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Effects of structural and textural properties of brittle cereal foams on mechanisms of oral breakdown and *in vitro* starch digestibility

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ABSTRACT

Structural and textural properties as well as the dietary fibre content of solid cereal foams influence the oral breakdown of structure, bolus formation and digestibility. The aim of this study was to investigate how structural differences of solid cereal foams (puffs vs. flakes) affect *in vivo* chewing and *in vitro* starch digestion. Four extruded puffs and flakes were produced from endosperm rye flour by extrusion processing without or with 10% rye bran (RB) addition. Extruded puffs and flakes were masticated by fifteen healthy females and the process was monitored using electromyography. Extruded puffs were more porous than flakes (97% vs 35%). The two products were also significantly different ($p < 0.05$) in their structural and textural properties such as expansion, hardness, density and crispiness. A negative correlation was observed between hardness and crispiness index ($p < 0.05$, $r = -0.950$) and density and porosity ($p < 0.05$, $r = -0.964$). Addition of 10% RB had a significant effect on structural, textural and mastication properties both for puffs and flakes. Mastication of puffs required less total work than flakes (204 vs. 456%) and they were degraded to smaller particles than flakes during mastication. Irrespective of the considerable differences in structure, texture and oral disintegration process, no significant ($p < 0.05$) differences were observed between puffs and flakes (86.4 vs. 85.1) in terms of starch hydrolysis index. RB addition increased the hydrolysis index of puffs and flakes to 89.7 and 94.5, respectively, which was probably attributable to the increased number of particles in the bolus.

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1. Introduction

Consumption of snack food products is globally high; the market for extruded products alone is expected to reach \$31 billion by 2019 (Markets and Markets, 2014). Consumer interest in extruded products is growing due to their on-trend texture and shape. However, extruded cereal snacks are often unhealthy due to their excessive starch content and lack of dietary fibre (Alam et al., 2016). Extruded products have the structure of solid foam. Open brittle foams such as extruded puffs consist of an interconnected porous structure with thin cell walls, offering a mechanically weaker and less dense structure compared to closed and dense structures such as extruded flakes (Dogan, Romero, Zheng, Cuitino, & Kokini, 2008).

Food macrostructure (such as porosity and relative density) and microstructure (cell diameter, cell distribution and cell wall thickness) play an important role in mastication, bolus formation, transportation of the bolus in the human gastrointestinal tract and in starch hydrolysis (Hoebler et al., 1998; Kong & Singh, 2008; Le Bleis, Chaunier, Della Valle, Panouillé, & Réguerre, 2013). Generally, the addition of insoluble fibre (e.g. bran) to extruded matrices interferes

with the macro-, micro- and textural properties by decreasing the expansion and crispiness and increasing hardness, cell wall thickness and density (Alam et al., 2014; Guan, Fang, & Hanna, 2004; Lue, Hsieh, Peng, & Huff, 1990; Mendonça, Grossmann, & Verh e, 2000; Moore, Sanei, Hecke, & Bouvier, 1990). These effects are more pronounced for coarse particle sized bran, particularly at higher addition levels (Alam et al., 2016).

Food is mechanically disintegrated to smaller fragments and enzymatically broken down by digestive enzymes during digestion (Bornhorst & Singh, 2012; Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Panouill e, Saint-Eve, D el eris, Le Bleis, & Souchon, 2014; Stokes, Boehm, & Baier, 2013). Oral processing of food, i.e. mastication, prepares the food into a lubricated and cohesive bolus by means of complex mechanical and chemical transformation (Le Bleis et al., 2013).

Textural properties of cereal products, such as hardness and crispiness, are also assumed to have a role in physiological responses (Kong & Singh, 2008; Turgeon & Rioux, 2011). Agrawal, Lucas, Prinz, and Bruce (1997) reported that the fracture events occurring during mastication of food (raw vegetables, nuts and cheese) are inversely related to the products' hardness; however the exact food properties determining mastication are not fully understood. The bolus is further broken down in the stomach by gastric secretions and muscular contractions and gradually released to the duodenum, when the particle size is $< 1\text{--}2$ mm (Thomas, 2006). The particle size distri-

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bution of a bolus depends on the original food structure and texture (Hoebler, Devaux, Karinthi, Belleville, & Barry, 2000; Jalabert-Malbos et al., 2007). Crispy and less hard products disintegrate more easily during mastication, thus producing smaller particles, whereas hard and less crispy products result in larger particles in the bolus (Pangborn & Lundgren, 1977). The textural properties of solid foams are related to matrix architecture, its composition, and the state and homogeneity of the solid matrix (Sozer, Bruins, Dietzel, & Kokini, 2011). However, the literature on how structural and mechanical properties of different brittle solid foams affect mastication, bolus formation and digestibility is scarce and is focused on commercial flakes made from refined flour (Hedjazi, Guessasma, Yven, Della Valle, & Salles, 2013; Hedjazi, Martin, Guessasma, Della Valle, & Dendievel, 2014; Yven, Guessasma, Chaunier, Della Valle, & Salles, 2010).

We have previously studied the *in vitro* starch digestibility and the structural and textural properties of rye extrudates (Alam et al., 2016), and showed that microstructure has a major role in the digestibility of high fibre extruded puffs. The aim of this study was to elucidate how structural differences in open and closed extruded rye matrices influence oral processing and *in vitro* starch digestibility. We hypothesised that closed solid cereal foams (flakes) prepared by extrusion would require more mastication, disintegrate into larger particles and have a lower hydrolysis index compared to open cereal foams (puffs).

2. Material and methods

2.1. Puffs and flakes

2.1.1. Feed material preparation

Rye endosperm flour (ERF) obtained from Helsingin Mylly Oy (Järvenpää, Finland) was used as base material during the extrusion processing. Commercial rye bran (RB) was purchased from Fazer Mills and Mixes Ltd. (Lahti, Finland) and milled to obtain a particle size of $D_{50} < 30 \mu\text{m}$. RB was ground by ultra-fine grinding equipment Ceren Miller DAU (Masuko Sangyo Ltd. Co., Kawaguchi, Japan). Directly puffed extruded snacks and flakes were prepared either using 100% ERF or with 10% RB added to increase the fibre content. Salt (0.8%) was added in the dry mix of all recipes. A spiral mixer (Diosna SP 24 D, Dierks & Söhne, Osnabrück, Germany) was used to mix the raw material for 8 min. For each extrusion trial, a 2 kg mixes from each recipe was used.

2.1.2. Particle size analysis

Particle size of the ultra-fine milled RB was analysed by a Beckman Coulter LS 230 (Beckman Coulter Inc., CA, USA) using a liquid module and ethanol as a carrier. The D_{50} and D_{90} values of the RB materials were: 23.7 ± 0.8 and $155.5 \pm 9.2 \mu\text{m}$, respectively.

2.1.3. Extrusion processing

Extruded puffs and flakes were prepared using a twin screw extruder (APV MPF 19/25, Baker Perkins Group Ltd., Peterborough, UK) with a constant feed rate of 60 g/min 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. For feeding of the flour mix a co-rotating twin screw feeder, K-Tron Soder, Niederlenz, Switzerland was used. Twin screw configuration with a 3 mm die was used in all extrusion trials. Feed rate calibration was performed for each recipe before running the extruder. 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, 30 min for puffs and 60 min for flakes at 100 °C. Recipe and extrusion process parameters are shown in Table 1. The extrusion trial was repeated for extruded puffs and flakes to observe whether extrusion processing affects the product quality if extruded in a different batch. The torque values were monitored and recorded during the extrusion. Specific mechanical energy (SME) was calculated using Eq. (1):

$$SME \text{ (kW h kg}^{-1}\text{)} = \frac{\omega}{\omega_r} \times \frac{\tau}{100} \times \frac{Z_r}{Q} \quad (1)$$

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).

2.2. Starch content and moisture

Analyses of the starch content of the extruded samples were made using total starch by the AACC method no. 76.13 (AACC, 1999), insoluble and soluble dietary fibre by the AOAC method no. 991.43 (AOAC, 1992), and moisture content by drying the samples in an oven at 130 °C for 2 h. Total starch and moisture content analysis were performed as triplicates.

2.3. Structure and texture analysis

2.3.1. Macro- and microstructure

The extruded samples of each extrusion experiment were collected, dried at 100 °C for 30 min for puffs and 45 min for flakes, and cooled to room temperature. The measurements of expansion rate, specific length and piece density were made from 20 replicates from each extrusion treatment using the method described by Alam et al. (2014). Microstructures of the extruded puffs and flakes were analysed using a desktop X-ray micro tomography (XMT) system (Model 1172, SkyScan, Aartselaar, Belgium) and a method described by Alam et al. (2014). Puffed extruded samples were made by cutting 10 mm pieces from the extrudate samples with an electric saw (Power ST-WBS800, Taiwan Sheng Tsai Industrial Co. Ltd., Taiwan) and the flaked samples were analysed as is. The diameter of the puffs was approximately 16–17 mm. A desktop XMT system (Model 1172, SkyScan, Aartselaar, Belgium) with a 12-bit cooled CCD camera (512×1024 pixels) was used to collect the X-ray data. The x-ray tube was operated at a voltage of 40 kV and a current of 250 μA in order to obtain optimum contrast between air cells and cell walls. The samples were rotated a total of 180° during the scanning process with a voxel size of $11.65 \mu\text{m} \times 11.65 \mu\text{m} \times 11.65 \mu\text{m}$. The total scanning time was 18 min. The initial X-ray radiographs or raw images were

Table 1
Extrusion recipes and process parameters.

	ERF 100% puffs	ERF 90% + RB 10% puffs	ERF 100% flakes	ERF 90% + RB 10% flakes
Screw speed (rpm)	345	345	250	260
Torque (%)	86–90	86–92	42	43
Feed rate (g/min)	60	60	60	60
SME (kW·h·kg ⁻¹)	0.330–0.345	0.330–0.353	0.117	0.124

ERF: endosperm rye flour; RB: rye bran; salt (0.8%) was added to all extrusion recipes.

obtained at every 0.7° of rotation. After scanning, reconstructed images were further analysed for total, open and closed porosity (%), cell wall thickness (mm) and cell diameter (mm) by Ctan (v. 1.12, SkyScan, Belgium) image analysis software. Five different measurements from different XMT samples were performed for both puffs and flakes and the results were reported as means \pm SD. Stereomicroscopic images were captured using the protocol published by Nikinmaa et al. (2016).

2.3.2. Texture

Texture analysis of extruded puffs was performed by uniaxial compression of the extrudates by 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. Puffed samples were prepared by cutting the extruded samples to 10 mm height. The samples were deformed at 70% strain with a test speed of 1 mm/s and an acquisition rate 200 points/s. The force-deformation curve was obtained to assess the textural properties of the snack samples. The analysis was performed with 30 replicates. Texture Exponent software v.5.1.2.0 (Stable Micro Systems, UK) was used to obtain values of maximum force, actual curve length, area under the force-deformation curve and number of peaks. The number of peaks represents the number of cell wall ruptures during compression and hardness is the maximum force needed to initiate cell wall crack. Hardness (F_{max}), crispiness work (C_w) and crispiness index (C_i) were calculated using the protocol published by Alam et al. (2014). 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.

Due to the thin and flat shape of the flakes, the texture analysis protocol used for puffs was not suitable. Instead, the mechanical stress was applied onto a thick bed of flakes using a Texture Analyser TA-HDi (HD3071, Stable Micro Systems, United Kingdom) equipped with a 250 kg load cell and a 5-bladed Kramer shear cell ($90 \times 66 \times 82 \text{ mm}^3$). Test mode was set to compression and cross head speed was 1 mm/s. The samples were deformed at 25% strain and the acquisition rate was 400 points/s. The weighed amount of the samples (as is) was 35 g (about a 20 mm thick bed in the cell) and 10 replicates were made for each flake sample. The test distance used was 25 mm (cross head return distance was calibrated for 22 mm) in order to ensure that the blades passed through the respective slots of the Kramer shear cell with a clearance of 3 mm. Data obtained from the force-deformation curve were analysed in the same way as described above.

2.4. Mastication trial

2.4.1. Participants

Fifteen young female participants aged between 20 and 40 years were recruited by circulating group emails and through the bulletin boards at the University of Eastern Finland. Only female participants were included in order to avoid unnecessary variation due to the gender effect, as it has been demonstrated in the literature that there is a gender-dependent difference in mastication time (Buschang, Hayasaki, & Throckmorton, 2000). The mean age of participants was 24.6 ± 4.4 years, and their mean Body Mass Index (BMI) was $22.0 \pm 1.4 \text{ kg/m}^2$. All participants were of normal weight, they had no missing teeth (except 3rd molars), and they had no diagnosed mastication problems. Smokers were excluded from the study. The participants gave written informed consent to their participation in the study. Ethical principles of good research and clinical practice described in the declaration of Helsinki were followed during the study.

Ethical approval was obtained from the Research Ethics Committee of the Hospital District of Northern Savo, Finland.

2.4.2. Procedure

The participants attended one study visit during which all extruded samples were masticated in three replicates. The experiments took place between 8 and 11 a.m., and the participants were instructed to eat breakfast 1 to 1.5 h beforehand. They were familiarised with the study procedure before the actual mastication trial. Four extruded samples of puffs and flakes were offered to each participant in random order. The samples were blind coded by using 3-digit numbers. Each product was served and masticated in three separate portions. Portion sizes represented mouthfuls of food: puff portions consisted of $2 \times 3.5 \text{ cm}$ (diameter: 1.6–1.7 cm) ribbons and flake portions contained 10.5 g of flakes, presented as 1 tablespoon (thickness: 0.26–0.32 cm and length: 2.2–2.3 cm). The three portions of puffs weighed on average $5.8 \pm 0.7 \text{ g}$ and puffs with RB weighed $5.3 \pm 0.3 \text{ g}$. The participant masticated each portion without swallowing until she felt ready to swallow the portion. Instead of swallowing, the bolus was expectorated to a plastic container which was kept on ice. The three portions each of puffs and flakes samples were masticated in direct succession and between different products there was a break of 2 min during which the mouth was rinsed with water.

2.4.3. Electromyography measurements

The mastication process was characterised by measuring the electrical activity of facial muscles using electromyography (EMG). EMG was measured with the NeurOne system (Mega Electronics, Kuopio, Finland) using disposable dermal Ag/AgCl electrodes. The skin was cleaned with 70% ethanol and bipolar electrodes were placed on the masseter and temporalis 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 the temporal record of the experiment. From the EMG time series, the onset, duration and amplitude of each chewing event was extracted by applying wavelet filtering for the elimination of high frequencies and background fluctuations, followed by squaring and smoothing of the data. Individual chews were recognized from the derivative curve. The parameters extracted for these data were number of chews, chewing time, EMG activity time, duty cycle, force/chew and total work (EMG activity time \times force/chew). All EMG data analysis was carried out using Matlab software (The MathWorks Inc., Natick, MA, USA).

2.5. Bolus analyses

2.5.1. Saliva impregnation

Bolus saliva impregnation was determined based on the moisture contents of extruded puffs, flakes and their bolus samples. Wet bolus samples were weighed and placed in an oven at 105 °C overnight and the dried bolus was weighed again. The saliva impregnation was determined by the difference between the water content of boluses and the water content of extruded puffs and flakes.

2.5.2. Particle size distribution

The bolus samples (from eight individuals out of fifteen participants) were diluted into 100 ml of water, mixed with magnetic stirring (220 rpm) for 25 min and left to stand for 5 min in order to allow larger particles to settle to 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 larger particles were poured onto petri dishes for imaging. Around 6 to 8 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. Particle areas were determined using Cell[^]P imaging software (Olympus, Germany). The analysis of bolus particle size distribution (in total: $15 \times 4 \times 3 = 180$ bolus samples) were time consuming thus grouped in three different chew types: six average chewers, one heavy and one light chewer (in total: $8 \times 4 \times 3 = 96$ bolus samples).

2.6. Starch hydrolysis index

In vitro starch hydrolysis index (HI) was determined using a method described by Sozer, Cicerelli, Heiniö, and Poutanen (2014). A ground, extruded puff or flake sample of about 1.5 g (to get 1 g starch in the sample) was used to obtain each starch hydrolysis curve. Two different buffer addition methods (either after 15 min of soaking or directly after adding water) were tested for HI analysis. The latter method was chosen to avoid clump formation. The area under the curve (A_{uc}) was obtained after 180 min incubation with porcine pancreatic α -amylase (A6255, DFP Treated, Type I-A, saline suspension, ≥ 1000 units/mg protein, Sigma-Aldrich Co. LLC, USA). The A_{uc} was calculated using the Sigmaplot 10.0 (Systat Software Inc., Point Richmond, CA, USA) program with the preloaded macro. HI values were calculated by comparing the areas under the curve to that of wheat bread using the formula: $HI = (A_{uc} \text{ of extrudates} / \text{Average } A_{uc} \text{ of white wheat bread}) \times 100$ and the results were reported as means \pm SD.

2.7. Statistical analyses

Overall differences between study products were assessed using one-way ANOVA, and LSD was used as a post hoc analysis for pair-

wise comparison. Multivariate analysis of variance (MANOVA) was performed in order to distinguish the effects of structural, textural, mastication and bolus properties on the samples, and a separate analysis of variance (ANOVA) was performed with Bonferroni adjustment (or correction) for p value. Principal component analysis (PCA) was performed using Oblimin rotation with Kaiser normalization in order to demonstrate the correlation between structural, textural, mastication and bolus properties individually and interaction between all the studied properties. Sampling adequacy was considered acceptable when Kaiser-Meyer-Olkin (KMO) values were over 0.60 and sphericity (Bartlett's p) test values were < 0.05 . Statistical analyses were conducted using SPSS Statistics 20 (SPSS Inc., Chicago, USA) software.

3. Results and discussion

3.1. Structural characteristics

There were notable structural differences between puffs and flakes (Fig. 1, Table 3). Flakes were significantly less porous, less expanded and had higher cell wall thickness compared to puffs (Table 3). Piece density is an indicator of a products' porosity and expansion. Both flakes had higher piece density compared to puffs, and RB addition increased the piece density for both puffs and flakes. Cell diameter was considerably greater (2.4 vs 0.2 mm) for puffs regardless of RB addition (Table 3). Puffs and flakes with added RB had higher fibre content compared to those without RB (Table 2). RB addition in each group (puffs and flakes) did not cause any significant change in microstructural properties such as porosity, average cell diameter and cell wall thickness, but macrostructural properties such as expansion, specific length and piece density were significantly different.

MANOVA results for structural (macro- and microstructure) properties showed that puffs and flakes samples were significantly different ($p < 0.05$) due to the combined effect (Table 4a) of expansion, specific length, piece density, porosity, cell diameter and

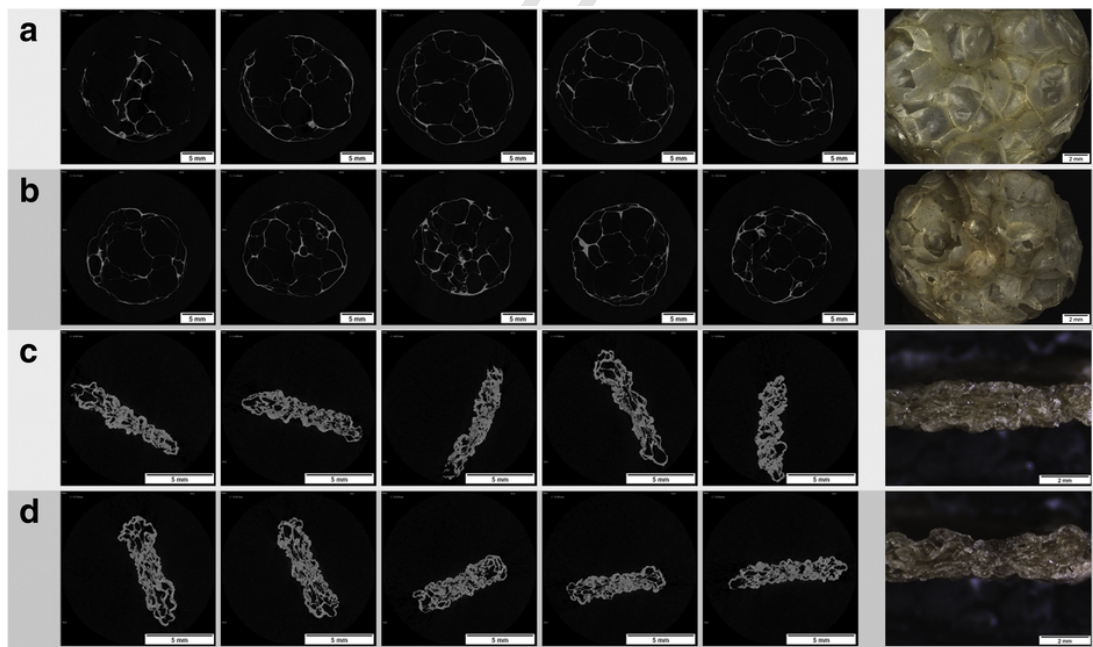


Fig. 1. 2D-XMT (images on the same line are taken from 5 different measurements from different samples; the white bar is 5 mm) and microscopy (the white bar is 2 mm) images of a) 100% endosperm rye flour puffs b) 90% ERF + 10% rye bran puffs c) 100% endosperm rye flour flakes and d) 90% ERF + 10% rye bran flakes.

Table 2

Total dietary fibre (TDF), insoluble (IDF) and soluble (SDF) dietary fibre and starch contents of extruded puffs and flakes.

	ERF 100% puffs	ERF 90% + RB 10% puffs	ERF 100% flakes	ERF 90% + RB 10% flakes
TDF (%)	5.34	8.00	6.45	8.90
IDF (%)	2.75	5.16	3.95	5.67
SDF (%)	2.59	2.85	2.50	3.23
Starch (% dry weight basis)	78.17	75.23	76.67	73.22

cell wall thickness. There was a significant difference ($p < 0.05$) between samples for each individual variable, with partial η^2 varying between 0.842 and 0.996. Expansion, porosity and piece density including DF had a slightly stronger effect on the extruded samples than specific length, cell diameter and the cell wall thickness. Contrast (simple, K matrix) results showed that all the variables played significant ($p < 0.05$) roles in making the puffs and flakes different, although expansion, porosity and cell diameter did not have significant ($p > 0.05$) effects when comparing 100% ERF flakes vs. 10% RB flakes. Moreover, all macro- and microstructural properties were jointly and individually influenced ($p < 0.05$) by fibre content (Table 4g). The PCA plot shows that a strong positive correlation was found between cell diameter and expansion and between cell wall thickness and density (Fig. 2a). Total porosity was positively correlated with expansion and cell diameter, and negatively correlated with piece density and cell wall thickness. On the other hand, increased fibre content increased specific length and piece density and decreased expansion and cell diameter.

3.2. Textural characteristics

Puffs both with or without RB were less hard and crispier (Table 3) than the flakes with similar composition. RB addition increased hardness and reduced crispiness for both puffs and flakes. Puffs and flakes were significantly different ($p < 0.05$) due to the combined (Table 4b) effect of hardness, crispiness work, crispiness index and fibre content. There was a significant difference ($p < 0.05$) between samples for each individual variable, with partial η^2 varying between 0.977 and 0.996. The differences between extruded products were more significant in terms of hardness and DF content than crispiness. Contrast results (simple K Matrix) showed that all variables played significant roles ($p < 0.05$) in making the puffs and flakes different, although crispiness index did not have a significant effect when comparing 100% ERF flakes with 10% RB-enriched flakes. Moreover, all textural properties were jointly (Table 4h) and individually influenced by fibre content ($p < 0.05$). The PCA plot shows that hard extruded puffs and flakes were less crispy (Fig. 2b). Increased fibre content increased the hardness and crispiness work and reduced the crispiness index.

3.3. Mastication properties

Flakes required more oral processing compared to puffs, reflected as significantly higher ($p < 0.05$) values in the number of chews, chewing time, EMG activity time, applied force/chew, and total work (Table 5). Samples were significantly different ($p < 0.05$) based on their mastication properties (data obtained from 15 different participants). A higher Wilk's Λ and low η^2 value indicate that although the difference between products observed based on the mastication properties was significant, the effect was not so strong enough to differentiate the products (Table 4c). There was a significant difference

($p < 0.05$) between samples for chewing time, number of bites, EMG activity time and total work, but not for duty cycle and force/chew. Contrast results (K Matrix) showed that all variables except the duty cycle played significant roles ($p < 0.05$) in differentiating the puffs and flakes. However, when comparing 100% ERF flakes and 10% RB-enriched flakes, none of the mastication properties have a strong effect indicating the difference ($p > 0.05$) between them. Moreover, all mastication properties were jointly and individually affected by the fibre content ($p < 0.05$) but force/chews did not show any significant effect (Table 4i). The PCA plot shows that number of chews and chewing time were strongly correlated with each other, and total work and force/chews also showed strong correlation (Fig. 2c). Duty cycle and fibre content were situated relatively far away in the PCA plot, and thus were not well correlated with any other mastication parameters.

3.4. Inter-individual variation in mastication and consistency of the participants

The participants were significantly different ($p < 0.05$) when jointly considering all mastication properties obtained from fifteen participants (Table 4l). There was a significant difference ($p < 0.05$) between participants for each chewing parameter except duty cycle for different products. The differences between the participants were more significant ($\eta^2 = 0.859-0.959$) in terms of total work, force/chew, chewing time and number of chews compared to EMG activity time and duty cycle ($\eta^2 = 0.309-0.749$). A PCA plot with high residual variance from the first two components (Fig. 2d) for all the variables indicates that the participants were not all consistent in explaining the mastication properties of puffs and flakes samples. There were 45% non-redundant residuals with absolute values > 0.05 .

Four extruded samples were significantly different ($p < 0.05$) when jointly considering chewing parameters (data obtained from fifteen participants) (Table 4d). Out of fifteen participants, 90% had significantly ($p < 0.05$) different total work, 67% had different chewing time and 60% had different numbers of chews and EMG activity time. Only 20% of participants had shown differences ($p < 0.05$) in force/chew, and the duty cycle was different for only one participant. Based on the above results it can be concluded that total work and chewing time were the most important parameters, followed by number of chews. More than 60% of participants were consistent in chewing/evaluating the extruded samples. In the PCA plot, since the individual residual variance from the first two components (Fig. 2e) for all the parameters was high, it may be concluded that not all participants were consistent in explaining the mastication properties of puffs and flakes samples. There were 40% non-redundant residuals with absolute values > 0.05 .

3.5. Bolus properties

Disintegration of the samples into particles was examined for the bolus samples ($n = 8$), grouped in three different chew types: six average chewers, one heavy and one light chewer (Fig. 3). A set of granulometric curves based on the data obtained from particle size measurements was produced to visualise the particle area distribution (Fig. 4). From the cumulative surface (%) vs particle area curve it is clear that puffs and flakes can be easily discriminated after mastication based on their particle size and particle area distribution. Visual examination of the photographs showed that puffs were masticated to smaller particles than flakes, and that RB addition increased the proportion of smaller particles (Figs. 4a-d). However, bolus samples with added RB also contained bigger particles than samples with

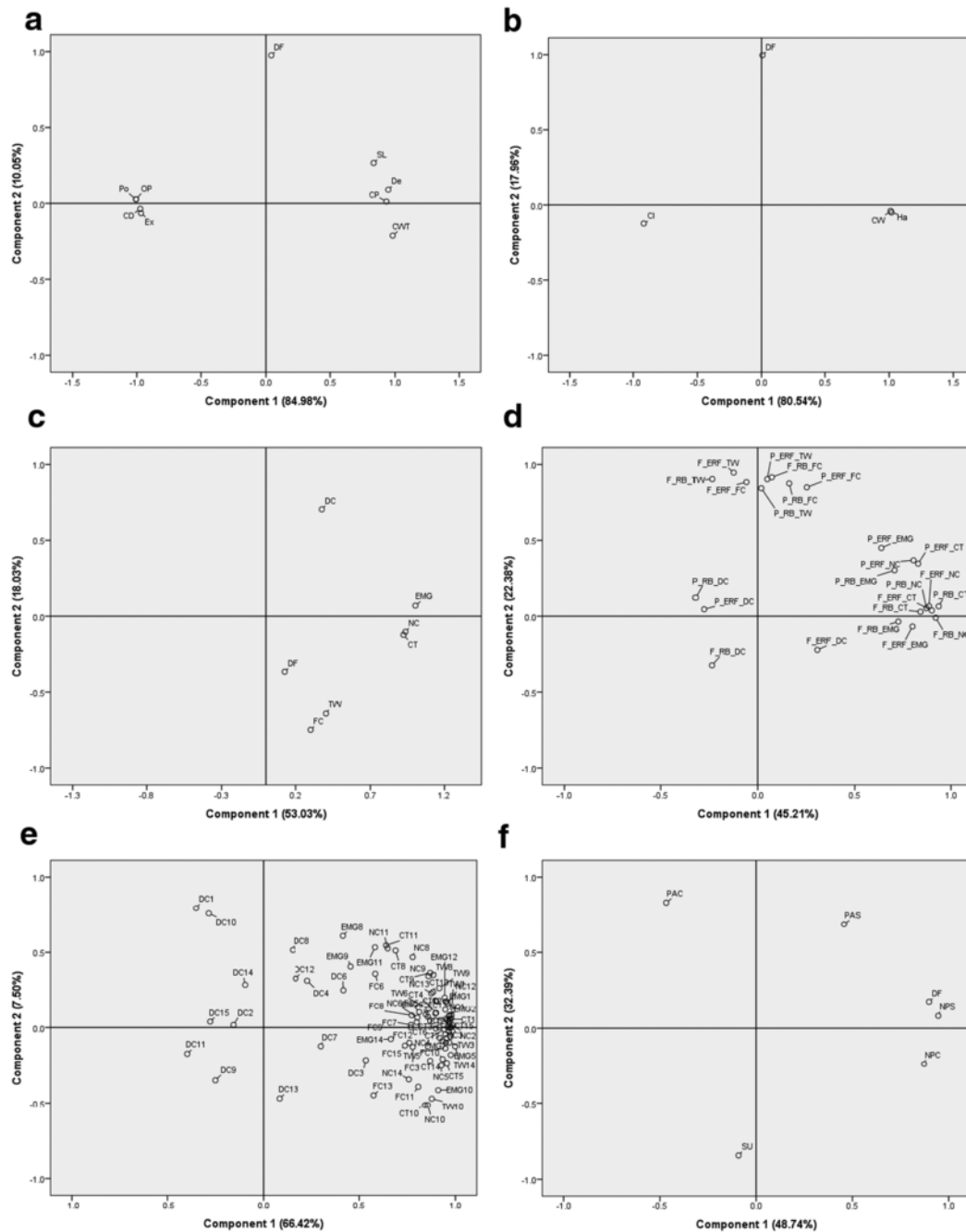


Fig. 2. PCA plots of **a)** macrostructural (Ex expansion ratio, De piece density and SL specific length) and microstructural properties (Po total porosity, OP open porosity, CP closed porosity) [KMO = 0.831 & Bartlett's $p = 0.000$], **b)** textural properties (Ha hardness, CW crispiness work, CI crispiness index) [KMO = 0.721 & Bartlett's $p = 0.000$], **c)** mastication properties (NC number of chews, CT chewing time, EMG EMG activity time, DC duty cycle, FC force/chew, TW total work) [KMO = 0.603 & Bartlett's $p = 0.000$], **d)** effects of mastication properties on puffs and flakes (P_ERF 100% rye flour puffs, F_ERF 100% rye flour flakes, P_RB 10% rye bran puffs and F_RB 10% rye bran flakes) [KMO = 0.681 with Bartlett's $p = 0.000$], **e)** inter-individual variation of the participants 1–15 [due to the large number of variables the matrix was not a positive definite, and thus KMO and Bartlett's tests were not performed] and **f)** bolus properties (NPS number of particles by sample, NPC number of particles by participants, SL saliva uptake, PAS particle area by sample and PAC particle area by participants) [KMO = 0.658 & Bartlett's $p = 0.000$]. DF total dietary fibre and HI *in vitro* hydrolysis index of puffs and flakes made of 100% endosperm rye flour (ERF) and 90% ERF + 10% rye bran (RB).

100% endosperm rye flour (ERF). These observations were confirmed by data on particle size distribution presented as granulometric curves showing the cumulative percentage of the total area occupied by particles (Fig. 4). Puffs and flakes with added RB had the highest share of small particles compared to corresponding products without RB. The presence of larger bolus particles in both flake samples was detected in the granulometric curves as a lower share of

particles < 100 μm^2 than in puffs. Both the removal of the smallest particles and the effect of swelling may have had an influence on the result. However, the effect of the removal of the smallest particles was the same for all the samples. As the analysis was carried out in water, the particles with less saliva were most probably swollen to some extent. Nevertheless, most of the differences in saliva uptake were not statistically significant. Mean particle area of the puffs

Table 3
Structural and textural properties of the extruded puffs and flakes.

	ERF 100% puffs	ERF 90% + RB 10% puffs	ERF 100% flakes	ERF 90% + RB 10% flakes
Macrostructure				
Expansion rate (%)	570.67 ± 17.13 ^d	531.60 ± 24.64 ^c	106.88 ± 21.93 ^b	87.83 ± 9.67 ^a
Specific length (m/kg)	37.82 ± 1.72 ^a	40.07 ± 3.57 ^a	59.90 ± 7.92 ^b	71.44 ± 5.05 ^c
Piece density (kg/m ³)	115.41 ± 9.18 ^a	126.79 ± 18.83 ^a	2229.98 ± 580.40 ^b	2632.76 ± 430.46 ^c
Microstructure				
Porosity (%)	96.78 ± 0.44 ^b	96.48 ± 0.62 ^b	33.72 ± 4.65 ^a	36.66 ± 4.57 ^a
Open porosity (%)	96.78 ± 0.44 ^b	96.47 ± 0.62 ^b	33.72 ± 4.87 ^a	35.74 ± 4.90 ^a
Closed porosity (%)	0.01 ± 0.01 ^a	0.17 ± 0.22 ^a	1.48 ± 0.41 ^b	1.41 ± 0.52 ^b
Mean cell diameter (mm)	2.43 ± 0.25 ^b	2.31 ± 0.21 ^b	0.18 ± 0.03 ^a	0.17 ± 0.04 ^a
Mean cell wall thickness (mm)	0.12 ± 0.01 ^a	0.12 ± 0.01 ^a	0.17 ± 0.01 ^c	0.15 ± 0.01 ^b
Texture				
Hardness (N)	25.64 ± 0.26 ^a	29.73 ± 1.0 ^b	1868.02 ± 80.42 ^c	2107.10 ± 96.93 ^d
Crispiness work (× 10 ⁻²) (Nmm)	63.22 ± 6.33 ^a	73.35 ± 5.72 ^b	4266.64 ± 307.72 ^c	4954.43 ± 398 ^d
Crispiness index (× 10 ⁻⁷)	258,199 ± 12,635 ^d	184,155 ± 29,363 ^c	6.38 ± 1.02 ^b	4.21 ± 0.39 ^a

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

Table 4
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 sample by				
a. Macro- and microstructure * DF	0.000006	F (27, 59) = 135.14	0.000	0.982
b. Texture * DF	0.000005	F (12, 66) = 550.13	0.000	0.983
c. Mastication properties * DF	0.293129	F (18, 145) = 4.37	0.000	0.336
d. Mastication properties (15 participants)	0.000001	F (24, 4) = 17.40	0.011	0.990
e. Bolus properties * DF (8 participants)	0.001760	F (12, 66) = 55.32	0.000	0.879
f. Macro- and microstructure * texture * mastication * DF * HI * bolus properties (8 participants)	0.000000	F (66, 22) = 151.76	0.000	0.998
Effect of DF on				
g. Macro- and microstructure	0.000362	F (32, 75) = 17.84	0.000	0.862
h. Texture	0.000709	F (12, 66) = 80.29	0.000	0.911
i. Mastication properties	0.317482	F (15, 143) = 4.95	0.000	0.318
j. Macro- and microstructure * texture * mastication * HI * bolus properties (8 participants)	0.000000	F (84, 30) = 23.70	0.000	0.984
Effect on HI by				
k. Expansion * hardness * piece density * porosity * crispiness index * DF	0.000004	F (66, 86) = 18.85	0.000	0.913
Effect on participants by				
l. Mastication properties (15 participants)	0.000000	F (336, 130) = 7.07	0.000	0.923

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

Table 5
Mastication parameters of the samples.

	ERF 100% puffs	ERF 90% + RB 10% puffs	ERF 100% flakes	ERF 90% + RB 10% flakes
Number of chews	17.7 ± 5.7 ^a	16.2 ± 5.50 ^a	29.2 ± 10.4 ^b	30.0 ± 7.4 ^b
Chewing time (s)	11.8 ± 4.2 ^a	10.7 ± 3.95 ^a	19.0 ± 6.7 ^b	19.9 ± 5.7 ^b
EMG activity time (s)	3.3 ± 1.1 ^a	3.0 ± 1.06 ^a	5.6 ± 2.6 ^b	5.6 ± 1.5 ^b
¹ Duty cycle	0.3 ± 0.04 ^a	0.3 ± 0.02 ^a	0.3 ± 0.03 ^a	0.3 ± 0.02 ^a
² Force/chew (%)	191.4 ± 68.6 ^a	193.6 ± 69.5 ^a	248.4 ± 74.0 ^b	254.5 ± 77.5 ^b
² Total work (%)	215.3 ± 118.6 ^a	193.3 ± 92.0 ^a	438.4 ± 175.7 ^b	473.4 ± 174.2 ^b
Saliva uptake (g/1 g puffs and flakes)	0.47 ± 0.10 ^a	0.45 ± 0.10 ^{ab}	0.38 ± 0.07 ^{ab}	0.37 ± 0.09 ^b
Moisture (%)	10.01 ± 0.01 ^c	10.02 ± 0.27 ^c	6.50 ± 0.01 ^a	7.49 ± 0.06 ^b

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

¹ EMG activity time/chewing time.

² Normalized to the corresponding values of a reference product (white wheat bread).

(0.568 and 0.325 mm² with and without RB, respectively) and flakes (1.134 and 0.521 mm² with and without RB, respectively) varied between 0.325 and 1.134 mm² and the average of all four puffs and flakes was 0.637 mm². Four extruded samples were significantly different ($p < 0.05$) when jointly (Table 4e) considering bolus properties and DF (data obtained from eight participant). The PCA plot shows that the total particle area ($p < 0.05$, $r = 0.566$) and the number of particles ($p < 0.05$, $r = 0.876$) were positively correlated with the fibre contents of the sample (Fig. 2f). Large inter-individual differences in bolus particle area of different participants masked the effect of the fibre effect. Peyron et al. (2011) found that at swallowing point, the mean particle size of cereal flakes was 1.52 mm. A higher number of chews (puffs < flakes) does not always result in a higher number of small particles, because of the loss of many small particles during intermediary swallows (Jalabert-Malbos et al., 2007).

3.6. Hydrolysis index of puffs and flakes

In the current study, extruded puffs and flakes had high hydrolysis indices (85.9%–94.5%) (Fig. 5). Previous studies also showed that extrudates had a high starch hydrolysis rate, regardless of their dietary fibre content (Anguita, Gasa, Martín-Orúe, & Pérez, 2006; Brennan, Merts, Monro, Woolnough, & Brennan, 2008). Both high shearing and high temperature profile of extrusion processing dis-

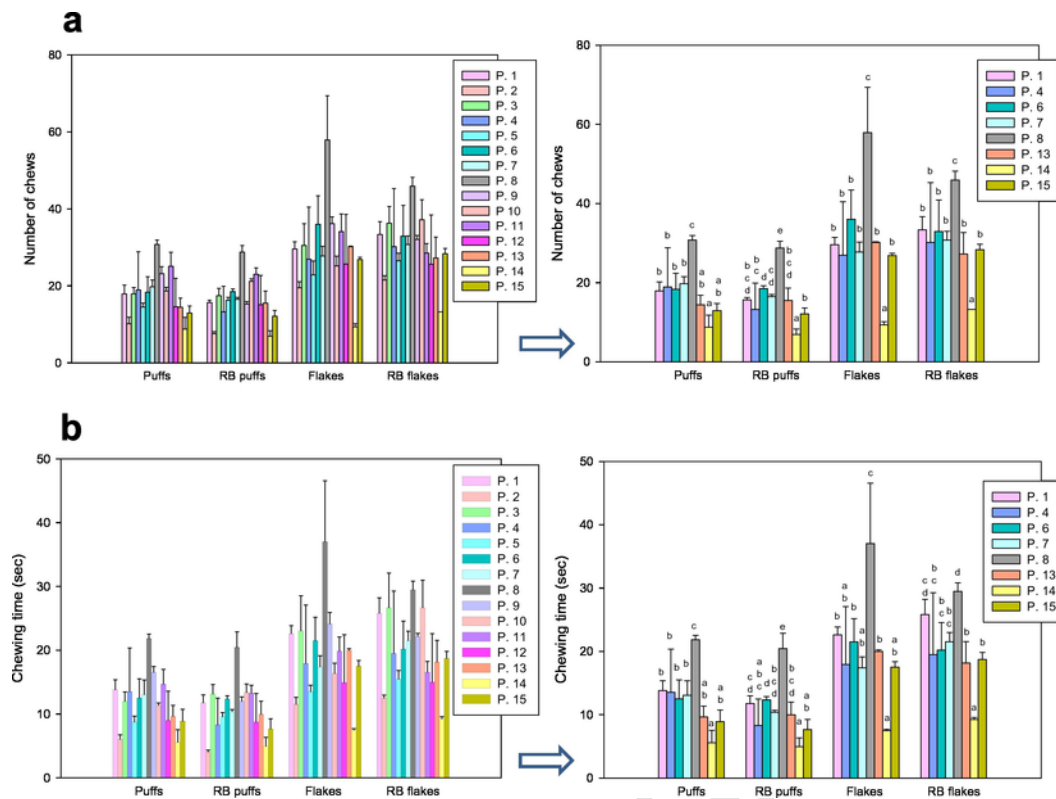


Fig. 3. Six average, one heavy and one light participants were chosen based on the mastication parameters of puffs and flakes chewed by 15 female participants: **a)** number of chews and **b)** chewing time. Data shown here are means of three replicates \pm SD. The bars marked with different letters within each samples were significantly different ($p < 0.05$).

rupts the structural integrity of starch granules and increases their susceptibility to enzymatic breakdown, leading to an increase in the hydrolysis rate. In the current study, no difference in HI was observed between puffs and flakes made with 100% ERF. However, the HI of both puffs and flakes with added RB was significantly higher than that of puffs and flakes made with 100% ERF. RB addition increased the rate of starch digestion already in the early phase. After 30 min of enzymatic incubation, RB-enriched puffs (71.4 ± 1.5) and flakes (71.3 ± 2.8) had higher percentages of hydrolysed starch compared to the puffs (63.7 ± 2.2) and flakes (64.1 ± 2.9) without bran addition.

Insoluble DF content was increased by RB addition, which caused significant changes in the structural and textural properties of puffs and flakes. The HIs of rye puffs and flakes were significantly different ($p < 0.05$) when jointly considering expansion, hardness, piece density, porosity, crispiness index and DF (Table 4k). There was a significant difference ($p < 0.05$) between samples for each individual variable, with partial η^2 varying between 0.957 and 0.997. DF content showed the strongest effect, followed by texture, macro- and microstructure, on the HI of the puffs and flakes. A strong positive correlation between DF and HI ($p < 0.05$, $r = 0.916$) indicates that increasing fibre content increased HI for puffs and flakes. The increased starch hydrolysis by bran addition might be associated with disruption in the starchy matrix due to the presence of insoluble bran particles. A similar phenomenon was earlier observed for bran-enriched pasta, in which higher *in vivo* glucose responses were observed after bran addition. The difference was concluded to result from the less dense structure of the pasta with bran addition, attributable to small fractures within the pasta matrix (Eelderink et al., 2015). There were clear structural and textural differences between

flakes and puffs, which ultimately created differences in particle size (both in the bolus samples but also in the milled samples for the *in vitro* starch digestibility tests). After *in vivo* or *in vitro* mastication (simulated by milling), flakes had more fine particles compared to puffs, which further increased with bran addition. The presence of fine particles might have increased the availability of starch for digestive enzymes. *In vitro* starch HI of RB-enriched extrudates might be dependent on the concentration of the bran addition. In a recent study (Alam et al., 2016), we observed that when the concentration of RB decreased from 30% to 15%, there was a significant increase in the HI (69.0 vs 74.6). Addition of finely milled RB ($D_{50} = 23.7 \mu\text{m}$) might also have an effect on increased HI. Significant increase ($p < 0.05$) in the HI was observed in 15% fine ($D_{50} = 28 \mu\text{m}$) RB-enriched extruded puffs (74.6%) compared to puffs made of coarse ($D_{50} = 440 \mu\text{m}$) RB (70.3%) with a similar bran concentration (Alam et al., 2016). According to these results, the use of coarse bran at higher addition levels looks like a good strategy to decrease the HI. However, as it significantly interferes with the structural and textural properties, we recommend the use of fine bran to create palatable high fibre extrudates.

On the other hand, Brennan et al. (2008) observed slightly reduced starch hydrolysis of the extrudates (TDF: 15%) when either guar gum (soluble fibre) or wheat bran (insoluble fibre) were added as DF source. However, the hydrolysis of guar gum-enriched extrudates was significantly lower compared to the extrudates made of wheat bran. Considering the results obtained in the current study, but also based on existing literature, it is clear that the effect of dietary fibre addition on HI is dependent on various factors such as the fibre type, concentration, particle size and subsequent effects of the fibre on structure.

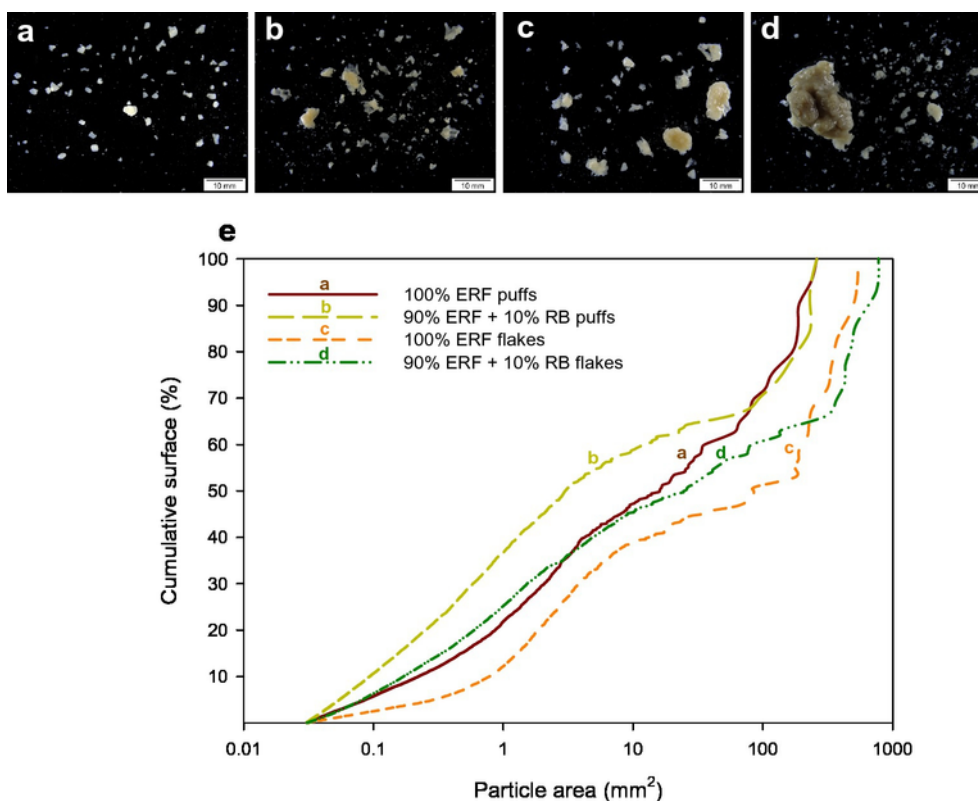


Fig. 4. Photographs of masticated samples of **a**) 100% ERF puff, **b**) 90% ERF + 10% RB puff, **c**) 100% ERF flake, **d**) 90% ERF + 10% RB flake (the white bar is 10 mm), and **e**) particle area distribution the same samples. The curves represent a cumulative percentage of the total area occupied by particles obtained from the boluses of 8 individual participants.

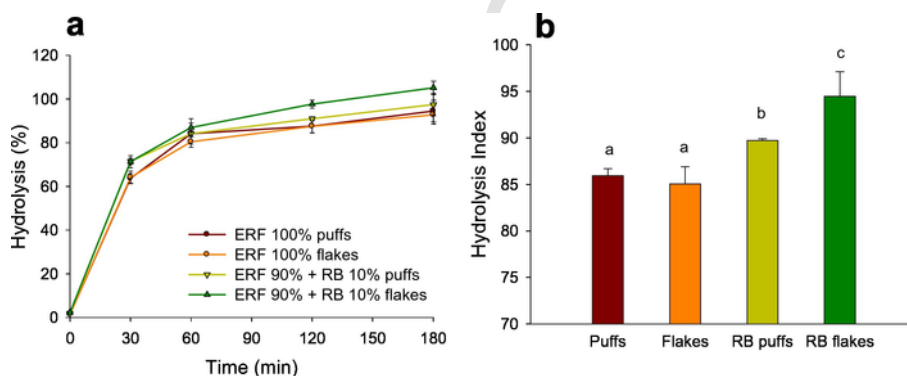


Fig. 5. **a**) *In vitro* starch hydrolysis (% of hydrolysed starch) of extruded puffs and flakes during 180 min incubation. **b**) Hydrolysis indices (HI = (A_{uc} of puffs or flakes / Average A_{uc} of white wheat bread) \times 100) of puffs and flakes. Values are means of three replicates \pm SD. The bars marked with different letters were significantly different ($p < 0.05$).

3.7. Interactions between structure, texture, mastication and bolus properties

Puffs and flakes were significantly different ($p < 0.05$) when all the studied properties were taken into account (Table 4f). All structural and textural properties and hydrolysis index were significantly different ($p < 0.05$) within the product, with a partial η^2 variation between 0.816 and 0.996. The differences between products were more significant in terms of hardness, piece density, expansion rate, porosity, number of particles by participants, number of particles by samples and DF content. In terms of mastication properties, the dif-

ferences between the products were significant ($p < 0.05$) only for total work (data obtained from 8 participants).

Contrast results (K Matrix) showed that all ($p < 0.05$) variables except EMG activity time, duty cycle, force/chew, average particle area by participants and saliva uptake played a significant role in making the puffs and flakes different when 10% RB puffs were compared to 10% RB flakes. When the 100% ERF and 10% RB-enriched flakes were compared, there were no statistically significant differences in the mastication. Hardness and piece density dominated the product structure but there was no significant effect ($p > 0.05$) for crispiness index, expansion, porosity or cell diameter. However, all studied properties except duty cycle, work per chew and saliva up-

take were jointly (Table 4j) and individually influenced by fibre content ($p < 0.05$).

The properties of puffs and flakes, representing hard and dense structure, respectively, were closely packed in the right side of the first component matrix, making a cluster, whereas the properties representing expanded and crispy structure were loaded in the left hand side of the first component matrix and closely packed together (Fig. 6). Mastication properties were weakly loaded in the first component matrix and formed a separate cluster. The number of particles by sample and by participants, hydrolysis index and total dietary fibre were loaded in the second component matrix. The component correlation matrix results with low coefficient value ($= 0.098$) indicated that the variables/parameters loaded in the first and second component were not correlated with each other. Mean particle area by participants and duty cycle were weakly loaded in the plot due to the low loading value and high residual values.

Increase in cell diameter makes both products less hard and vice versa. Increased average cell diameter resulted in an increase in the C_i and decrease in the hardness and C_w of both extruded puffs and flakes, indicating that extrudates with lower hardness values were more expanded and crispier. We have previously reported that crispiness increased (high C_i and low C_w) with the increase of average cell diameter, indicating that extrudates with larger air cells were less hard and crispier (Alam et al., 2014). Increased crispiness with reduced hardness was also reported for bran-enriched oat extrudates in a previous study (Sibakov et al., 2015). High dietary fibre content in extruded snack products by RB addition has also earlier resulted in high hardness, low crispiness and poor microstructural properties of extruded snacks based on corn, wheat, rye and oat (Heiniö et al., 2016).

Flakes required more work for mastication and more force/chew than puffs, owing to their dense structure, low cell diameter and thick cell walls. The results are in line with a previous study in which bread with higher bulk density required higher total work and force/chew compared to lower density wheat bread matrices (Pentikäinen et al., 2014). Flakes had a hard, crunchy texture, whereas puffs had an easily broken, crispy texture. Food texture plays a major role in disintegration. Hard food products, in general, require longer mastication time and more chewing cycles for insalivation to produce a lubricated bolus (Jalabert-Malbos et al., 2007). Increased food hardness has earlier been shown to correlate positively with the number of masticatory cycles in different natural foods such as salted and roasted peanuts, raw and peeled carrots and raw and pickled gherkins (Jalabert-Malbos et al., 2007).

Rye bran addition increased hardness and decreased crispiness of both flakes and puffs. However, this variation observed in instrumental textural properties was not sufficient to induce changes in mastication behaviour. Jalabert-Malbos et al. (2007) reported that within different food categories (such as vegetable, meat, cheese and egg), product texture had an effect on disintegration but the disintegration still varied depending on the individual participants. Fontijn-Tekamp et al. (2000) and Le Bleis, Chaunier, Montigaud, and Della Valle (2016) reported that extended chewing time had very little effect on particle size reduction but it rather allowed more saliva uptake for agglomeration of the fractured particle to form a suitable bolus. This is in agreement with our study, in which the flakes had a bigger share of large particles and a longer chewing time compared to the puffs. Therefore, both particle size reduction and saliva uptake are crucial during mastication.

Crispier and less hard extrudates disintegrated rather easily and produced more particles in the bolus (number of particle for puffs vs

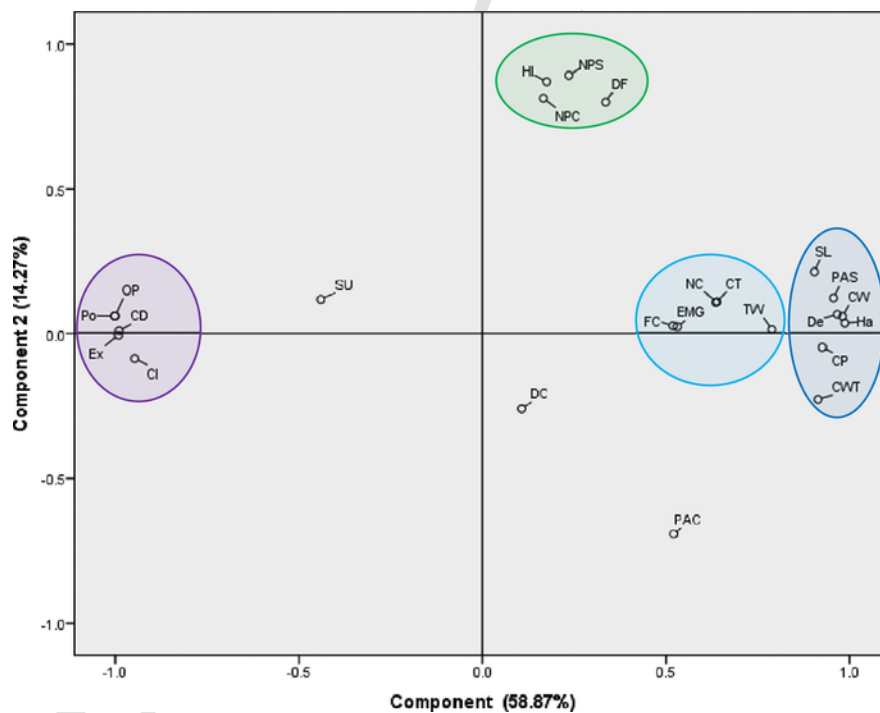


Fig. 6. PCA [KMO = 0.722 & Bartlett's $p = 0.000$] plot showing the correlation between macro- (Ex expansion ratio, De piece density and SL specific length) and micro- (Po total porosity, OP open porosity, CP closed porosity) structural and textural properties (Ha hardness, CW crispiness work, CI crispiness index), fibre content (DF total dietary fibre), *in vitro* hydrolysis (HI hydrolysis index) of extruded puffs and flakes and their mastication (NC number of chews, CT chewing time, EMG EMG activity time, DC duty cycle, FC force/chew, TW total work) and bolus properties (NPS number of particles by sample, NPC number of particles by participants, SL saliva uptake, PAS particle area by sample and PAC particle area by participants). Mastication and bolus properties were obtained from 8 individual participants.

flakes: 718 vs 387/g of bolus sample; RB puffs vs RB flakes: 1406 vs 916/g of bolus sample). This led to increased total surface area of the disintegrated particles, which required more saliva for bolus formation and particle agglomeration. Saliva impregnation in masticated samples was on average 0.46 and 0.37 g saliva/g puffs and flakes, respectively. Inter-individual variation in saliva uptake was considerable, as was that in mastication pattern. Saliva uptake was negatively correlated (Fig. 6) with hardness ($p < 0.05$, $r = -0.422$) and positively with crispiness ($p < 0.05$, $r = 0.399$). A similar result was reported for crispy bread by Pangborn and Lundgren (1977), who reported that small particles of crisp bread required more saliva for lubrication and agglomeration to form a cohesive bolus which was easy to swallow.

In our study, it was noticeable that RB addition increased the number of smaller particles both for puffs and flakes. Reduction in starch content and increase in TDF content (Table 2) by RB addition made the matrices less porous and denser, resulting in a harder and less crispy product. Textural properties such as hardness have a significant influence on chewing and bolus formation. Chen, Khandelwal, Liu, and Funami (2013) observed that increased food hardness tends to result in smaller bolus particles, which is in line with our results within product categories. Boluses of relatively hard foods (e.g. nuts and cheese) were shown to have a smaller mean particle size compared to the boluses obtained from soft food (e.g. jelly and peach). It can be hypothesised that RB made the puffs and flakes less cohesive, and thus cluster formation of numerous small particles was less likely during bolus formation. The presence of smaller particles in RB-enriched puffs and flakes increased the susceptibility of starch to digestive enzymes and led to increased HI. Singh, Dartois, and Kaur (2010) reported in their review that increase in surface area by lowering the particle size distribution of the starch granule leads to a higher degree of starch hydrolysis.

The dominating factor in the variation of mastication process, particle size distribution and saliva uptake was inter-individual variability, e.g. some persons chewed for a longer period of time, and their bolus samples had uniform small particles, whereas others chewed for shorter periods of time and were ready to swallow already when some particles were still significantly large and uneven in shape. In earlier studies a wide variation between individuals has also been reported for mastication parameters and particle size distribution (Foster, Woda, & Peyron, 2006; Jalabert-Malbos et al., 2007). Le Bleis et al. (2016) reported that particle size of extrudate bolus before swallowing varied between 0.7 and 2.66 mm due to inter-individual differences. Therefore, inclusion of a higher number of participants in this study would possibly have led to the detection of small differences in the mastication patterns due to minor structural differences between samples within each sample type.

4. Conclusions

Puffs and flakes were notably different in their structure and texture and the differences were reflected in their mastication properties. Puffs with less hard and crispy structure degraded to smaller particles in oral processing and required less work for bolus formation than flakes, but the wide inter-subject variation in mastication prevented the detection of the effects of such subtle structural adjustment on mastication properties. Bran addition resulted in increased hardness and reduced crispiness for both puffs and flakes, but significant increase in density was observed only for flakes. Hardness and density dominated the mastication properties, rather than crispiness. The harder puffs or flakes required more mastication. In contrast, when the product become expanded and crispy it required less mastication.

Despite the striking differences in structure and texture, the hydrolysis index of puffs and flakes did not differ. However, addition of rye bran increased the number of smaller particles in the bolus as well as the hydrolysis rate, which was noticeable in the early phase of digestion at 30 min, indicating that the disintegration process and consequently the particle size of the bolus had a significant role on the starch digestion rate. Therefore, a small increase in fibre content in an extruded matrix may not always reduce HI but, as seen in these data, the effect may even be opposite.

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