210 min follow-up period. Average appetite (Average appetite = [desire to eat + hunger + (10–fullness) + prospective food consumption]) was calculated according to Anderson, Catherine, Woodend, and Wolever (2002) in order to provide a general view of desire to eat, hunger, fullness and prospective food consumption. Eating times were assessed by giving the participants timers and asking them to mark down the time spent on eating.

Test products

Test breakfasts consisted of biscuits and juice. There were two kinds of biscuits: normal wheat biscuits (“biscuits”) with no β -glucan and oat bran biscuits (“enriched biscuits”) with 5.1 g of β -glucan per 100 g). The average weight of one biscuit was 13 g. Oat bran concentrate (Oatwell22, CreaNutrition) was added to the dough in order to provide the targeted amount of β -glucan in enriched biscuits.

Similarly, there were two kinds of juices: normal orange juice (“juice”) and orange juice with added oat bran having high β -glucan content (“enriched juice”). Normal juice consisted of 55% orange juice and 45% water and contained no β -glucan.

Table 1
Baseline characteristics of the study subjects.

	Mean	SEM	Range	Reference values
Age (y)	24.3	0.7	20.7–37.3	
BMI (kg/m ²)	21.7	0.3	18.3–25.2	18.5–25
Waist circumference (cm)	75.9	0.9	64.0–83.5	<90
Systolic blood pressure (mmHg)	114.9	1.4	101–130	<120
Diastolic blood pressure (mmHg)	71	1.5	57–88	<80
<i>Three Factor Eating Questionnaire</i>				
“Cognitive restraint”	8.9	0.7	2–15	
“Uncontrolled eating”	4.9	0.4	2–11	
“Hunger”	4.7	0.5	1–10	

Table 2
Nutrient contents of the test products.

Nutrient contents of the test products/100 g	Biscuits	Enriched biscuits	Juice	Enriched juice
Energy (kcal)	468	420	25	38
Carbohydrates (g)	77.2	69.5	6.25	9.1
Starch ^a	59.7	40.6	–	–
Fibre (g) ^a	2.3	13.2	–	1.9
Of which β -glucan (g) ^a	0.16	5.1	–	1
Free sugars ^a	15.2	15.7	–	–
Protein (g) ^a	8.2	12.4	–	0.9
Fat (g) ^a	14.6	13.2	–	0.2

^a Analysed values.

Enriched juice was otherwise the same, but oat bran concentrate was added to provide 1 g of β -glucan per 100 g. Oat bran concentrate (Oatwell22, CreaNutrition) was mixed into the juice. To prevent excessive thickening of the product, each portion was mixed individually immediately before consumption. The nutrient contents of the test products are presented in [Table 2](#).

Test breakfasts

The test products formed four different combinations of biscuits + juice which were used as test breakfasts. Each breakfast contained 78.4 g of biscuits (on average 6 biscuits) and 400 g of orange juice : (1) biscuits + juice, (2) enriched biscuits + juice, (3) biscuits + enriched juice, (4) enriched biscuits + enriched juice, and provided 0 g, 4 g, 4 g or 8 g of oat β -glucan, respectively. The energy contents of the test breakfasts were 467 kcal, 429 kcal, 526 kcal and 488 kcal, respectively. We assumed 1 g of dietary fibre to contain 2 kcal of energy. Nutrient contents and weights of the test breakfasts are presented in [Table 3](#).

In vitro viscosity measurements

The viscosities of the test breakfasts were measured in artificial digestion conditions, using an *in vitro* digestion tool consisting of a rheometer combined with a titration station (Aymard & Wahl, 2010). The set-up combines a high performance rheometer (MCR 300, Anton Paar, Physica) with a titration station. The rheometer

Table 3
Nutrient values and weights of the test breakfasts.

	Biscuits + juice	Enriched biscuits + juice	Biscuits + enriched juice	Enriched biscuits + enriched juice
Energy (kcal)	467	429	526	488
Carbohydrates (g)	85.5	79.5	98.7	92.7
Total fibre (g)	1.8	10.3	9.8	18.4
β -glucan (g)	0.1	4.0	4.0	8.0
Protein (g)	6.4	9.7	10.2	13.5
Fat (g)	11.4	10.3	12.2	11.1
Weight (g)	478	478	497	497

is equipped with a double-jacketed glass vessel maintained at 37 °C and a calibrated helix geometry, which allows blending the content of the vessel while measuring its viscosity. The titration station consists of a workstation Titralab 856 with 4 burettes controlling the addition of water, HCl, NaOH and pepsin solution, and a peristaltic pump controlling the addition of a concentrated suspension of bile salts and pancreatin. Biscuits (biscuits or enriched biscuits) were ground and blended with the beverage (juice or enriched juice) directly in the rheometer, using the same quantity as ingested, i.e. 78.4 g of biscuit powder into 400 ml of beverage. This was followed by pH adjustment to a value of 2.0 ± 0.1 and addition of a pepsin solution to mimic gastric conditions. After 15 min at high shear rate (150 s^{-1}), the evolution of the viscosity was monitored for 70 min at low shear rate (10 s^{-1} , which has been suggested to mimic flow conditions in the gut). The bolus was then brought to small-intestine conditions by addition of NaOH (to obtain a pH of 6.3 ± 0.1), pancreatin and bile salts. The bolus was again stirred under high shear during 20 min to ensure homogeneity, before reverting to low shear rate and monitoring of viscosity for 90 min. The viscosity values reported below were taken at the end of the gastric and small-intestine phases (at 10 s^{-1}).

Statistical analyses

The results are expressed as mean \pm standard error of the mean (SEM) with a *p* value < 0.05 (2-sided) as a criterion for statistical significance, unless otherwise specified. The values for each time point are calculated as a change from baseline. This was done in order to take into account possible small differences in baseline values among the test sessions even if significant differences were not observed. Hunger- and satiety-related sensations are reported as graphical curves describing the changes in the sensations as a function of time and as bars describing the calculated areas under the curve (AUC, $\text{cm} \times \text{min}$). Correspondingly, the calculated average appetite scores are presented as comparable graphical curves.

SPSS software (version 14.0, SPSS Inc. Chicago, IL, USA) was used for statistical analyses. Linear mixed-effects modelling was used to compare the effects of the test breakfasts on hunger and satiety-related sensations and average appetite scores. The method takes into account the sources of variation when the participant is used as a random factor and product, time and product \times time as fixed factors. When a significant main effect of a product or product \times time interaction was observed, post hoc analyses were performed using the Sidak correction for multiple comparisons in order to identify the significant differences among the test breakfasts. Repeated measures ANOVA was used to compare the calculated areas under the curves.

Results

Viscosity measurements

Viscosity increased during the 60 min of the test and almost reached a plateau value at the end of the gastric phase. The

subsequent development during the small-intestine phase was very limited. Table 4 shows the viscosity obtained at the end of the gastric and small-intestine phases. A marked impact of β -glucan concentration was observed. The control product had very low viscosity (0.05 Pa s). Both test products containing 4 g β -glucan gave rise to similar viscosities (between 6.3 and 8.2 Pa s in small intestinal conditions) and the breakfast containing 8 g β -glucan resulted in the highest viscosity (22.7 Pa s). The results confirm that addition of β -glucans in the breakfast led to a marked dose-dependent increase in viscosity. This indicates that viscosity in intestinal conditions is governed by the amount of β -glucans incorporated, independently of the food form.

Eating times and pleasantness of the test breakfasts

The average time for eating the test breakfasts was 9.5 min (1.6 SD). For individual breakfasts, it was 8.7 min (2.0 SD) for biscuits + juice, 9.5 min (1.7 SD) for enriched biscuits + juice, 9.1 min (1.5 SD) for biscuits + enriched juice and 10.5 min (2.2 SD) for enriched biscuits + enriched juice. The eating time of enriched biscuits + juice was significantly longer than that of biscuits + juice ($p = 0.003$) and the eating time of enriched biscuits + enriched juice was significantly longer than the eating times of the 3 other test breakfasts ($p < 0.05$).

The breakfast with enriched biscuits and enriched juice was evaluated as less pleasant than the other breakfasts ($p < 0.01$), the mean rating being 2.1 (SD 1.9). The other breakfasts did not significantly differ from each other in the pleasantness ratings (biscuits + juice: 4.4 (SD 2.1); enriched biscuits + juice: 3.8 (SD 0.4) and biscuits + enriched juice: 3.8 (SD 2.3)).

Appetite ratings

The mean VAS ratings for satiety, fullness, hunger, desire to eat and prospective food consumption and the calculated values for average appetite scores are presented in Fig. 1. The breakfast with 8 g of β -glucan (enriched biscuits + enriched juice) increased satiety more than the control breakfast (biscuits + juice) and the breakfast with 4 g of β -glucan in biscuits (enriched biscuits + juice) ($p < 0.001$, $p = 0.007$, respectively). The breakfast with 4 g of β -glucan in juice (biscuits + enriched juice) also increased satiety more than the control (biscuits + juice). The feeling of fullness was significantly higher after the breakfasts with 8 g of β -glucan (enriched biscuits + enriched juice) and 4 g of β -glucan in juice (biscuits + enriched juice) as compared to the control (biscuits + juice) and to the breakfast with 4 g of β -glucan in biscuits (enriched biscuits + juice) (for all $p \leq 0.001$). The breakfast with 8 g of β -glucan (enriched biscuits + enriched juice) decreased the desire to eat significantly more than that with 4 g of β -glucan addition in juice (biscuits + enriched juice) ($p = 0.003$). Enriched biscuits + enriched juice also decreased ratings of prospective food consumption more than the control (biscuits + juice) ($p = 0.01$) and the breakfast with β -glucan only in juice (biscuits + enriched juice) ($p = 0.02$). There were no significant differences between the test breakfasts either regarding the feeling of hunger or the average

appetite score. The VAS ratings for thirst were significantly lower for enriched biscuits + juice compared to biscuits + enriched juice and enriched biscuits + enriched juice ($p < 0.001$ and $p < 0.005$, respectively). Control breakfast (biscuits + juice) also scored lower for thirst than biscuits + enriched juice ($p = 0.038$) (data not shown).

The means of the areas under the curves \pm SEM are presented in Table 5. There were significant differences in fullness ($p = 0.002$), with the breakfast including enriched biscuits + enriched juice inducing greater fullness than control breakfast ($p = 0.008$) or breakfast with enriched biscuits + juice ($p = 0.019$). There were also significant differences among the test breakfasts in satiety ($p = 0.042$), but no pairwise differences were found.

Discussion

The findings of this study indicate that oat bran addition in breakfast is effective in increasing satiety and fullness and decreasing hunger-related sensations in young, healthy female subjects. The effect was especially evident when using a high dose of β -glucan (8 g) which was achieved by adding oat bran with high β -glucan concentration to both biscuits and juice.

The amount of β -glucan in the experimental breakfasts varied from 0 g to 4 g or 8 g. In previous studies examining the impact of oat bran on satiety, the amount of β -glucan has varied from 2 g to about 6 g per serving (Beck et al., 2009; Hlebowicz et al., 2008; Juvonen et al., 2011; Lyly et al., 2009, 2010). Some positive satiety effects have been observed already at a dose of 2.2 g β -glucan per serving (Beck et al., 2009). On the other hand no significant effects on satiety were observed in the studies of Juvonen (30 g of oat bran containing approximately 2.5 g of β -glucan) or Hlebowicz (4 g β -glucan), suggesting that in addition to the fibre content, other factors in the foods or study procedure also determine the final response. A possible explanation for these results might be that the products used in these two studies had high consistency (pudding and yoghurt). It has been observed that thick and creamy mouth feel of a product induces greater and more prolonged reduction in hunger compared to thinner products (Bertenshaw, Lluch, & Yeomans, 2013; Mattes & Rothacker, 2001). This effect is suggested to occur through an oro-sensory mechanism (Yeomans & Chambers, 2011), and could mask the potential additional satiating effect of added dietary fibre.

The β -glucan concentration had a marked influence on bolus viscosity in the current study. Viscosity of the control breakfast was very low, the viscosities of the breakfasts containing 4 g β -glucans were higher and similar to each other, and the viscosity of the breakfast containing 8 g β -glucan was markedly higher than that of the other breakfasts. According to one review concerning fibres and satiety, the satiety-enhancing effect of soluble fibres results substantially from viscosity (Wanders et al., 2011). Even small increases in food viscosity have been found to reduce post-prandial hunger ratings (Mattes & Rothacker, 2001). The viscosity of the test meals measured *in vitro* is expected to induce a slowing down of the digestion rate in the upper tract that could ultimately be related to the difference in the satiety-enhancing impacts of the different breakfasts. The results of the current study support the

Table 4
Viscosities of the test breakfasts measured in artificial digestion conditions.

	Gastric		Intestine	
	Start viscosity (Pa s)	End viscosity (Pa s)	Start viscosity (Pa s)	End viscosity (Pa s)
Biscuits + juice	0.04	0.06	0.03	0.05
Enriched biscuits + juice	2.92	7.77	7.30	8.16
Biscuits + enriched juice	2.11	4.80	5.80	6.29
Enriched biscuits + enriched juice	19.89	21.34	24.90	22.73

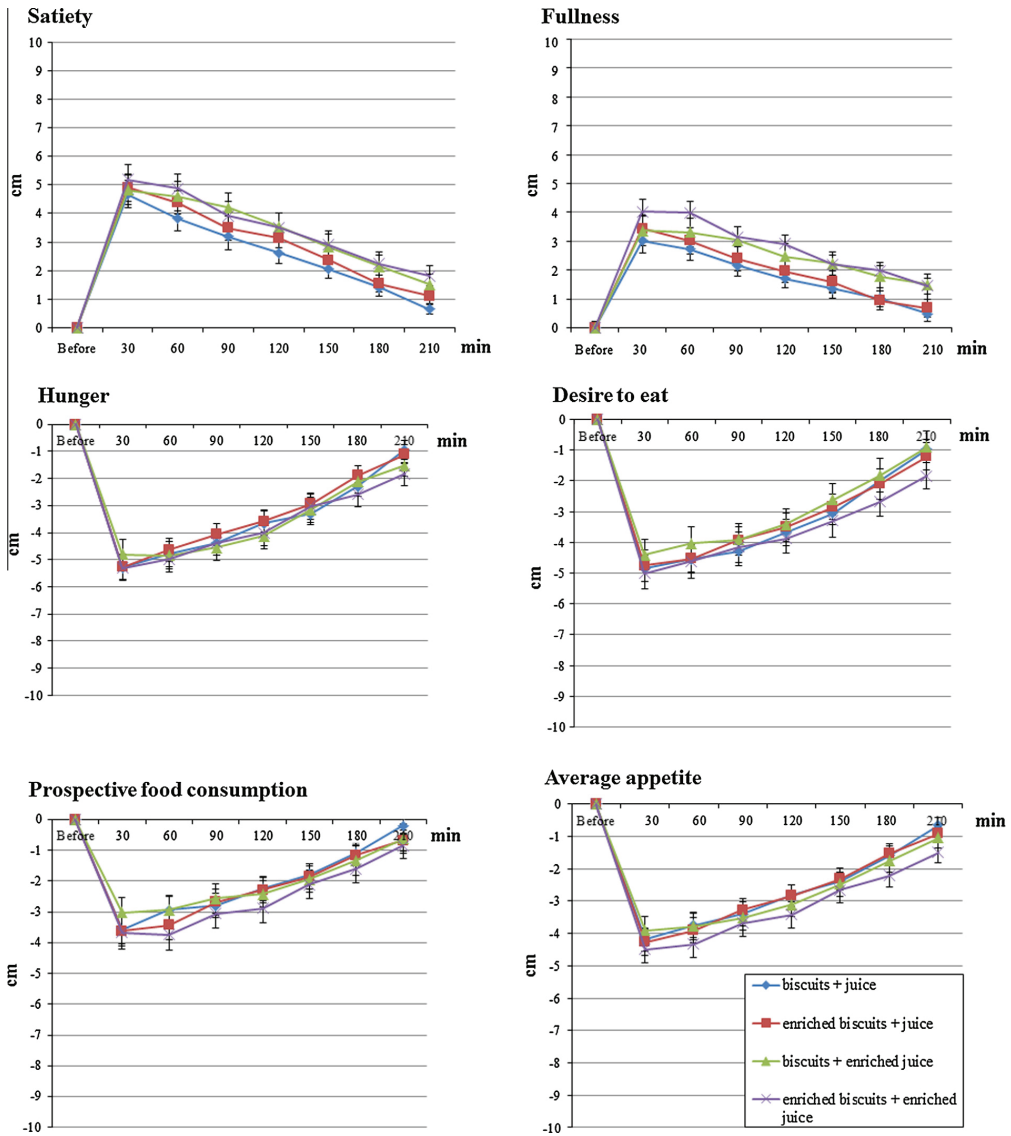


Fig. 1. Changes in the subjective VAS ratings for satiety (time $p < 0.001$, product $p < 0.001$, time \times product $p = 0.998$), fullness (time $p < 0.001$, product $p < 0.001$, time \times product $p = 0.986$), hunger (time $p < 0.001$, product $p = 1.43$, time \times product $p = 0.955$), desire to eat (time $p < 0.001$, product $p = 0.006$, time \times product $p = 1.0$), prospective food consumption (time $p < 0.001$, product $p = 0.073$, time \times product $p = 0.904$) and average appetite score (Anderson et al., 2002) (time $p < 0.001$, product $p = 0.20$, time \times product $p = 0.85$) during the 210 min postprandial period in young women consuming normal biscuits and normal juice (biscuits + juice, \blacklozenge), β -glucan biscuits and normal juice (enriched biscuits + juice, \blacksquare), normal biscuits and β -glucan juice (biscuits + enriched juice \blacktriangle) or β -biscuits and β -glucan juice (enriched biscuits + enriched juice, \blackcross). Values are means \pm SEM.

Table 5

Areas under the curve (AUC) for satiety, fullness, hunger, desire to eat, prospective food consumption and average appetite score. All values are $\bar{x} \pm$ SEM.

AUC (cm \times min)	Biscuits + juice	Enriched biscuits + juice	Biscuits + enriched juice	Enriched biscuits + enriched juice
Satiety	550.8 \pm 63.7	619.5 \pm 62.8	690.2 \pm 79.7	717.2 \pm 80.9
Fullness	374.5 \pm 58.0	421.9 \pm 60.2	504.9 \pm 71.2	570.7 \pm 70.7
Hunger	-733.6 \pm 73.1	-696.6 \pm 67.0	-738.3 \pm 83.1	-767.6 \pm 83.7
Desire to eat	-697.5 \pm 67.2	-683.6 \pm 78.2	-648.2 \pm 83.4	-744.1 \pm 89.7
Prospective food consumption	-456.7 \pm 63.9	-481.6 \pm 69.8	-458.4 \pm 77.2	-542.0 \pm 83.6
Average appetite score	-520.1 \pm 52.6	-520.9 \pm 54.3	-530.2 \pm 59.4	-591.4 \pm 62.9

conception that bolus viscosity is an important factor in the development of satiety. Thus, possibilities to increase the development of bolus viscosity should be considered when designing foods to promote weight maintenance.

In the current study the same amount of oat bran (providing 4 g β -glucan) was added in biscuits or juice in otherwise similar meal settings. The food matrix itself has been found to affect postprandial satiety. Solid foods in general are considered to contribute to greater satiety compared to liquids (DiMeglio & Mattes, 2000; Mourao, Bressan, Campbell, & Mattes, 2007). This observation may be a consequence of differences between solid and liquid matrix in required oral processing, gastric emptying time, oro-cecal transit time, and also of the expectation that eating solid food would result in greater satiety than drinking liquids (Cassady, Considine, & Mattes, 2012). The time point of observation also appears to be important: liquids might contribute to greater satiety in short term and solids in the long term (Almiron-Roig, Chen, & Drewnowski, 2003). Whether oat bran addition in biscuits or juice is more effective is a complex question. Oat bran addition in biscuits might result in longer oral processing time and thus in stronger oro-sensory cues for perceived satiety (Wanders et al., 2011). On the other hand adding oat bran to liquid matrix (juice) facilitates hydration. According to the review by Wanders et al. (2011), dietary fibres provided in liquid form may have stronger appetite-reducing effects compared to dietary fibres ingested as part of solid foods, due to the rate of fibre hydration. Furthermore, adding oat bran concentrate to liquid makes the mouthfeel of the product thicker. It has been observed that thicker or creamier mouthfeel increases subjective satiety, regardless of the energy content of the ingested food. In line with previous observations, in this study β -glucan addition in juice (biscuits + enriched juice) increased satiety and fullness compared to the control (biscuits + juice), whereas there was no such effect of β -glucan added only in biscuits (enriched biscuits + enriched juice).

There were some differences between breakfasts in their energy contents resulting from modifications in the oat bran content of biscuits and juice. It has been shown that volume, in addition to energy content, macronutrient content and sensory properties, is an important factor for post meal satiety and food intake (Rolls et al., 1998) and that both energy density and portion size modulate energy intake (Kral & Rolls, 2004). In this study the portion size of biscuits and juice was matched and the energy content between breakfasts varied. However, even a much greater difference in the energy contents of the test meals (i.e. 700 kJ (167 kcal)) has been shown not to change the short term satiety responses at a constant level of fibre (10 g dietary fibre, 5 g β -glucan) (Lyly et al., 2010). Despite this, we cannot rule out the possibility that the variation in energy content might have had some effect on the results.

The breakfasts in this study were evaluated as rather unpleasant (2.1–4.4 on a scale of 1–10). Low pleasantness ratings might have been at least partly due to the relatively large amount of biscuits that the subjects had to consume within a limited time: the subjects were instructed to eat the portion of biscuits (78.4 g) in 10 min. Secondly, biscuits were served as a breakfast, which is not usual in Finland. The breakfast with enriched biscuits + enriched juice was evaluated the most unpleasant of the test breakfasts. Highly palatable foods have been found to be less satiating, as indicated by increased intake in a meal (Yeomans, Weinberg, & James, 2005). It is controversial whether lower palatability of food increases only satiation (occurring during the meal) or also satiety (de Graaf, De Jong, & Lambers, 1999; Yeomans, Lee, Gray, & French, 2001; Sorensen, Moller, Flint, Martens, & Raben, 2003). Lower palatability has been suggested to be one possible explanation for the satiety-enhancing effect of fibre-enriched foods (Burton-Freeman, 2000). However, it must be considered that the satiety-increasing effect of breakfast with enriched bis-

cuits + enriched juice might partly have been due to its lower palatability. In any case, in the current study the breakfasts which included 4 g of β -glucan were evaluated as equally pleasant and thus potential differences in the satiety-producing effects between them were probably not due to the differences in palatability.

Consumption of foods rich in dietary fibre usually requires longer oral processing time than that of low fibre foods, which has also been suggested to promote satiety (Zijlstra, Mars, de Wijk, Westerterp-Plantenga, & de Graaf, 2007). Longer exposure time in the mouth induces a stronger cephalic phase response (de Graaf, 2012). Thus, longer consumption time of the test breakfast containing 8 g of β -glucans might have contributed to its higher satiating effect.

The participants of this study were lean women. Lean individuals were chosen in order to have a homogenous group of subjects. It has previously been found that measurement of an appetite response may vary according to the body mass index of an individual (Blundell et al., 2010). Women were recruited since they were more likely to be a target group for the products studied. 24 Subjects has been found to be an adequate number to detect a 10 mm difference in the VAS evaluations with a study power of 0.9 and using a paired design (Flint et al., 2000). Thus 30 subjects as used in this study should be adequate.

It is noteworthy that the dietary fibre contents including the β -glucan doses in the study breakfasts were rather high. Including this large amount of β -glucan in the studied products was technologically possible by using an oat bran ingredient with very high β -glucan concentration (22%). In Western countries the intakes of whole grain and dietary fibre are lower than the recommendations for fibre intake. Several countries and organizations, including the American Dietetic Association, recommend a fibre intake of 25–35 g per day. However, according to a National Health and Nutrition Examination Survey, American adult males consume approximately 18 g of fibres per day and females only 14 g per day (U.S. Department of Agriculture, Agricultural Research Service, 2010). In Europe the consumption of fibres has been found to vary between different areas, ranging between 18 g and 29 g in males and between 15 g and 25 g in females (Cust et al., 2009). Breakfast and snacks have been recognised as important meal occasions to fill in the current gap in fibre intake (Clemens et al., 2012). Thus, the kind of products that were used in this study would have a potential to increase dietary fibre intake; addition of only one portion of biscuits and juice enriched with β glucans per day would increase the total fibre intake by 18 g and thus would increase the intake of dietary fibre to a sufficient level.

Conclusions

This study indicates that 4 or 8 g of β -glucan consumed at breakfast enhances satiety and related feelings in young, healthy females. In the studied meal setting (biscuits + juice), oat bran enrichment (4 g of β -glucan) was more efficient in enhancing satiety when added in juice than in biscuits. The most evident enhancement of satiety was observed when a double dose of oat bran was used (enriched biscuits + enriched juice). Viscosity development in gastrointestinal conditions is suggested to be a mediator of enhanced satiety.

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Title	How does cereal food structure influence digestion and satiety - <i>In vitro</i> and <i>in vivo</i> approaches
Author(s)	Saara Pentikäinen
Abstract	<p>The current study explored the impact of cereal food structure on digestion and satiety. Food structure is important for acceptability, functionality and health effects. Cereal foods contribute significantly to energy and nutrient intakes in the diets worldwide. The benefits of consuming a diet rich in whole grain and dietary fibre (DF) are evident, and cereal DF complex is most likely behind the beneficial effects.</p> <p>The first part of the study investigated the effect of bread structure on mastication-induced structure disintegration, starch hydrolysis and dissolution of compounds from bread matrices. Despite the structural differences among the studied rye and wheat breads, there were only small differences in mastication processes. However, rye breads disintegrated to smaller particles than wheat bread and starch tended to hydrolyse at a slower rate by salivary α-amylase. A large array of compounds was dissolved from masticated breads to saliva. Specifically, peptides and amino acids were dissolved from rye breads and sugars from wheat bread. The relevance of food structure to satiety was explored in the second part of the study. Among rye products with different structures and similar chemical compositions, portions of wholemeal rye bread or extruded wholemeal rye puffs and juice were more effective than the portion of extruded wholemeal rye flakes and juice to maintain some aspects of satiety. Intense oral processing did not relate to satiety response but perceived pleasantness and satiety expectations did. Less pleasant food portions resulted in enhanced satiety as well as those that were anticipated already prior to ingestion to be satiating. Oat bran added to juice was more effective in maintaining the feelings of satiety and fullness compared to oat bran incorporated in biscuit matrix.</p> <p>The results showed that disintegration of bread structure and the release of compounds differed between bread types already in mastication. The current study was the first to explore the dissolution of compounds from food, namely bread, after mastication using non-targeted metabolomics approach. The significance of the released compounds warrants further research. The study also showed that food structure is of importance for the postprandial satiety responses of high fibre cereal foods. Perceptions of food, such as liking and expectations, as well as interactions of solid and liquid components (hydration and dissolution) of meal in stomach, are suggested to explain the observed differences in satiety responses.</p>
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Nimeke	Viljatuotteiden rakenteen vaikutus ruoansulatukseen ja aterianjälkeiseen kylläisyyteen <i>in vivo</i> ja <i>in vitro</i> -menetelmin tutkittuna
Tekijä(t)	Saara Pentikäinen
Tiivistelmä	<p>Tässä tutkimuksessa tutkittiin runsaskuituisten viljatuotteiden rakenteen vaikutusta ruoansulatukseen ja kylläisyyteen. Ruoan koostumuksen lisäksi rakenne vaikuttaa ruoan hyväksyntään ja funktionaalisiin ominaisuuksiin. Viljatuotteet ovat tärkeä osa ruokavaliota kaikkialla maailmassa. Runsaasti täysjyväviljaa ja ravintokuitua sisältävä ruokavalio on terveydelle edullinen.</p> <p>Työn ensimmäisessä osassa tutkittiin leivän rakenteen vaikutusta pureskelun aiheuttamaan rakenteen hajoamiseen, tärkkelyksen hydrolyysiin sekä leipämatriisista sylkeen liukeneviin yhdisteisiin. Vaikka tutkitut ruis- ja vehnäleivät olivat rakenteeltaan erilaisia, niiden pureskeluprosesseissa havaittiin vain pieniä eroja. Ruisleivät kuitenkin hajosivat pureskelun myötä pienemmiksi partikkeleiksi vehnäleipään verrattuna ja syljen amylaasientsyymi hydrolysoi niiden sisältämää tärkkelystä hitaammin.</p> <p>Pureskelun myötä leivistä liukeni sylkeen runsaasti erilaisia yhdisteitä. Ruisleivistä liukeni erityisesti peptidejä ja aminohappoja ja vehnäleivästä sokereita.</p> <p>Toisessa osassa tutkittiin ruoan rakenteen vaikutusta aterianjälkeiseen kylläisyyteen. Rakenteeltaan erilaisista, mutta kemialliselta koostumukseltaan toisiaan vastaavista täysjyväruoatuotteista täysjyväruisleipä ja ekstruusiomenetelmällä valmistetut naksut, mehun kanssa nautittuna, ylläpitivät kylläisyyttä joitain osin paremmin kuin ekstruusiomenetelmällä valmistetut täysjyväruislastut yhtäläisesti mehun kanssa nautittuna. Tuotteiden koettu miellyttävyyden ja kylläisyysodotukset vaikuttivat aterianjälkeiseen kylläisyydentunteeseen, toisin kuin tuotteiden rakenteellisten ominaisuuksien johdosta vaihdellut pureskelun intensiteetti. Paremman kylläisyydentunteen saivat aikaan ne annokset, jotka arvioitiin vähemmän miellyttäväiksi, sekä ne, joiden tutkittavat henkilöt arvioivat etukäteen aikaansaavan paremman kylläisyyden. Kauraleseen ja mehun sekoitus ylläpiti kylläisyyttä ja täydenolon tunnetta paremmin kuin keksi, johon oli leivottu vastaava määrä kauralesettä, mehun kanssa nautittuna.</p> <p>Tulokset osoittivat, että erityyppisten leipien rakenne hajosi pureskelun myötä eri tavoin ja että niistä liukeni sylkeen erilaisia yhdisteitä. Tutkimuksessa hyödynnettiin ensimmäisen kerran kohdentamatonta metabolomiikka-analyysia ruoasta pureskelun myötä sylkeen liukenevien yhdisteiden tutkimiseen. Ruoasta jo suussa liukenevien yhdisteiden merkitys ruoansulatukseen vaatii lisätutkimuksia. Tutkimus osoitti, että täysjyväviljatuotteiden rakenne on yksi tekijä aterianjälkeisten kylläisyyden tuntemusten määrätymisessä. Tutkimustulosten perusteella esitetään, että aterianjälkeisessä kylläisyydessä havaittuja eroja selittävät ruoan koettu miellyttävyyden ja ennako-odotukset sekä kiinteiden ja nestemäisten aterianosien vuorovaikutukset (vettyminen ja liukeneminen) mahassa.</p>
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How does cereal food structure influence digestion and satiety

- *In vitro* and *in vivo* approaches

Cereal foods contribute significantly to energy and nutrient intakes in the diets worldwide. Not only the composition of food but also food structure is important for acceptability, functionality and health effects of cereal foods. This study investigated high fibre cereal foods: breads, extruded products, biscuits and cereal smoothies. The effect of bread structure on mastication-induced structure disintegration, starch hydrolysis and dissolution of compounds from bread matrices were investigated in the first part of this study. The relevance of cereal food structure to satiety was explored in the second part of the study.

The results showed that disintegration of bread structure and the release of compounds differed between bread types already in mastication. The study also showed that food structure is of importance for the postprandial satiety responses of high fibre cereal foods.

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