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Alam, Syed Ariful

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PAPER

The effect of structure and texture on the breakdown pattern during mastication and impacts on *in vitro* starch digestibility of high fibre rye extrudates

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Syed Ariful Alam,^a Saara Pentikäinen,^a Johanna Närväinen,^a Kati Katina,^b Kaisa Poutanen^a and Nesli Sozer^a

The snack product category is lacking palatable, high dietary fiber containing products. This study explored how the addition of native or fermented rye bran influences the texture and sensory properties of endosperm rye flour based extrudates. In addition, mastication and bolus properties ($n = 26$), and *in vitro* starch digestibility were assessed. Three high fiber extrudates based on endosperm rye flour (EF) were produced with addition of either 40% native rye bran (NBE) or 40% fermented rye bran (FBE), and with no added bran (EFE) to achieve two pairs of extrudates to compare. EFE and FBE had different composition but resembled each other regarding macrostructure and the second pair (NBE vs. FBE) had similar core composition but different structure due to bran fermentation. The fermentation of bran was performed using exopolysaccharide (EPS)-producing strain *Weissella confusa*, which led to 3% (3 g per 100 g bran; dry weight) *in situ* dextran production. The compositionally similar extrudates (NBE vs. FBE) varied in both structure and instrumental texture: FBE were less dense, less hard and crispier than NBE. The extrudates with different composition (EFE vs. FBE) varied regarding instrumental texture: FBE were less hard and crispier than EFE. There were also subtle structural differences FBE being somewhat denser than EFE. NBE and FBE differed regarding sensory texture while textures of EFE and FBE were perceived similar. Mastication properties of the different products did not exhibit remarkable differences. There was a large number of smaller particles in both NBE and FBE bolus samples. The fragile structure of FBE, and its lower bolus viscosity, led to high *in vitro* starch digestibility. The results demonstrate that the structural attributes of the extrudates, rather than the core composition, dictate the breakdown pattern during mastication and *in vitro* starch digestibility. The extrudates with similar composition may be digested at different rates depending on their structural attributes. Although FBE had higher *in vitro* starch digestibility, its high DF content, palatable texture and improved sensory properties were important determinants underlying eating quality and therefore it could be a promising product to snack food category.

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Introduction

Rye (*Secale cereale* L.) is a widely cultivated cereal grain in Northern and Eastern Europe. Rye has the highest content of dietary fiber (DF) among cereal grains, and is typically consumed as wholegrain including the DF rich bran (39–48% DF, mostly insoluble). Rye bran is also rich in starch (13–28%), protein (14–18%), pentosans (23%) and β -glucan (3.5–5.3%).^{1–3}

Rye bran could be utilized as a raw material in the manufacture of DF- and protein-enriched extruded products. Addition of wheat bran, guar gum and inulin into extruded products at DF levels of 5–15%,⁴ and of 15% fenugreek polysaccharide into chickpea and rice blend,⁵ reduced both *in vitro* starch digestibility rate and *in vivo* glycaemic responses. A similar effect has been shown (*in vitro*) by adding 30% rye bran into rye flour based extrudates.⁶ However, bran addition interfered with the palatability of extrudates *via* reduced expansion and crispiness and increased hardness, limiting its use particularly at addition levels above 5% of flour.^{6–9}

Processability of wheat and rye bran was improved by fermentation with lactic acid bacteria (LAB) and yeast. Fermentation-induced modification of wheat and rye bran has

^aVTT Technical Research Centre of Finland Ltd, Tietotie 2, P.O. Box 1000, FI-02044 VTT, Finland. E-mail: ariful.alam@vtt.fi; Tel: +358 40 578 9984
^bDepartment of Food and Nutrition, Agnes Sjöbergin katu 2, P.O. Box 66, 00014 University of Helsinki, Helsinki, Finland

been shown to improve microstructure, texture, flavor and shelf-life of high DF breads.^{10–12} Many LAB belonging to the species *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Weissella* are capable of producing an inducible dextranucrase enzyme, which converts sucrose ($C_{12}H_{22}O_{11}$) to dextran ($C_6H_{10}O_5$)_n and fructose ($C_6H_{12}O_6$).^{13–15} Dextran is a large group of α glucans, in which the main polymeric backbone chain consists of α -(1 → 6)-glycosidic linkages (>50%) with α -(1 → 2)-, α -(1 → 3)-, and/or α -(1 → 4)-branched linkages.^{15–18} *Weissella confusa* is reported to be efficient in the production of linear dextrans with molecular weights around 1.8×10^7 g mol⁻¹, with mainly 97% α -(1 → 6) and a very few (3%) α -(1 → 3)-linkages.^{14,19–21} Dextran produced during *in situ* fermentation has been shown to have technological benefits over externally added dextran.²² Addition of 20% rye bran into rye flour-based extrudates with 5% commercial dextran did not improve the structural properties compared to untreated polished bran extrudates.²² Polysaccharides produced during *in situ* fermentation were also effective in improving bread structure compared to externally added polysaccharides.²³

Although fermentation of rye bran has been shown to have technological benefits in baking, its applicability has not been studied widely in extruded products. In a previous study²² rye bran fermentation with EPS-producing *Weissella confusa* improved the structural and textural properties of extrudates, although the effects on mastication, *in vitro* starch digestibility and product viscosity were not investigated. During mastication of food, contraction of the jaw muscles occur and a bio-electrical signal propagates to the adjacent muscle cells, to help to coordinate muscle contraction. This bioelectrical signal could be measured by using Electromyography (EMG), which is a commonly used technique in studying the relationships between oral processing and food texture.²⁴ Food structure and texture have a prominent role in mastication. Mastication is the first step of digestion, during which food is broken down to small particles and mixed with saliva, preparing the bolus for swallowing. The size of particles in the bolus varies depending on food structure and texture.^{25,26} The rate of food breakdown is inversely related to hardness.²⁷ Hard and dense extruded products required more intense mastication than crispy and expanded extrudates.^{28,29} The proportion of small particles in the bolus increased as a result of bran addition, which further increased *in vitro* starch digestibility.²⁸ However, further research is needed to understand the mechanisms and factors explaining the structure of cereal solid foams and their disintegration in the mouth and during digestion.

The aim of this study was to evaluate the effect of structure, texture and process-related factors (*e.g.* bran modification) in oral processing, bolus formation and *in vitro* starch digestibility of rye extrudates with varying structures and compositions. Therefore, two different pairs of extrudates were prepared; the first pair had similar macrostructure and expansion but different composition (EFE vs. FBE), and the second pair had similar core composition but varied in terms of structure and texture (NBE vs. FBE). The targeted variation of these properties is illustrated in Fig. 1.

Fig. 1 Schematic overview showing the structural differences between EFE, NBE and FBE.

2 Materials and methods

2.1. Raw materials

Endosperm rye flour (EF) was obtained from Helsingin Mylly (Järvenpää, Finland). Rye bran with high DF content was prepared by milling and air classification of native rye kernels (Jalon Mylly, Kouvola, Finland) with a protocol published by Nikinmaa *et al.*^{22,30} Native rye kernels were milled by 100 UPZ-lb fine impact mill (Hosokawa Alpine AG, Germany) using a stainless steel pin discs (17 800 rpm), followed by air classification (speed 3500 rpm, airflow 220 m³ h⁻¹, feed rate 50 kg h⁻¹) (British Rema Minisplit, Chesterfield, UK). Repeated milling and air classification was performed in order to produce a coarse fraction (34%) of the material. After the second air classification the coarse fraction (23%) was collected for extrusion and termed as native bran (NB).

2.1.2. Bioprocessing of bran. Bioprocessing of native bran was performed to obtain fermented bran (FB) by using EPS producing *Weissella confusa* (VTT E-133279) from the VTT Culture Collection (VTT Technical Research Centre of Finland, Espoo) using a protocol published by Nikinmaa *et al.*²² Starter cultures of *Weissella confusa* were prepared revitalizing in MRS (de Man, Rogosa and Sharpe) broth (Oxoid LTD, Basingstoke, Hampshire, United Kingdom), before subculturing in GEM (general edible medium, containing 2 g per 100 ml glucose and sucrose, 3 g per 100 ml soy peptone, 0.7 g per 100 ml yeast extract, 0.1 g per 100 ml MgSO₄·7H₂O in 0.01 mol l⁻¹ pH 6.3 potassium phosphate buffer). Fermentation was performed with a bran : water ratio of 22 : 78. Sucrose was used as a substrate for dextran production thus 10 g per 100 g of the bran was replaced with sucrose. Sucrose was dissolved in water

(25 °C) in a large glass beaker and cell suspension was added and mixed together. The native bran was added later in the mixture and carefully mixed to avoid lump formation (no further mixing during fermentation). The beaker was then covered with aluminum foil and incubated at constant temperature of 25 °C for 20 hours. Fermentation of bran sample were performed in duplicate.

2.1.3. Chemical analysis of bran. Acidification of bran, before and after fermentation were carried out as described by Kajala *et al.*³⁰ Samples for pH and Titratable Acidity (TTA) analysis were taken at the beginning and the end of fermentation and were performed in duplicate. The values of pH were determined on-line by a pH meter (Model HI 99161, Hanna Instruments, Woonsocket, RI, USA) using a food penetration probe. Samples for final pH and TTA analysis were collected at 20 hours of fermentation. Final pH and TTA was determined by adding 10 g of fermented bran in 100 ml of distilled water and was titrated with 0.1 M NaOH using an automatic titrator (EasyPlus Titrator, Mettler-Toledo, Schwerzenbach, Switzerland). The TTA was expressed as the amount of NaOH used (ml) for titration.

2.1.4. Microbiological analysis of bran. The microbiological analyses were carried out of the bran material (in duplicate) at the beginning and at the end of fermentation as described by Kajala *et al.*³⁰ Bran samples (10 g) were homogenized with sterile saline (90 ml) in a Stomacher 400 lab blender (Seward Medical, London). Serial dilutions were made and the enumeration of LAB was carried out by plating on MRS (de Man, Rogosa and Sharpe) agar. From plate count agar (PCA, Difco Laboratories, Detroit, MI, USA), aerobic heterotrophic bacteria were determined. Cycloheximide (0.001%) was added to the PCA plates to prevent fungal growth. A yeast mold (YM) agar (Difco Laboratories) was used to determine the growth of yeasts and molds. To prevent bacterial growth, 0.01% of chloramphenicol and chlortetracycline were added to YM agar and to limit the spreading of fungal colonies, 0.02% of Triton-X 100 was used. The plates for MRS agar was incubated anaerobically at 30 °C for 2–3 days and PCA and YM agars were incubated at 25 °C for 2–3 days. After fermentation, fermented bran sample were dried in a Christ Epsilon 2–25 freeze drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and ground using 100 UPZ-lb Fine impact mill (Hosokawa Alpine AG, Germany) with stainless steel pin discs (17 800 rpm).

2.1.5. Dextran content analysis. Dextran content in fermented bran was analysed by an enzyme-assisted method as described by Kajala *et al.*³⁰ Dextran was measured from the duplicate bran fermentations and also for the control native bran. A high-performance anion-exchange chromatography coupled with pulsed amperometric detector (HPAEC-PAD) was used to quantify the glucose formed after enzyme hydrolysis. The amount of dextran formed was estimated as the sum of anhydroglucose and was corrected with a recovery factor of 1.5. The recovery factor was determined with preliminary experiments as described by Katina *et al.*,³¹ to take into account the recovery of dextran in bran matrices. The dextran yields were

calculated using eqn (1) and all amounts are given as percentages of dry weight (% d.w.). The amount of anhydroglucose in sucrose was calculated by multiplying with (0.4737), which is the molar mass ratio of anhydroglucose to sucrose.

$$\text{Dextran yields} = \frac{\text{FD}}{(\text{IS} - \text{FS}) \times 0.4737} \quad (1)$$

where IS is the initial sucrose (endogenous sucrose in the raw material + added sucrose), FS is the final sucrose after fermentation and FD is the final dextran. Dextran contents (in %) of the FB and control NB was 2.96 ± 0.13 (dry weight) and 0.84 ± 0.06 (dry weight). Native rye bran has some sucrose in itself. Therefore, there is some dextran formed also in the control bran.

2.1.3. Particle size analysis of the bran material. Particle size of the bran material (NB and FB) was analysed with a Beckman Coulter LS 230 (Beckman Coulter Inc., CA, USA) using liquid module and with ethanol as a carrier as described by Alam *et al.*³² Milling of rye bran was optimized in order to obtain similar particle size distributions of NB and FB (median particle sizes were: $D_{50} = 361 \pm 49 \mu\text{m}$). Particle size analysis was performed in duplicate.

2.2. Extrusion processing

Feed materials for extrusions were prepared by adding 40% FB or 40% NB into EF in order to obtain the high fiber extrudates, while 100% EF was used to obtain extrudates for reference. The extrusion processing was performed using the processing parameters described by Nikinmaa *et al.*²² Small amount of salt (0.8% of the total mass of all recipes) was added in the bran-flour mixture. Extrudates were prepared using a twin screw extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, UK) with a constant screw speed of 350 rpm, feed rate of 60 g min^{-1} and a temperature profile increasing gradually from 80 to 120 °C from feed inlet to die exit in the following order: 80–95–110–120 °C (sections 1 to 4). A co-rotating twin screw feeder (K-Tron Soder, Niederlenz, Switzerland) was used for feeding of the flour-bran mix. Water ($2.0\text{--}3.5 \text{ g min}^{-1}$) was injected into the section 1 of the extruder barrel in order to obtain the desired moisture contents in the extrudates. A pair of twin screws and a 3 mm die were used in all extrusion trials. Feed rate calibration was performed for each recipe prior to extrusion. During extrusion, extrudates were collected from the die exit and dried in an oven for 30 min at 100 °C. Extrusion processing for each recipes was performed in duplicate. Torque values were recorded during extrusion (variation was between 70 and 80%), and the specific mechanical energy (SME)²⁷ was calculated using eqn (2):

$$\text{SME}(\text{kW h/kg}) = \frac{\omega}{\omega_r} \times \frac{\tau}{100} \times \frac{Z_r}{Q} \quad (2)$$

where ω is the screw speed (rpm), ω_r is the maximum screw speed of the extruder used (500 rpm), τ is the torque (%), Z_r is the maximum power capacity of the extruder (2 kW) and Q is the feed rate (kg h^{-1}). The calculated SME values were varied between $0.27\text{--}0.31 \text{ kW h kg}^{-1}$.

2.3. Compositional analysis of the extrudates

Extruded samples were analysed for total starch content by AACC method no. 76.13,³³ insoluble and soluble dietary fiber by AOAC method no. 991.43,³⁴ total protein content by AACC method no. 46-11A³⁵ and fat by AOAC method no. 922.06.³⁶ Moisture content of the extrudates was determined by drying the samples in an oven at 130 °C for 2 hours. All the analysis were performed in triplicate and result reported as mean $\pm$ SD of three replicates.

2.4. Properties of the extrudates

2.4.1. Macrostructural analyses. The measurements of expansion rate, specific length and piece density were made from 30 replicates (15 from each extrusion batch) of each extrusion recipe using the method described by Alam *et al.*³² A Vernier calliper was used to obtain the length and diameter of extrudate samples. The diameter was measured at three different points of an extrudate ribbon (9.7–10.5 cm long) and the average value was obtained, which represented the diameter of each sample.

Expansion rate was calculated with eqn (3):

$$\text{Expansion rate (\%)} = \frac{D_e}{D_d} \times 100\% \quad (3)$$

where D_e is the diameter (mm) and D_d is the diameter of the die (3 mm).

Specific length was calculated with eqn (4):

$$\text{Specific length (m/kg)} = \frac{L_e}{m_e} \quad (4)$$

where L_e is the length of the sample (m) and m_e is the mass of the sample (kg).

Piece density was calculated with eqn (5):

$$\text{Piece density (kg/m}^3\text{)} = \frac{4 \times m_e}{\pi \times (D_e)^2 \times L_e} \quad (5)$$

where m_e is the mass of the sample (kg), D_e is the diameter sample (m) and L_e is the length of the sample (m).

2.4.2. Textural properties analyses. A uniaxial compression test was used to determine the instrumental texture of the extrudates using a TA.XT2 Texture analyser (Stable Micro System Ltd, Godalming, United Kingdom) equipped with a 30 kg load cell and 25 mm diameter cylinder aluminium probe. Texture analysis samples were prepared by cutting the extrudate ribbon to 10 mm length (10.31–18.05 mm diameter) and placed vertically between the texture analyser platform and the aluminium probe. The samples were deformed at 70% strain with a test speed of 1 mm s⁻¹ and with an acquisition rate of 200 points per s. Trigger force was set to 5 g and trigger type was selected to 'Auto (Force)' in the settings window. The force–displacement (f – d) curves were obtained (Fig. 2) in order to assess the textural properties of the extrudate samples. The force (in y -axis) and time (in x -axis) threshold was 0.049 N and 1 s, respectively, and was remain constant for all TA analysis. The analysis was performed with 80 replicates

(40 from each extrusion batch) of each extrusion recipe using the method described by Alam *et al.*³² In f – d curve, the number of peaks represents the number of cell wall ruptures during compression and hardness ($F_{\max}$) is the maximum force needed to initiate cell wall crack. Texture Exponent software v.5.1.2.0 (Stable Micro Systems, UK) and a predefined macro was used to obtain the values of hardness ($F_{\max}$), crispiness work (C_w) and crispiness index (C_i). The calculation was performed using the formulas published by Alam *et al.*³² High C_i and low C_w values indicate high crispiness, whereas low C_i and high C_w values indicate low crispiness of the sample.

Crispiness work (C_w) was calculated with eqn (6) (Van Hecke *et al.*³⁷)

$$C_w (\text{N mm}) = \frac{A}{N} \quad (6)$$

where A is the area under the f – d curve (Nmm) and N is the number of peaks.

Crispiness index (C_i) was calculated with eqn (7) (Heidenreich *et al.*³⁸)

$$C_i = \frac{L_N}{A \times F_{\text{mean}}} \quad (7)$$

where L_N is the normalized curve length (length of actual curve/ $F_{\max}$), A is the area under the f – d curve (Nmm) and F_{mean} is the sum of the actual force values in the data file divided by the number of data points (N).

2.4.3. Sensory analysis. A trained sensory panel ($n = 12$) of VTT Technical Research Centre of Finland profiled the sensory attributes of the extrudates. Members of the sensory panel have passed the basic colour vision test, odour test and taste test and they have been trained for sensory profiling method. A training session was organized where the panellists familiarized themselves with the products and the key attributes relevant to the product category were defined. The defined sensory attributes were hardness, crispiness, coarseness, thickness, sliminess and intensity of overall flavour. Descriptive sensory profile analysis was conducted.³⁹ All descriptors were verbally anchored and reference samples were used for most attributes to define the extremes. Sensory attributes were evaluated using a 10 cm line scale anchored from “not at all = 0” to “extremely = 10”. During the evaluation sessions the lightning was adjusted to hide the colour of the products. Samples were offered as 2 cm pieces. The panellists were instructed on how to treat the sample during the evaluation (for example “Chew the sample using your back teeth until the sample is ready to be swallowed and assess the properties”) (Table 1). Sensory profiling of the samples was conducted in duplicate sessions in two consecutive days by all the panellists. The presentation order of the samples was randomized within each test day and the samples were blind-coded by 3-digit numbers. Water was served for cleaning the palate between the different samples. During the session the scores were collected and recorded using software (Compusense Five, Ver 5.4.15, CSA Computerized Sensory Analysis System, Compusense Inc., Guelph, ON, Canada).

Fig. 2 Representative “force–displacement” curves obtained from (a) EFE, (b) NBE and (c) FBE to assess the mechanical characteristics.

Table 1 Definition of the sensory attributes and instruction given to the sensory panel (n = 12)

Properties	Instruction to sensory panel	Reference product
Crispiness	“Place the sample vertically between molars and evaluate crispiness from the first bite” Breaking sound (first bite) of a crispy product is sharp, and clear. The sound of a crispy product fades fast during the first bites. Crispy product disintegrate into fine particles fast	Rye flakes are not crispy (= 0) Baby snacks are crispy (= 10)
Hardness	“Place the sample vertically between molars and break the sample down with your molars” The more force need to break down the sample the harder it is	Baby snacks are not hard (= 0) Rye flakes are hard (= 10)
Coarseness	“Chew the sample until swallowing point and press the bolus (masticated sample) with tongue against palate” Large particle between tongue and the palate indicate coarseness	Baby snacks are not coarse (= 0) Rye flakes are coarse (= 10)
Thickness	“Chew the sample until swallowing point and evaluate the thickness of the bolus”	Berry juice is not thick (= 0) Rye smoothie is thick (= 10)
Sliminess	“Chew the sample until swallowing point and evaluate the sliminess of the bolus (mixture of the sample and saliva)”	Berry juice is not slimy (= 0) Viili (processed sour whole milk from Finland, similar to yoghurt) is slimy (= 10)
Intensity of overall flavour	“Chew the sample and hold it in your mouth and evaluate the intensity of overall flavour”.	

2.4.4. Viscosity. The viscosity of the ground extrudate sample was analysed using a Rapid Visco Analyzer (RVA-Super4, Newport Scientific, Warriewood, Australia). Extrudate samples were ground using a Retsch mill at 6000 rpm speed with a 1 mm sieve. For each experiment, about 5 g of sample was dispersed into 25 ml of distilled water in order to obtain a homogeneous and viscous slurry. Experiment time was set for 180 min with initial stirring speed was 960 rpm (10 s) and later constant stirring speed of 120 rpm. A constant temperature (37 °C) was used during the experiment. Viscosity

by RVA depend very strongly on the dry matter concentration. Slight changes in the amount of water or sample may introduce error in the final viscosity. Therefore, 14% (commonly used % moisture in standard RVA protocol) water was kept constant for all samples. For this purpose, the amount of distilled water and sample was calculated prior to experiment using pre-installed calculator with Thermocline software (TCW3). The adjusted weight of the sample (4.63–4.71 g) and water (25.29–25.34 g) was varied depending on their moisture contents (7.11–8.61%) of the extrudates. Weight of the sample

and water was determined by analytical balance with 0.001 accuracy. The water was weighed in an empty canister and the carefully weighed ground sample was slowly dispersed in the water. To avoid lump formation, RVA paddle was pumped back and forth for 10 times before placing the canister into the RVA equipment. The pH of the distilled water was adjusted to 6.9 (either by 0.1 mol l⁻¹ NaOH or HCl) before performing the experiment. Thermocline software (TCW3) was used to obtain the final viscosity (cP) after cooling to 25 °C. The measurements were performed in triplicate and the results were reported as mean ± SD.

2.4.5. Hydrolysis index. Starch hydrolysis index (HI) of the extrudate samples was determined using a protocol published by Sozer *et al.*⁴⁰ and Alam *et al.*²⁸ Extrudate samples were ground (Retsch Ultra Centrifuge Mill ZM 200, 6000 rpm, 1 mm sieve) to mimic the particle size after mastication (visually observed). Ground extruded samples of about 1.4–2.0 g (to get 1 g starch in the sample) were taken in a beaker and same amount of distilled water (1:1) was added. A 100 ml 0.05 mol l⁻¹ sodium potassium phosphate buffer (KH₂PO₄-Na₂HPO₄·2H₂O, pH = 6.9) was added in the beaker and pH of the solution was adjusted to 6.9 with 0.1 mol l⁻¹ NaOH or HCl. Two different buffer addition methods (either after 15 minutes soaking or directly after adding water) were tested. The latter method was chosen to avoid lump formation. The solution was then incubated for 180 min at 37 °C with porcine pancreatic (110 U, 1 ml per 1 g starch) α-amylase (A6255-25MG, DFP Treated, Type I-A, saline suspension, ≥units per mg protein, 0.59 ml, 42 mg protein per ml (UV), 1151 units per mg protein, Sigma-Aldrich Co. LLC, USA). Samples were taken every 30 min for maltose analysis. After 180 min incubation with porcine pancreatic α-amylase, the solution was uniform and no lump was observed. The area under the curve (*A_{uc}*) was calculated using Sigmaplot 10.0 (Systat Software Inc., Point Richmond, CA, USA) using the pre-loaded macro. Incremental *Auc* was calculated using the formula of trapezoidal area at 0 min = $\frac{1}{2} \times (\text{abs}_0 + \text{abs}_{30}) \times (t_{30} - t_0)$ followed by 30, 60, 120 and 180 min (here, abs = mg of maltose per 1 g soluble starch). HI values were calculated for each time interval (0, 30, 60, 120 and 180 min) and the results were reported as mean value of three replicates. Air dried white wheat bread was ground and was used as a reference product. HIs of extruded samples were calculated by comparing the areas under the curve to that of wheat bread (average of three analyses) using the formula: HI = (Auc of extrudates/Average Auc of white wheat bread) × 100. The HI analyses were performed in triplicate and the results were reported as means ± SD.

2.5. Mastication trial

2.5.1. Participants. Participants (*n* = 26) for the mastication trial with electromyography (EMG) measurements were enrolled through an email and public advertisement near the study location. The eligibility of the participants was checked through screening questionnaire. The eligibility criteria included women aged between 20 and 40 years, BMI between

Table 2 Characteristics of the participants for mastication trial (*n* = 26)

	Mastication trial (<i>n</i> = 26)	
	Avg. ± SD	Range
Age	31.7 ± 7.5	19–50
BMI	22.2 ± 1.9	19.1–27.3
Eating behaviour ^a		
Cognitive restraint	45.7 ± 16.6	11–72
Uncontrolled eating	27.6 ± 10.3	11–48
Emotional eating	33.3 ± 24.7	0–89

^a 18-Item three-factor eating questionnaire (Karlsson *et al.*⁴²) was used to measure eating behaviour.

18.5 and 25 kg m⁻², with stable body weight (±4 kg during the previous year) and a habit of eating breakfast. Persons with missing teeth (except 3rd molars) or with diagnosed acute temporomandibular disorders (self-reported), persons with abnormal eating behavior (according to the Eating Disorder Diagnostic Scale; Stice *et al.*⁴¹), pregnant or lactating women, and persons with dietary restrictions (*e.g.*, celiac disease, allergies or aversions to cereal foods or high carbohydrate foods) and smokers were excluded. Young healthy females were recruited to avoid the possible variation in mastication pattern. The interested participants who fulfilled the inclusion criteria were invited to an information visit. The participants who decided to participate in mastication trial, signed an informed consent form. In total 26 female participants were included in mastication trial and were conducted during October–December 2015. Characteristics of the female participants are described in Table 2. To compensate time and effort, one movie ticket per study visit was offered to all the participants. The coordinating Research Ethics Committee of the Helsinki and Uusimaa Hospital District has approved the study protocol. The mastication trial was conducted according to the ethical principles of good research and clinical practice as described in the declaration of Helsinki. The trials were registered in ClinicalTrials.gov (NCT02554162).

2.5.2. Procedure. Mastication trials for electromyography (EMG) measurements of extrudate products were performed using a protocol published by Pentikäinen *et al.*²⁹ All trials were conducted using a crossover and single-blind design, in which the participants attended one study visit and masticated three extrudates samples in random order. All extruded samples were masticated in three replicates. Participants were instructed to eat breakfast 1–1.5 h before the study visit, which was scheduled 8–11 in the morning. Participants were familiarised with the study procedure by going through the protocol with bread samples before the actual mastication trial. The coded extrudate samples were served in a random order and three portions of each extrudate sample were masticated in a row. Each portion included two pieces (2 cm × 1–1.8 cm) of extrudate samples (1 g). The participants were instructed to masticate each portion of sample until they feel the portion is ready to swallow and then expectorate the bolus in a plastic cup. There was a break between different extrudate products,

during which the mouth was rinsed with water. After completion of the mastication of all the study products, the participant was offered three pieces of chewing gum and was asked to chew each for 20 s. During the mastication trials, oral processing was characterised by measuring the electrical activity of facial muscles using electromyography. Although the force generated by four muscle was linearly relative to the measured voltage, the calibration varies between different subjects. Therefore, individual data on oral processing of chewing gum²⁹ was used as a reference for force parameter, to get an indication of the relative force needed to masticate each of the samples. All the mastication sessions were video recorded in order to support the data analyses.

2.5.3 Mastication properties with electromyography (EMG). Electrical activities of masticatory (masseter and temporal) muscles during mastication were measured with an EMG equipment (Mega Electronics, Kuopio, Finland) using disposable dermal Ag/AgCl electrodes. Masseter and temporal muscles were identified by touching when the participant gritted her teeth. The skin was cleaned with 70% ethanol and bipolar electrodes were placed on the muscles of both sides of the face. A reference electrode was placed on the cervical vertebra. EMG activity was measured and recorded continuously throughout the mastication session. In the EMG acquisition system, the data blocks for each chewing period were isolated for analysis using markers added to the EMG data. Experiment minute records and the video recording of each mastication session were kept to support the data analysis. The elimination of high frequencies and background fluctuations of the EMG time series was performed by applying chemometric techniques on the extracted onset, duration and amplitude of each chewing event.⁴³ Chewing force and work parameters were normalized to mastication data obtained from chewing gum trial. A Matlab® (The MathWorks Inc., Natick, MA, USA) software was used to analyze EMG data. The calculation of EMG activity time, chewing time, duty cycle (EMG activity time/chewing time), number of chews, chewing force and work were calculated for each extrudate. Relative chewing force (highest EMG amplitude normalized to highest EMG amplitude for chewing gum) and relative work (EMG activity time × relative chewing force) parameters were calculated with relation to the chewing process of chewing gum. It is worth noting that the force and work computed this way are descriptive mastication parameters and not force and work in strict physical sense. Although the force generated by four muscle is linearly⁴⁴ relative to the measured voltage, the calibration varies between different subjects. The linear relationship between force generated by muscles and measured voltage was not measured in this study but this is a known correlation, which was first demonstrated by Prum *et al.*⁴⁴ All the parameters were computed separately for all four muscles monitored and the mean values were used in data analysis.

2.6. Bolus sample analyses

2.6.1 Saliva uptake. Saliva uptake of the bolus was determined based on the moisture contents of extrudates and their

bolus samples.²⁸ Wet bolus samples were collected from 26 individual participants during mastication trial. A 0.5 g bolus from each participants was weighed in an aluminum moisture cup without mixing with each other. Moisture cups containing bolus samples were then placed in an oven at 105 °C. After overnight drying, bolus was weighed again. Saliva uptake was determined as the difference between the moisture content of boluses and the moisture content of extrudates. The data first calculated (average of three replicates) for each individual participants, and then reported as mean ± SD of 26 participants for each extruded product.

2.6.2. Particle size distribution. Disintegration of the extrudates into particles was examined for the bolus samples ($n = 26$) using the protocol published by Alam *et al.*²⁸ The bolus samples (from 26 individuals participants) were diluted in a beaker with 100 ml of water, mixed with constant magnetic stirring of 220 rpm for 25 min. Diluted solution is then left to stand for 5 min in order to allow larger particles to settle to the bottom. Turbid liquid containing the smallest particles that could not be imaged was removed. A 100 ml of water was added again to increase the sample volume. The liquids containing bolus particles were poured onto Petri dishes for imaging. Around 7 to 8 Petri dishes were needed depending on the sample. The position of the particles were adjusted by moving/dragging them (when needed, if touched each other) over Petri dishes so that they were as little as possible in contact with each other. Digital images were taken of each Petri dish. Particle areas were determined using Cell[^]P imaging software (Olympus, Germany). Particle area distribution was visualized by a set of granulometric curves.

2.6.3. Viscosity. The viscosity of the bolus sample was analysed according to the method described for ground extrudates in section (2.4.3. Viscosity). Bolus samples were collected from a single chewer, who was not one of the 26 mastication trial participants. About 8 g of bolus (obtained from same person) sample was added to 25 ml of distilled water to reach the desired concentration (14% moisture basis). The adjusted weight of the sample (8.38–8.89 g) and water (21.11–21.62 g) was varied due to their moisture contents (48.66–50.42%). The data reported as mean ± SD values of three replicates.

2.7. Statistical analyses

Overall differences between study products were assessed using one-way ANOVA, and HSD Tukey was used as a *post hoc* analysis for pairwise comparison. Multivariate analysis of variance (MANOVA) was performed in order to see the joint and individual effect of each variables. Strongest effect indicated by Wilks Λ value close to zero, partial η^2 value close to unity and p less than 0.05. Linear correlations between different variables were calculated using 2-tailed Pearson bivariate correlation with significance level of 0.05. Statistical analyses were conducted using SPSS Statistics 24 (SPSS Inc., Chicago, USA) software.

3. Results

3.1. Chemical composition

The chemical compositions of the extrudates are shown in Table 3. The two extrudates with added rye bran (FBE and NBE) had almost identical compositions, and were clearly different from the EFE. EFE had 8% total DF, 6.5% protein and 78% starch. Both the extrudates with added rye bran had 22% total DF and 10% protein, whereas FBE had slightly less (54% vs. 57%) starch ($p < 0.05$). FBE had slightly lower insoluble (14% vs. 16%) DF ($p < 0.05$) and higher soluble DF (5.9% vs. 8.0%) compared to NBE. FBE had lower pH ($p < 0.05$) as compared to NBE and EFE (4.4 vs. 6.3 and 6.1, respectively). TTA varied between 1.5 (EFE) and 13 (FBE), whereas TTA of NBE was 2.5 ml.

3.1.1. Acidity, microbial and dextran analysis. Before fermentation, the measured pH of the bran was 6.3, while TTA was 2.0 (ml). The LAB counts in MRS agar was $6.4 \log \text{cfu g}^{-1}$, cell densities of yeasts and molds (YM agar) were less than $2 \log \text{cfu g}^{-1}$, cell densities of aerobic heterotrophic bacteria or fungi (PCA agar) were $3.0 \log \text{cfu g}^{-1}$ and total spore forming aerobic bacteria count was less than $2 \log \text{cfu g}^{-1}$. After 20 h of fermentation at 25 °C, the pH and TTA of the bran was 4.1 and 9.7 (ml), respectively. The observed LAB count was $9.3 \log \text{cfu g}^{-1}$, yeasts and molds counts were less than $4 \log \text{cfu g}^{-1}$, aerobic heterotrophic bacteria or fungi were less than $3.0 \log \text{cfu g}^{-1}$ and total spore forming aerobic bacteria count was $2 \log \text{cfu g}^{-1}$. The dextran content of fermented rye bran was 3% (3 g per 100 g bran; dry weight). Dextran was also formed in the control bran 0.8% (0.8 g per 100 g bran; dry weight) due to the free sucrose available in native rye bran.

3.2. Structural properties

FBE were notably more expanded than the NBE ($p < 0.05$), even though they had similar bran-flour ratios (Table 4). EFE were more expanded than both bran-added NBE and FBE ($p < 0.05$). The specific length of FBE was significantly higher than that of NBE ($p < 0.05$). Overall, bran addition resulted in reduced expansion and increased density, but this effect was

Table 3 Compositional analysis of the extrudates made from 100% endosperm rye flour (EFE), 40% native rye bran (NBE) and 40% EPS fermented rye bran (FBE)

	EFE	NBE	FBE
Moisture (%)	8.9 ± 0.0^c	8.0 ± 0.0^b	7.0 ± 0.1^a
Protein (%)	6.5 ± 0.1^a	10 ± 0.0^b	10 ± 0.1^b
Starch (%)	78 ± 0.2^c	57 ± 0.0^b	54 ± 0.3^a
Total dietary fiber (%)	8.0 ± 0.3^a	22 ± 0.9^b	22 ± 0.0^b
Insoluble dietary fiber (%)	4.5 ± 0.1^a	16 ± 0.0^c	14 ± 0.2^b
Soluble dietary fiber (%)	3.5 ± 0.2^a	5.9 ± 0.9^b	8.0 ± 0.2^b
Fat (%)	0.61 ± 0.00^a	1.5 ± 0.0^c	1.4 ± 0.0^b
pH	6.1 ± 0.1^b	6.3 ± 0.2^b	4.4 ± 0.0^a
TTA (ml)	1.5 ± 0.0^a	2.5 ± 0.0^b	13 ± 0.0^c

Based on dry weight; TTA: titratable acidity by 0.1 M NaOH. Values followed by different letters (a–c) in the same row were significantly different ($p < 0.05$).

Table 4 Structural and textural properties of 100% endosperm rye flour (EFE), 40% native rye bran (NBE) and 40% EPS fermented rye bran (FBE)

	EFE	NBE	FBE
Macrostructure			
Expansion rate (%)	525 ± 32^c	369 ± 17^a	414 ± 18^b
Specific length (m kg^{-1})	48 ± 3.9^a	49 ± 2.4^a	63 ± 3.9^b
Piece density (kg m^{-3})	109 ± 13^a	213 ± 19^c	133 ± 11^b
Texture			
Hardness (N)	36 ± 4.7^b	49 ± 5.5^c	19 ± 2.3^a
Number of peaks	96 ± 3^b	85 ± 3^a	125 ± 3^c
Crispiness work (Nmm)	1.6 ± 0.3^b	2.2 ± 0.3^c	0.48 ± 0.07^a
Crispiness index ($\times 10^{-3}$)	4.7 ± 1.2^b	2.3 ± 0.4^a	43 ± 10.1^c

Values followed by different letters (a–c) in the same row were significantly different ($p < 0.05$).

less pronounced in FBE. MANOVA results showed that extrudates were significantly different due to the combined effect of expansion, specific length, piece density and DF content ($p < 0.05$), (Table 6a). There was a significant difference ($p < 0.05$) between samples for each individual variable with partial η^2 varying between 0.809 and 0.996 (data not shown in Table 6). DF content had the strongest effect on the product followed by piece density and expansion rate than specific length. Moreover, all structural properties were jointly and individually influenced ($p < 0.05$) by DF content (Table 6f). Expansion rate was negatively influenced by insoluble DF ($r = -0.83$, $p < 0.05$), indicating that the higher the insoluble DF in the extrudates, the lower the expansion was.

3.3. Textural and sensory properties

NBE had the highest instrumental hardness (49 N), which was dramatically reduced by bran fermentation, even to a lower level than EFE (19 N vs. 36 N) (Table 4). As expected, rye bran addition resulted in lower crispiness ($C_i = 0.002$ vs. 0.005, $C_w = 2.2$ Nmm vs. 1.6 Nmm) in NBE compared to EFE. However, EPS-fermentation significantly increased the crispiness ($C_i = 0.043$ and $C_w = 0.48$ Nmm) of FBE compared to EFE and NBE ($p < 0.05$). Extrudates were significantly different ($p < 0.05$) due to the combined (Table 6b) effect of hardness, crispiness work, crispiness index and DF content. There was a significant difference ($p < 0.05$) between extrudates for each individual variable, with partial η^2 varying between 0.973 and 0.996 (data not shown in Table 6). Furthermore, all textural properties were jointly (Table 6g) and individually influenced by DF content ($p < 0.05$). EFE and FBE did not differ in perceived hardness and crispiness, whereas NBE was perceived to be the hardest and least crispy sample ($p < 0.05$), (Fig. 3). FBE was perceived as less coarse but had similar sliminess when compared to NBE. In MANOVA analysis, a combined effect of all sensory properties and DF content was observed (Table 6c). The samples were differed regarding all the variables except sliminess and thickness ($p < 0.05$) with partial η^2 varying between 0.238 and 0.476 (data not shown in Table 6). Intensity

Fig. 3 Sensory attributes of the EFE, NBE and FBE evaluated by the sensory panel. The different letters indicate statistically significant differences between products ($p < 0.05$).

of the overall flavour and DF content had the most significant effect (partial η^2 of 0.705 and 0.996, respectively).

3.4. Mastication properties

All extrudates had similar mastication profiles (Table 5). None of the mastication properties were significantly different ($p < 0.05$). The number of chews and chewing time were similar for all the studied products. Mastication properties and DF content had combined influence on the samples (Table 6d) but none of the mastication properties and DF content had individual effect ($p > 0.05$). Participants of mastication trial had significant effect on the mastication properties (Table 6m) indicating the existence of inter-individual variation. All the mastication parameters were significantly different based on

the participants with partial η^2 varying between 0.843–0.929 (data not shown in Table 6).

3.5. Viscosity of the ground extrudates and boluses

When ground extrudate dispersions were compared, FBE were significantly less viscous ($p < 0.05$) than EFE and NBE, and EFE was the most viscous (Fig. 4). In the case of bolus samples, FBE was less viscous than EFE or NBE, but the difference was not statistically significant between EFE and NBE (Fig. 4). A pH drop was seen for EFE (6.0 vs. 4.3) and NBE (6.3 vs. 5.1) bolus samples when compared to dispersions of ground extruded products. However, the pH of both ground extruded products and bolus samples of FBE was below 5 (Fig. 4). In bolus samples, it took 2 hours to reach a constant viscosity level, after which all the clumps had been broken down and fully dissociated into the slurry.

3.6. Bolus particle size

There were more small particles in the bran-containing FBE and NBE boluses compared to the EFE bolus (Fig. 5). Crispy and low viscous FBE extrudates disintegrated rather easily during mastication, resulting in a larger number of small particles (Fig. 5d). The largest particle in the FBE bolus was 142 mm², whereas EFE and NBE had comparatively bigger particles, 218 mm² and 283 mm², respectively (Fig. 5d). The proportion of smaller particles (<10 mm²) in FBE bolus was higher than in the EFE and NBE bolus. FBE had the highest share of small particles ($\approx 80\%$) compared to EFE ($\approx 57\%$) and NBE ($\approx 66\%$). Mean particle area of all boluses varied between

Table 5 Mastication properties of 100% endosperm rye flour (EFE), 40% native rye bran (NBE) and 40% EPS fermented rye bran (FBE)

	EFE	NBE	FBE
EMG activity time (s)	3.9 ± 1.5	4.3 ± 1.9	3.7 ± 1.7
Chewing time (s)	7.8 ± 3.4	8.4 ± 4.1	7.2 ± 3.7
Number of chews	11 ± 3.9	13 ± 5.9	11 ± 4.7
Duty cycle ^a (%)	52 ± 5.2	53 ± 5.0	53 ± 5.4
Relative total work ^b (%)	30 ± 13.1	34 ± 14.7	26 ± 12.6
Relative force/chew ^b (%)	76 ± 21.2	79 ± 24.6	71 ± 23.8
Saliva uptake (g per 100 g)	43 ± 7.4	43 ± 6.9	47 ± 8.8

The mean differences are NOT significant at the 0.05 level. ^aEMG activity time/chewing time. ^bNormalized to the corresponding values of a reference product (chewing gum).

Table 6 Multivariate analysis of variance results of the studied properties

	Wilk's Λ	Degree of freedom (hypothesis df, error df)	p	Partial η^2
Effect on products by:				
a. Macrostructure $\times$ DF	0.000412	$F(8, 144) = 869$	0.000	0.980
b. Texture $\times$ DF	0.000034	$F(8, 144) = 3075$	0.000	0.994
c. Sensory properties $\times$ DF	0.001367	$F(14, 54) = 100$	0.000	0.963
d. Mastication properties $\times$ DF	0.002682	$F(14, 138) = 181$	0.000	0.948
e. Bolus properties $\times$ DF	0.003342	$F(8, 144) = 293$	0.000	0.942
Effect of DF on:				
f. Macrostructure	0.011049	$F(15, 194) = 53$	0.000	0.777
g. Texture	0.001103	$F(15, 194) = 139$	0.000	0.897
h. Sensory properties	0.069424	$F(30, 102) = 3.2$	0.000	0.413
i. Mastication properties	0.563836	$F(30, 270) = 1.4$	0.093	0.108
j. Bolus properties	0.615383	$F(15, 194) = 2.5$	0.002	0.149
Effect on HI by:				
l. Macrostructure	0.008603	$F(33, 189) = 23.1$	0.000	0.795
k. Texture	0.000534	$F(33, 189) = 68.3$	0.000	0.919
Effect of participants on:				
o. Sensory properties	0.001332	$F(66, 107) = 3.9$	0.000	0.668
m. Mastication properties	0.000028	$FF(150, 283) = 9.4$	0.000	0.825
n. Bolus properties	0.067112	$F(75, 150) = 2.9$	0.000	0.594

Strongest effect indicated by Wilks Λ value close to zero and partial η^2 value close to unity, $p < 0.05$.

Fig. 4 Viscosity of ground (a, b, and c) and bolus (d, e, and f) samples of EFE, NBE and FBE.

0.027 and 0.034 mm². The extruded products did not differ in saliva uptake. Extrudates were significantly different due to the combined effect of bolus particle area, number of particles in the bolus, saliva uptake and fibre content ($p < 0.05$), (Table 6e). Among all the bolus properties, particle area had the most significant effect. Furthermore, all but saliva uptake were jointly (Table 6j) and individually influenced by fibre content ($p < 0.05$) with weak Wilks Λ and low partial η^2 values. However, all the bolus properties were significantly different

based on the participants with partial η^2 varying from 0.502–0.678 (data not shown in Table 6), where saliva uptake had the strongest effect. There was a strong positive correlation between total particle area of each product and insoluble DF ($r = 0.99$, $p < 0.05$), suggesting that bran addition increased the number of particles in the boluses.

3.7. *In vitro* starch digestibility of the extrudates

The extent of starch hydrolysis in FBE was higher than in EFE and NBE ($p < 0.05$) (Fig. 6). Although containing 40% bran, NBE had a similar hydrolysis index to that of EFE. It was noticeable that the use of fermented rye bran in FBE resulted in higher starch digestion in the early phase. After 30 minutes of enzymatic incubation, FBE had a higher percentage of hydrolysed starch (80%) compared to EFE (70%) and NBE (73%). Macrostructure and texture both had significant effect on HI either jointly (Table 6l and k) or by individually with partial η^2 varying from 0.837–0.926 and 0.977–0.991 for macrostructure and texture, respectively (data not shown in Table 6). Interestingly, increase in DF content increased the HI ($r = 0.56$, $p < 0.05$), while increase in starch content reduced the HI ($r = -0.71$, $p < 0.05$).

4. Discussion

In the current study the use of fermented rye bran in rye extrudates resulted in a less hard and crispier texture, compared to native bran-containing extrudates. The positive effect of fermented rye bran on the textural properties of rye extrudates was also reported by Nikinmaa *et al.*,²² who explained that when rye bran was fermented with dextran producing *Weissella confusa*, the bran surface was covered by a dextran layer. This might have reduced the premature air bubble ruptures by smoothing the surface of bran particles. We have shown in our previous paper Nikinmaa *et al.*,²² that fermentation with *Weissella confusa* increased soluble pentosans from 1.5% to 2.8%. Solubilisation of DF from corn⁴⁵ and wheat bran⁴⁶ increased expansion and reduced density, respectively, whereas production of dextrans influenced product texture due to their ability to influence viscosity.³¹ *In situ* dextran production (11–16 g kg⁻¹) in wheat sourdough increased the viscosity of the dough and improved the volume (up to 10%) and crumb softness (25–40%) of the corresponding bread. Therefore, both dextran production and solubilisation of DF might explain the improved structure observed in FBE.

Two high-DF (22%) rye extrudates had similar mastication properties to those of the extrudate (EFE) with less (8%) DF. Mastication properties of the three extrudates varying in structure and composition were not significantly different, although subtle differences were observed in the mastication effort measured as relative total work and relative force/chew. Although the product category was the same (*i.e.* all were dry puffed rye products), it was expected that the dense structure and hard texture of NBE would cause higher force/chew and longer chewing time. Probably the high inter-individual vari-

Fig. 5 Representative images of the masticated samples of (a) EFE (b) NBE and (c) FBE (the white bar is 10 mm). Particle area distributions of the samples are shown in (d), where the curves represent a cumulative percentage of the total area occupied by particles obtained from the boluses of 26 individual participants [color indication: red = 0.001–0.2 mm², green = 0.2–0.5 mm², blue = 0.5–1.0 mm², yellow = 1.0–10.0 mm², and cyan blue = 10–1000 mm²].

Fig. 6 (a) *In vitro* starch hydrolysis (% of hydrolysed starch) of EFE, NBE and FBE during 180 min incubation. (b) Hydrolysis indices (HI = (A_{uc} of extrudates/Average A_{uc} of white wheat bread) $\times$ 100) of extrudates. Values are means of three replicates $\pm$ SD. The bars marked with different letters were significantly different ($p < 0.05$).

ation overrode the possible small differences between products. We have previously reported that hard and dense extruded products required more mastication effort. However, in that study,²⁸ the effect of structure and texture on mastication

was not observed within the same product type (puffs vs. rye bran added puffs). Significant differences in the mastication properties were only seen between different product types (puffed vs. flaked products). Proper insalivation and lubrication

tion of the food is required in order to produce a swallowable bolus. It is assumed that hard and less crispy food products require longer mastication, which allows the food to be sufficiently lubricated with the saliva. However, in the current study, clear differences in hardness between the samples was not reflected in the mastication time or the number of chewing cycles.

Viscosity of the ground samples was 16–62% higher compared to bolus samples, demonstrating that the presence of salivary alpha amylase in bolus samples caused the main reduction. The hydrolysis of starch by salivary amylase was the main reason for this viscosity reduction. In this study, the low pH probably reduced the swelling capacity of ground FBE (pH = 4.4) compared to ground EFE (pH = 6.0) and NBE (pH = 6.3), which resulted in reduced viscosity. This is in agreement with the results of Okafor *et al.*⁴⁷ who observed that the swelling capacity of defatted black bean flour decreased when pH decreased from 6 to 4. Furthermore, FBE samples were crispy and mechanically weaker than EFE and NBE. It has been shown in our previous study that the crispy extrudates had low water solubility index (the amount of soluble components released from starch) compared to less crispy extrudates.⁶

Disintegration of the food during chewing, hydration, swelling and solubilization of different components, affects the viscosity of the bolus. In this study, FBE bolus was less viscous than the EFE and NBE boluses. The EFE and NBE boluses were more compact (not loose) than FBE boluses, indicating that more cohesive (visually observed) starch and protein phase was formed during mastication, which is in line with the study of Johansson *et al.*⁴⁸ and thus prevented swelling. In bolus samples, pH varied between 4.3–5.1. The activity of α -amylase reaches its peak at pH range 5.5–6.5 (Evans *et al.*).⁴⁹ Below this range enzyme activity starts to reduce and become close to zero at pH 4.5. Therefore, low viscosity of EFE bolus samples (pH = 4.3) than NBE bolus (pH = 5.1) could be explained by its low pH and in turn reduced swelling capacity. Moreover, EFE had the crispier structure than NBE, which probably disintegrated rather easily during constant stirring and due to the activity of α -amylase. For the same reason, crispiest FBE had the lowest viscosity. The mechanical energy of the gelatinised starch granules nearly lost when they come in contact with salivary α -amylase (Evans *et al.*).⁴⁹ The loss of mechanical energy make the granules weaker and accelerate disintegration. Amylases not only attack the surface of the granules but also the starch chains throughout the granule, which caused a drop in viscosity of the overall bolus due to a progressive weakening of granule structure.

Extrudates with added rye bran were disintegrated to smaller particles in the resulting bolus. The number of fine particles in the bolus was greater in NBE than in EFE, and a further increase of fine particles was seen in FBE. Sensory results also confirmed that the FBE bolus was perceived as less coarse than NBE. Rye bran addition made the extrudates less cohesive, probably due to incorporation of insoluble particles, which may have restricted the cluster formation during mastication. This further led to formation of smaller particles in the

bolus. FBE bolus was paste like and loose, with the highest saliva uptake (47 g per 100 g of sample), whereas NBE as well as EFE boluses were compact and slightly dry (43 g per 100 g of both sample). Dissolution of polymers might have increased adhesion of saliva to bolus particles, resulting in loose bolus structure.⁵⁰ On the other hand, dryness in NBE and EFE was caused by the presence of comparatively intact starch-protein matrices which absorbed less saliva.

The hydrolysis index of the extrudates varied between 93 and 104, which is rather high compared to baked food such as bread. High HI is expectable after extrusion processing, as the starch present in the food matrix is totally degraded due to high shearing and temperature.^{6,28,51,52} Increasing DF content from 8 to 22% using native bran in NBE (94) did not change HI compared to EFE (93), but using fermented bran did (104). Juntunen *et al.*⁵³ observed a decreasing trend in starch hydrolysis rate with an increased amount of DF in rye breads. The hydrolysis rate of starch was lower for high DF (29% DF) rye breads compared to traditional (15.2% DF) and endosperm rye bread (6.1% DF). By contrast, Rosen *et al.*⁵⁴ reported that although having less DF, the HI of endosperm rye bread (6.7% DF) was lower than that of whole grain rye (9.6% DF) and rye bran added (12.3% DF) bread. However, accessibility of starch to digestive enzymes may differ depending on processing methods and ingredients of food,⁴³ their structure and their particle size.⁵⁵ Structural differences within the products influenced the particle size of the ground extrudates as well as of the bolus samples. HI was positively influenced by crispiness index ($r = 0.98$, $p < 0.05$) and number of particles ($r = 0.85$, $p < 0.05$). We reported in our previous study²⁸ that the availability of starch for digestive enzymes in rye bran supplemented extrudates increased with the number of fine particles in the bolus. Insoluble bran particles probably disrupted the starch matrices in both NBE and FBE, but crispy texture in FBE led to an increase in smaller particles. Crispy extrudates were easily disintegrated, which enhanced the release of starch granules from the matrix, making them easily accessible to α -amylases. The presence of smaller particles in FBE increased the surface area, which led to increased susceptibility of starch granules to digestive enzymes, which is in agreement with the recent studies of Alam *et al.*⁶ and Singh *et al.*⁵⁶ Therefore, texture, *i.e.* hardness and crispiness, had a prominent role in bolus formation and starch digestibility. We have previously reported that the use of coarse ($D_{50} = 440 \mu\text{m}$) rye bran decreased the HI compared to finely milled ($D_{50} = 28 \mu\text{m}$) rye bran.⁶ However, in the current study coarse ($D_{50} = 365 \mu\text{m}$) rye bran was used in both NBE and FBE. Furthermore, comparatively low viscosity of FBE might also have influenced the HI by promoting diffusion in the digestive medium and consequently enhanced the susceptibility of enzymic breakdown of starch into sugars. A negative correlation was found between viscosity and HI ($r = -0.957$, $p < 0.05$), suggesting that the higher the viscosity, the lower the HI. Although EPS fermentation of the bran in the present study hindered the HI decreasing effect of coarse rye bran, it improved the palatability by means of providing better textural and structural properties. High DF content and good

textural properties of FBE make it a good alternative in the snack food category as a DF carrier.

5. Conclusion

The extrudates studied in this work were grouped into two pairs; the first (EFE and FBE) had similar macrostructure but distinct composition and the second pair (NBE and FBE) had distinct macrostructure but similar gross composition. The difference between EFE and FBE in instrumental crispiness was not reflected as a difference in perceived crispiness. This was probably due to the inter-subject variation, which prevented the detection of subtle textural differences. Possibly small differences in crispiness, detectable instrumentally, are not large enough to influence the perceived crispiness in general. Fermentation of bran induced compositional changes (e.g. production of dextran and solubilization of DF), which resulted in structural changes making the FBE extrudates more fragile. This further led to reduced bolus particle size and higher *in vitro* starch digestibility. Although, there was less starch in the fermented bran product compared to the unfermented product, the structure-texture interplay overruled this difference by resulting in a more fragile, easy to digest structure. A snack product with high dietary fibre content and palatable texture was achieved with fermented rye bran addition. Compared to NBE, FBE had less hard and more expanded and crispy texture that is considered beneficial in this product category. From nutritional point of view, FBE had a disadvantage of having higher *in vitro* starch digestibility compared to EFE and NBE. However, a portion (30 g) of FBE provides as much as 6.6 g of DF and only 16.2 g starch. Therefore, the glycemic load is reasonably low while the portion contributes significantly to the recommended daily DF intake. Considering the palatable texture and high DF content, FBE is a potential healthy alternative to the snack product category.

Abbreviations

C_i	Crispiness index
C_w	Crispiness work
DF	Dietary fibre
EF	Endosperm rye flour
EFE	Endosperm rye flour extrudates
EMG	Electromyography
EPS	Exopolysaccharide
FB	Fermented rye bran
FBE	Fermented rye bran extrudates
F_{max}	Hardness
HI	Starch hydrolysis index
NB	Native bran
NBE	Native rye bran extrudates
TTA	Titrateable acidity

Conflicts of interest

■■■■

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