VTT Technical Research Centre of Finland

The role of rye bran acidification and in situ dextran formation on structure and texture of high fibre extrudates

Nikinmaa, Markus; Kajala, Ilkka; Liu, Xia; Nordlund, Emilia; Sozer, Nesli

Published in:
Food Research International

DOI:
10.1016/j.foodres.2020.109438

Published: 01/11/2020

Document Version
Publisher's final version

Food Research International 137 (2020) 109438

Contents lists available at ScienceDirect

Food Research International

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

The role of rye bran acidification and *in situ* dextran formation on structure and texture of high fibre extrudates

Markus Nikinmaa[a,*], Ilkka Kajala[a,1], Xia Liu[b], Emilia Nordlund[a], Nesli Sozer[a]

[a] VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 Espoo, Finland
[b] General Mills, United States

ARTICLE INFO

Keywords:
High fibre extrusion
Bran
Exopolysaccharides
Dextransucrase
Lactic acid bacteria fermentation

ABSTRACT

High insoluble dietary fibre content causes challenges with structure and texture in extrusion. This paper focused on studying the structure of extrudate enriched with rye bran modified in different ways. Fermentation of rye bran with dextran-producing *Weissella confusa* (with 10 g/100 g, 5 g/100 g and 0 g/100 g added sucrose as substrate for dextran production), *in situ* enzymatic production of dextran in the bran and chemical acidification of bran with lactic acid were compared in extrusion trials. Endosperm rye flour was the base in extrusion, of which 32 g/100 g was substituted for rye bran. Fermentation with dextran production showed similar improvement in extrudate expansion as chemically acidified bran samples (489 and 493%), in comparison with native bran (420%). Similarly, these treatments decreased extrudate hardness and increased crispiness index (CI) (16 N, 0.06 and 14 N, 0.071 respectively) compared to the control (39 N, 0.008). Enzymatically produced dextran did not affect expansion, although it decreased hardness (26 N) and increased CI compared to the control (0.023). Chemical changes in the fermented and acidified rye bran included reduction in insoluble dietary fibre (DF) (19 g/100 g → 17 g/100 g) and increase in soluble DF (5.17 g/100 g → 5.51–7.19 g/100 g), as well as soluble protein (8 g/100 g → 11 g/100 g) content. Lactic acid bacteria fermentation or acidification is therefore a promising method to increase the functionality of rye bran in extrusion.

1. Introduction

Extruded cereal products, commonly produced with refined flour, are staple snack foods and breakfast items. Due to the use of refined flours, these products contribute little to the recommended daily intake of dietary fibre (DF) of ca 25 g/day. Enriching extruded cereal foods with DF by including cereal bran, would improve the nutritional value of these products. However, bran addition is associated with negative effects on extrudate expansion and texture due to reduction in starch content, its effect on water distribution by competing for water with the starch matrix, reducing melt viscoelasticity and hindering bubble formation (Pai, Blake, Hamaker, & Campanella, 2009; Robin et al., 2012; Yanniotis, Petraki, & Soumpasi, 2007). Especially the insoluble dietary fibre (IDF) component of the bran has a negative effect on expansion. Bran also contains a relatively high amount of protein (ca 17–25 g/100 g Karppinen, Kiiliöinen, Liukkonen, Forssell, & Poutanen, 2001; Nilsson et al., 1997). High protein content can affect expansion of extrudates both negatively and positively depending on the protein characteristics and concentration, e.g. by diluting the starch, affecting water availability for the starch gel, as well as taking part in various reactions (Moraru & Kokini, 2003).

Several studies have investigated different physical and bioprocessing methods to improve the behaviour of bran in extrusion. Processing strategies such as ultrafine milling (Alam et al., 2014) and enzymatic treatment (Santala, Kiran, Sozer, Poutanen, & Nordlund, 2014) improved expansion of high-DF bran-containing extrudates. In the former study, the effect was hypothesized to be due to DF reduction observed, while in the latter solubilisation of DF was the main effect. Lesser particle size reduction than in Alam et al. (2014), of wheat bran (317–224 μm) did not improve expansion of wheat bran extrudates (Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011b). As with enzymatic solubilisation, solubilisation of arabinoxylan with alkali treatment of corn bran increased the expansion of corn bran containing extrudates, (Pai et al., 2009). Our previous study (Nikinmaa et al., 2017) found that fermentation of rye bran with dextran-producing *Weissella confusa* improved expansion of bran supplemented extrudates. Addition of the same amount of dextran as produced *in situ* by *W. confusa* did not improve expansion compared to the control. We

* Corresponding author.
E-mail address: markus.nikinmaa@vtt.fi (M. Nikinmaa).
[1] Deceased January 30th, 2019.

https://doi.org/10.1016/j.foodres.2020.109438
Received 6 April 2020; Received in revised form 4 June 2020; Accepted 11 June 2020
Available online 20 June 2020

hypothesized that *in situ* produced exopolysaccharides (EPS) coats the bran particles, reducing their negative effect in the starch matrix. However, the effect of acidification or bran modification by microbial or endogenous enzymes was not eliminated. Acidification of rye by lactic acid bacteria activates endogenous enzymes, e.g. xylanases and proteases, as well as possibly inducing mild acid hydrolysis (Boskov Hansen et al., 2002). Xylanolytic and proteolytic enzymes are concentrated in the outer layers of rye, and therefore, present and activated during lactic acid fermentation of rye bran (Brijs, Bleukx, & Delcour, 1999; Gys, Courtin, & Delcour, 2004).

The aim of this study was to investigate the bran modification mechanisms of bioprocessing, specifically elucidate the role of acidification and EPS in high-DF extrusion. To achieve this, chemical acidification using lactic acid and *in situ* enzymatic dextran production were examined and compared to fermentation with LAB in relation to extrudate structure and texture.

2. Materials and methods

2.1. Production of dextransucrase

The production of dextransucrase was essentially carried out as described previously by Kajala et al. (2015). The *Lactococcus lactis* strain harbouring the dextransucrase gene of *W. confusa* was cultivated in 10 L of M17 medium, supplemented with 5 g/L glucose, for 18 h at 30 °C in the presence of 4 ng/mL of the nisin inducer, which was added at an OD_{600} of ~0.1–0.3.

The supernatant was concentrated by ultrafiltration to ~1 L (6 ft^2, Prep/Scale TFF, cut-off 10 kDa; Millipore) and 1 L of 10 mM Na-citrate buffer, pH 5.4 was added. The concentration–dilution was repeated five times and the final sample was concentrated to ~500 ml.

Dextransucrase activity was measured using the Nelson-Somogyi-method (Nelson, 1944), as described by Kajala et al. (2015).

2.2. Bran processing

Commercial rye bran (Fazer Mills, Finland) was milled with a pin disc mill (Hosokawa-Alpine 100UPZ, Augsburg, Germany) at 17,800 rpm. The milled material was air classified (British Rema Minisplit, Chesterfield, UK) at 3000 rpm, 220 m^3/h to reduce starch content. The coarse fraction (50% of the total input) obtained was used in the subsequent trials.

Brans were fermented with dextran-producing *W. confusa* VTT E-143403 (VTT Culture Collection). Starter culture preparation was performed as described by Nikinmaa et al. (2017). To obtain samples with different dextran contents, 0 g/100 g, 5 g/100 g and 10 g/100 g of bran was replaced by sucrose as substrate for dextran production in fermentations. Fermentations were incubated for 20 h at 25 °C at 20 g/100 g dry matter content. Dextransucrase treatment was performed in the same conditions for 6 h, with addition of 10 g/100 g sucrose and 0.8 U/g dextransucrase enzyme. Native bran, soaked bran, as well as a chemically acidified bran were used as controls. Chemical acidification was carried out by addition of lactic acid in order to mimic acidification during fermentation. This sample was first incubated at native pH for 10 h, followed by adjustment to pH 5.5 for 5 h. Finally, the pH was adjusted to 4.3 for a further 5 h. During chemical acidification, microbial growth was controlled by addition of 0.01 g/100 g chloramphenicol and cycloheximide. After treatment, all samples were freeze dried (Parker Freeze Dry, Inc, Pulaski, WI, USA)

Following freeze drying, the material was pin disc milled at 17,800 rpm. The non-freeze dried native bran was also milled again with the same parameters. Particle sizes were measured by laser diffraction using a Coulter LS230 (Beckman Coulter, Fullerton, USA). Chemically acidified, *W. confusa* 0% sucrose, soaked and native brans were milled again with a pin disc mill at 17,800 rpm to achieve similar particle sizes for all bran samples.

2.3. Extrusion

Endosperm rye flour was used as the starch base in extrusion. The amount of bran added to each sample was 32 g/100 g by weight, which corresponded to a DF content of ca 18 g/100 g. The samples were extruded using a twin-screw extruder (APV MPF 19/25, Baker Perkins Group LTD, Peterborough, UK). The feed rate was 60 g/min whereas the water feed varied between 3.5 and 4.5 g/min. The temperature profile was 120-110-95-80 °C (die to feed) and the screw speed was constant at 350 rpm. Duplicate extrusion trials on separate days were performed.

2.4. Macrostructural analysis

The physical dimensions of the extrudates were measured as described by Nikinmaa et al., 2017. The diameter of extrudates was measured in three places for each replicate sample and each sample was weighed. Expansion rate and density were calculated for the extruded samples based on the measured values.

2.5. Instrumental textural analysis

Texture of the extruded samples was measured by uniaxial compression using a TA.XT2i Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) with a 30 kg load cell and a cylindrical 25 mm aluminium probe. Cross-sectional samples of 10 mm were cut using an electric saw (Power ST- WBS800, Taiwan Sheng Tsai Industrial Co LTD, Taiwan). The samples were deformed at 70% strain at 1 mm/s as described by Alam et al. (2014). Hardness, crispiness work (CW) and crispiness index (CI) values were obtained from the force-deformation curves. Hardness is defined as the peak force value of the force-deformation curve.

Crispiness work is calculated with Eq. (1) (Van Hecke, Allaf, & Bouvier, 1995)

$$CW\,(Nmm) = \frac{A}{N} \tag{1}$$

where A is the area under the force-defomation curve (Nmm) and N is the number of peaks

Crispiness index is calculated according to Eq. (2) (Heidenreich, Jaros, Rohm, & Ziems, 2004).

$$CI = \frac{L_N}{AxF_{mean}} \tag{2}$$

Where L_N is the normalised curve length, A is the area under the force deformation curve and F_{mean} is the sum of force values divided by the number of data points.

2.6. Chemical analysis

Total DF and SDF content of extrudates was analysed using AOAC method 991.43 (AOAC, 1995). Ash content was measured gravimetrically after combustion at 550 °C.

Dextran content of brans was analysed essentially as described by Katina et al. (2009). 100 mg of bran sample was washed three times with 50 vol-% ethanol to remove sugars and oligosaccharides and centrifuged at 10,000*g* for 10 min. The supernatant was discarded and the pellet was resuspended in 4.5 ml 50 mM pH 5.5 sodium citrate buffer to dissolve dextran. Next, transglucosidase and dextranase enzymes were added to hydrolyze dextran to glucose. After enzyme addition, samples were incubated for 48 h at 30 °C. After thorough mixing 1 ml samples were taken. Enzymes were inactivated by keeping the tubes in 100 °C for 10 min. Samples were centrifuged and glucose content was measured spectrophotometrically with a glucose oxidase peroxidase kit (D-Glucose Assay Kit - GOPOD Format, Megazyme, Bray, County Wicklow, Ireland). Samples were compared with a bran control

Table 1
Pasting properties of bran-flour mixtures as analysed by Rapid ViscoAnalyzer. Results are the mean of 3 samples, letters a-d indicate significant differences.

Sample	Peak viscosity	Hold viscosity	Breakdown	Final viscosity	Setback viscosity	Pasting temperature
Native	1574 ± 59^{d}	539 ± 30^{bc}	1036 ± 30^{d}	1231 ± 78^{bc}	693 ± 48^{bc}	65 ± 1^{a}
Soaked	1508 ± 10 ^{cd}	507 ± 12^{ab}	1001 ± 14^{d}	1143 ± 33^{ab}	636 ± 22^{ab}	66 ± 1^{a}
W. confusa 0% sucrose	1284 ± 27^{b}	556 ± 18^{bc}	728 ± 10^{b}	1251 ± 45^{bc}	695 ± 28^{bc}	66 ± 0^{a}
W. confusa 5% sucrose	1314 ± 12^{b}	594 ± 11 ^{cd}	721 ± 2^{ab}	1314 ± 15 ^{cd}	720 ± 13^{bcd}	67 ± 1^{a}
W. confusa 10% sucrose	1369 ± 25^{b}	643 ± 28^{d}	726 ± 10^{b}	1430 ± 71^{d}	787 ± 44^{d}	66 ± 1^{a}
Chemical acidification	1131 ± 21^{a}	456 ± 36^{a}	675 ± 15^{a}	1012 ± 77^{a}	556 ± 41^{a}	67 ± 1^{a}
Dextransucrase treated	1482 ± 34^{c}	583 ± 12 ^{cd}	899 ± 27^{c}	1352 ± 17 ^{cd}	769 ± 7 ^{cd}	65 ± 0^{a}

without dextranase as well as a pure 5 mg/ml *W. confusa* dextran control.

pH and TTA of rye extrudates and brans were measured by weighing 5 g of sample and adding 50 g of water. TTA of samples was measured by titrating to pH 8.5 with 0.1 M NaOH using an automatic titrator (T50, Mettler Toledo, Columbus, Ohio, USA).

In order to investigate the effect of the treatments on the state of protein in the bran, soluble protein analysis of brans was performed using a DC Protein assay kit (Bio-rad Laboratories Inc., USA). Bran was diluted 1:10 in water, mixed carefully, and then centrifuged. The supernatant was recovered and analysed spectrophotometrically at 750 nm using the reagents provided in the kit.

Water binding of brans was measured by a centrifuge-based method as described by (Silventoinen, Sipponen, Holopainen-Mantila, Poutanen, & Sozer, 2018) with an addition of determining the dry matter content of the supernatant.

2.7. RVA analysis

Pasting properties and viscous properties of flour mixtures for extrusion were analysed using a Rapid Visco Analyzer (RVA Super4, Newport Scientific, Australia). 3.5 g of flour (DM basis) was weighed in aluminium cups and 25 g of water was added. The suspension was heated to 50 °C, and held at this temperature while stirring at 160 rpm, followed by heating to 95 °C in 3 min 42 s. The temperature was held at 95 °C for 2 min 30 s and then cooled to 50 °C in 3 min 48 s, after which the sample was held at 50 °C for 2 min. Peak viscosity, final viscosity, setback viscosity, breakdown viscosity and pasting temperature were determined from the pasting curve using Thermocline v. 2.2 software

2.8. Light microscopy

Light microscopy was performed for 4 bran samples, soaked bran, chemically acidified bran, *W. confusa* 0% sucrose bran and *W. confusa* 10% sucrose bran, and 3 extrudates (soaked bran, chemically acidified bran, *W. confusa* 10% sucrose bran). To enable their handling, samples were first embedded in 2% (w/v) agar and then fixed in 1% (v/v) glutaraldehyde in 0.1 M Na-K phosphate buffer (pH 7.0), dehydrated in a graded ethanol series, and embedded in hydroxyethyl methylacrylate resin as recommended by the manufacturer (Leica Historesin embedding kit, Leica Microsystems, Heidelberg, Germany). Polymerized samples were sectioned (2 μm sections) in a rotary microtome HM 355S (Microm Laborgeräte GmbH, Walldorf, Germany) using a tungsten carbide knife. The sections were transferred onto glass slides and stained.

Protein and beta-glucan were visualised with 0.1 g/100 ml Acid Fuchsin 0.01 g/100 ml Calcofluor White as described by Hemery et al. (2011). In exciting light (excitation, 390–420 nm; emission, > 450 nm), intact cell walls stained with Calcofluor appear blue and proteins stained with Acid Fuchsin appear red. Starch is unstained and appears black. Protein and starch were, in turn, visualised with 0.1 g/100 ml Light Green SF and starch with 1:10 diluted Lugol's iodine solution according to Andersson et al. (2011). When imaged in brightfield, protein stained with Light Green appears green. Iodine stains the amylose component of starch blue and amylopectin brown. The stained sections were examined with a Zeiss AxioImager M.2 microscope (Carl Zeiss GmbH, Göttingen, Germany). Micrographs were obtained using a Zeiss Axiocam 506 CCD colour camera (Zeiss) and the Zen imaging software (Zeiss). Representative images were selected for publication

2.9. Statistical analysis

IBM SPSS Statistics 22 (IBM Corporation, Somers, NY, USA) was used for statistical analysis. One-way ANOVA with Tukey HSD (significance level of 0.05) was used to assess the significance of the differences between samples, with treatments as independent variables and measurement results as dependent variables

3. Results and discussion

The dextransucrase activities were determined as 1.5 U (cultivation A) and 12 U (cultivation B). The values are only approximate, since the Nelson-Somogyi method used is not specific for the fructose released in the reaction, but the by-products of the reaction and other carbohydrates present in the sample gave a positive signal as well (Kajala et al., 2015).

3.1. Particle size adjustment and extrusion processing.

Particle sizes of the brans differed from each other after freeze drying and milling. Chemically acidified bran and control brans had larger particle size (D50: 300–400 μm) than the dextran containing samples (D50: ca 180 μm). In order to exclude the effect of particle size on extrusion, an attempt was made to bring particle sizes of rye brans to a similar D50 range of ca 160–180 μm for all samples by milling the samples again as needed. The D50 of the native, as well as the soaked bran remained slightly larger than the acidified and fermented samples (216 μm and 291 μm respectively, compared to 160–180 μm for fermented and acidified samples). This indicates that the acidification made the material more brittle, as all the acidified materials achieved smaller particle size than the soaked bran. The reason for the particle size adjustment was that large differences in particle size might affect the extrusion performance, as observed by Alam et al. (2014) and Santala et al. (2014). The differences after adjustment were in the same range as Robin et al. (2011b) or as between the medium and large particle sizes in Alam et al. (2014), who observed no difference in expansion between these particle sizes.

During extrusion, small differences in torque and water distribution were evident, as with acidified samples lower water addition was possible without burning. Torque values of up to 75–90% corresponding to a specific mechanical energy (SME) of 291–350 kW/kg was observed with the native and soaked brans, while generally lower torque (70–85%) and SME (272–330 kW/kg) was observed with the fermented and treated brans despite lower water addition. Pai et al. (2009) and Robin, Bovet, Pineau, Schuchmann, and Palzer (2011a) observed increase in melt viscosity with addition of bran to corn and wheat extrudates, respectively. The equal or lower torque, despite lower water content, observed in fermented or acidified bran

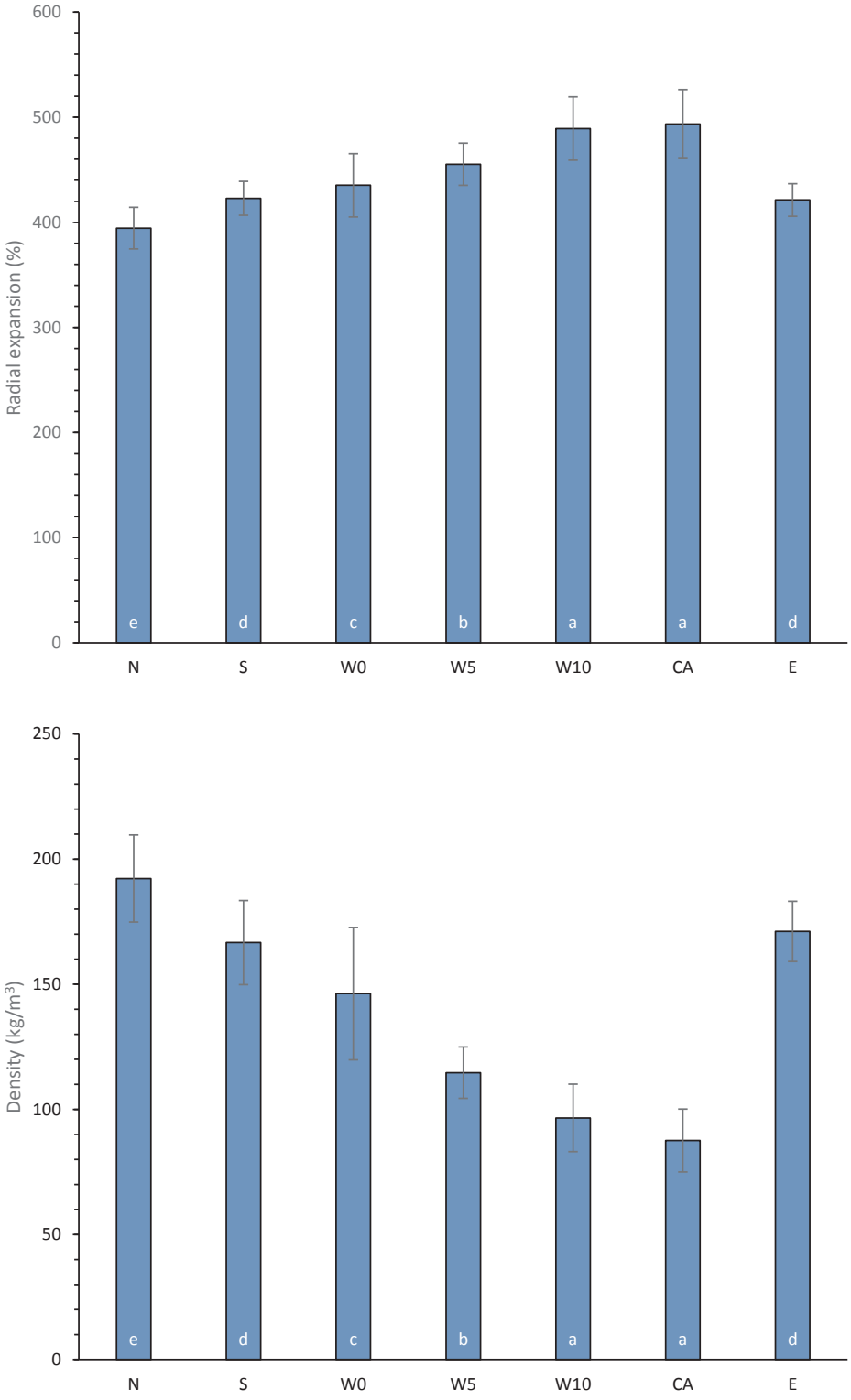

Fig. 1. Macrostructural properties of extrudates. Letters indicate statistical sameness of samples. Sample acronyms: N = Native bran, S = Soaked bran, W0-W10 = *W. confusa* fermented samples with 0–10% sucrose, CA = Chemically acidified, E = dextransucrase enzyme treated.

containing extrudates, suggests that these treatments reduced the melt viscosity compared to untreated bran-containing extrudates.

3.2. Chemical composition and acidity of brans and extrudates

Fermentations lowered the pH of the brans from 6.3 to ca 4.3 (Table 1). Correspondingly, TTA rose to ca 30–35 ml 0.1 M NaOH, compared to 9.8 for native bran. Chemically acidified bran had similar pH (4.39) and TTA (29.7) to the fermented samples. *W. confusa* 10% sucrose had lower TTA, despite the pH also being lower. This may be because part of the bran was replaced by sucrose, and thus, there was less buffering capacity in the sample. Compared to Nikinmaa et al., 2017, pH was slightly higher for *W. confusa* fermented samples (4.3 compared to 4.1) and TTA is lower if adjusted to the same bran

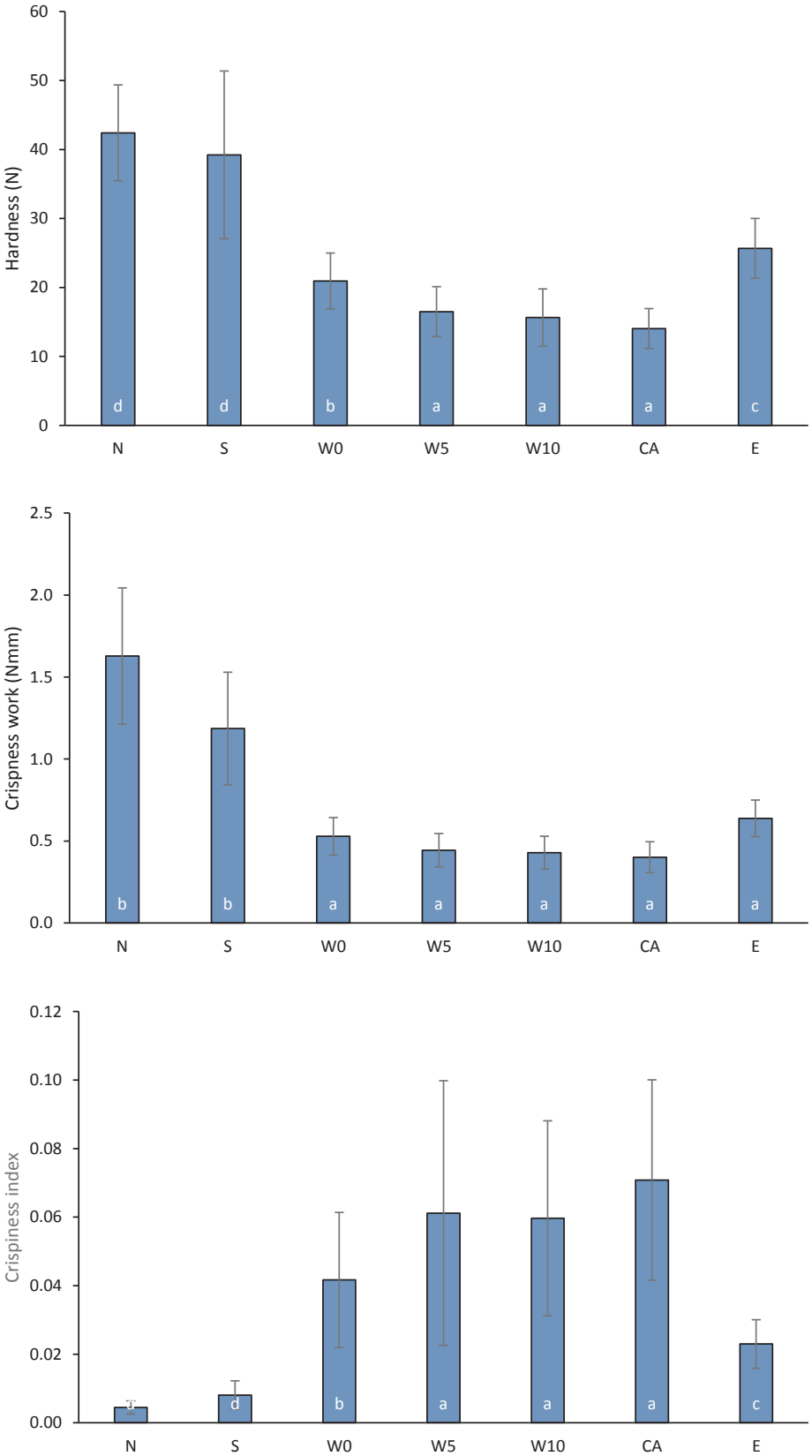

Fig. 2. Instrumental texture analysis results of extrudates. Letters indicate statistical sameness of samples. Sample acronyms: N = Native bran, S = Soaked bran, W0-W10 = *W. confusa* fermented samples with 0–10% sucrose, CA = Chemically acidified, E = dextransucrase enzyme treated.

concentration in analysis (6.6–7.5 ml compared to 8.7 and 9.7 ml).

Reduction in total DF content and increase in the proportion of SDF was observed in extrudates with fermented and acidified brans, compared to the controls (Table 1). The *W. confusa* 10% sucrose extrudate had the highest content of SDF (7.2 g/100 g) compared to the soaked bran extrudate (5.3 g/100 g) which is due to the dextran

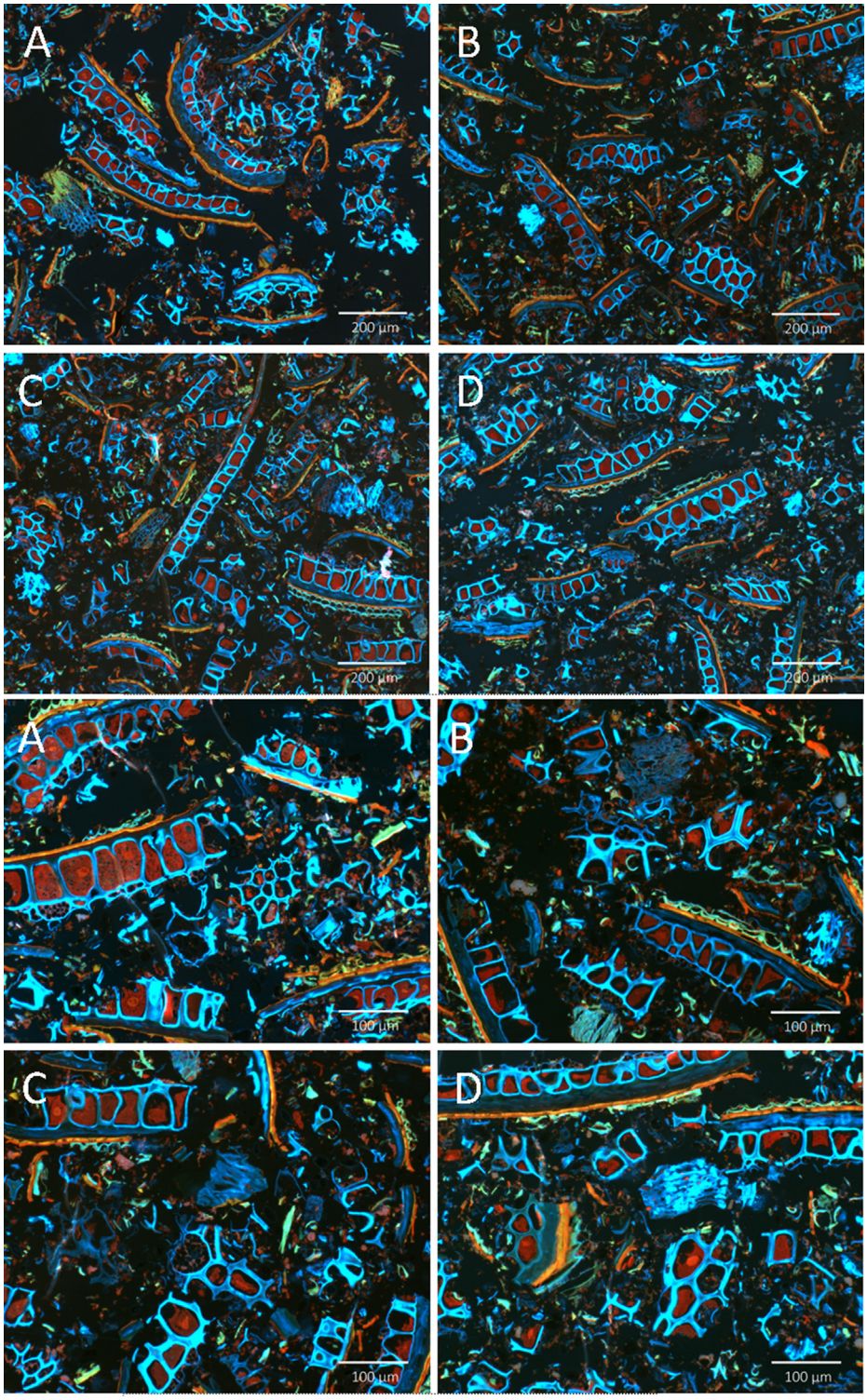

Fig. 3. Light microscopy of rye bran samples at two different magnifications with acid fuchsin and calcofluor staining. A = Soaked bran (control), B = Bran treated with *W. confusa* (with 0% Sucrose), C = Chemically acidifed bran, D = Bran treated with *W. confusa* (with 10% sucrose).

produced (i.e. SDF). In all samples with sucrose addition, part of the DF reduction was due to replacement of bran with sucrose, as only the glucose is converted into dextran. As observed by Boskov Hansen et al. (2002) the content of SDF did not increase in samples without dextran production, while the total DF decreased. As endogenous xylanolytic enzymes exhibit activity specifically at the pH levels observed in this study, in all likelihood their efficient action caused the reduction in IDF content. Further, the reduction in total DF may be caused by endogenous β-xylosidase removing monomeric xylose from the end of the xylan backbone (Boskov Hansen et al., 2002; Rasmussen, Boskov Hansen, Hansen, & Melchior Larsen, 2001). Both fermentation and dextransucrase treatment were successful for dextran production (Table 1). Rye bran contains some sucrose, which means that even the W0 bran contained some dextran (0.8 g/100 g). W5 and W10 brans correspondingly had a higher dextran content (2.4 g/100 g and 4.7 g/100 g respectively). Dextransucrase treatment produced 4.3 g/100 g

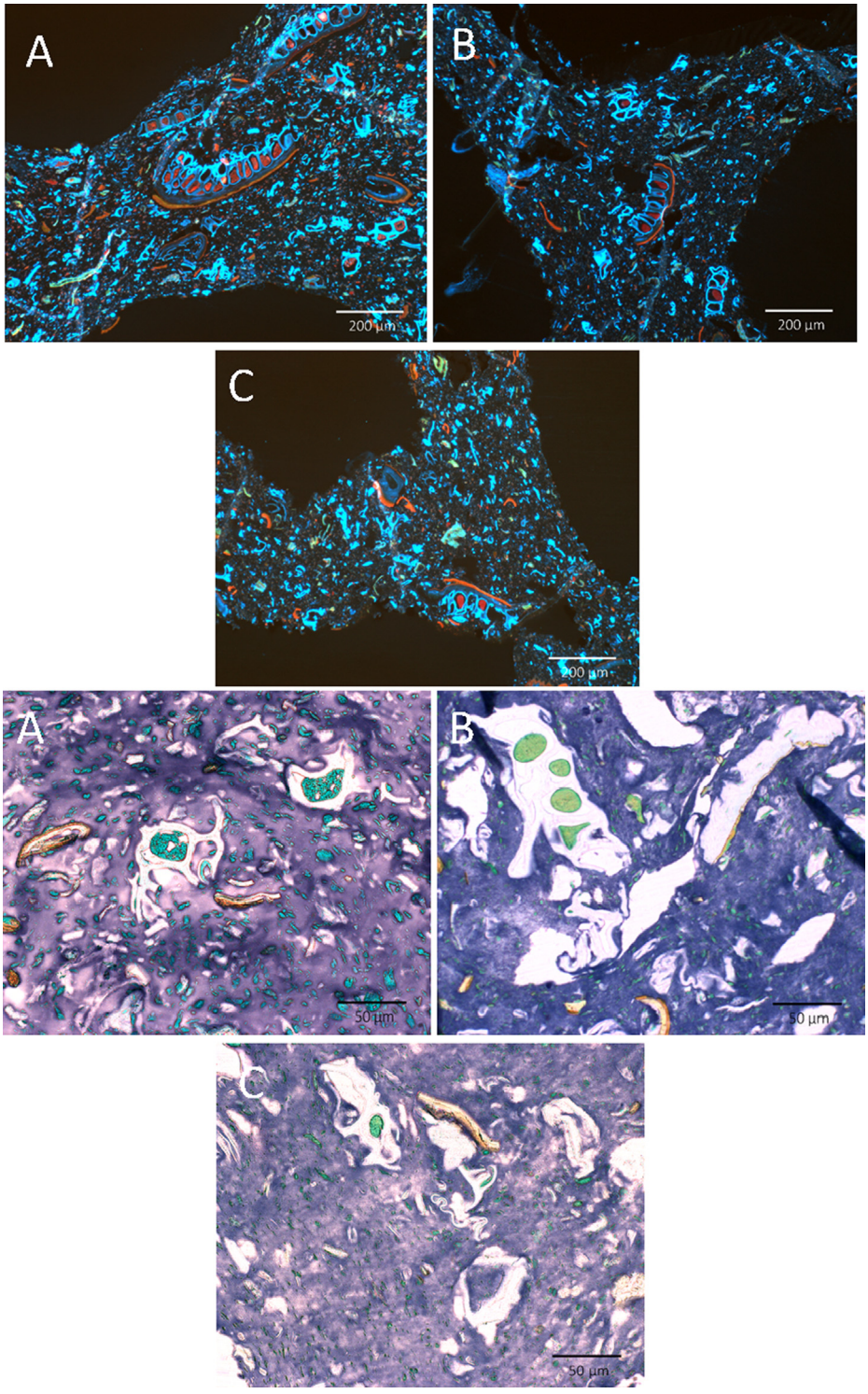

Fig. 4. Microscopy images of extrudates, with acid fuchsin and calcofluor staining (Above) and Lugol's Iodine + light green staining (below). A = Extrudate with 32 g/100 g soaked bran (control), B = Extrudate with g/100 g rye bran treated with *W. confusa* (10 g/100 g Sucrose), C = Extrudate with g/100 g (dm) chemically acidifed rye bran. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dextran.

Analogous to DF hydrolysis, the amount of soluble protein significantly increased ($P > 0.05$) in fermented and acidified bran samples compared to the control. The highest soluble protein content was found in the *W. confusa* 10% bran (11.0 g/100 g DW), followed by the 5% sucrose (10.8 g/100 g), 0% sucrose (10.6 g/100 g) and chemically acidified brans (10.3 g/100 g), although the difference between these samples were not significant. The soaked control had a soluble protein content of 8.2 g/100 g. As with DF degrading enzymes, proteolytic enzymes also show increased activity at low pH - whereas at native pH no protease activity is present (Brijs et al., 1999).

Fermentation or chemical acidification did not reduce the water holding capacity of the brans compared to the control, despite this being commonly observed during enzymatic hydrolysis of e.g. arabinoxylan (Santala, Nordlund, & Poutanen, 2013). Probably, the water binding of the starch still present in the bran (ca. 20 g/100 g) increased

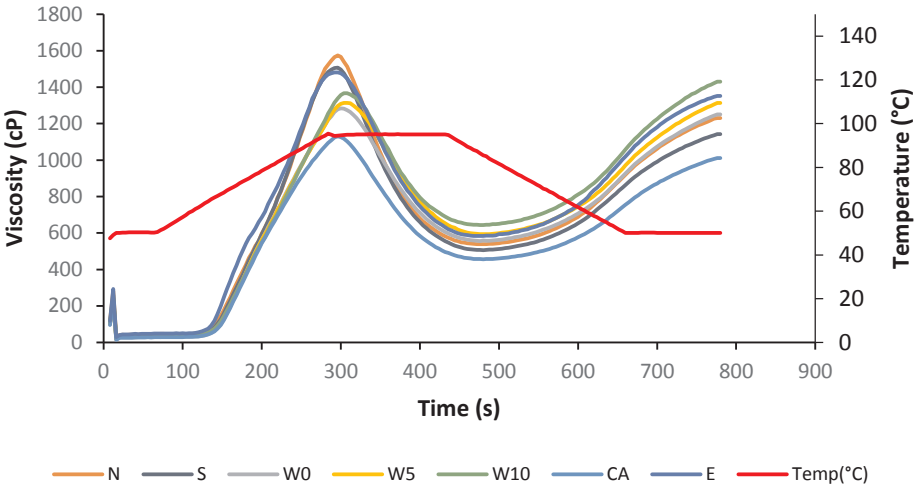

Fig. 5. RVA curves of flour-bran mixes. Sample acronyms: N = Native bran, S = Soaked bran, W0-W10 = *W. confusa* fermented samples with 0–10% sucrose, CA = Chemically acidified, E = dextransucrase enzyme treated.

during the acidification, which has been observed in e.g. sourdough baking (Hammes and Gänzle, 1997). The water binding of all dextran containing bran samples decreased, which is most likely because of the replacement of a part of the bran with a mixture of dextran and fructose which is extracted into the supernatant.

3.3. Macrostructure and instrumental texture analysis of extruded samples

Both fermentation and chemical acidification of rye bran increased the expansion and decreased the density of extruded samples (Fig. 2). The highest radial expansion was observed in the pH treated (493% expansion) and *W confusa* 10% sucrose treated bran sample (489%), compared to the soaked control (422%). Significant increase in the expansion was also observed between the *W. confusa* 5% and 0% sucrose samples (455 and 435%, respectively). Densities of all acidified samples (87–146 kg/m^3), Fig. 1) were significantly lower than the soaked control (166 kg/m^3), the largest decrease evident in the chemically acidified and *W. confusa* 10% samples. Soaking of bran also increased expansion compared to the non-treated native bran, possibly because freeze-drying transforming the bran into a more porous and fragile state. Dextransucrase treatment of bran did not significantly ($P > 0.05$) affect the radial expansion or density of bran-enriched extrudates when compared to the soaked control bran. Thus, neither added dextran, as shown in our previous study (Nikinmaa et al., 2017), nor *in situ* enzymatically produced dextran alone appear to improve expansion or density of extruded samples.

Chemical acidification was most effective at reducing extrudate hardness (14.0 N) and crispiness work (0.40 Nmm) while increasing the CI (0.07), compared to the soaked control (hardness: 39.2 N, crispiness work: 1.19 Nmm, CI: 0.008). The hardness of the extrudates made with *W. confusa* 10% sucrose, *W. Confusa* 5% sucrose and *W. confusa* 0% sucrose bran samples was measured as 15.6 N, 15.5 N and 20.9 N respectively, while the crispiness work was 0.43 Nmm, 0.44 Nmm and 0.53 Nmm respectively. CI values for the extrudates made with fermented bran samples were 0.06, 0.06 and 0.04, respectively. Therefore, the dextran containing fermented samples resulted in significantly better extrudate texture than the fermented sample without dextran, which corresponds with the differences in expansion and density between the samples. In addition, statistically significant improvement compared to the soaked control was also found in the enzyme treated sample (hardness: 25.7 N, crispiness work: 0.64 Nmm and CI: 0.02). Thus, dextransucrase treatment also had an effect on the texture of the extrudates, however, the effect could be because some IDF was replaced by SDF and sugar, and not specifically induced by dextran, as SDF has shown a positive effect on extrudate textures compared to bran (Brennan, Monro, & Brennan, 2008; Yanniotis et al., 2007). Paula and Conti-Silva (2014) analysed the texture of three commercial cylindrical extrudates and found the compression force to be between 21.6 N and 34.2 N. Although the results are not absolutely comparable due to some methodological differences, the hardness values of the extrudates tested in this study were on a comparable level.

3.4. Bran and extrudate microscopy

Microstructure of brans (Fig. 3) and extrudates (Fig. 4) with different magnifications were analysed to further understand how the treatments affected the different bran components. When bran samples were compared, greater disruption of cell walls was observed in acidified and fermented samples compared to the control (Fig. 3). This was also evident in the extrudates where there were smaller bran fragments present in the fermented/acidified samples than the soaked control (Fig. 4). In addition, in extruded samples stained with Lugol's iodine and light green, larger protein particles were observed in the soaked control than in the fermented or chemically acidified extrudates (Fig. 4), which was in line with a higher amount of soluble protein in the acidified samples. These results, although not entirely quantitative because of the microscopy method, indicate that acidified bran particles were more fragile than the soaked control bran both during milling and extrusion and thus perhaps cause less physical interference during extrusion (see Fig. 5).

3.5. RVA results

RVA-analysis was undertaken in order to obtain information about the effect of the bran treatment on pasting properties of bran-flour mixes, and thus give indications on the viscosity of the mixes in extrusion. Acidification appeared to lower the peak viscosity (Table 2) of the bran-flour mixture, as all the fermented samples and the pH treated sample had lower peak viscosities than the native (1574 cP) and the soaked (1508 cP), whereas dextransucrase enzyme treatment did not result in a significant effect on the peak viscosity (1482 cP). The chemically acidifed sample had the lowest peak viscosity (1131 cP), followed by W0 (1284 cP), W5 (1314 cP) and W10 (1369) cP). On the other hand, dextran production increased hold viscosity and final viscosity of the samples, as the dextran containing fermented samples and the dextransucrase treated sample exhibited higher values for these parameters than the control brans. Increase in viscosity with increasing EPS content has previously been observed in sourdoughs (Abedfar,

Table 2
Chemical analysis results for extrudates and corresponding bran samples. Total dietary fibre is the sum of insoluble dietry fibre (IDF) and soluble dietary fibre (SDF). Soluble protein measurement was performed in duplicates. The pH, titratable acid (TTA) and dextran are the average of three replicate measurements (letters indicate statistical difference). NA = not analysed.

	Extrudate samples					Bran Samples			
Treatment	Total DF (g/100 g)	IDF (g/100 g)	SDF (g/100 g)	pH	TTA (% lactic acid equivalents)	pH	TTA (% lactic acid equivalents)	Dextran content (g/100 g)	Soluble protein (g/100 g)
Native	18.0	12.8 ± 0.03	5.2 ± 0.03	NA	NA	NA	NA	NA	NA
Soaked	18.7	13.4 ± 0.26	5.3 ± 0.58	6.4 ± 0.07^{b}	0.6 ± 0.07^{a}	6.5 ± 0.07^{c}	1.8 ± 0.06^{a}	N/A	8.2 ± 0.3^{a}
W. confusa 0% sucrose	17.3	11.6 ± 0.16	5.7 ± 0.57	4.7 ± 0.14^{a}	2.1 ± 0.04^{c}	4.4 ± 0.03^{ab}	6.4 ± 0.20^{c}	0.8 ± 0.02^{a}	10.6 ± 0.2^{b}
W. confusa 5% sucrose	17.4	11.4 ± 0.21	5.9 ± 0.08	4.7 ± 0.14^{a}	2.1 ± 0.00^{c}	4.3 ± 0.03^{ab}	6.3 ± 0.51^{c}	2.4 ± 0.06^{b}	10.8 ± 0.2^{b}
W. confusa 10% sucrose	18.3	11.1 ± 1.13	7.2 ± 0.43	4.5 ± 0.14^{a}	2.1 ± 0.01^{c}	4.3 ± 0.03^{a}	5.7 ± 0.13^{b}	4.7 ± 0.20^{d}	11 ± 0.3^{b}
Chemical acidification	17.2	11.7 ± 0.69	5.5 ± 0.31	4.7 ± 0.08^{a}	2.0 ± 0.02^{b}	4.3 ± 0.00^{b}	5.4 ± 0.02^{b}	NA	10.3 ± 0.1^{b}
Dextransucrase treated	NA	NA	NA	NA	NA	NA	NA	4.3 ± 0.06^{c}	NA

Hosseininezhad, & Rafe, 2020; Katina et al., 2009). The effects observed are probably a combined effect of amylase activity during the initial stages of fermentation as well as solubilisation of bran components during acidification. Acid hydrolysis of starch could also play a role in the viscosity reduction of the starch, as hypothesized by Sriburi and Hill (2000), in both RVA and during extrusion processing. Because only a minor part of the starch in the flour-bran mixture had undergone treatment, the major effect in RVA was because of the effects on the non-starch components. Previously, Park, Fuerst, and Baik (2019) have observed viscosity reduction due to enzymatic arabinoxylan and cellulose hydrolysis, when SDF content remained constant compared to a non-treated control and IDF was reduced. RVA is a higher moisture system than extrusion, but reduced viscosities in RVA might suggest that the melt viscosity was lower for fermented and acidified samples than the soaked control.

4. Conclusions

Acidification of rye bran improved structure and texture in a similar way to fermentation with dextran producing *W. confusa,* while dextran production alone had a minor effect. This indicates that during LAB fermentation of rye bran the major effects relevant in extrusion are pH related. These effects include activation of proteolytic and xylanolytic enzymes, as well as possibly mild acid hydrolysis.

Most likely the cause of improvement of the characteristics of the bran in extrusion cannot be isolated to one single change, but is due to a multitude of effects. The hydrolysis of DF and protein is likely to have reduced the water binding of these components, making more water available for the starch. Plausibly acid production can also induce hydrolysis of the starch during extrusion, which may affect starch gelatinization. In addition, the more soluble protein can have a foam stabilising effect as rye bran protein has high foam capacity and stability even after heating. Possibly, the increased water binding of the starch caused by the acidification could also improve the behaviour in extrusion.

Based on this study, LAB fermentation is a potential method to improve the structure of bran enriched high DF extrudates. Assuming a serving size of 30 g, the extrudates in this study would provide over 20% of the recommended 25 g DF intake, while retaining a crispy expanded structure.

CRediT authorship contribution statement

Markus Nikinmaa: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing - original draft, Visualization. **Ilkka Kajala:** Methodology, Investigation, Resources. **Xia Liu:** Supervision, Writing - review & editing. **Emilia Nordlund:** Supervision, Writing - review & editing, Funding acquisition. **Nesli Sozer:** Conceptualization, Supervision, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no conflict of interests.

Acknowledgements

Antti Nyyssölä is acknowledged for assistance with enzyme production. Ulla Holopainen-Mantila is acknowledged for microscopy imaging. Riitta Pasanen and Leila Kostamo are thanked for their skilled technical assistance.

This project was funded by General Mills.

References

Abedfar, A., Hosseininezhad, M., & Rafe, A. (2020). Effect of microbial exopolysaccharide on wheat bran sourdough: Rheological, thermal and microstructural characteristics. *International Journal of Biological Macromolecules, 154*, 371–379. https://doi.org/10.1016/j.ijbiomac.2020.03.149.

Alam, S. A., Järvinen, J., Kirjoranta, S., Jouppila, K., Poutanen, K., & Sozer, N. (2014). Influence of particle size reduction on structural and mechanical properties of extruded rye bran. *Food and Bioprocess Technology, 7*(7), 2121–2133. https://doi.org/10.1007/s11947-013-1225-2.

Andersson, A. A. M., Landberg, R., Söderman, T., Hedkvist, S., Katina, K., Juvonen, R., ... Åman, P. (2011). Effects of alkylresorcinols on volume and structure of yeast-leavened bread. *Journal of the Science of Food and Agriculture, 91*, 226–232.

AOAC (1995). *AOAC Official Method 991.43 Total, Soluble, and Insoluble Dietary Fibre in FoodsOfficial Methods of Analysis* (16th ed.). Association of Official Analytical Chemists.

Boskov Hansen, H., Andreasen, M. F., Nielsen, M. M., Larsen, L. M., Bach Knudsen, K. E., Meyer, A. S., ... Hansen, A. (2002). Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread-making. *European Food Research and Technology, 214*(1), 33–42. https://doi.org/10.1007/s00217-001-0417-6.

Brennan, M. A., Monro, J. A., & Brennan, C. S. (2008). Effect of inclusion of soluble and insoluble fibres into extruded breakfast cereal products made with reverse screw configuration. *International Journal of Food Science and Technology, 43*(12), 2278–2288. https://doi.org/10.1111/j.1365-2621.2008.01867.x.

Brijs, K., Bleukx, W., & Delcour, J. A. (1999). Proteolytic activities in dormant rye (Secale cereale L.) grain. *Journal of Agricultural and Food Chemistry, 47*(9), 3572–3578. https://doi.org/10.1021/jf990070t.

Gys, W., Courtin, C. M., & Delcour, J. A. (2004). Reduction of xylanase activity in flour by debranning retards syruping in refrigerated doughs. *Journal of Cereal Science, 39*(3), 371–377. https://doi.org/10.1016/j.jcs.2004.01.003.

Hammes, W. P., & Gänzle, M. G. (1998). Sourdough breads and related products. In B. J. B. Wood (Ed.), Microbiology of Fermented Foods (pp. 199–216). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4613-0309-1_8.

Heidenreich, S., Jaros, D., Rohm, H., & Ziems, A. (2004). Relationship between water activity and crispness of extruder rice crisps. *Journal of Texture Studies, 35*(6), 621–633. https://doi.org/10.1111/j.1745-4603.2004.35513.x.

Hemery, Y., Chaurand, M., Holopainen, U., Lampi, A.-M., Lehtinen, P., Piironen, V., ... Rouau, X. (2011). Potential of dry fractionation of wheat bran for the development of food ingredients, part I: Influence of ultra-fine grinding. *Journal of Cereal Science, 53*(1), 1–8. https://doi.org/10.1016/j.jcs.2010.09.005.

Kajala, I., Shi, Q., Nyyssölä, A., Maina, N. H., Hou, Y., Katina, K., ... Juvonen, R. (2015). Cloning and characterization of a Weissella confusa dextransucrase and its application in high fibre baking. *PLoS ONE, 10*(1), 1–19. https://doi.org/10.1371/journal.pone.0116418.

Karppinen, S., Kiiliöinen, K., Liukkonen, K., Forssell, P., & Poutanen, K. (2001). Extraction and in vitro fermentation of rye bran fractions. *Journal of Cereal Science, 34*(3), 269–278. https://doi.org/10.1006/jcrs.2001.0413.

Katina, K., Maina, N. H., Juvonen, R., Flander, L., Johansson, L., Virkki, L., ... Laitila, A. (2009). In situ production and analysis of Weissella confusa dextran in wheat sourdough. *Food Microbiology, 26*(7), 734–743. https://doi.org/10.1016/j.fm.2009.07.008.

Moraru, C. I., & Kokini, J. L. (2003). Nucleation and expansion during extrusion and microwave heating of cereal foods. *Comprehensive Reviews in Food Science and Food Safety, 2*(4), 147–165. https://doi.org/10.1111/j.1541-4337.2003.tb00020.x.

Nelson, N. (1944). A Photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry, 153*(2), 375–380.

Nikinmaa, M., Alam, S. A., Raulio, M., Katina, K., Kajala, I., Nordlund, E., & Sozer, N. (2017). Bioprocessing of bran with exopolysaccharide producing microorganisms as a tool to improve expansion and textural properties of extruded cereal foams with high dietary fibre content. *LWT - Food Science and Technology, 77*, 170–177. https://doi.org/10.1016/j.lwt.2016.11.041.

Nilsson, M., Åman, P., Härkönen, H., Hallmans, G., Bach Knudsen, K. E., Mazur, W., & Adlercreutz, H. (1997). Content of nutrients and lignans in roller milled fractions of rye. *Journal of the Science of Food and Agriculture, 73*(2), 143–148. https://doi.org/10.1002/(SICI)1097-0010(199702)73:2<143::AID-JSFA698>3.0.CO;2-H.

Pai, D. A., Blake, O. A., Hamaker, B. R., & Campanella, O. H. (2009). Importance of extensional rheological properties on fiber-enriched corn extrudates. *Journal of Cereal Science, 50*(2), 227–234. https://doi.org/10.1016/j.jcs.2009.05.007.

Park, E. Y., Fuerst, E. P., & Baik, B.-K. (2019). Effect of bran hydration with enzymes on functional properties of flour–bran blends. *Cereal Chemistry, 96*, 273–282.

Paula, A. M., & Conti-Silva, A. C. (2014). Texture profile and correlation between sensory and instrumental analyses on extruded snacks. *Journal of Food Engineering, 121*(1), 9–14. https://doi.org/10.1016/j.jfoodeng.2013.08.007.

Rasmussen, C. V., Boskov Hansen, H., Hansen, Å., & Melchior Larsen, L. (2001). pH-, temperature- and time-dependent activities of endogenous endo-β-D-xylanase, β-D-xylosidase and α-L-arabinofuranosidase in extracts from ungerminated rye (Secale cereale L.) grain. *Journal of Cereal Science, 34*(1), 49–60. https://doi.org/10.1006/jcrs.2000.0376.

Robin, F., Bovet, N., Pineau, N., Schuchmann, H. P., & Palzer, S. (2011a). Online shear viscosity measurement of starchy melts enriched in wheat bran. *Journal of Food Science, 76*(5), https://doi.org/10.1111/j.1750-3841.2011.02193.x.

Robin, F., Dubois, C., Pineau, N., Schuchmann, H. P., & Palzer, S. (2011b). Expansion mechanism of extruded foams supplemented with wheat bran. *Journal of Food Engineering, 107*(1), 80–89. https://doi.org/10.1016/j.jfoodeng.2011.05.041.

Robin, F., Dattinger, S., Boire, A., Forny, L., Horvat, M., Schuchmann, H. P., & Palzer, S. (2012). Elastic properties of extruded starchy melts containing wheat bran using online rheology and dynamic mechanical thermal analysis. *Journal of Food Engineering, 109*(3), 414–423. https://doi.org/10.1016/j.jfoodeng.2011.11.006.

Santala, O., Kiran, A., Sozer, N., Poutanen, K., & Nordlund, E. (2014). Enzymatic modification and particle size reduction of wheat bran improves the mechanical properties and structure of bran-enriched expanded extrudates. *Journal of Cereal Science, 60*(2), 448–456. https://doi.org/10.1016/j.jcs.2014.04.003.

Santala, O., Nordlund, E. A., & Poutanen, K. S. (2013). Treatments with Xylanase at High (90%) and Low (40%) water content have different impacts on physicochemical properties of wheat bran. *Food and Bioprocess Technology, 6*(11), 3102–3112. https://doi.org/10.1007/s11947-012-0967-6.

Silventoinen, P., Sipponen, M. H., Holopainen-Mantila, U., Poutanen, K., & Sozer, N. (2018). Use of air classification technology to produce protein-enriched barley ingredients. *Journal of Food Engineering, 222*, 169–177. https://doi.org/10.1016/J.JFOODENG.2017.11.016.

Sriburi, P., & Hill, S. E. (2000). Extrusion of cassava starch with either variations in ascorbic acid concentration or pH. *International Journal of Food Science and Technology, 35*(2), 141–154. https://doi.org/10.1046/j.1365-2621.2000.00360.x.

Van Hecke, E., Allaf, K., & Bouvier, J. M. (1995). Texture and structure of crispy puffed food products II: mechanical properties in puncture. *Journal of Texture Studies, 29*(1), 617–632. https://doi.org/10.1111/j.1745-4603.1995.tb00781.x.

Yanniotis, S., Petraki, A., & Soumpasi, E. (2007). Effect of pectin and wheat fibers on quality attributes of extruded cornstarch. *Journal of Food Engineering, 80*(2), 594–599. https://doi.org/10.1016/j.jfoodeng.2006.06.018.