



## 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  $\alpha$ -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/crumb 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  $\mu$ 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  $\mu$ 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  $\beta$ -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  $\beta$ -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/m<sup>2</sup>. 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  $\pm$  2.4 g, 15.1  $\pm$  1.0 g, 9.6  $\pm$  0.8 g, and 10.9  $\pm$  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.

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