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Citation for the original published paper (version of record):
Lu, J., Hansson, H., Johansson, D. et al (2024). Microstructure and viscosity of in vitro-digested rye and wheat food products. Food Hydrocolloids, 154. http://dx.doi.org/10.1016/j.foodhyd.2024.109990

(article starts on next page)

Food Hydrocolloids 154 (2024) 109990

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Microstructure and viscosity of in vitro-digested rye and wheat food products

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ARTICLE INFO

Keywords:
Grain bread
Microstructure
In vitro digestion
Rheology
Glucose response

ABSTRACT

Understanding rye and wheat digestion is vital for evaluating impacts on nutrient availability and glycaemic responses. This study investigated the disintegration of processed high-fibre rye foods and refined wheat products during simulated intestinal digestion, aiming to link product characteristics with nutrient liberation. The overarching aim was to elucidate how these rye products contribute to the observed benefits in human intervention studies, particularly regarding satiety, weight loss, and metabolism.

Analysis included four wholegrain rye products and three refined wheat products, spanning yeast-fermented breads and un-leavened cereal products. Microstructure examination revealed larger, partially intact digesta particles in wholegrain rye products after 120 min of digestion, alongside more aggregated and less degraded starch granules compared to refined wheat bread. Fermented rye bread exhibited greater degradation of sub-aleurone cell wall fragments than un-fermented rye bread. Viscosity assessments indicated lower viscosity for wheat products than for rye products, with yeast-fermented soft rye bread and rye crispbread showing notably lower viscosity than unfermented rye products. Post-digestion carbohydrate analysis uncovered higher glucose and maltose release during digestion for wheat products. PCA analysis confirmed negative correlations between glucose and maltose release and rye products, characterized by larger post-digestion bolus particles and higher dietary fibre. The elevated cell wall content in rye products acted as a protective barrier for starch granules, mitigating swelling and amylose release, explaining the observed viscosity differences between wheat and rye products and potentially influencing starch digestibility. Consequently, rye products undergo slower and less complete digestion than wheat, aligning with findings from human intervention studies.

1. Introduction

Non-communicable diseases (NCDs) represent a significant cause of global morbidity and mortality. Among NCDs, cardiovascular diseases and type 2 diabetes are the most prevalent and are increasing in both developed and developing countries (Balakumar, Maung-U, & Jagadeesh, 2016; Nazir, 2017). Modifiable risk factors including diet play a crucial role for their prevention (Joseph et al., 2017). Epidemiological studies consistently show that a high intake of whole grain is associated with lower risk of developing type 2 diabetes, cardiovascular diseases (Hu et al., 2020; Reynolds et al., 2019; Shivakoti et al., 2022).

Among whole grains, rye has the highest content of dietary fibre and whole grain rye has been shown to increase satiety compared with refined wheat (Forsberg, Åman, & Landberg, 2014, pp. 1–9; Johansson, Lee, Risérus, Langton, & Landberg, 2015). Rye also contains dietary fibre with different properties compared with other grains (Jonsson, Andersson, Erik, et al., 2018). For example, arabinoxylan is the most abundant fibre and has higher solubility than in wheat (Glitsø et al., 1999). Among a wide variety of bioactive compounds, the content of lignans, alkylresorcinols, phytosterols and benzoxazinoids is reported to be higher in rye than in wheat (A. A. M. Andersson, Dimberg, Åman, & Landberg, 2014). A recent intervention study found that a high intake of high-fibre whole grain rye foods significantly reduced body weight and fat mass compared to refined wheat after a 12-week hypocaloric intervention (Iversen, Jonsson, & Landberg, 2022). A diet rich in rye has also been shown to increase gut fermentation products in the circulation and in feces (Landberg, 2022; Liu et al., 2021) beneficial postprandial glycemic responses (Iversen et al., 2022) and increased energy excretion in feces (Isaksson et al., 2013), all of which may contribute to weight management and have beneficial metabolic effects. However, research

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https://doi.org/10.1016/j.foodhyd.2024.109990
Received 30 October 2023; Received in revised form 29 February 2024; Accepted 11 March 2024
Available online 2 April 2024

has shown that the specific structural and fibre characteristics of cereals are affected by different processing techniques, such as fermentation and extrusion, leading to the transformation of starch, protein, non-starch polysaccharides, with implications for appetite and metabolic responses in humans (Johansson et al., 2015, 2018; Zamaratskaia et al., 2017). Refined wheat bread typically has a porous structure, as wheat gluten proteins form a viscoelastic network that traps gas generated by yeast fermentation (Collar, 2018; Delcour et al., 2012). In contrast, rye bread is generally fermented by lactic acid bacteria, resulting in a more solid and dense foam structure with fewer pores (Alam, 2020). In rye bread, the continuous network is formed by a protein-starch matrix, leading to smaller pores and a higher number of large particles (Pentikäinen et al., 2014). As a result, the structure of rye bread is typically stiffer than that of wheat bread, with weaker gas-holding properties. This can be mainly attributed to the water-binding substance arabinoxylan, which has a greater effect on bread rheology and gas retention properties than protein (Föste, Verheyen, Jekle, & Becker, 2020; Heiniö et al., 2016).

Digestion of wheat and rye foods is a complicated process that involves multiple enzymes and is influenced by fibre content (Amaral, Guerreiro, Gomes, & Cravo, 2016). Rye contains high-molecular-weight fibre that can slow absorption of glucose and affect hormonal responses *in vivo* (Juvonen et al., 2009), whereas refined wheat bread is rapidly broken down in the small intestine, leading to a rapid increase in blood glucose and insulin levels (Seal, Courtin, Venema, & de Vries, 2021; Svihus & Hervik, 2016). Fermented rye bread produced with lactic acid bacteria can reduce post-meal responses (Jalili, Nazari, & Magkos, 2023), but the underlying mechanisms, apart from slower gastric emptying at high acid concentrations (Holm, Hagander, Bjorck, Eliasson, & Lundquist, 1989), are not fully understood. Controlled dietary interventions in humans provide the strongest causal evidence of nutrient release and absorption but have limitations in revealing the underlying mechanisms. *In vitro* methods are often used to study the behaviour of food components during digestion and could provide a good model system to monitor the release of carbohydrates from cereal products, microstructural changes and the relationships between them (Bohn et al., 2018; Brodkorb et al., 2019; Minekus et al., 2014). However, most previous *in vitro* studies have focused on a single product type and its processing parameters, with comparative studies of different food products and processes currently lacking. In particular, the relationships between product properties, disintegration during *in vitro* digestion, end-product food structure and dynamic liberation of nutrients have not been thoroughly examined.

The objective of this study was to examine the disintegration process of differently processed high-fibre rye foods in comparison to their corresponding refined wheat foods during simulated intestinal digestion. Our aim was to assess the connection between product characteristics, including composition and microstructure, and the liberation of nutrients *in vitro*. This investigation sought to gain insights into the underlying factors contributing to the observed beneficial effects of these rye products on satiety, weight loss, and metabolism in human intervention studies. Four high-fibre rye-based food products processed in different ways and three correspondingly processed refined wheat products, all differing in protein/starch matrix microstructure and the distribution and integrity of dietary fibre, were selected for the study. Digesta viscosity, microstructural properties of the digesta and the release of glucose and maltose from these products during *in vitro* digestion were analysed. Multivariate analysis was used to identify relationships between bread characteristics, intestinal phase bolus viscosity and sugar release.

2. Material and methods

2.1. Food products and chemicals

The rye and wheat products analysed were: two extruded products, made from wheat (W-ext) and rye (R-ext); two yeast fermented crispbreads, made from wheat (WCB) and rye (RCB); one unfermented rye crispbread (uRCB) for which no yeast, sourdough or leavening agent have been used and (uRCB); and two yeast fermented soft breads, one of baguette type made from refined wheat flour (WB) and a wholegrain rye bread made from wholegrain rye flour (RB). The extruded products and yeast fermented soft breads were provided by Lantmännen, while the crispbreads were supplied by Wasabröd. Ultrapure water from a MilliQ Advantage A10 was used for all preparations and samples. Chemicals used were human salivary α-amylase (cat. No. 1031), porcine pepsin (P7012), bovine bile (B3883), porcine pancreatin (P7545), haemoglobin from bovine blood (H6525), p-toluene-sulfonyl-L-arginine methyl ester (TAME; T4626), a Bile Acid Assay Kit (MAK 309), and NaHCO3 (6329), MgCl2(H2O)6 (9272), (NH4)2CO3 (207,861), and Tris-HCl buffer (T3253) (all obtained from SigmaAldrich); CaCl2(H2O)2, NaOH (50% Emsure), KCl, trichloroacetic acid and maltose (all obtained from Merck); hydrochloric acid, KH2PO4, and NaCl (obtained from VWR International); 3,5-dinitrosalicyclic acid (obtained from AlfaAesar); sodium potassium tartrate (obtained from FisherScientific); soluble potato starch (obtained from AGROS Organics); and a Total Starch HK kit (obtained from Megazyme). Glutaraldehyde, ruthenium red, OsO4, ethanol and LR white were also used in the study.

2.2. Sampling and analytical methods

2.2.1. In vitro digestion

All rye and wheat products were subjected to *in vitro* gastrointestinal digestion. The INFOGEST protocol for static *in vitro* simulation of gastrointestinal food digestion was followed (Brodkorb et al., 2019), with some modifications. To prepare simulated salivary fluid (SSF), gastric fluid (SGF) and intestinal fluid (SIF), the method described in the INFOGEST protocol (Brodkorb et al., 2019) was employed. The solution for the simulated intestinal phase was prepared from bile acids (20 mM) and pancreatin (equivalent to 100 U trypsin activity/mL). Dry matter content of the different products was calculated by measuring the weight before and after 24 h of drying at 110 °C. Samples of 1.5 g of each product, based on dry matter content, were used in analyses. For WB and RB, only the soft inner parts of the bread were used. For each of the seven products, triplicate samples were prepared for three sampling time points during the simulated intestinal digestion phase (0 min, 30 min, 120 min) giving 63 tubes of samples in total (nine per product). For the simulated oral phase, the samples were first ground (pestle and mortar) in dry state and then transferred to 50-mL plastic conical centrifuge tubes together with SSF (3 mL) containing 150 U/mL human salivary amylase activity. The samples were mixed using a Vortex and then incubated at 37 °C for 120 s with continuous mixing in a water bath. Oral/salivary digestion was stopped after 120 s by adding a pre-determined volume of 5 M hydrochloric acid to reach pH 3 in the samples, which were then immediately put on ice. This way of performing a simulated oral phase was selected to manage 63 samples with less downstream variation between samples given that no advanced techniques for simulating an oral disintegration were available in the lab. The 1:2 ratio for the oral phase is a deviation from the 1:1 ratio recommended in the INFOGEST method (Brodkorb et al., 2019). Five of the seven products were very dry (crispbreads and extruded products) and this ratio was tested out as a minimum ratio, still being able to mix the food product with the SSF to obtain a bolus. To simulate the gastric phase, 4.5 mL SGF containing 4000 U/mL pepsin activity were added to the samples from the simulated oral phase and incubated at 37 °C for 2 h in a shaking incubator at 400 rpm. Gastric digestion was stopped by adding a pre-determined volume of 5 M sodium hydroxide to reach pH 7 in the samples, which were then put on ice. For the simulated intestinal phase, 9 mL SGF containing 100 U/mL trypsin activity from pancreatin were added to the samples from the simulated gastric phase to a final volume of 18 mL. To represent the intestinal phase at time point zero, three samples of each product were immediately removed and

submerged in an ice-salt-water bath. The remaining 42 samples underwent the same incubation process as the gastric phase and were kept at 37 °C. At the 30-min mark, three sample tubes of each product were removed from the incubator and placed in an ice-salt-water bath. After an additional 90 min, the remaining 21 samples were removed from the incubator and placed in an ice-salt-water bath.

2.2.2. Microstructure analysis of product and digesta with microscopy

Samples collected at 0, 30 and 120 min during simulated intestinal digestion were immediately embedded for further microscopic analysis and characterisation of microstructure. The embedding protocols used were according to Johansson, Gutiérrez, et al. (2018) with slight modifications. Samples, kept in ice-salt-water bath after the sampling, were directly after the digestion fixed in 5% glutaraldehyde containing 0.2% ruthenium red at a ratio of 1:1 (v/v) in Eppendorf tubes for 12 h at 4 °C. After two rinses (0.1 M phosphate buffer, pH 6.8), the supernatant was removed and replaced with 1% OsO_4 for fixation for at least 2 h at room temperature. The fixed digested samples and cold gelling agar (1:1) were first embedded using a plate mould and then dehydrated in a series of aqueous ethanol solutions of increasing ethanol concentration, ending with infiltration using LR white resin. To ensure representative digestive samples, triplicate samples were embedded. An ultra-microtome (Leica EMUC6, Leica, Austria) was used to acquire embedded sections (0.8 μm thick). Multiple staining was applied to the sections. Lugol's solution (0.05 g/L iodine) to detect starch and protein, calcofluor white (0.1 g/L) for cell walls. Bright field and epifluorescence images were obtained using a Nikon Eclipse Ni–U microscope with 20× (N.A. 0.75, W.D. 1 mm) and 40× (0.95 NA) objectives. The images were captured with a Nikon Digital Sight DS-Fi2 camera with resolution 2560 × 1920 pixels. The ImageJ software (available at Fiji. se/Fiji) was utilized to adjust brightness and contrast in light microscopy graphs and split channels in epifluorescence images. All images were in RGB colour and saved as TIFF files. (Nordlund, Katina, Mykkänen, & Poutanen, 2016; D. P. Johansson, Gutiérrez, et al., 2018).

2.2.3. Bolus viscosity analysis

Viscosity of the samples after 120 min of simulated intestinal digestion was determined immediately after the sampling in particle-free solutions using a strain-controlled rheometer (ARES-G2, TA Instruments, DE, USA) with 40 mm plate-plate geometry. The instrument was calibrated in terms of friction, inertia and rotational mapping prior to the measurements and the temperature was set to 37 °C. In total, 1.5 mL of whole content of each sample was transferred to Eppendorf tubes, kept in salt-ice-water bath, and centrifuged at 4 °C for 10 min at 13,400 rpm in an Eppendorf Centrifuge 5430 R. A 0.63 mL portion of the supernatant was placed on the bottom plate, after which the upper plate was lowered while rotating slowly. Each sample measurement began with a 60-s hold to equilibrate the temperature to 37 °C, followed by a logarithmic sweep (10-100 s^{-1}, 5 data points/10s^{-1} increase) and then a reversed logarithmic sweep (100-10 s^{-1}, 5 data points/10 s^{-1} decrease). Data were analysed and viscosity was calculated using TA Instrument Trios software v.5.0.0.44608.

2.2.4. The release of glucose and maltose

Immediately after the sampling at 0, 30 and 120 min, 1.5 mL of the whole content of each sample was transferred to Eppendorf tubes, kept in salt-ice-water bath, and centrifuged at 4 °C for 10 min at 13,400 rpm in an Eppendorf Centrifuge 5430 R. The supernatants were transferred to new tubes and kept in −20 °C freezer until analysis. For analysis, samples were carefully thawed in ice-cold water, diluted 20-fold (time point 0) or 500-fold (time points 30 and 120 min) with MilliQ water, then put in vials and kept at 4 °C in the autosampler for analysis. Quantification of glucose release in each sample was performed using ion chromatography. Carbohydrates in samples were analysed and quantified using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a Dionex ICS3000 with a PA1 separation column. Sample volume used was 5 microL and flow rate 0.25 mL/min. Eluents were: A MilliQ, B 20 mM NaOH and C 200 mM NaOH. The sequence was: isocratic elution from time −2 min (prior to injection) to +5 min, a gradient to 10% B at +35 min, isocratic elution at 10% B to +45 min followed by regeneration (gradient to achieve 100% C at time point 50 min and then immediate change to 100% A with isocratic elution to time point 55.1 min). Post-column addition of 0.3 NaOH for detection using PAD was performed at a flow of 0.125 mL/min. Wave form used was the Standard Quad for carbohydrates and standards used for quantification were 2.5, 5, 10, 25 and 50 mg/L of glucose and of maltose.

2.3. Principal component analysis (PCA)

Differences in viscosity and total soluble sugar release (glucose and maltose) between the differently processed breads were analysed by one-way ANOVA, followed by Tukey's post-hoc test using Minitab software (Minitab Inc., State College, PA, USA) for comparisons, with $P < 0.05$ considered statistically significant.

Multivariate principal component analysis (PCA) was used to screen for similarities, differences and distinct groupings of the samples and measurements. Bi-plots of the PCA score and loading plots were created to reveal the location of the differently processed breads and measured values in a two-dimensional plot that showed variation in the values for differently processed breads by compressing it to the most two dominant factors. Mean values of all measurements were used for PCA analysis. The validity of the PCA model, shown as R^2X, indicated the proportion of X variation explained by the model and Q^2 values showed goodness of prediction of the model. All multivariate analyses were performed using SIMCA-17.0 (Umetrics, Sweden).

3. Results and discussion

3.1. Composition and microstructural characterisation of wheat and rye products

Table S1 shows the chemical composition of the products used in human intervention studies conducted by Iversen et al. (2021). As expected, notable differences were found in carbohydrate, protein and dietary fibre content between wheat and rye products. In products processed by fermentation and extrusion, wheat-based products contained higher amounts of carbohydrates and proteins, while rye-based products contained higher amounts of dietary fibre. Among products within the same category, total fibre content ranged from 3.53 g (WB) to 5.56 g (WCB) in wheat-based products, and from 10.62 g (RB) to 19.27 g (uRCB) in rye-based products, i.e. it was affected by the processing method used. The yeast fermented soft wheat bread (WB) had the lowest fibre content (3.53 g), including arabinoxylans (1.24 g) and fructans (0.15 g). The arabinoxylan content varied slightly between crispbread (WCB) and extruded product (W-ext) (2.56 and 2.36 g, respectively). A similar pattern was observed for the rye-based products, with the lowest arabinoxylan content found in RB and relatively similar levels in RCB and R-ext. Unfermented rye crisp bread (uRCB) had the highest total dietary fibre content (19.27 g) and arabinoxylan content (8.43 g), while the extruded rye product (R-ext) contained the highest amount of fructans (3.85 g).

The microstructural features of the cereal products were significantly affected by type of raw material (wheat or rye) and form of processing (extrusion or fermentation). In WB and WCB, the continuous protein phase was distinguishable as yellow areas (iodine-stained) throughout the bread fragments (Fig. 1a). Swollen and elongated starch granules were observed, and starchy granules were oriented and partly fused with neighbouring granules, but most still retained their granular identity. Iodine-stained amylose and amylopectin in WB and WCB appeared blue and brown, respectively, and were non-uniformly distributed in the granules. Amylose-accumulation areas appeared as elongated regions in

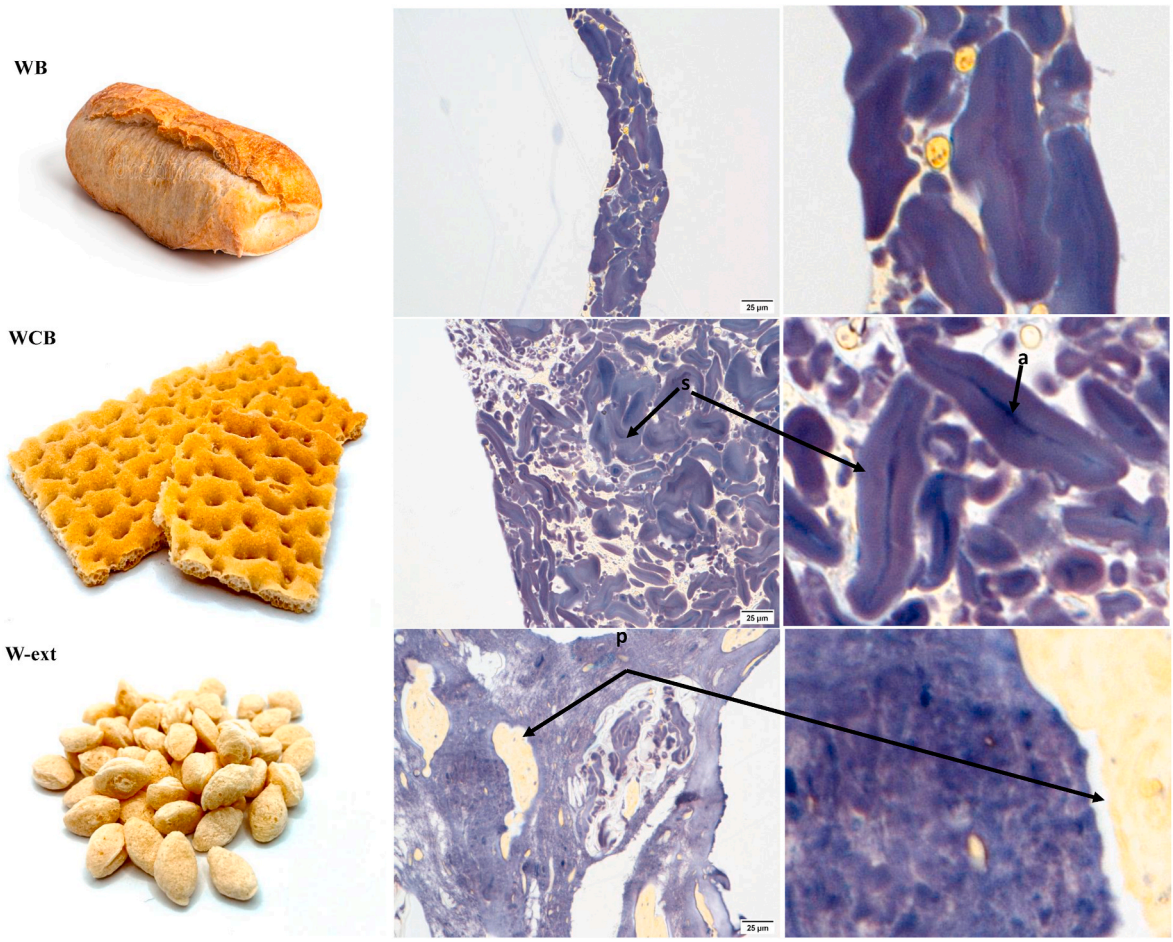

Fig. 1a. Product images and light microscopy micrographs stained with iodine showing the microstructure of undigested wheat bread with the magnification (20×, 40×). Protein (p) appears yellow, starch (s) purple, amylopectin brown and amylose (a) blue. Cell walls (cw) are unstained. WB: wheat soft bread. WCB wheat crisp bread. W-ext: wheat puffs (extruded).

the inner of the large starch granules, while amylopectin accumulation areas occurred in the surrounding phase, along the starch protein interface. In contrast, the rye bread products RB and RCB were characterized by a continuous phase composed of highly swollen and tightly packed starch granules encapsulating proteins, which were packed into discrete aggregates. There was also more prevalent leakage of amylose from the starch granules, which was visible in micrographs as blue particles in the centre of the swollen granules and between the granules, especially for RB (Fig. 1b). Large bran pieces were present and there also appeared to be a higher content of aleurone layers, encapsulating mainly protein in the rye bread products, especially uRCB. Unfermented rye bread had a dense and compact structure, with small air pockets. In contrast, RCB had a more open and airy crumb structure, with larger and irregular air pockets from yeast fermentation. In the extruded products (W-ext and R-ext), processing led to loss of integrity of starch granules and formation of a homogenous starch phase with entrapped protein aggregates and fibre particles.

Epifluorescence analysis revealed the presence and location of proteins and cell walls in the wheat (Fig. 2a) and rye products (Fig. 2b). Proteins appeared in magenta (stained with acid fuchsin) and cell walls in deep blue (Calcofluor white) (left column in Fig. 2, the right column shows the same sections in black and white (b-w)). For both colour and b-w images, the background had to be adjusted to compensate for low-level binding of acid fuchsin to the plastic resin. In all products, small fragments of cell walls were detected in the aqueous phase, together with detached starch granules and protein aggregates. The aleurone and subaleurone layers of unfermented rye bread contained a high concentration of fibre, with larger and thicker pieces of intact cell wall fragments distributed throughout the matrix. In contrast, the cell walls of yeast-fermented rye bread were less dense and more porous, with a thinner outer layer and more air pockets. Under the microscope, the cell wall structure of unfermented rye bread appeared intact, thick and tightly packed. The blue fluorescence of the starch endosperm was weaker and lost continuity in deeper layers, indicating lower dietary fibre content and cell wall damage in those areas.

These results provided insights into the microstructure and chemical composition of rye and wheat products and the effects of processing techniques on structural features of the starch/protein matrix in these products. Microstructural analysis of the rye products revealed higher density of cell walls compared with the wheat products, which indicates higher dietary fibre content, especially arabinoxylan. Arabinoxylan is known to be a primary component of non-starch polysaccharides in cell walls (Ain et al., 2018; Frølich, Åman, & Tetens, 2013) and rye products are an excellent source of dietary fibre (Jonsson, Andersson, Bach Knudsen, et al., 2018).

As expected, our analyses revealed that the production process significantly affected the structural properties of the wheat and rye food products. In fermented products, hydration of cereal flour at elevated temperatures can activate endogenous enzymes and lead to the degradation of dietary fibre, such as arabinoxylans, resulting in a lower fibre content (Rakha, Åman, & Andersson, 2011). Hydration of flour also occurred during the production of unfermented rye crispbread (uRCB), although degradation of the aleurone and subaleurone layers appeared to be less pronounced than in the fermented rye products (RB, RCB).

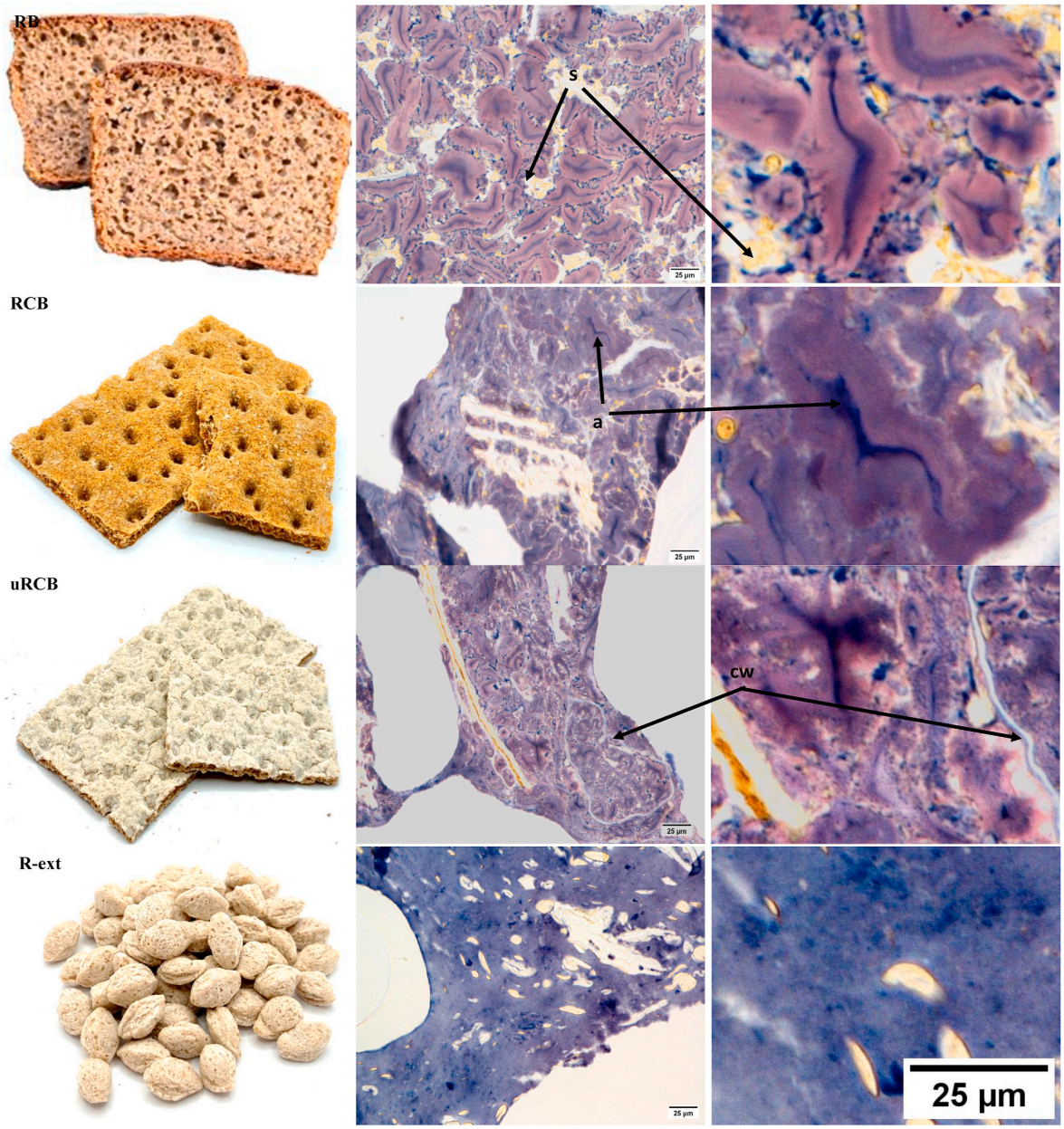

Fig. 1b. Product images and light microscopy micrographs stained with iodine showing the microstructure of undigested rye products with the magnification (20×, 40×). Protein (p) appears yellow, starch (s) purple, amylopectin brown and amylose (a) blue. Cell walls (cw) are unstained. RB: rye soft bread. RCB: rye crisp bread. uRCB: unfermented rye crisp bread. R-ext: rye puffs (extruded).

This observation may be explained by the endogenous arabinoxylan-degrading enzymes being mainly active at higher temperatures and lower pH. Thus, the lower pH during fermentation may promote degradation of arabinoxylan, which does not occur in the production process for unfermented rye products (Johansson, Gutiérrez, et al., 2018; Loponen et al., 2009; Rakha, Åman, & Andersson, 2010). For rye products, it has been shown that extrusion increases the content of extractable arabinoxylans (Wang, Klopfenstein, & J.G.Ponte, 1993)) and disintegration of cell wall structures, resulting in increased solubility of arabinoxylans (Maina et al., 2021, pp. 1–29). Our observations confirmed these results in that processing technique had a significant impact on the composition and structural features of cereal products in ways that can affect their nutritional and functional properties.

3.2. *Effect of digestion on microstructure*

The microstructure of the digested residuals after static *in vitro* digestion of the seven wheat and rye products (WB, WCB, W-ext, RB, RCB, uRCB and R-ext) was analysed using bright field microscopy after 30 and 120 min of simulated intestinal digestion, but also directly after the simulated gastric phase (0 min of intestinal digestion) (Fig. 3a and b). During the oral and gastric digestion phases, the protein/starch matrix of both wheat and rye products was affected to some extent, forming pieces of bolus containing aggregates of starch granules and protein. The digested particles of refined wheat bread (WB and WCB, Fig. 3a) were broken down and partially fractured into different size of fragments. The protein network of the wheat bread residuals was hydrolysed and freely distributed starch granules were evident in the sample. Compared with the wheat breads, the digested particles of rye breads (RB, RCB, uRCB, Fig. 3b) seemed less disintegrated, retaining the

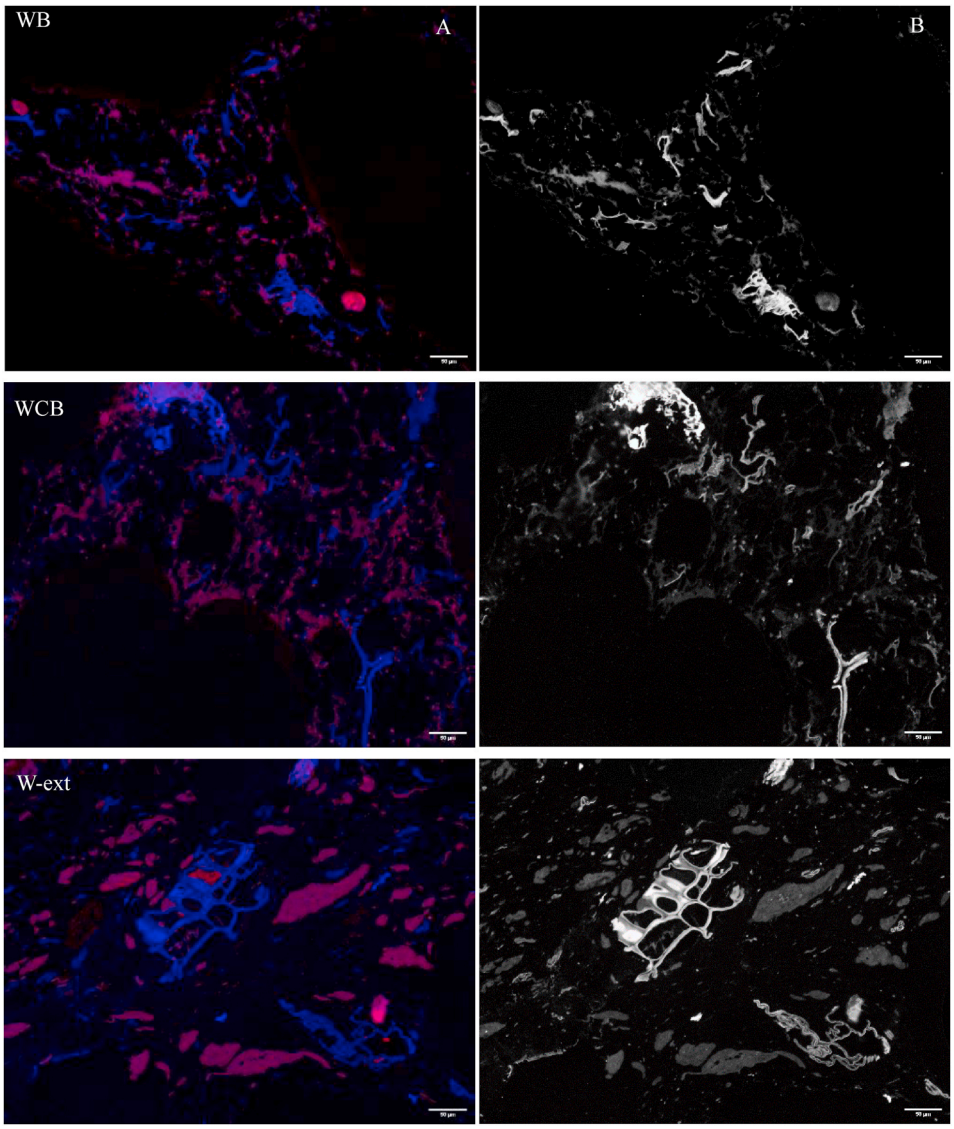

Fig. 2a. Epifluorescence images (column A) showing the microstructure of protein stained with acid fuchsin (purple fluorescence) and cell walls stained with calcofluor white (blue fluorescence). Images of column B showing cell wall structure in blue channel stained with Calcofluor white (20×). WB: wheat soft bread. WCB wheat crisp bread. W-ext: wheat puffs (extruded).

original structural matrix of the raw product with a continuous network formed by aggregated starch granules and with the protein acting as a filler. The aleurone layer appeared mainly intact and protein (coloured yellow by iodine) was clearly visible inside the aleurone cells, associated with the (weakly stained) starch phase. In the extrusion products (W-ext and R-ext), the protein/starch matrix appeared to be compacted into aggregates, whereas no significant degradation of protein and starch granules seemed to occur during the oral and gastric phases. Large pieces of digesta particles and some intact cells were visible in the extrusion product bolus. Differences in the microstructure of refined wheat and wholegrain rye bread digesta particles were apparent already after the oral and gastric phase (0 min intestinal digestion) but were more pronounced after the intestinal phase, especially at the endpoint of intestinal digestion (120 min) (Fig. 3). In refined wheat bread (WB and WCB), the gluten protein network was extensively hydrolysed. There was also a noticeable transition in WB and WCB during the intestinal digestion phase, from larger pieces of digested residuals containing aggregates of starch granules and proteins to smaller aggregates and an increasing proportion of freely dispersed starch granules. In contrast, the rye bread digesta particles resembled intact fragments of food matrix in millimetre size. In addition, some amylose leaked out from the starch granules to form aggregates in the rye bread residuals. Compared with the beginning of the intestinal phase (0 min) (Fig. 3), it was apparent that intestinal digestion also caused some degradation of the aleurone cell walls adjacent to the endosperm part of the rye grain (i.e. the inner part of the aleurone cell), although intact bran particles were still present. There were indications of protein degradation, with less yellow colour in the micrographs from time point 120 min compared with time point 0 min (Fig. 3). There were also differences between the rye bread products, as fragments of subaleurone cell walls were degraded to a greater extent in the fermented rye breads (RB and RCB) compared with the unfermented rye bread (uRCB).

During simulated gastrointestinal digestion, the physical characteristics of the original food products had a significant impact on the digestion behaviour. Comparisons of rye and wheat products revealed that larger particles containing intact proteins and starch granules were present in the digesta of rye products. This can be attributed to the partially intact botanical structure of wholegrain rye products (cell walls), which prevents enzymatic hydrolysis of the continuous starch phase during digestion (Li, Chen, Bui, Xu, & Dhital, 2021). In contrast,

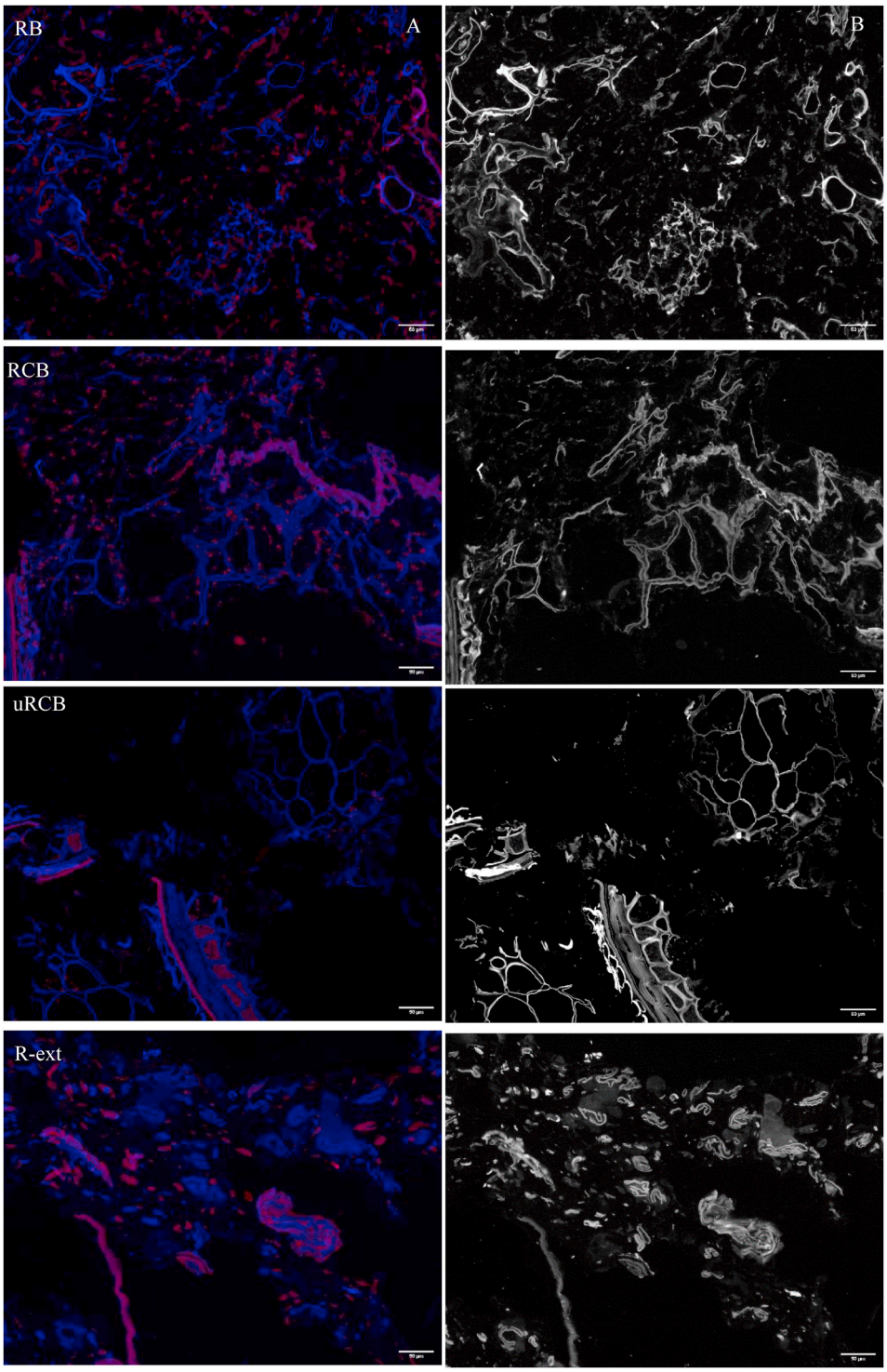

Fig. 2b. Epifluorescence images (column A) showing the microstructure of protein stained with acid fuchsin (purple fluorescence) and cell walls stained with calcofluor white (blue fluorescence). Images of column B showing cell wall structure in blue channel stained with Calcofluor white (20×). RB: rye soft bread. RCB: rye crisp bread. uRCB: unfermented rye crisp bread. R-ext: rye puffs (extruded).

the protein network in wheat products was more readily hydrolysed during digestion, leading to lower presence of intact proteins and freely distributed starch in the digesta. Thus enzymatic degradation of protein is likely to be more critical for wheat products than for rye products, which is consistent with previous findings by Nordlund et al. (2016) and Johansson, Gutiérrez, et al. (2018). In the extruded products (W-ext and R-ext), digestion may have been impacted by the extrusion process, which altered its physical structure. The high temperature and pressure involved in extrusion can lead to gelatinisation of starch granules in the flour and cause changes in their crystalline structure (Y. Wang et al., 2021). Our results indicated that the extruded samples had a more compact and uniform structure than traditionally baked bread, which could affect the accessibility of starch molecules to digestive enzymes and thus the rate of starch digestion. However, a previous study observed no larger starch fragments at any point during gastric digestion, indicating rapid disintegration of starch (D. Johansson, 2016).

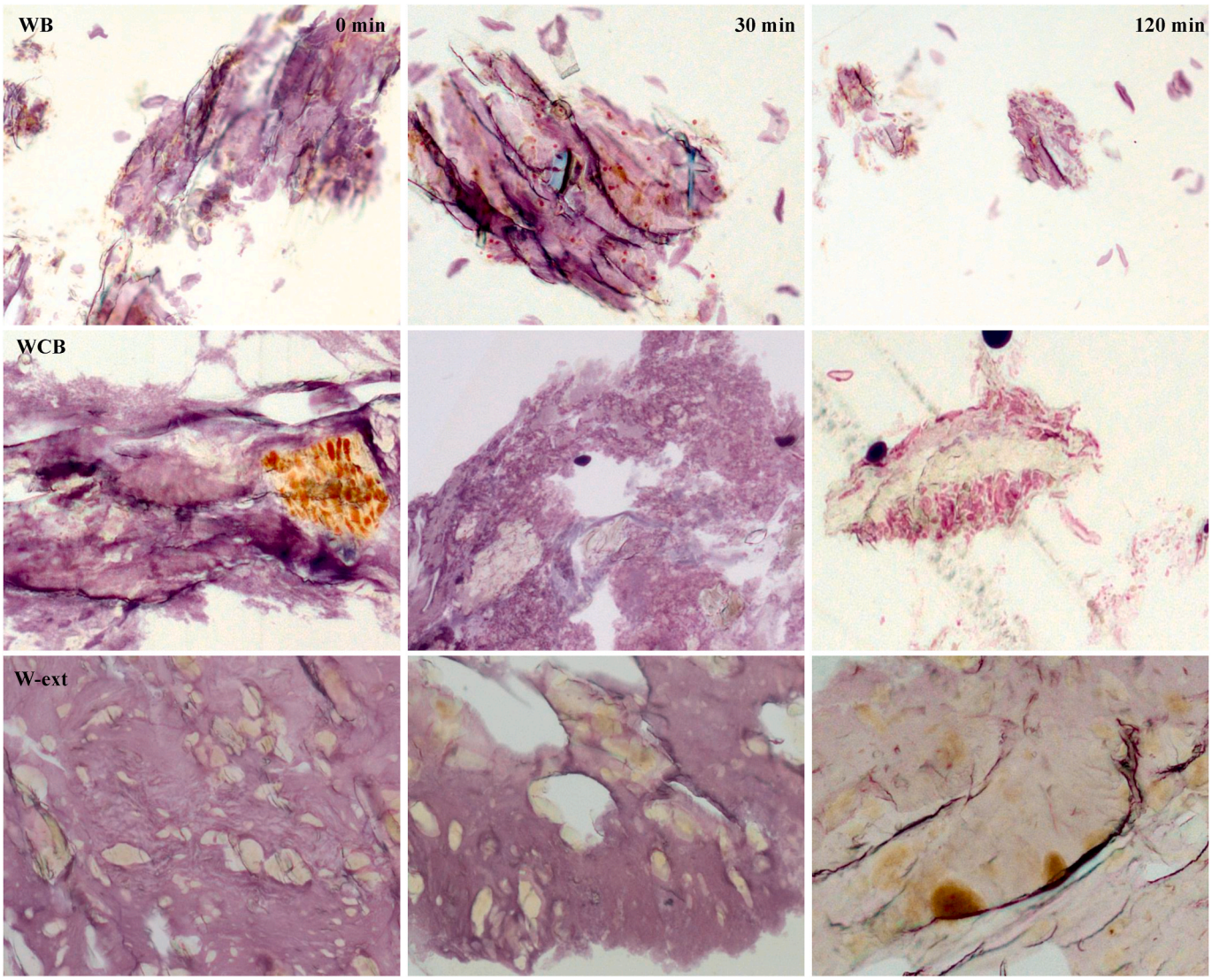

Fig. 3a. Light microscopy micrographs stained with iodine showing the microstructure of differently processed bread collected at 0 min, 30 min and 120 min during small intestinal (SI) digestion. Protein appears yellow, starch purple, amylopectin brown and amylose blue. Cell walls are unstained. WB: wheat soft bread. WCB wheat crisp bread. W-ext: wheat puffs (extruded).

3.3. Effect of digestion on viscosity

Viscosity measured after 120 min of simulated intestinal digestion is shown in Fig. 4 for each of the products, with the error bars indicating the variation between replicate samples. The highest viscosity after simulated intestinal digestion was seen for the extruded rye product R-ext, followed by uRCB. The lowest viscosity was seen for WB, although all three wheat products had similarly low values. The measurements indicated a clear difference between wheat and rye products, where all three wheat products had lower viscosity after 120 min of intestinal digestion than the rye products, but also between yeast fermented and unfermented rye products, where RB and RCB formed an intermediate group. In wholegrain rye flour, the main contributor to variation in the viscosity of water-soluble dietary fibre is water-soluble arabinoxylan (Bengtsson, Andersson, Westerlund, & Åman, 1992). The rye products used all have a higher total fibre content than the used wheat products. Thus, the higher viscosities recorded for the different rye products, compared with the wheat products, are best explained by the presence of more soluble fibre, mainly arabinoxylans, after the digestion.

In a previous study comparing different crispbreads, it was found that the viscosity increased during simulated gastric (*in vitro*) digestion for unfermented rye crispbread (Zamaratskaia et al., 2017). It has been shown for rye and oat products that the fermentation process results in reduced molecular weight (Mw) of soluble arabinoxylans and β-glucans, which is suggested to depend on endogenous enzymes activated during fermentation (Andersson, Fransson, Tietjen, & Åman, 2009; Johansson, Gutiérrez, et al., 2018; Rakha et al., 2010; Zamaratskaia et al., 2017). In unfermented bread, this effect should be less pronounced, resulting in higher Mw soluble dietary fibre and thus higher viscosity. This is also a plausible explanation for the higher viscosity observed for uRCB and R-ext than for the fermented product RCB and the soft RB. In a recent study on arabinoxylan and wheat bread, both endogenous and added arabinoxylans retained their structural integrity throughout a simulated *in vitro* digestion process (Zhang et al., 2023). However, based on the xylanase activity of the wheat flour used, those authors concluded that endogenous enzymes were unlikely to have affected the arabinoxylan content during fermentation of the bread. Higher viscosity of the digesta has been shown to reduce gastric emptying rate and to reduce the absorption rate of nutrients due to lower retention time. This has implications for release of satiety hormones and self-reported satiety. Furthermore, viscosity is known to be a major determinant in modulation of postprandial glucose responses and blood lipid profiles after

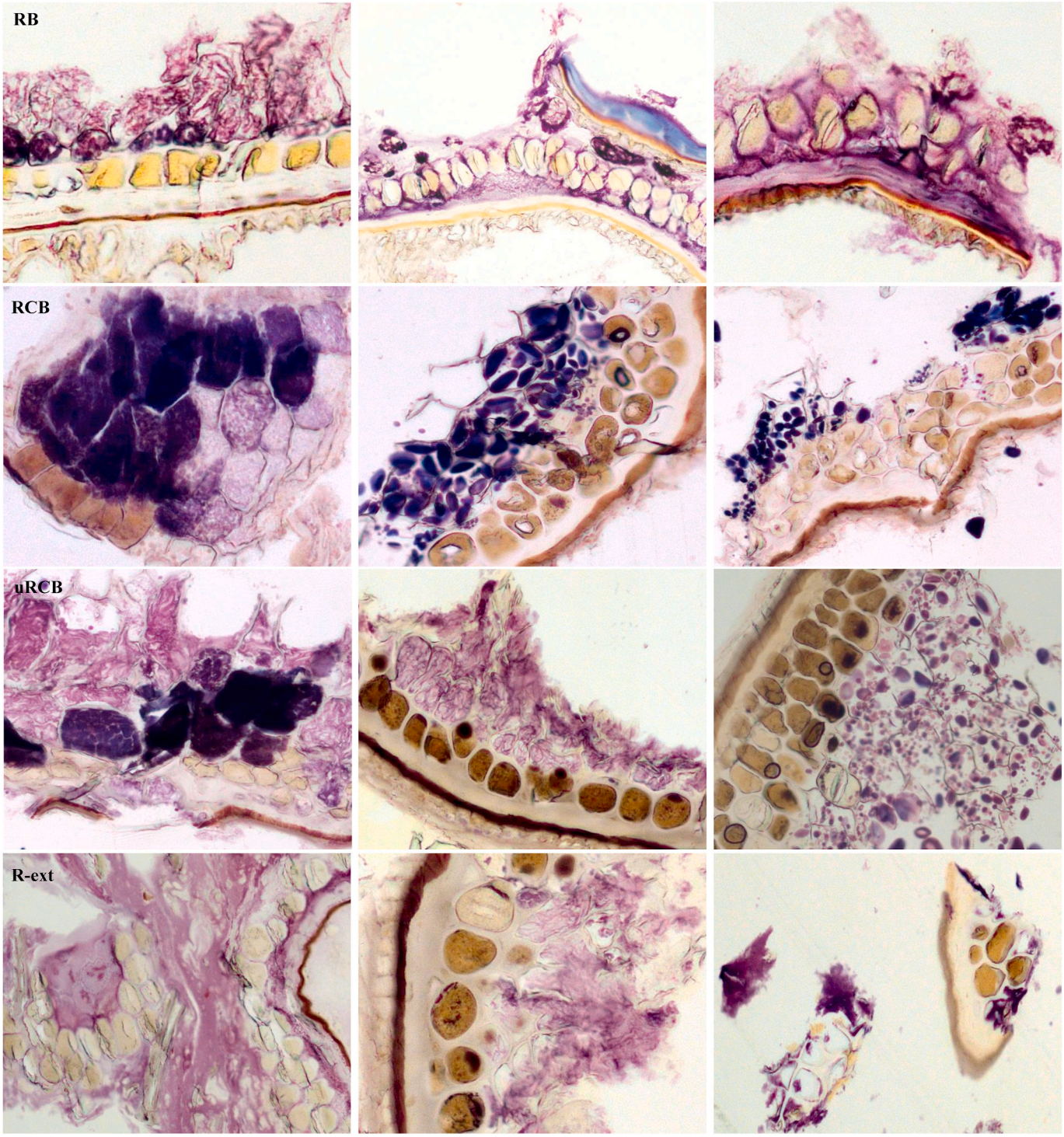

Fig. 3b. Light microscopy micrographs stained with iodine showing the microstructure of differently processed bread collected at 0 min, 30 min and 120 min during small intestinal (SI) digestion. Protein appears yellow, starch purple, amylopectin brown and amylose blue. Cell walls are unstained. RB: rye soft bread. RCB: rye crisp bread. uRCB: unfermented rye crisp bread. R-ext: rye puffs (extruded).

consumption of beta-glucans, with two approved health claims in Europe (Wolever et al., 2010). The higher viscosity observed for rye products after simulated intestinal digestion in the present study could thus be one reason why rye products have favourable effects on satiety (Forsberg et al., 2014; Isaksson et al., 2009, 2012, pp. 1–9; Johansson et al., 2015) and glycemia and insulinaemia (Iversen et al., 2022).

3.4. Release of glucose and maltose

Release of glucose and maltose during simulated digestion was determined for all products and sampling time points (Fig. 5). Wheat and rye products differed in that more glcuose and maltose were released from all three wheat products compared with the rye products. Other sugar species were detected, but not identified, in the digested samples of the rye products (data not shown). The difference in release

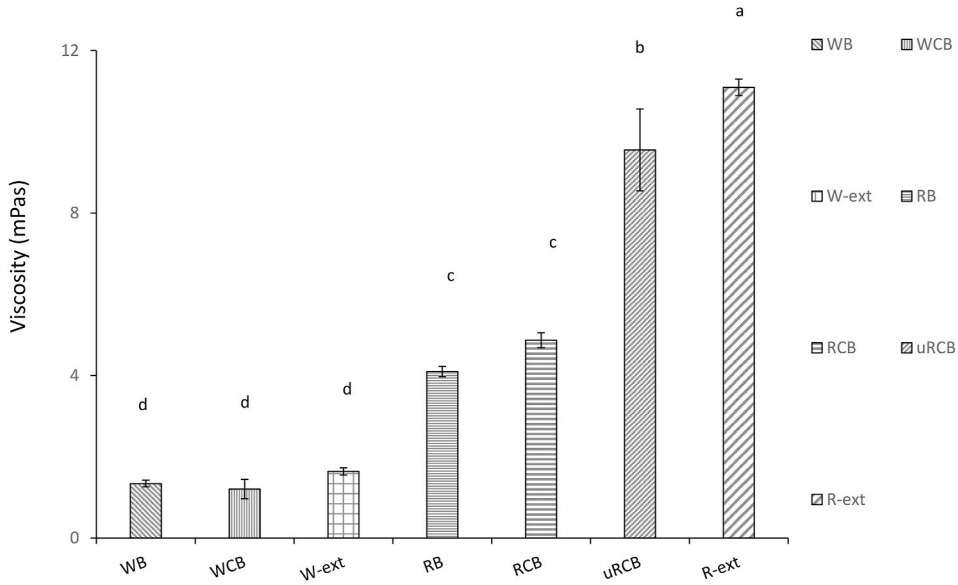

Fig. 4. Viscosity of the soluble part after 120 min of simulated intestinal digestion.

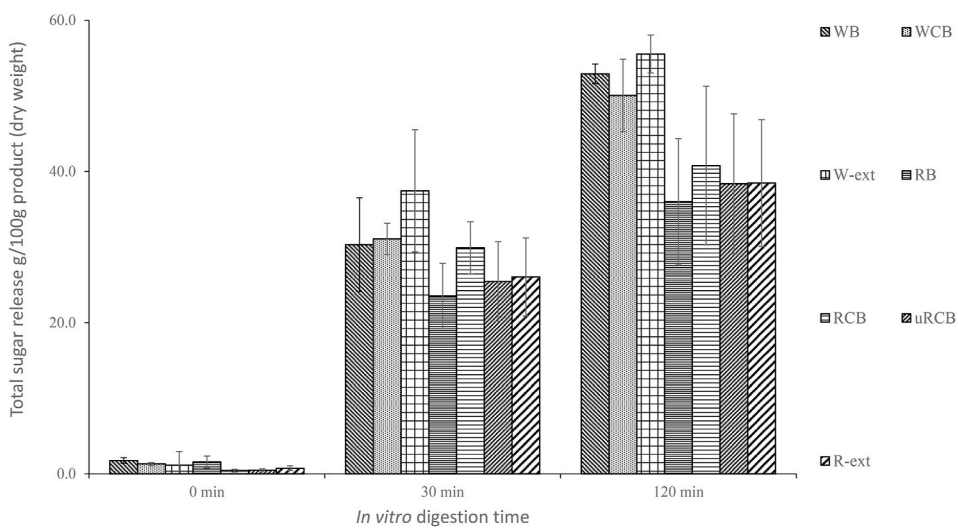

Fig. 5. Total sugar release (glucose + maltose) during the simulated intestinal digestion. Glucose and maltose concentration in the soluble part of the sample were quantified separately at all three sampling time points of the in-vitro digestion.

of glucose and maltose between wheat and rye products may reflect a higher total carbohydrate content of the wheat products and the difference in availability of carbohydrates for digestion between refined wheat and wholegrain rye products. This difference was noticeable already after 30 min of simulated intestinal digestion, indicating that starch digestion is slower in rye products.

3.5. Principal component analysis (PCA)

The PCA plots provided an overview of the relationships between chemical composition, viscosity and total sugar release during *in vitro* digestion of the different processed products. (Fig. 6). The two principal components (PC1, PC2) accounted for 85.2% of total variation between the samples. PC1 (68%) effectively distinguished between rye bread and wheat bread, with WCB, WB, and W-ext situated to the left and RCB, uRCB, and R-ext situated to the right of the bi-plot (Fig. 6). Total sugar release, which included glucose and maltose, was grouped with three different time retentions. Wheat samples, particularly WB, showed a positive correlation with sugar release during the entire duration of *in vitro* intestinal digestion, resulting in higher sugar release. Sugar release was strongly inversely correlated with both the amount of total dietary fibre of the cereal products and the measured viscosity after *in vitro* digestion (Fig. 6). These viscosities and the amount of total dietary fibre was positively correlated to each other. The higher measured viscosities in the digesta supernatant of rye products vs. wheat products, as a consequence of released soluble dietary fibres, was described above. But it also seems that a higher dietary fibre content can affect the sugar release during the *in vitro* intestinal digestion, which the correlations indicate. In the rye bread products, the cell walls exhibited limited breakdown during the digestion. This may not only restrain the access to starch but it also resulted in the formation of a denser and more cohesive mass of undigested material. The viscosities were only measured on the soluble part though this undigested material should be able to contribute to an even higher viscosity of the whole digesta. Conversely, the cell walls in wheat bread underwent more extensive degradation during digestion, leading to a less cohesive and more dispersed mass of digested material. This is likely connected to a better access to the starch and a facilitated release of sugars from the bread, explaining the faster rate of

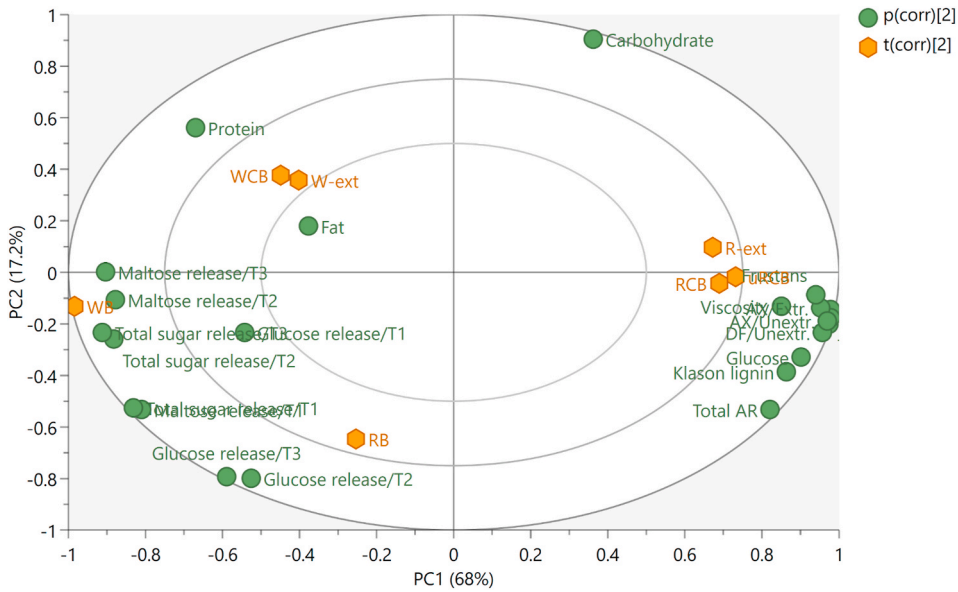

Fig. 6. Results from principal component analysis (PCA) plots measuring chemical composition of seven differently processed breads, viscosity after 120 min of simulated intestinal digestion and total sugar release during in-vitro digestion gathered at 3 time intervals (T1:0 min; T2:30min; T3:120min). The first principal component describes 68% and the second component explains 17.2% of the total variance. In the bi-plot picture, differently processed bread was shown as orange hexagon and the scattering of all variables were shown as green circle.

sugar release during digestion.

The relationship observed between viscosity, sugar release and dietary fibre content in wheat and rye bread during *in vitro* intestinal digestion is likely to have important implications for human appetite and post-prandial metabolic responses, and may account for the clear differences observed in such responses in previous studies. Prolonged release of sugars may contribute to a more sustained feeling of fullness and reduced hunger, which may result in greater weight loss from consumption of rye products compared with refined wheat products, as observed in a recent weight-loss trial performed with the same products as used in the present study (Iversen et al., 2021). The rate of sugar release during digestion is important for glucose regulation and glycaemic response (Brennan, 2005). In the *in vitro* digestion setup used, the release of sugar is faster for wheat products with lower dietary fibre content than for more fibre rich rye products. This resembles both beneficial and negative effects on metabolic health that has been shown for similar products on e.g. glycaemia. (Jonsson, Andersson, Bach Knudsen, et al., 2018). The dietary fibre content of bread can also influence postprandial metabolic responses, including lipid metabolism and insulin sensitivity (Giacco et al., 2014).

4. Conclusions

The rye products analysed in this study all disintegrated more slowly and to a lesser degree than the compared refined wheat products. The wheat products had few non-degraded particles after 120 min of simulated intestinal digestion, while the rye products had more non-degraded particles remaining in digesta. In general, both the wheat and rye product samples contained some starch after 120 min of digestion, but digesta of the rye products contained more untouched starch granules. This is most likely the effect of digestion in the rye products being hindered by e.g. bran particles and of the starch being more resistant to digestion, which may also be linked to slower release of maltose in the rye products. After simulated intestinal digestion, viscosity was higher for the rye products than the wheat products and fermentation appeared to play a role in the type of soluble fibre contributing to viscosity. These effects may in part explain why high-fibre rye foods have beneficial effects and in some cases on blood lipid profile and inflammation markers. The results from this study on the effects of product processing and digestion behaviour can be used to explain observations in human diet studies using the same products.

Funding

This work was supported **by** Formas [grant number 2019-02200].

CRediT authorship contribution statement

Jing Lu: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Henrik Hansson:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Daniel P. Johansson:** Investigation, Formal analysis. **Rikard Landberg:** Writing – review & editing. **Maud Langton:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2024.109990.

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