Selective modification of wheat bran affects its impact on gluten-starch dough rheology, microstructure and bread volume

Yamina DE BONDT[a*], Wisse HERMANS[a], Paula MOLDENAERS[b] and Christophe M. COURTIN[a]

[a] Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

[b]Soft Matter, Rheology and Technology, Department of Chemical Engineering, KU Leuven, Celestijnenlaan 200F, Box 2424, B-3001 Leuven, Belgium

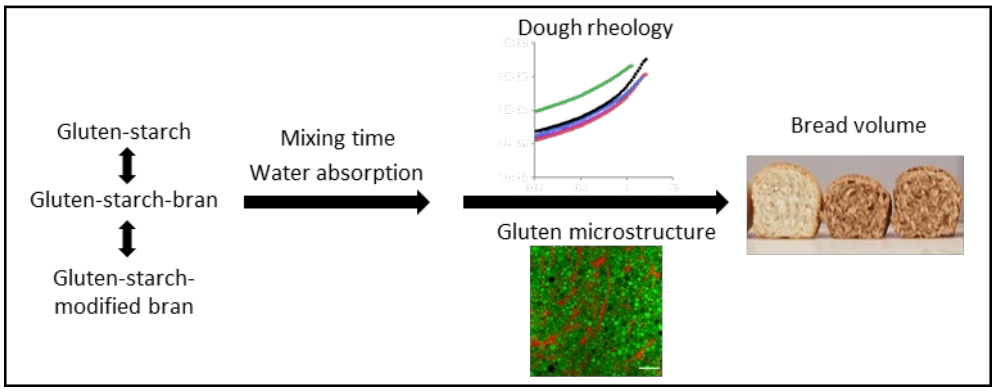
[*] Corresponding author. Phone: (+32)-16-372542

E-mail: yamina.debondt@kuleuven.be

Abstract

Wheat bran incorporation in bread has multiple health benefits, but also a detrimental effect on overall bread quality. Bran is hypothesised to withdraw water from gluten, resulting in less optimal viscoelastic dough properties and a lower gas retention capacity, in turn resulting in a decreased bread loaf volume. In this study, wheat bran samples having different water retention capacities were produced and used to investigate this hypothesis. Gluten-starch model systems were used and the effect of substitution of part of the starch by bran in combination with different water absorptions and mixing times was evaluated. The properties of the gluten network in the doughs were investigated using rheological and microstructural analyses and these properties were linked to the final bread loaf volume. A proper gluten network microstructure, as visualised with CLSM, could be achieved in the presence of wheat bran. However, significant effects of the type of wheat bran, water absorption and mixing time on dough rheology and loaf volume were observed. Wheat bran addition decreased the strain hardening of dough despite optimisation of water absorption and mixing time. The deleterious effect of wheat bran on dough rheology increased by adding modified wheat bran with high water retention capacity and surface area. The results indicate that dynamic water redistribution after mixing and gas cell incorporation can have an effect on dough rheology when wheat bran is added to dough. The strain hardening behaviour of dough proved itself a valuable predictor of bread loaf volume also in the presence of (modified) wheat bran.

Graphical abstract



Keywords

Wheat bran; dough rheology; gluten; CLSM; bread; water retention

1 Introduction

Because of the high dietary fibre content and rich nutritional profile, consumption of bran-rich food products is, from a health perspective, more interesting than consumption of products based on refined wheat flour. Indeed, consumption of wheat bran has been associated with health benefits such as improved gastrointestinal health and a decreased risk for the development of cardiovascular diseases, cancer and metabolic diseases (Stevenson et al., 2012). However, consumption of wheat bran enriched foods remains low, because most consumers prefer products made with refined flour. The incorporation of bran in cereal-based foods poses significant challenges during processing and on product quality (Hemdane, Jacobs, et al., 2016). Because bread is an important staple food in many countries, insight into the effect of wheat bran in bread making is crucial to increase whole wheat bread consumption. However, there is a lack of insight into the mechanism(s) responsible for the deleterious effect of wheat bran. This hampers the development of strategies for improvement.

The incorporation of wheat bran in bread has been associated with unwanted effects on dough properties, bread loaf volume, colour, texture and taste. Different mechanisms have been proposed to explain the effect of wheat bran on bread loaf volume (Hemdane, Jacobs, et al., 2016). It has been demonstrated that the lower bread loaf volume in the presence of wheat bran is not caused by a decrease in the gassing power of the dough but by a decrease in the gas retention capacity (Pomeranz et al., 1977; Sanz Penella et al., 2008). Several studies ascribe this effect to the competition for water between bran and gluten, which may cause considerable modifications to the gluten network (Bock et al., 2013; Hemdane et al., 2017; Hemdane, Langenaeken, et al., 2016; Lai et al., 1989; Li et al., 2012). Indeed, the strong water-binding properties of bran may negatively affect dough rheology (Hemdane et al., 2018). Bock *et al.* (2013) and Li *et al.* (2012) stated that because of bran induced redistribution of water in dough, the secondary structure of gluten changes and this is the basis of the poor quality of whole wheat bread. Nevertheless, a comprehensive study into the combined effect of wheat bran and water binding on the gluten network development is lacking.

During the entire bread-making procedure, dough is submitted to stress and deformation. The rheological properties of dough are involved in its functional behaviour and final product quality. Strain hardening of gluten proteins in dough promotes equal growth of gas cells and delays their coalescence and disproportionation during late fermentation and early baking (Meeus et al., 2019). Recent work (Meerts et al., 2017a, 2017b) has shown the value of pure uniaxial extensional measurements for studying the rheology of wheat doughs at large deformations in the non-linear regime. In literature, the effect of wheat bran on dough rheology has to the best of our knowledge, however, only been studied with empirical methods using the Farinograph (Sanz Penella et al., 2008; Zhang & Moore, 1997), Extensigraph (Zhang & Moore, 1997), Alveograph (Gómez et al., 2011) and Rheofermentometer (Gómez et al., 2011; Sanz Penella et al., 2008). Besides, the effect of water content is not clear, as dough development conditions are often not optimised. Jacobs *et al.* (2016) indicated the importance of the water absorption and mixing time on the ability of dough to retain gases during fermentation and baking and consequently bread loaf volume. They reported that for wheat bran addition, baking absorptions as determined by Farinograph and Mixograph do not correspond to the baking absorptions that coincide with the maximal loaf volume. This makes studying the behaviour of bran in bread extremely complicated, as it is challenging to disentangle the effects of altered dough formulations and direct effects of wheat bran.

Against this background, more insight into the effect of wheat bran hydration properties on gluten network development is needed. Moreover, the effect of dough water absorption and mixing time should be included when examining the effect of wheat bran in bread. Therefore, in this study, the hypothesis that the high water binding capacity of wheat bran causes a modification of the microstructure of the gluten network and/or its rheological properties and, therefore, a lower loaf volume was evaluated. This was done by creating wheat bran samples with different water binding capacities. The effect of these wheat bran samples on the viscoelastic gluten network in dough was investigated at different water absorptions and mixing times and linked to the effect on bread loaf volumes. A gluten-starch mixture as a simplified model system for flour was used. It allowed us to

keep the gluten content constant when wheat bran is added and excludes the effect of gluten dilution. By using different water absorptions and mixing times, we could evaluate if, under optimal conditions, a similar gluten network with good gas retention capacity could be developed and contribute to a high bread loaf volume. The development of the gluten network was investigated by studying the gluten network microstructure. The viscoelastic properties of the dough were evaluated using a fundamental rheological technique rather than empirical ones. The effect of the different water absorptions and mixing times combined with the wheat bran samples with different water binding capacities will inform us of the effect of wheat bran hydration properties during bread making.

2 Materials and methods

2.1 Materials

Commercial wheat (*Triticum aestivum* L.) bran was provided by Dossche Mills (Deinze, Belgium). The wheat bran consisted of 25.0 ± 2.8% AX, 11.4 ± 1.0% starch, 18.1 ± 0.5% proteins, 5.4 ± 0.5% lipids and 6.4 ± 0.1% ash as described by De Bondt *et al*. (2020). Vital wheat gluten (ca. 75% proteins) and wheat starch were from Tereos Syral (Aalst, Belgium). Sugar, salt and fresh compressed baker's yeast (Koningsgist, AB Mauri, Dordrecht, The Netherlands) were purchased at a local supermarket. The dyes used for microscopy were from Sigma Aldrich (Bornem, Belgium).

2.2 Wheat bran modification

Three different wheat bran samples were obtained by dry milling (DM) of commercial wheat bran: coarse (C_{DM}), fine (F_{DM}) and ultrafine (UF_{DM}) wheat bran. C_{DM} was obtained by milling the commercial wheat bran using a Cyclotec 1093 Sample mill (FOSS, Höganäs, Sweden) with a sieve with mesh size 2 mm. The F_{DM} and UF_{DM} wheat bran were obtained by milling the commercial wheat bran for 30 seconds and 2 min, respectively, in a vibratory disc mill RS 200 (Retsch, Haan, Germany) equipped with a stainless steel grinding jar of 250 mL (20 g dm wheat bran). With wet milling (WM), an

additional wheat bran sample with an ultrafine particle size was obtained (UF_{WM}). Herefore, a suspension of bran in deionised water (20% dm) was milled for 6 min in the vibratory disc mill RS 200. After milling, the suspension was freeze-dried before further use.

2.3 Particle size distribution

The particle size of the modified wheat bran samples was measured with the wet module of an LS 13 320 Laser Diffraction Analyser (Beckman Coulter, Suarlée, Belgium). Approximately 50 mg of bran was suspended in 20 mL water and sonicated (20 kHz, amplitude 40%) for 1 minute to avoid aggregation. This sample was analysed in quadruplicate and the particle size was calculated using the Fraunhofer Theory.

2.4 Hydration properties of wheat bran

The total water retention capacity (TWRC) was measured in triplicate based on the method described by Jacobs et al. (2015) with some slight modifications. Wheat bran (1.000 g) was weighed in a falcon tube and 30 mL deionised water was added. The falcon tube was shaken for 30 min (150 rpm, 7°C) and centrifuged for 10 min (4000 *g,* 7°C) after which the supernatant was discarded from the pellet. The residue was weighed and reweighed after drying overnight in an oven at 90°C. The water held by the sample was expressed on the initial dry mass of the sample, as shown by this equation:

$$Total\ water\ retention\ capacity\ \left(\frac{mL}{g}\right) = \frac{mass\ pellet\ after\ centrifugation - mass\ pellet\ after\ drying}{inital\ dry\ mass\ of\ sample}$$

The strong water retention capacity (SWRC) was determined in triplicate with drainage centrifugation based on the method described by Jacobs *et al.* (2015) and optimised by De Bondt *et al.* (2020). Wheat bran (50 mg) was added to the upper part of a QIAprep Spin Miniprep Columns (Qiagen, Hilden, Germany) and 700 µl deionised water was added. Samples were hydrated for 1 h

and centrifuged for 10 min at 15000g. Afterwards, the samples were weighed and reweighed after drying overnight at 90°C. A blanc with water was used to correct for the amount of water held by the filter. The SWRC was calculated as the amount of water held by the initial dry mass of the sample:

$$Strong\ water\ retention\ capacity\ \left(\frac{mL}{g}\right) = \frac{m_{centr} - m_{dry} - m_{filter}}{m_{initial\ dry}}$$

With $m_{initial\ dry}$ the initial dry mass of the sample, m_{centr} the mass of the material in the column after centrifugation, m_{dry} the mass of the material in the column after drying and m_{filter} the mass of water held by the filter.

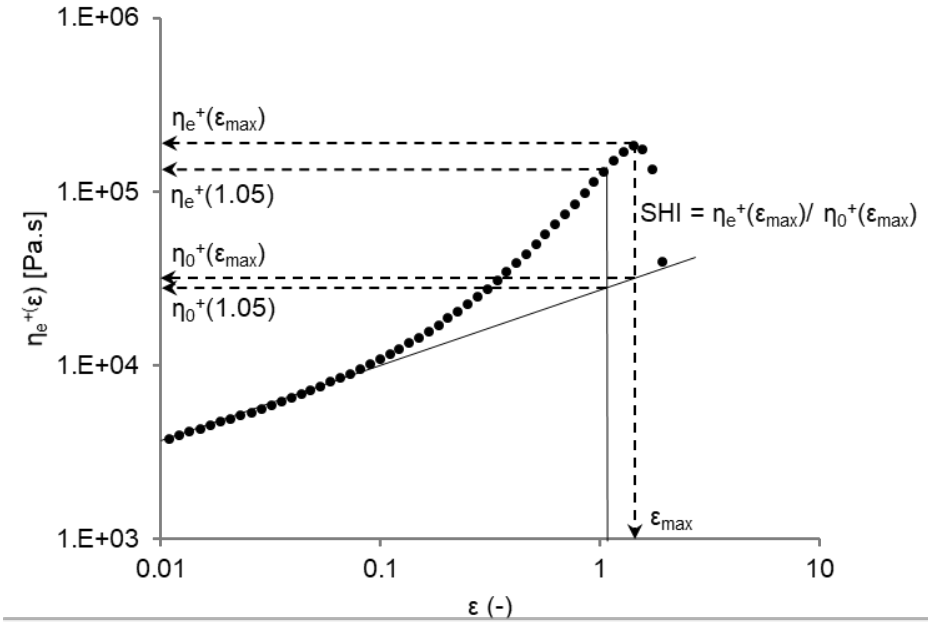
2.5 Nitrogen physisorption

The specific surface area of the wheat bran samples was measured using the BET Surface Area Analyser (3P meso 222, 3P Instruments, Odelzhausen, Germany) based on nitrogen gas sorption at 77 K. Samples were freeze-dried and 1 to 2 g of sample was used for analysis. Degassing was done for 6 hours at 60°C, and the measurements were done at a dose amount of 0.5ml/g until a relative pressure (p/p0) of 0.3 and 2.0 ml/g until a relative pressure of 0.99.

2.6 Dough rheology

Dough rheology was measured according to Meerts *et al.* (2017b) with some slight modifications. Dough was prepared as described in 2.2.1, i.e. including yeast, sugar and salt. After kneading, the dough was pressed between two plates for 1 min to obtain a sample thickness of 4 mm. Dough strands with fixed dimensions (length = 12.5 mm, height = 4.0 mm, thickness = 4.0 mm) were prepared using a hollow stamp and analysed without resting. The strands were attached to the extensional viscosity fixture (EVF) mounted on an ARES-G2 rheometer (TA Instruments, New Castle, DE, USA) using two clamps. Extension took place at a constant nominal rate of 0.12 s^{-1}, resulting in an effective extension rate of 0.10 s^{-1} due to slip phenomena until a Hencky strain (ε(t)) of 2.89 was reached. The reported data are means of measurements on seven dough strands from two separate

dough batches. In Figure 1 the different parameters derived from the extensional viscosity curves are shown alongside the symbols used. In the other figures, the measuring points after breakage of the dough strands are removed for clarity of the graphs.



Symbol	Definition	Terminology
ε	Uniaxial extensional Hencky strain	Strain
η_e^+	Transient extensional viscosity	Viscosity
ε_{max}	Maximal uniaxial extensional Hencky strain before strand breaks	Strain at the breakpoint
η_{e0}^+ (1.05)	Extrapolated transient extensional viscosity at strain 1.05	Linear viscosity at strain 1.05
η_{e0}^+ (ε_{max})	Extrapolated transient extensional viscosity at maximal strain	Linear viscosity at maximal strain
η_e^+ (1.05)	Transient extensional viscosity at strain 1.05	Viscosity at strain 1.05
$\eta_e^+(\varepsilon_{max})$	Maximal transient extensional viscosity	Maximum viscosity
SHI	$[\eta_e^+(\varepsilon_{max})/\eta_{e0}^+(\varepsilon_{max})]$	SHI = strain hardening index

Figure 1. An example of an extensional viscosity curve that is obtained using the setup that is used and the parameters derived from it.

2.7 Confocal laser scanning microscopy (CLSM)

Dough was prepared as described in 2.2.1, but part of the water was replaced by solutions containing calcofluor (2.00 ml; 0.1 mg/ml), fluoresceïne (0.43 ml; 0.1 mg/ml) and rhodamine B (0.43 ml; 0.1 mg/ml), such that the dough contained 23 ppm calcofluor and 5 ppm fluoresceine and rhodamine B

each on flour basis. After kneading, the dough was squeezed between slides and and the fluorescently stained components visualised with a 20x objective on a Fluoview 1000 confocal laser scanning microscope (Olympus). Images were obtained by sequentially scanning with different laser beams and emission filters. Calcofluor was excited with a laser of 405 nm and detected between 410 and 490 nm. Fluoresceine and Rhodamine B were excited with a laser of 488 and 561 nm respectively and dectected between 495-540 nm and 575-620 nm. Images of 1024 x 1024 pixels were made with a speed of 4 µs/pixel and size 635.9 µm x 635.9 µm with the use of the Kalman filter.

For better visualisation, the contrast of the pictures was adjusted with ImageJ. We choose to adjust the contrast up to 1% saturated pixels with histogram stretching using the 'enhance contrast' function. The image of the red channel shows the gluten strands and is further analysed with AngioTool (see further). The green channel shows both proteins and starch. To visualise the proteins and starch simultaneously, the red channel was subtracted from the green channel to create a new green image representing only fluorescent starch. Afterwards, this channel was merged with the red channel.

Protein network analysis was performed as described by Bernklau *et al.* (2016). The red channel of the CLSM pictures was analysed with AngioTool64 version 0.6a (National Cancer Institute, National Institute of Health, Maryland, USA). The vessel diameter was set to 2 and 3 and the low and high threshold intensity to 15 and 255. Small particles were removed under 10 and the function ‘fill holes’ was deactivated. Calibration was set to 1.6 pixels/µm. Doughs were made in triplicate and from each dough, eight images were made, resulting in 24 images for each dough type. The gas cell area was determined by calculating the area of pixels with a brightness value below 300.

2.8 Bread making

The gluten-starch mixture consisted of 12% vital gluten and 88% starch, while the gluten-starch-bran mixture consisted of 12% gluten, 73% starch, 15% bran.

192 Bread was made according to the straight dough procedure of Shogren and Finney (1984). The
193 following formula was used: 10.0 g gluten-starch or gluten-starch-bran mixture (14.0% moisture
194 content), 5.3% compressed fresh yeast, 6.0% sucrose, and 1.5% salt. Water absorption and kneading
195 time were varied. The ingredients were mixed in a 10 g pin bowl mixer (National Manufacturing,
196 Lincoln, NE, USA). Dough fermentation was performed in a fermentation cabinet (National
197 Manufacturing) at 30 °C and 90% relative humidity. Punching of the dough was done after 52 and 77
198 min of fermentation. After 90 min of fermentation, the dough was punched, moulded, and proofed
199 for an additional 36 min. The dough was baked for 13 min at 232 °C in a rotary oven (National
200 Manufacturing, Lincoln, NE, USA). Loaf volume was determined with a Volscan Profiler (Stable Micro
201 Systems, UK).

202

203 ### 2.9 Statistics

204 For dough rheology, seven measurements were performed on two dough samples and for CLSM
205 eight measurements were performed on three dough samples. A mixed model was constructed to
206 identify significant differences in parameters, and means were compared with a Tukey test. In this
207 way, both the variability in the strands and in the dough samples was taken into account.

208 # 3 Results

209 ### 3.1 Hydration properties of dry-milled and wet-milled wheat bran

210 Wheat bran was milled in different ways to obtain four wheat bran samples with different hydration
211 properties (Table 1). Three wheat bran samples were obtained by dry milling (DM): coarse (C_{DM}), fine
212 (F_{DM}) and ultrafine (UF_{DM}) wheat bran. One wheat bran sample was obtained by wet milling (WM):
213 ultrafine (UF_{WM}).

214 C_{DM}, obtained after dry milling with a Cyclotec 1093 Sample mill, had a median particle sizes (d_{50}) of
215 963.7 µm. Dry milling with a vibratory disc mill for 30 seconds and 2 minutes resulted in bran with a

d_{50} of 193.5 µm (F_{DM}) and 28.5 µm (UF_{DM}), respectively. A similar ultrafine particle size (d_{50} of 28.4 µm) was obtained by wet milling wheat bran for 6 minutes, resulting in the UF_{WM} wheat bran.

Particle size reduction by dry milling slightly increased the specific surface area of the wheat bran, but the UF_{WM} bran had a much higher surface area (1.49 m^2/g dm) than the UF_{DM} bran (0.48 m^2/g dm). On the one hand, the SWRC of the samples followed the same trend as the surface area. On the other hand, the TWRC decreased during progressive dry milling. Wet milling also reduced the TWRC in comparison with the C_{DM} bran. The decrease was smaller for the UF_{WM} bran (3.93 ml/g dm) than for the UF_{DM} bran (3.22 ml/g dm).

Table 1 The physical properties of coarse bran (C_{DM}), fine bran (F_{DM}) and ultrafine bran obtained by dry-milling (UF_{DM}) and wet milling (UF_{WM}). Means in the same row with a different letter are significantly different ($p<0.05$).

	C_{DM}	**F_{DM}**	**UF_{DM}**	**UF_{WM}**
d_{50} (µm)	963.7 ± 80.5^a	193.5 ± 60.8^b	28.5 ± 0.9^c	28.4 ± 0.3^c
BET surface area (m^2/g dm)	0.35 ± 0.02^c	0.33 ± 0.05^c	0.48 ± 0.02^b	1.49 ± 0.06^a
Total water retention capacity (ml/g dm)	4.96 ± 0.27^a	3.37 ± 0.04^c	3.22 ± 0.0^c	3.93 ± 0.02^b
Strong water retention capacity (ml/g dm)	0.90 ± 0.02^c	0.97 ± 0.02^c	1.09 ± 0.05^b	1.39 ± 0.04^a

3.2 Bread loaf volume of gluten-starch mixtures with and without modified wheat bran

Breads prepared from gluten-starch mixtures with and without modified wheat bran were made with different water absorptions and mixing times. The optimal conditions for dough development for the gluten-starch mixture were determined by Mixograph and Farinograph and were 4.0 min mixing time and 51.4% Farinograph water absorption, respectively (results not shown). In the presence of wheat bran, Farinograph and Mixograph do not provide the processing conditions that correspond with an optimal bread loaf volume. It was shown that the higher the water absorption is, the higher the bread loaf volume will be, with dough handling properties as the limiting factor (Roels et al., 1993).

Therefore, the maximal water absorption of a starch-coarse bran-water mixture that still gives a manageable dough was manually determined (61.6%). For the mixtures with modified wheat bran, the same maximal water absorption was used. Based on these values and some preliminary dough making trials, a range of manageable water absorptions and mixing times was chosen for gluten-starch and gluten-starch-bran bread-making (Figure 2).

Figure 2 shows the specific loaf volume of bread made with gluten-starch or gluten-starch-bran mixtures for different water absorptions and mixing times. The largest loaf volume (2.95±0.13 ml/g) was achieved with the gluten-starch mixture at a water absorption of 51.4% and mixing time 4.0 min. With the addition of bran, a maximal specific volume of only 2.50±0.10 ml/g could be achieved. Particle size reduction with dry milling always resulted in smaller bread loaf volumes. Bread made with UF_{WM} bran did not rise at all and was therefore very small. Changing the water absorption or mixing time did not have any effect on the dough made with UF_{WM} bran.

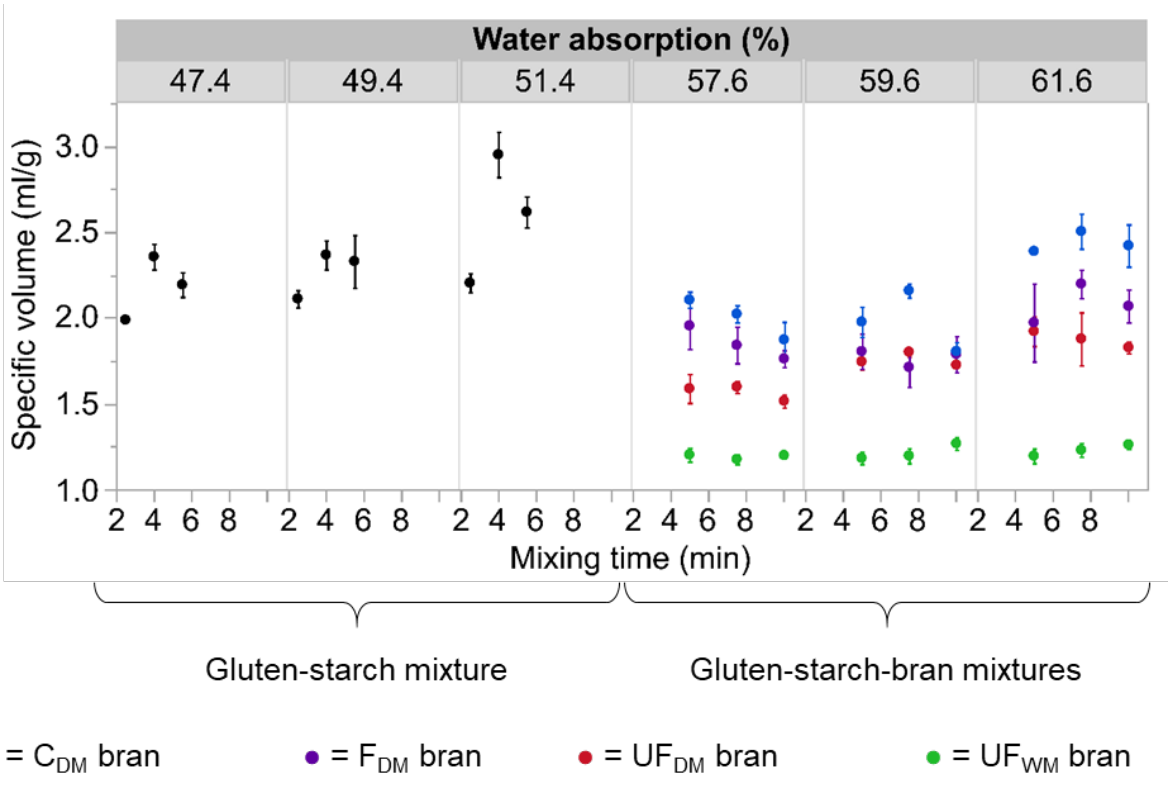


Figure 2. The specific volume of bread made with gluten-starch(-bran) mixtures with different water absorptions and mixing times. Coarse bran (C_{DM}), fine bran (F_{DM}) and ultrafine bran obtained by dry-milling (UF_{DM}) and wet milling (UF_{WM}) were used. Means of triplicate measurements are given with standard deviations.

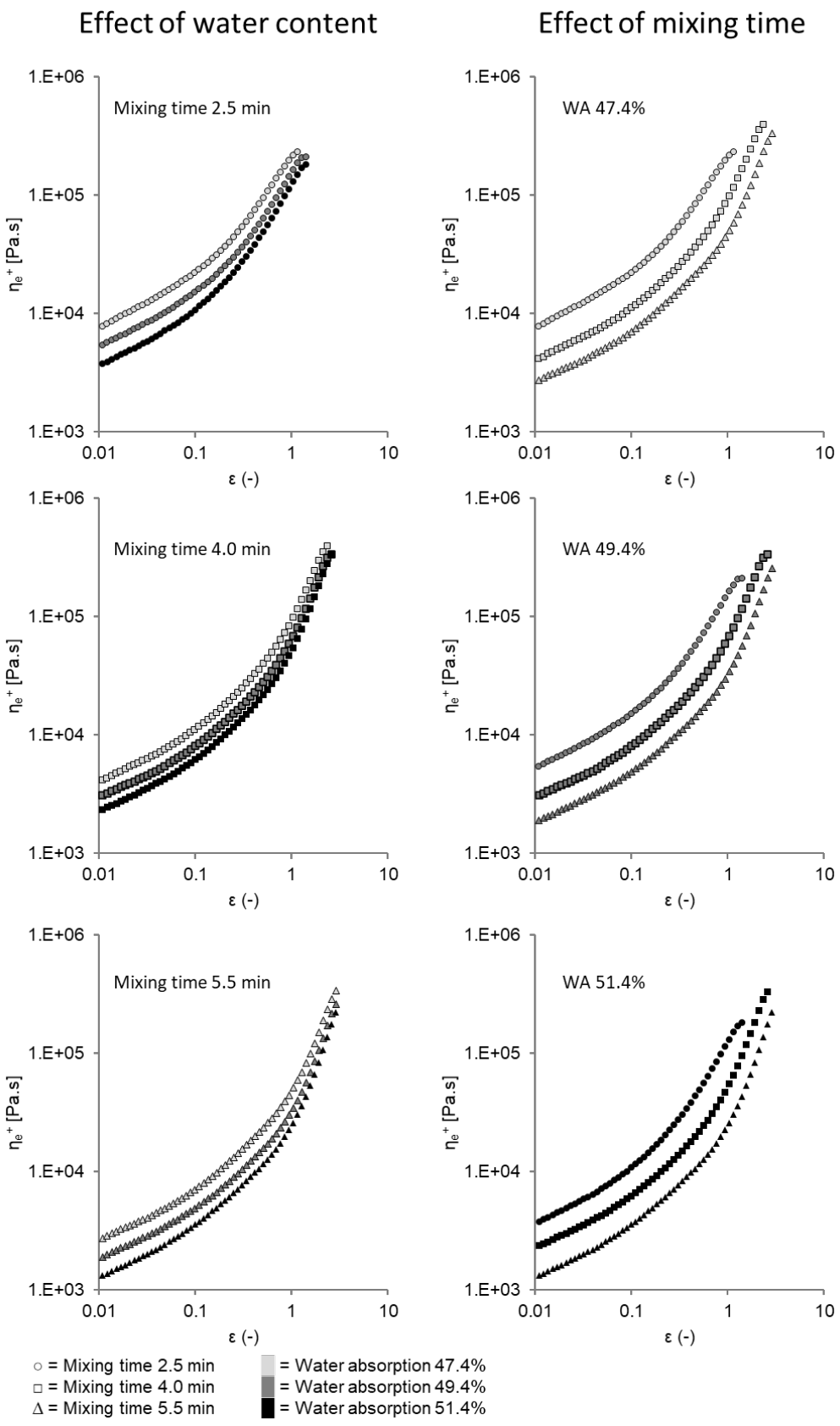
3.3 Rheology of gluten-starch dough at different water absorptions and mixing times

The effect of water content and mixing time on the rheology of gluten-starch dough is shown in Figure 3. For all curves, the transient extensional viscosity η_e^+ increased linearly between a strain of 0.01 and 0.1. At higher strains, η_e^+ increased super-linearly, which reflects the strain hardening behaviour of the viscoelastic gluten network. When the mixing time was increased, the whole curve shifted downwards. On the contrary, for increasing water contents, the linear part of the curve shifted downwards whereas at larger strains the curves at different water contents increase until a similar viscosity.

Several values can be deduced from the curves and used to compare them (Figure 4) quantitatively (Figure 1). The minimal strain at which the dough strands in this study break is 1.05. Therefore, the **viscosity at strain 1.05** is used to compare all dough strands in the non-linear part at the same viscosity. The extrapolation of the linear part of the curve (strain 0.01-0.1) to strain 1.05 gives a good indication of the behaviour of the dough in the linear part because it takes into account the absolute value and the slope of the linear part. This value is named the **linear viscosity at strain 1.05**. The **strain at the breakpoint** and the **maximum viscosity** are also given, in order to be able to quantify the full non-linear part of the different dough strands. The strain hardening behaviour of the dough is quantified by the **SHI**, which is the ratio of the maximum viscosity on the linear viscosity at the maximal strain.

The linear viscosity, the viscosity at strain 1.05 and the strain at breakpoint are well correlated with water absorption and mixing time. Increasing the water content or increasing the mixing time, decreased the linear viscosity at strain 1.05 and the viscosity at strain 1.05. The strain at the breakpoint**,** which is a measure for the resistance of dough against extension, was not influenced by the water absorption but was determined by the mixing time. The maximum viscosity gave a very large variability and, therefore, it was not possible to see significant differences in maximum viscosity

for the different conditions. However, there was a trend that the maximum viscosity decreases when water absorption increases. For a mixing time of 4 minutes, considered optimal, the maximum viscosity was higher than for 2.5 or 5.5 minutes. The SHI shows an increasing trend in function of water content and mixing time.



283

284 *Figure 3. Extensional viscosity curves for gluten-starch doughs prepared at different water contents and mixing*
285 *times. Mixing times are 2.5 min (○), 5.0 min (□) or 7.5 min (Δ) and water absorption (WA) 47.4% (light grey),*
286 *49.4% (grey) or 51.4% (black).*

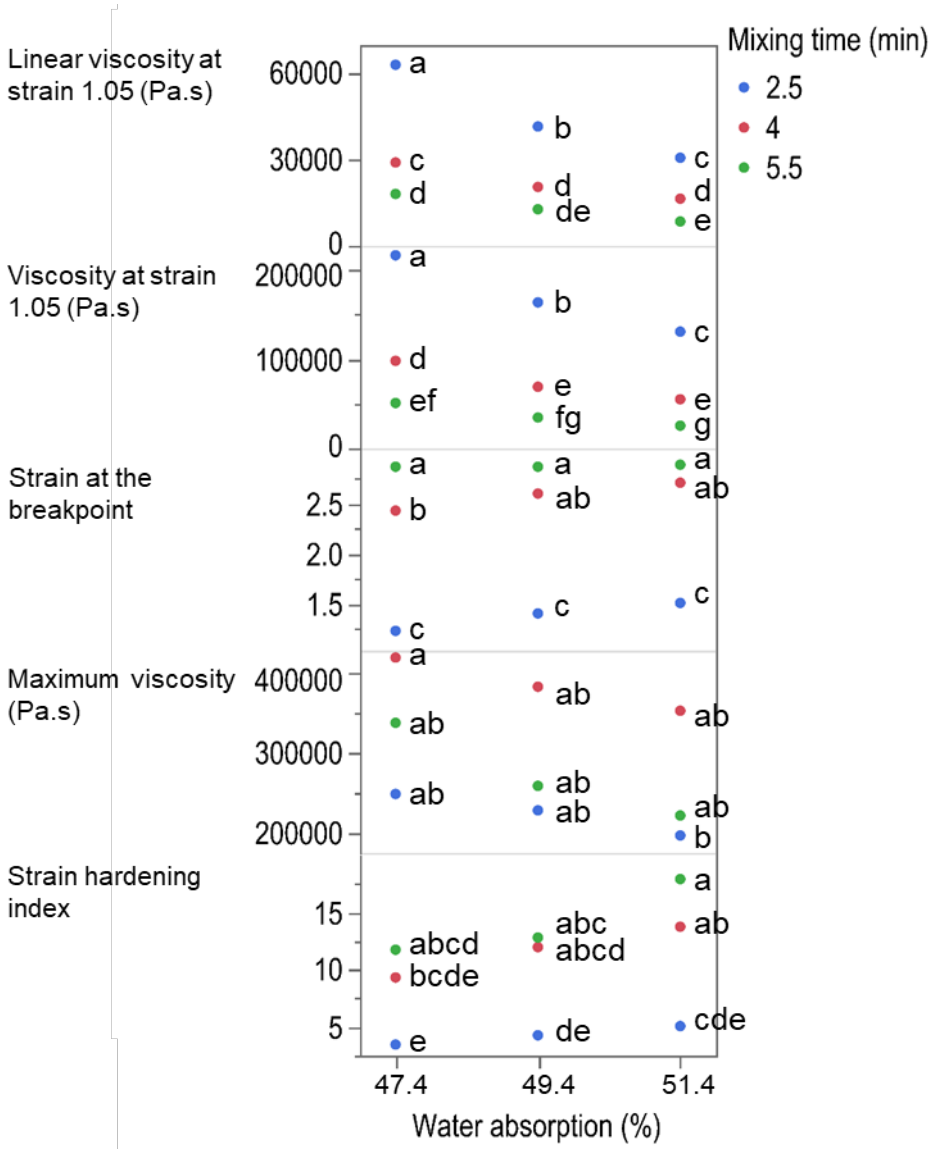


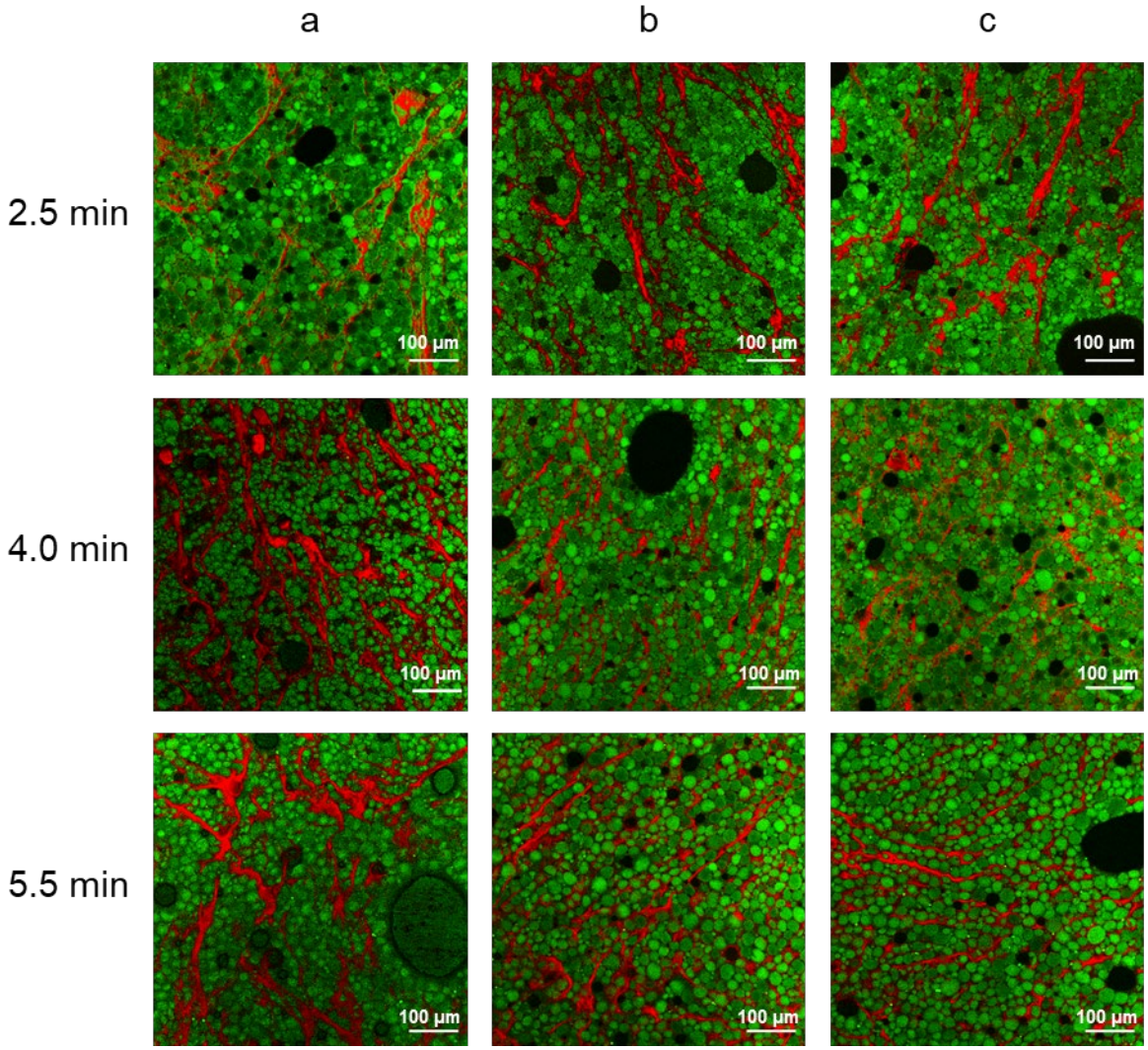
Figure 4 Rheological parameters of gluten-starch dough made with different water absorptions and mixing times, deduced from extensional viscosity curves. Means are shown. For each parameter, different letters indicate significant differences ($p<0.05$).

3.4 Gluten network microstructure in gluten-starch doughs at different mixing times

In Figure 5, the effect of mixing time on the gluten network microstructure of a gluten-starch dough is shown. At a mixing time of 2.5 min, fewer but bigger gluten strands are visible. At a mixing time of 4.0 and 5.5 min, the gluten network seems to be more distributed, surrounding the starch granules. The protein network analysis (Figure 6) indeed shows that the protein width and lacunarity (which is a measure for the presence of irregular structures and large holes) are larger and the protein length

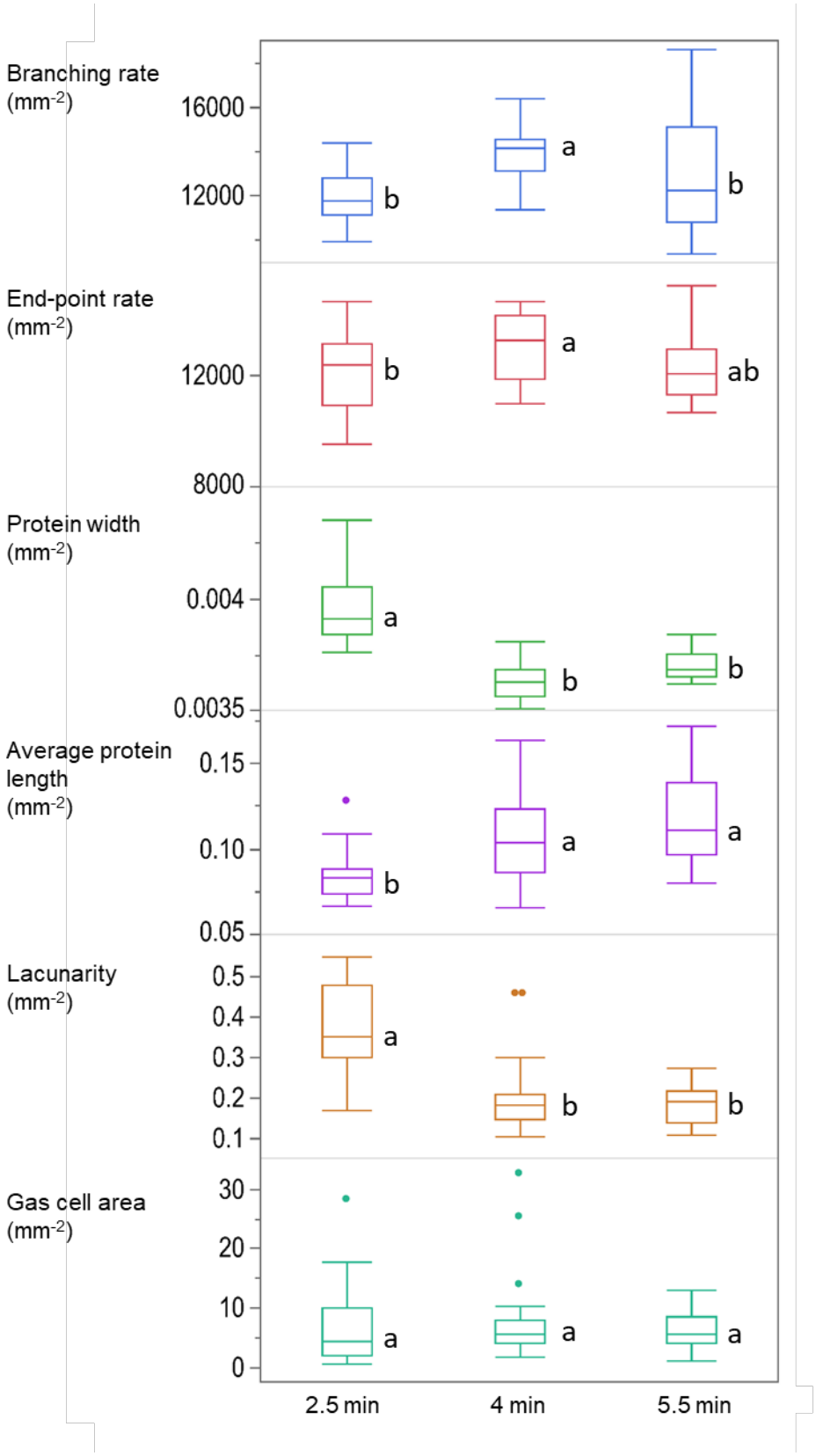
298 smaller for the dough that was mixed for 2.5 minutes than for dough mixed 4.0 or 5.5 minutes.

299 Besides, the end-point and branching rate are maximal at 4.0 minutes of mixing.



300

301 *Figure 5. Influence of mixing time on dough microstructure of gluten-starch dough. Doughs were prepared in*
302 *triplicate (a, b and c) at a water absorption of 51.4% and at different mixing times (2.5, 4.0 and 5.5 minutes).*
303 *Doughs were stained with rhodamine B to visualise proteins (red) and with fluoresceine to visualise starch*
304 *granules (green) and analysed with CLSM.*



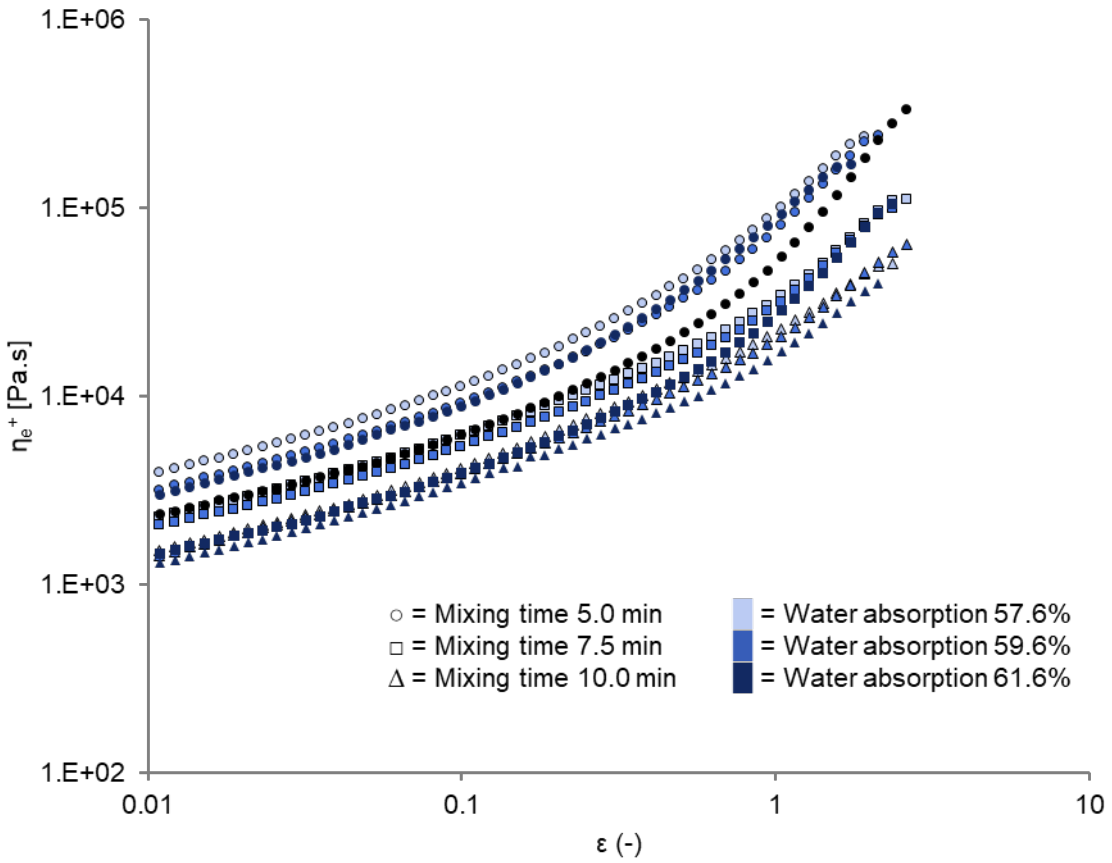
305

306 *Figure 6. Influence of mixing time on dough gluten network microstructure as analysed with AngioTool. Doughs*
307 *were prepared in triplicate (a, b and c) at a water absorption of 51.4% and at different mixing times (2.5, 4.0*
308 *and 5.5 minutes) and each dough was analysed 8 times (24 measurements). For each parameter, different*
309 *letters indicate significant differences ($p<0.05$).*

3.5 Rheology of gluten-starch doughs with coarse bran at different water absorptions and mixing times

In Figure S1 and Figure S2, the effect of water absorption and mixing time on the rheology of dough made from a mixture of gluten, starch and coarse bran is shown. There were only small effects of the water absorption on the dough rheological parameters. The effect of the mixing time on the linear viscosity at strain 1.05, the viscosity at strain 1.05 and the strain at breakpoint was similar as for dough without bran. The maximum viscosity decreased when mixing time increased, so there was no optimum as was seen with dough without bran (Figure 4).

The extensional viscosity curve of the gluten-starch dough at the conditions that correspond to the highest bread loaf volume (51.4% water absorption and 4.0 min mixing time) was compared with that of gluten-starch-coarse bran doughs with different water absorptions and mixing times (Figure 7). This shows that it is possible to get a similar viscosity in the linear part in the presence and absence of bran by changing water content and mixing time. However, in the presence of bran, the viscosity does not increase as much as in the gluten-starch dough. Besides, most gluten-starch-bran doughs break faster than the dough without bran.

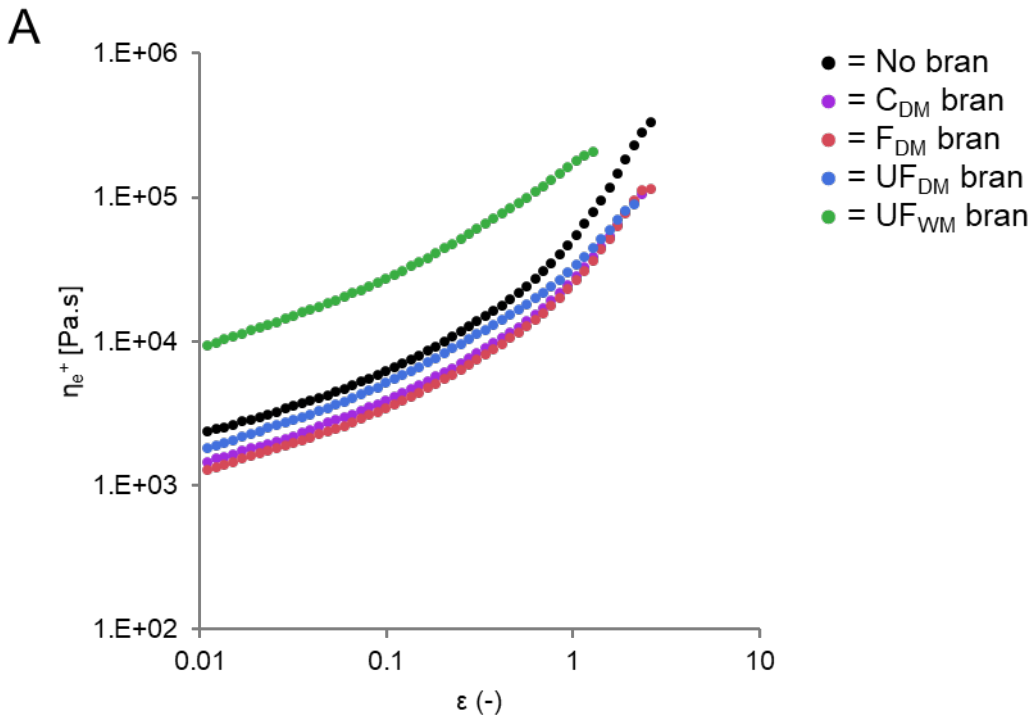


326

327 *Figure 7. Extensional viscosity curves for the optimal gluten-starch dough, prepared at 51.4% water absorption*
328 *and 4.0 minutes of mixing (black dots), together with gluten-starch-bran doughs prepared at different water*
329 *contents and mixing times.*

330 ## 3.6 Rheology of gluten-starch doughs with modified wheat bran

331 In Figure 8, the effect of the different modified wheat bran samples on the dough rheology of a
332 gluten-starch mixture is shown. For all doughs, the water absorption and mixing time that
333 correspond to the maximum bread loaf volume are used. This was a water absorption and mixing
334 time of 51.4% and 4.0 minutes or 61.6% and 7.5 minutes, for the gluten-starch and the gluten-starch-
335 bran doughs, respectively. The wheat bran-containing doughs had a significantly lower SHI (2.4-8.7)
336 than the dough without wheat bran (13.8) (Figure 8B). The dough rheology in the three dry milled
337 wheat bran samples is similar. However, the wet-milled wheat bran shows a complete different
338 rheological behaviour. The viscosity of the entire curve is much higher, and almost no strain
339 hardening could be observed. Besides, the strain at breakpoint is significantly lower than in the other
340 samples.



B

	Bread volume (ml/g)	Linear viscosity at strain 1.05 (10^4 Pa.s) $\eta_{e0}^+(1.05)$	Viscosity at strain 1.05 (10^4 Pa.s) $\eta_e^+(1.05)$	Strain at the breakpoint ε_{max}	Maximum viscosity (10^4 Pa.s) $\eta_e^+{}_{max}$	Strain hardening index SHI
No bran	2.95^a	1.6^b	5.5^b	2.71^a	35.3^a	13.76^a
C_{DM} bran	2.50^b	1.1^c	2.8^c	2.53^a	13.2^c	6.71^{bc}
F_{DM} bran	2.20^c	0.9^c	2.6^c	2.60^a	12.9^c	8.71^b
UF_{DM} bran	1.88^d	1.4^c	3.0^c	2.53^a	10.0^c	4.40^{cd}
UF_{WM} bran	1.26^e	7.6^a	16.3^a	1.48^b	21.5^b	2.37^d

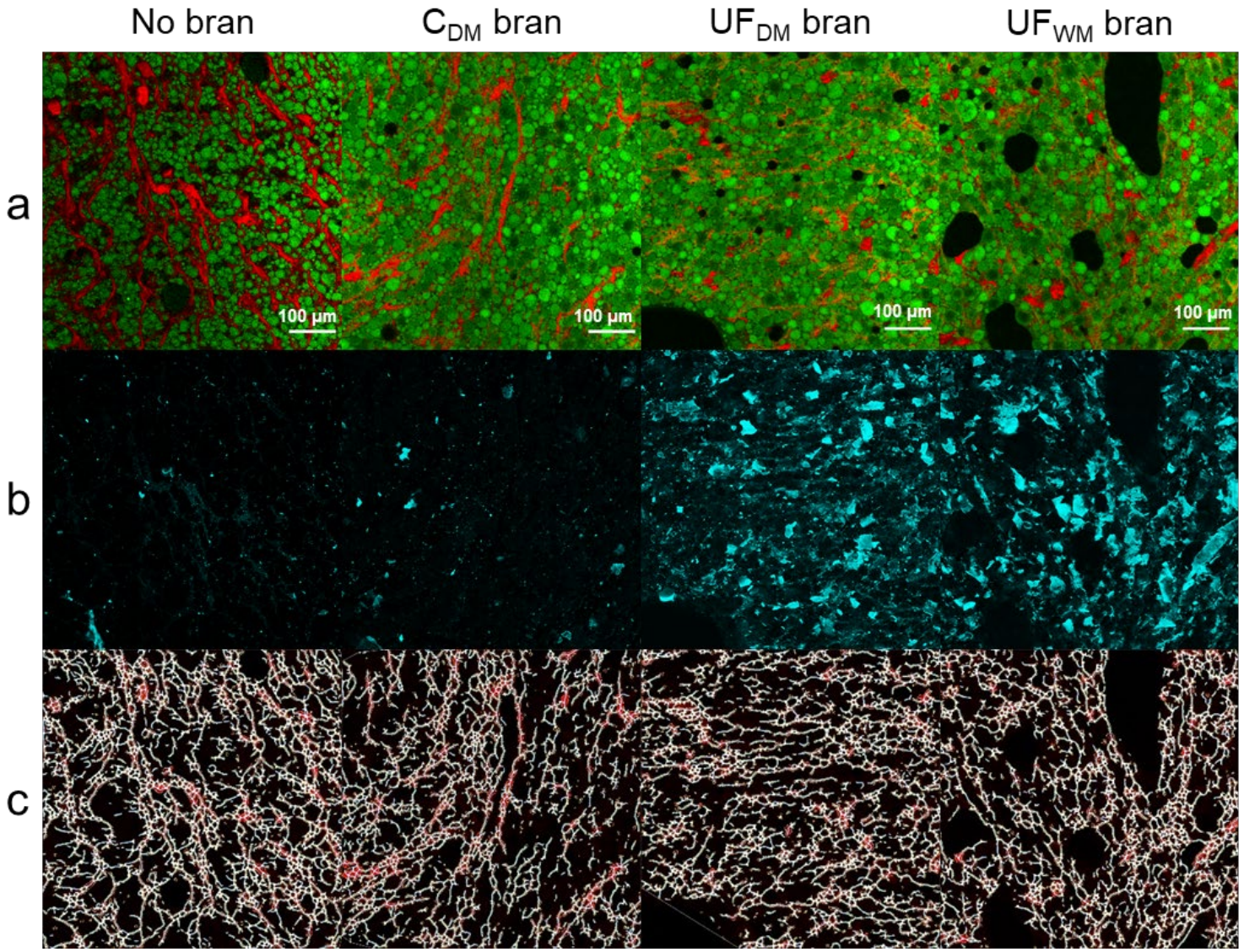
Figure 8. (A) Extensional viscosity curves for gluten-starch dough with or without modified bran. Coarse bran (C_{DM}), fine bran (F_{DM}) and ultrafine bran obtained by dry-milling (UF_{DM}) and wet milling (UF_{WM}) were used. (B) Rheological parameters corresponding to the extensional viscosity curves. Means are shown. For each column, different letters indicate significant differences ($p<0.05$).

3.7 Gluten network microstructure in gluten-starch doughs with modified wheat bran

The microstructure of dough was visualised with CLSM (Figure 9 and Figure S3). For all doughs, the water absorption and mixing time that correspond to the maximum bread loaf volume are used. In

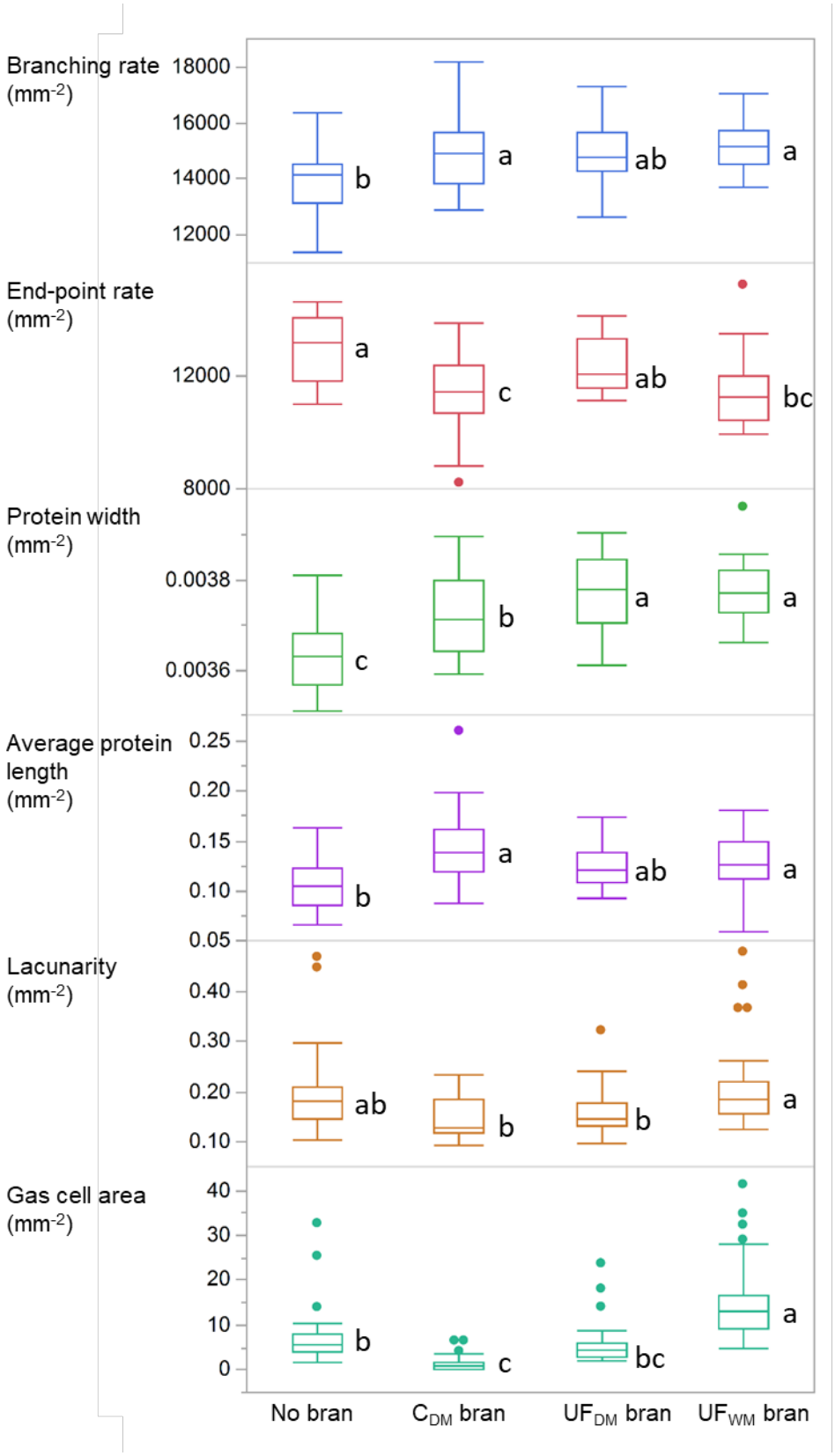
352 Figure 9A, the gluten strands are visualised in red and the starch granules in green. In Figure 9B, the
353 fluorescence of calcofluor is shown, which corresponds mainly to the wheat bran particles. It is not
354 possible to visually see differences in the gluten structure between the dough without bran and the
355 doughs with coarse, UF_{DM} and UF_{WM} bran. In the microscopy pictures of UF_{DM} and UF_{WM} bran, much
356 more wheat bran particles are present than in the coarse bran. A rough calculation shows that the
357 volume of one sphere of 200 µm (d_{50} of C_{DM} bran) is the same as around 300 spheres of size 30 µm
358 (d_{50} of UF_{DM} and UF_{WM} bran). This shows that it is possible that much more particles are seen in the
359 UF wheat bran. In Figure 9, there is no bran particle present in the microscopy picture of the C_{DM}
360 bran.

361 Protein network analysis was used to quantify the gluten network. In Figure 9C, the network
362 obtained with AngioTool is shown, and the results are shown in Figure 10. Dough with wheat bran
363 shows a higher protein strand width and length relative to a dough without bran. Besides, the
364 branching rate is higher and the end-point range is lower, which indicates that the network is more
365 interconnected. The different wheat bran samples show similar behaviour, only the lacunarity and
366 gas cell area of the UF_{WM} bran is significantly higher than for the other bran samples.



367

368 *Figure 9. Gluten microstructure of dough made with a mixture of gluten and starch (water absorption 51.4% and mixing time 4.0 min) or a mixture of gluten, starch and (modified)*
369 *bran (water absorption 59.6% and mixing time 7.5 min). Coarse bran (C_{DM}) and ultrafine bran obtained by dry-milling (UF_{DM}) and wet milling (UF_{WM}) were used. Doughs were*
370 *stained with rhodamine B to visualise proteins (A-red), with fluoresceine to visualise starch granules (A-green) and with calcofluor to visualise bran particles (B-blue) and analysed*
371 *by CLSM. The protein network was analysed with Angiotool (C). The scale bar has a size of 100 µm.*



372

373 *Figure 10. Gluten microstructure data as obtained with AngioTool analysis of pictures of gluten and starch*
374 *dough (water absorption 51.4% and mixing time 4 min) or gluten, starch and (modified) bran dough (water*
375 *absorption 59.6% and mixing time 7.5 min). Coarse bran (C_{DM}) and ultrafine bran obtained by dry-milling (UF_{DM})*
376 *and wet milling (UF_{WM}) were used. Doughs were prepared in triplicate, and each dough was analysed eight*
377 *times (24 measurements). For each parameter, different letters indicate significant differences ($p<0.05$).*

3.8 Correlation between dough rheology and bread loaf volume in the presence and absence of wheat bran

We investigated if there is a correlation between bread loaf volume and dough rheology. For dough without bran and dough with C_{DM}, F_{DM} and UF_{DM}, the 9 different conditions of water absorption and mixing time were used. The UF_{WM} bran was only included at a water absorption of 61.6% with mixing times 5, 7.5 and 10 minutes. Lower water contents did not allow proper measurement of dough rheology. In total, 39 samples were used. In Table 2, the correlation coefficients between loaf volume and the rheological parameters are shown. No significant correlation could be found between the loaf volume and the viscosity at strain 1.05 or the maximum viscosity. A weak negative correlation between the linear viscosity at strain 1.05 and the loaf volume and a weak positive correlation between loaf volume and strain at breakpoint was observed. The strongest correlation with bread loaf volume was found with the strain hardening index. This correlation is also shown in Figure S4.

Table 2. Correlation coefficients between bread loaf volume and dough rheology parameters. (p<0.01, **p<0.001, ***p<0.0001)*

	Loaf volume
Linear viscosity at strain 1.05	-0.53**
Viscosity at strain 1.05	-0.20
Strain at breakpoint	0.43*
Maximum viscosity	0.18
Strain hardening index	0.69***

4 Discussion

4.1 The effect of dry and wet milling of wheat bran on its hydration properties

Four wheat bran samples with different hydration properties were obtained using different milling techniques (Table 1). The **dry milling** process decreases particle size and TWRC, as was also seen by

Jacobs *et al.* (2015). However, the SWRC increased. Jacobs *et al.* (2015) found that the SWRC of wheat bran is independent of particle size until 77 µm. In this study, we show that this statement is no longer true for smaller particle sizes. The specific surface area increased from 0.34 m^2/g to 0.48 m^2/g for the C_{DM} and UF_{DM} bran, respectively. In comparison, Jacobs *et al.* (2015) observed an increase in specific surface area from 0.38 to 0.72 m^2/g, when the average particle size of wheat bran was reduced from 1687 to 77 µm. The difference with our study probably stems from the different milling technique used.

Dry and **wet milling** to a similar particle size resulted in entirely different properties of wheat bran. UF_{WM} bran had a much higher surface area (1.49 m^2/g) than the UF_{DM} bran (0.48 m^2/g). This could indicate that the real particle size of the UF_{WM} bran is actually smaller, but that during particle size measurement aggregates are formed. A more plausible explanation is that the sample is more porous because during wet milling water penetrates in the wheat bran structure and in the subsequent freeze-drying step, more pores are obtained. The high SWRC of the UF_{WM} bran can be associated with its high specific surface area.

4.2 The effect of water absorption and mixing time on gluten network microstructure, dough rheology and bread loaf volume in a gluten-starch dough

It is well known that water absorption and mixing time of dough are crucial parameters in the bread-making process. The effect of wheat bran is often attributed to competition for water between wheat bran and other dough ingredients and to improper development of the gluten network. To investigate this hypothesis, it is important to look first at how dough behaves at different water absorptions and mixing times to make comparison with dough with (modified) bran possible.

In a gluten-starch dough at each water absorption, a mixing time of 4.0 minutes resulted in higher bread loaf volumes than 2.5 or 5.5 minutes (Figure 2). It is known in the literature that both an underdeveloped and an overdeveloped gluten network can lead to a lower gas retention capacity.

Increasing the water absorption also increased the bread loaf volume, which was expected based on the study of Roels *et al.* (1993). Therefore, the optimal bread-making conditions for the gluten-starch mixture were a water absorption of 51.4% and a mixing time of 4.0 minutes.

Water absorption and mixing time had a clear effect on the dough rheology. Changes in water absorption mainly resulted in shifts of the viscosity curves at small strains. This was also observed by Meerts *et al.* (2017a). In agreement with the results of Meerts *et al.* (2017a) the SHI showed an increasing trend in function of the water absorption. However, the mixing time had a much larger effect on the strain hardening of the gluten network. This is in contradiction with the results of Meerts *et al.* (2017a), who showed that mixing time did not affect the viscosity at small strains. However, Meerts et al. (2017a) used dough made from flour while in this study, a mixture of starch and commercial purified gluten was used. During purification of gluten, the gluten network is developed and dried afterwards. Therefore commercial purified gluten can show different behaviour than gluten in flour. This is illustrated by the fact that in the study of Meerts et al. (2017a), dough strands did not break before the maximal strain of 2.7, while breakage at lower strains was observed in our study. The large protein width and small protein length of the dough at 2.5 minutes show that the gluten network is not yet fully developed at short mixing times (Figure 5 & Figure 6). This can indicate that commercial gluten needs a longer mixing time to show comparable behaviour than gluten in flour. Peighambardoust *et al.* (2006) also describe that at the early stages of mixing, a coarse and heterogeneous gluten structure is formed. Afterwards, the kneading process extends the gluten structure.

4.3 The effect of coarse wheat bran on gluten network microstructure, dough rheology and bread loaf volume

The effect of C_{DM} wheat bran on bread-making was investigated by substituting starch with wheat bran in a gluten-starch model system. By using a gluten-starch mixture as a model system for flour, it was possible to keep the gluten content constant. However, the obtained bread loaf volumes are

446 small in comparison with bread made from flour. The difference in loaf volume between gluten-
447 starch dough could be caused by the absence of the water-extractable fraction of flour. Besides, we
448 should keep in mind that the behaviour of isolated gluten may differ from its behaviour in wheat
449 flour.

450 Wheat bran can bind large amounts of water, which is reflected in a significant increase in dough
451 water absorption when part of the flour is substituted by wheat bran (Seyer & Gélinas, 2009). In this
452 study, the water absorption of the dough needed to be increased from 47.4-51.4% to 57.6%-61.6% to
453 get a manageable dough. However, even by adjusting the water absorption and mixing time, the
454 substitution of starch by wheat bran always resulted in lower bread loaf volumes (Figure 2). This can
455 be linked to the decreased strain hardening of the dough in the presence of wheat bran, which
456 causes a decreased gas retention capacity. Indeed, the SHI could be correlated with bread loaf
457 volume (Table 2). This is in accordance with the conclusions of Meeus et al. (2019) and Van Vliet et al.
458 (2008) that the strain hardening behaviour of dough during extension is a valuable indicator of bread
459 quality. We showed that this is also the case in a gluten-starch mixture in the presence of wheat
460 bran. By adjusting water absorption and mixing time, the viscosity in the linear part could be
461 equalised between dough with and without bran, but the strain hardening behaviour of the dough
462 with bran was always lower. This indicates that although the same amount of gluten is present in
463 dough with and without bran, wheat bran always decreases dough strength. Zhang *et al.* (1997) also
464 observed that wheat bran decreases dough strength, as indicated by a decrease in maximum
465 resistance and the area under the Extensigraph curve.

466 CLSM analysis of the dough also showed that wheat bran affects the gluten microstructure. However,
467 the gluten network is clearly well-developed because it is not comparable to the structure of an
468 underdeveloped dough (Figure 5 & Figure 6). According to the study of Lucas *et al*. (2018), a weak
469 gluten network is characterised by a low branching rate, high protein strand width, high end-point
470 ratio and low lacunarity. The addition of wheat bran increases the branching rate and decreases the

end-point rate of the gluten network. According to Lucas et al. (2018), this would be indicative of a stronger, more crosslinked network being formed. However, if this were the case, it was not reflected in the dough rheology analyses. Although it is reasonable to assume that not all microstructural alterations to the gluten network would necessarily imply rheological changes, these results do show that the interpretation of the AngioTool output parameters remains somewhat challenging. The visualisation of gluten strands with CLSM does not inform us of the type of interactions between proteins, and this can probably have a large effect on the viscoelastic properties.

4.4 The effect of modified wheat bran on gluten network microstructure, dough rheology and bread loaf volume

Wheat bran with different water retention capacities was incorporated in dough to investigate the effect of the water-binding on gluten network formation. Decreasing the particle size of wheat bran and consequently increasing the SWRC resulted in lower bread loaf volumes for all different water absorptions and mixing times. In contrary, Jacobs *et al.* (2016) observed that bread volume is independent of wheat bran particle size until an average particle size of 77 µm if optimal water absorption and mixing times are used. However, they did not see any differences in strong water binding capacity. In our study, the wheat bran samples had different SWRC and a higher SWRC clearly was correlated with lower bread loaf volumes. Bread made with UF_{WM} bran (SWRC = 1.39 ml/g dm) did not rise at all and was therefore very small. The very low bread loaf volumes of the UF_{WM} bran could indicate that the viscoelastic gluten network is not fully developed and therefore cannot retain air during fermentation and baking. However, visualisation of the dough microstructure (Figure 9) and quantification of the gluten network properties (Figure 10) did not show large differences between the different bran samples. The high number of wheat bran particles in the UF wheat bran (Figure 9b) apparently does not disturb the development of the gluten network. The only difference was that the number of gas cells in the dough with UF_{WM} bran was larger than in the other doughs,

496 which also influences the lacunarity. This can indicate that the more porous structure of the UF_{WM}
497 bran can function as gas nuclei in the dough. The difference in loaf volume between the UF_{WM} and
498 UF_{DM} bran can therefore not be attributed to a difference in the gluten microstructure as visualised
499 with CLSM. However, the dough rheology of the UF_{WM} bran was completely different from the other
500 bran samples. The viscosity of dough with UF_{WM} bran was much higher than those prepared with dry
501 milled bran or without bran (Figure 8). The effect in the linear part is similar to the effect of
502 decreasing the WA of a gluten-starch dough (Figure 3 and Figure 4) and could indicate the effect of
503 the high SWRC and specific surface area of this bran. This shows that the microstructure of the dough
504 does not directly affect its rheological properties and that it is possible that, after mixing, water
505 redistribution from gluten to the bran affects dough rheology. Hemdane *et al.* (2017) also observed
506 that wheat bran addition resulted in water immobilisation after mixing.

507 5 Conclusion

508 This study highlights the effect of water binding and mixing time on dough rheology, gluten
509 microstructure and bread loaf volume and indicates the importance of optimisation of bread-making
510 conditions in the presence of wheat bran. It was shown that a proper gluten network microstructure,
511 as visualised with CLSM, can be achieved in the presence of wheat bran. However, wheat bran
512 addition always decreases the strain hardening of dough despite the constant gluten content and
513 optimisation of water absorption and mixing time. The deleterious effect of wheat bran on dough
514 rheology increased by adding modified wheat bran with a high SWRC and surface area. The effect of
515 wheat bran with a high water retention capacity on dough rheology was similar to that of decreasing
516 the water absorption. This indicated that water redistribution after mixing and gas cell incorporation
517 could be important. The strain hardening behaviour of dough was observed to be a valuable indicator
518 of bread volume also in the presence of (modified) wheat bran.

519 **Acknowledgements**

Yamina De Bondt acknowledges the Research Foundation – Flanders (FWO – Vlaanderen, Brussels, Belgium) for a position as PhD fellow (Grant number: 1199919N). The authors wish to thank Rik Nuyts for providing assistance with the CLSM measurements and Pieter De Wever for assistance with the N_2 physisorption measurements.

Conflict of interest

The authors declare that they do not have any conflict of interest.

Credit author statement

Conceptualisation: YD, WH and CMC; Methodology: YD, WH, PM and CMC; Investigation: YD and WH; Funding acquisition: YD and CMC; Resources: PM and CMC, Writing – original draft: YD and WH; Writing – review & editing: YD, PM and CMC

Supplementary material

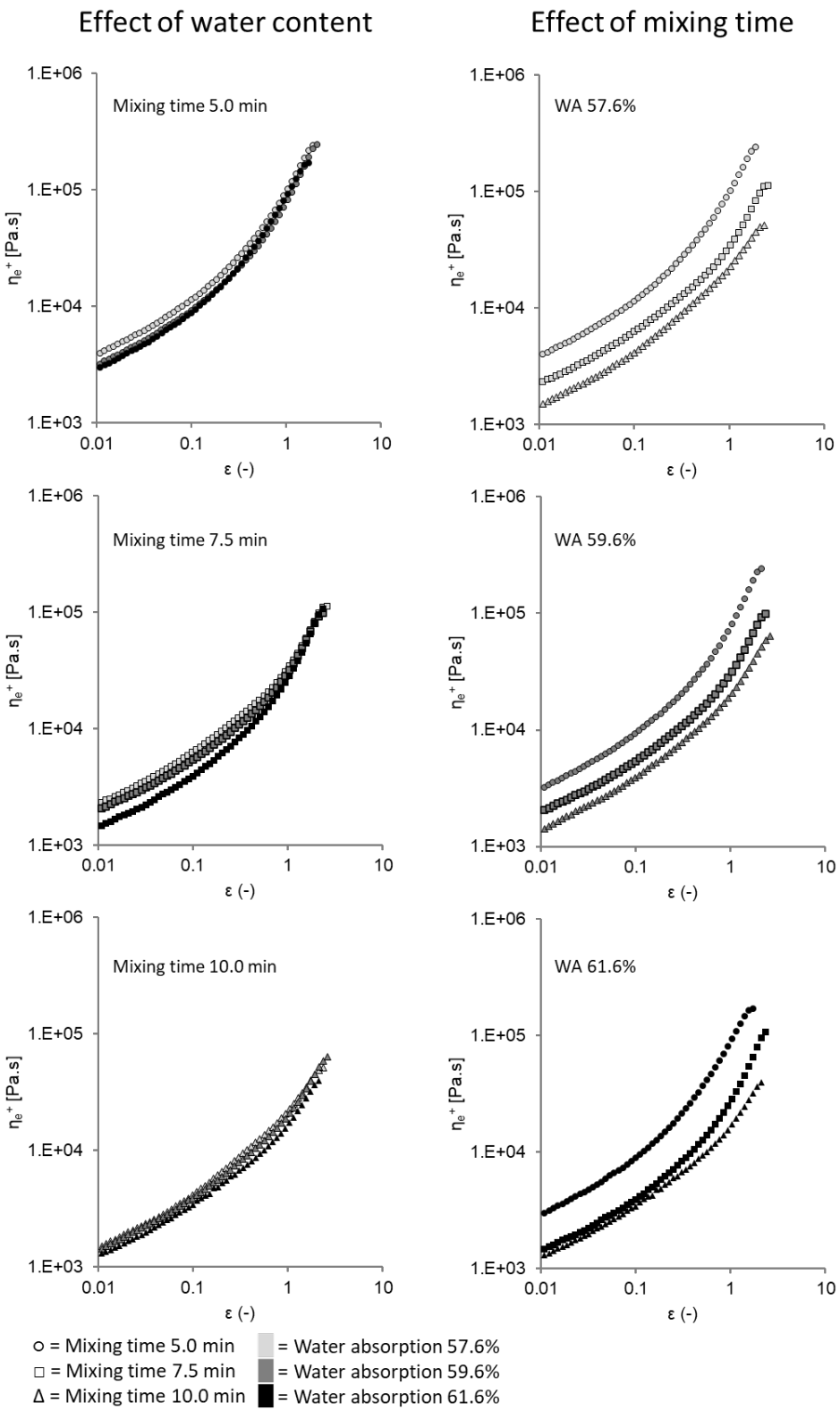
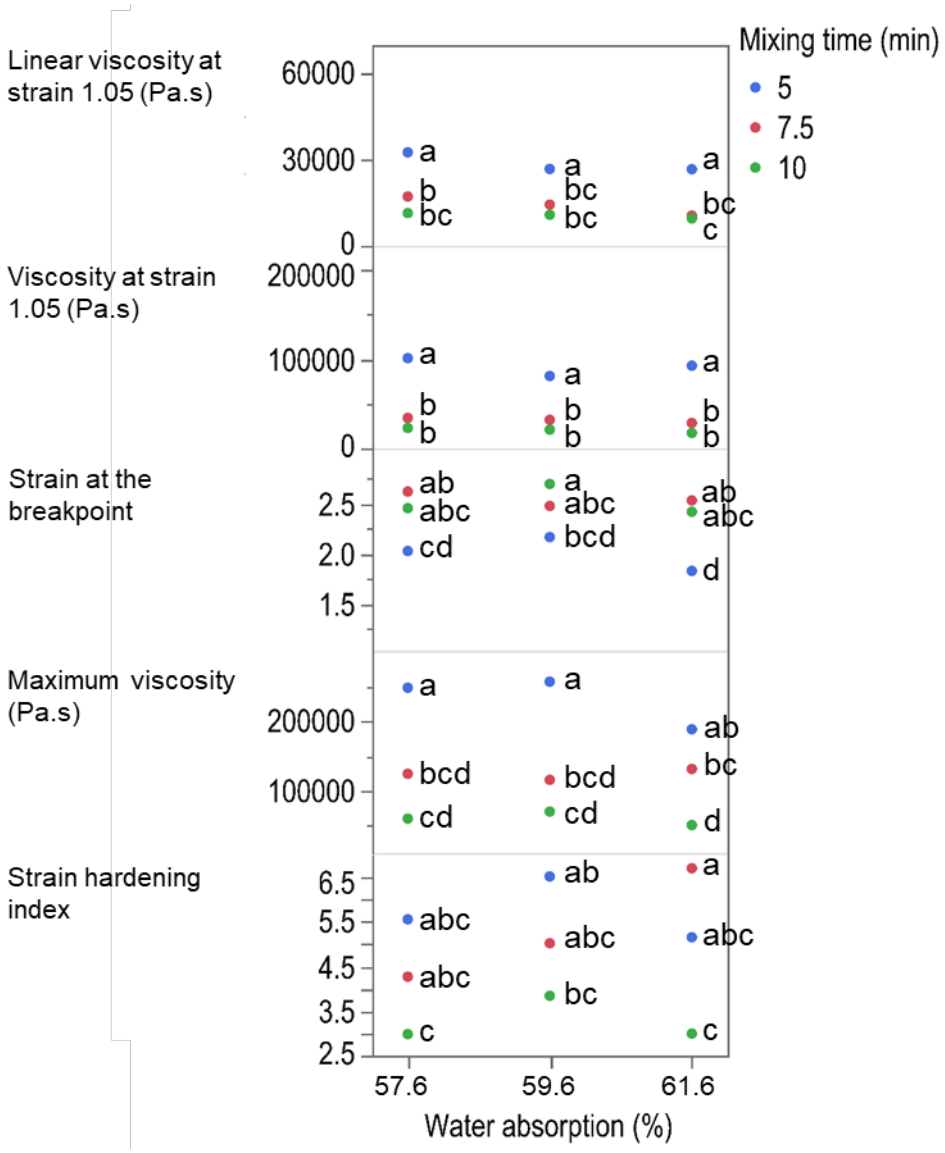


Figure S1. Extensional viscosity curves for gluten-starch-coarse bran doughs prepared at different water contents and mixing times. Mixing time 5.0 min (○), 7.5 min (Δ) or 10.0 min (□) and water absorption 57.6% (light grey), 59.6% (grey) or 61.6% (black).



535

536 *Figure S2. Rheological parameters of gluten-starch-coarse bran dough made with different water absorptions*
537 *and mixing times. Means are shown, and for each parameter, different letters indicate significant differences*
538 *(p<0.05)*

539

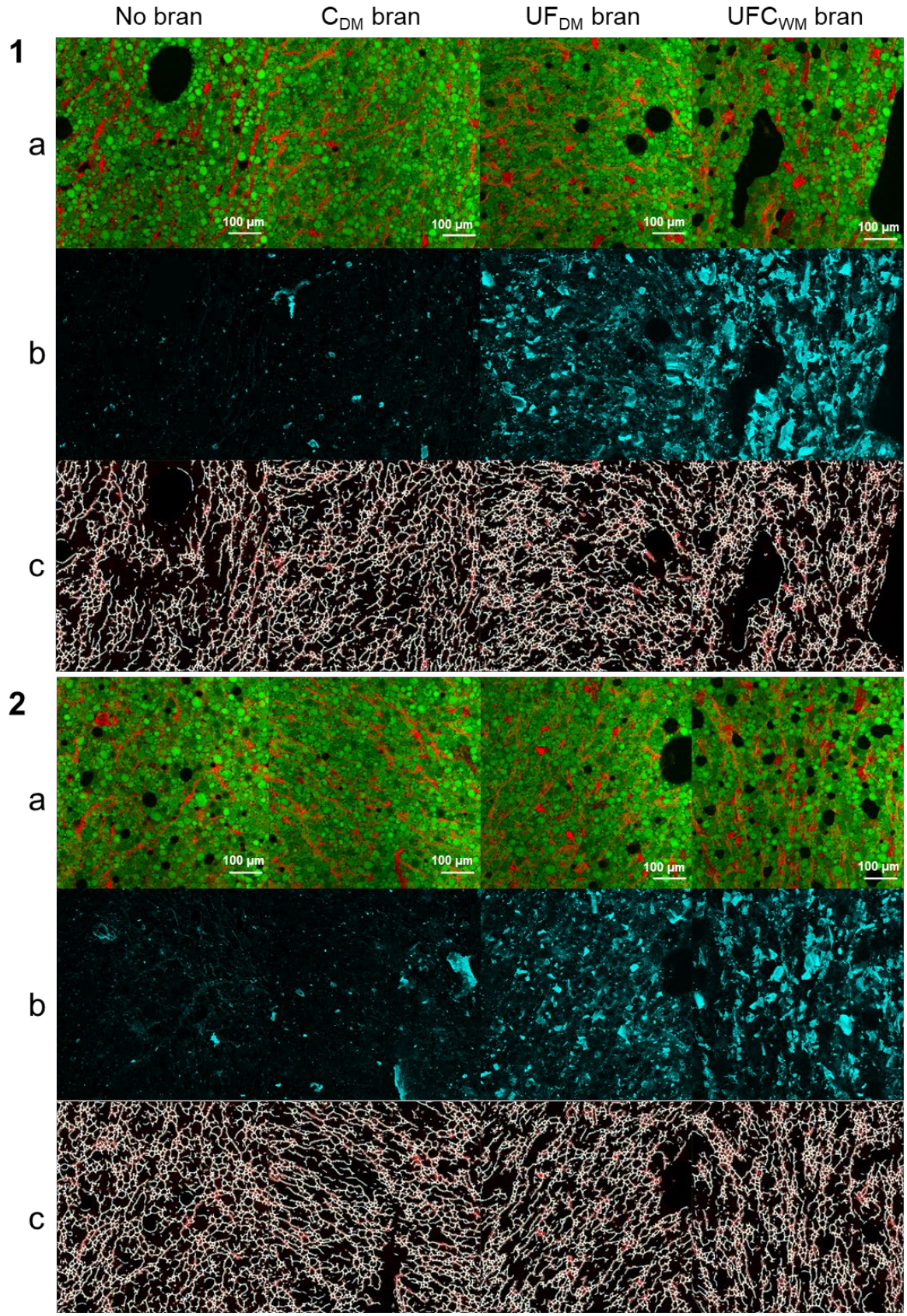


Figure S3. Gluten microstructure of dough made with a mixture of gluten and starch (water absorption 51.4% and mixing time 4.0 min) or a mixture of gluten, starch and (modified) bran (water absorption 59.6% and mixing time 7.5 min). Coarse bran (C_{DM}) and ultrafine bran obtained by dry-milling (UF_{DM}) and wet milling (UF_{WM}) were

used. Doughs were stained with rhodamine B to visualise proteins (A-red), with fluoresceine to visualise starch granules (A-green) and with calcofluor to visualise bran particles (B-blue) and analysed by CLSM. The protein network was analysed with AngioTool (C). The scale bar has a size of 100 µm. Two different doughs (1 & 2) are shown for each sample.

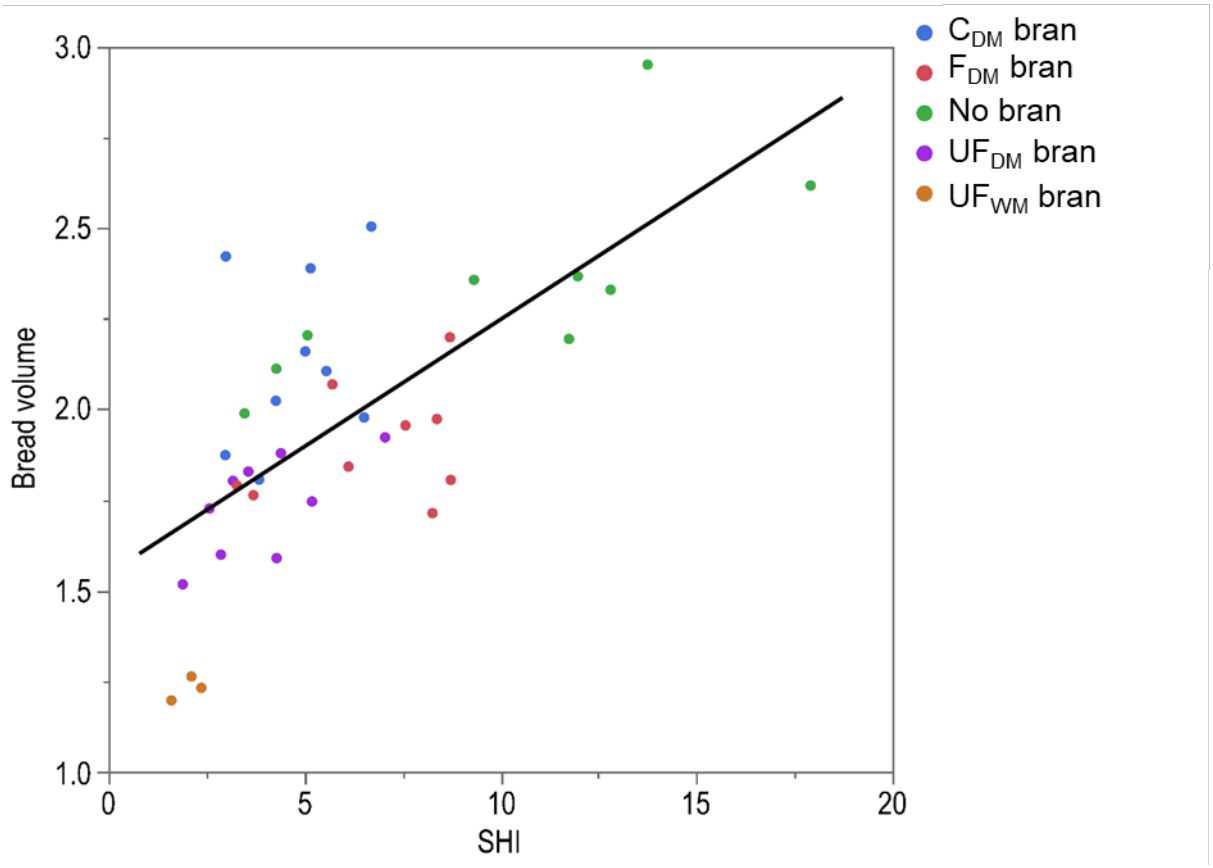


Figure S4. Correlation between bread loaf volume and the strain hardening index. Dough and bread made of a mixture of gluten and starch or a mixture of gluten, starch and (modified) bran were used at different water absorptions and mixing times. Coarse bran (C_{DM}), fine bran (D_{DM}) and ultrafine bran obtained by dry-milling (UF_{DM}) and wet milling (UF_{WM}) were used.

References

Bernklau, I., Lucas, L., Jekle, M., & Becker, T. (2016). Protein network analysis — A new approach for quantifying wheat dough microstructure. *Food Research International*, *89*, 812–819. https://doi.org/10.1016/j.foodres.2016.10.012

Bock, J. E., Connelly, R. K., & Damodaran, S. (2013). Impact of bran addition on water properties and gluten secondary structure in wheat flour doughs studied by attenuated total reflectance fourier transform infrared spectroscopy. *Cereal Chemistry*, *90*(4), 377–386. https://doi.org/10.1094/CCHEM-01-13-0008-FI

De Bondt, Y., Rosa-Sibakov, N., Liberloo, I., Roye, C., Van de Walle, D., Dewettinck, K., Goos, P., Nordlund, E., & Courtin, C. M. (2020). Study into the effect of microfluidisation processing parameters on the physicochemical properties of wheat (Triticum aestivum L.) bran. *Food Chemistry*, *305*. https://doi.org/10.1016/J.FOODCHEM.2019.125436

Gómez, M., Jiménez, S., Ruiz, E., & Oliete, B. (2011). Effect of extruded wheat bran on dough rheology and bread quality. *LWT - Food Science and Technology*, *44*(10), 2231–2237. https://doi.org/10.1016/j.lwt.2011.06.006

Hemdane, S., Jacobs, P. J., Bosmans, G. M., Verspreet, J., Delcour, J. A., & Courtin, C. M. (2017). Study on the effects of wheat bran incorporation on water mobility and biopolymer behavior during bread making and storage using time-domain 1H NMR relaxometry. *Food Chemistry*, *236*, 76–86. https://doi.org/10.1016/j.foodchem.2017.01.039

Hemdane, S., Jacobs, P. J., Dornez, E., Verspreet, J., Delcour, J. A., & Courtin, C. M. (2016). Wheat (Triticum aestivum L.) bran in bread making: A critical review. *Comprehensive Reviews in Food Science and Food Safety*, *15*(1), 28–42. https://doi.org/10.1111/1541-4337.12176

Hemdane, S., Langenaeken, N. A., Jacobs, P. J., Verspreet, J., Delcour, J. A., & Courtin, C. M. (2016). Study of the intrinsic properties of wheat bran and pearlings obtained by sequential debranning

578 and their role in bran-enriched bread making. *Journal of Cereal Science*, *71*, 78–85.
579 https://doi.org/10.1016/j.jcs.2016.08.003

580 Hemdane, S., Langenaeken, N. A., Jacobs, P. J., Verspreet, J., Delcour, J. A., & Courtin, C. M. (2018).
581 Study of the role of bran water binding and the steric hindrance by bran in straight dough bread
582 making. *Food Chemistry*, *253*, 262–268. https://doi.org/10.1016/j.foodchem.2018.01.152

583 Jacobs, P. J., Bogaerts, S., Hemdane, S., Delcour, J. A., & Courtin, C. M. (2016). Impact of wheat bran
584 hydration properties as affected by toasting and degree of milling on optimal dough
585 development in bread making. *Journal of Agricultural and Food Chemistry*, *64*(18), 3636–3644.
586 https://doi.org/10.1021/acs.jafc.5b05958

587 Jacobs, P. J., Hemdane, S., Delcour, J. A., & Courtin, C. M. (2016). Dry heat treatment affects wheat
588 bran surface properties and hydration kinetics. *Food Chemistry*, *203*, 513–520.
589 https://doi.org/10.1016/j.foodchem.2016.02.062

590 Jacobs, P. J., Hemdane, S., Dornez, E., Delcour, J. A., & Courtin, C. M. (2015). Study of hydration
591 properties of wheat bran as a function of particle size. *Food Chemistry*, *179*, 296–304.
592 https://doi.org/10.1016/j.foodchem.2015.01.117

593 Lai, C. S., Hoseney, R. C., & Davis, A. B. (1989). Effects of wheat bran in breadmaking. *Cereal*
594 *Chemistry*, *66*(3), 217–219.

595 Li, J., Kang, J., Wang, L., Li, Z., Wang, R., Chen, Z. X., & Hou, G. G. (2012). Effect of water migration
596 between arabinoxylans and gluten on baking quality of whole wheat bread detected by
597 magnetic resonance imaging (MRI). *Journal of Agricultural and Food Chemistry*, *60*(26), 6507–
598 6514. https://doi.org/10.1021/jf301195k

599 Lucas, I., Becker, T., & Jekle, M. (2018). Gluten polymer networks - A microstructural classification in
600 complex systems. *Polymers*, *10*(617). https://doi.org/10.3390/polym10060617

601 Meerts, M., Cardinaels, R., Oosterlinck, F., Courtin, C. M., & Moldenaers, P. (2017a). The impact of

water content and mixing time on the linear and non-linear rheology of wheat flour dough. *Food Biophysics*, *12*(2), 151–163. https://doi.org/10.1007/s11483-017-9472-9

Meerts, M., Cardinaels, R., Oosterlinck, F., Courtin, C. M., & Moldenaers, P. (2017b). The interplay between the main flour constituents in the rheological behaviour of wheat flour dough. *Food and Bioprocess Technology*, *10*(2), 249–265. https://doi.org/10.1007/s11947-016-1810-2

Meeus, Y., Janssen, F., Wouters, A. G. B., Delcour, J. A., & Moldenaers, P. (2019). Linear and non-linear rheology of bread doughs made from blends of wheat (Triticum aestivum L.) and rye (Secale cereale L.) flour. *Food and Bioprocess Technology*, *13*, 159–171. https://doi.org/10.1007/s11947-019-02393-w

Peighambardoust, S. H., Van Der Goot, A. J., Van Vliet, T., Hamer, R. J., & Boom, R. M. (2006). Microstructure formation and rheological behaviour of dough under simple shear flow. *Journal of Cereal Science*, *43*(2), 183–197. https://doi.org/10.1016/j.jcs.2005.10.004

Pomeranz, Y., Shogran, M. D., Finney, K. F., & Bechtel, D. B. (1977). Fiber in breadmaking - effects on functional properties. *Cereal Chemistry*, *54*(1), 25–41.

Roels, S. P., Cleemput, G., Vandewalle, X., Nys, M., & Delcour, J. A. (1993). Bread volume potential of variable-quality flours with constant protein level as determined by factors governing mixing time and baking absorption levels. *Cereal Chemistry*, *70*(3), 318–323.

Sanz Penella, J. M., Collar, C., & Haros, M. (2008). Effect of wheat bran and enzyme addition on dough functional performance and phytic acid levels in bread. *Journal of Cereal Science*, *48*(3), 715–721. https://doi.org/10.1016/j.jcs.2008.03.006

Seyer, M. È., & Gélinas, P. (2009). Bran characteristics and wheat performance in whole wheat bread. *International Journal of Food Science and Technology*, *44*(4), 688–693. https://doi.org/10.1111/j.1365-2621.2008.01819.x

Shogren, M. D., & Finney, K. F. (1984). Bread-making test for 10 grams of flour. *Cereal Chemistry*,

626 *61*(5), 418–423.

627 Stevenson, L., Phillips, F., O'Sullivan, K., & Walton, J. (2012). Wheat bran: Its composition and
628 benefits to health, a European perspective. *International Journal of Food Sciences and Nutrition*,
629 *63*(8), 1001–1013. https://doi.org/10.3109/09637486.2012.687366

630 van Vliet, T. (2008). Strain hardening as an indicator of bread-making performance: A review with
631 discussion. *Journal of Cereal Science*, *48*(1), 1–9. https://doi.org/10.1016/j.jcs.2007.08.010

632 Zhang, D., & Moore, W. R. (1997). Effect of wheat bran particle size on dough rheological properties.
633 *Journal of the Science of Food and Agriculture*, *74*(4), 490–496.
634 https://doi.org/10.1002/(SICI)1097-0010(199708)74:4<490::AID-JSFA822>3.0.CO;2-0

635