Amorphisation and chain length reduction of cellulose affect its impact on the water distribution, dough rheology and loaf volume in bread making

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Abstract

The unfermentable nature of cellulose limits its physiological benefits as a dietary fibre. Recently, we drastically increased the fermentability of microcrystalline cellulose by human colon microbiota by reducing its crystallinity and polymer chain length (Thielemans et al., 2023). Here, the techno-functional impact of incorporating amorphised cellulose, depolymerised cellulose and amorphised depolymerised cellulose on bread making is investigated. Microcrystalline cellulose and its modified

counterparts could be incorporated into bread at a 5% substitution level without significant volume losses. At a 20 w/w% substitution level, microcrystalline cellulose incorporation caused a loss of 36.4 ± 2.9% in bread loaf volume, while depolymerised celluloses reduced bread volume much less (16.2 ± 1.9%). In-depth characterisation of cellulose samples and ^{1}H-NMR and rheological measurements on bread dough revealed that the modified celluloses affected the water balance and gluten hydration in dough less than microcrystalline cellulose. These results complement insights on the importance of water-insoluble dietary fibre structure and hydration behaviour on bread (dough) quality.

Keywords: Cellulose, Dietary fibre, Fibre fortification, Bread quality, Dough hydration

Declarations of interest: None

1. Introduction

The average daily dietary fibre intake in Western countries remains significantly lower than recommended (European Commission, 2021). Enriching processed foods with dietary fibre is one of several strategies to close this fibre gap. The term dietary fibre refers to a group of carbohydrates and lignin resistant to digestive enzymes, and thus neither hydrolysed nor absorbed in the small intestine (European Union, 2011). Dietary fibres can be categorised as water-soluble and water-insoluble fibre, and their physiological effects partly depend on this characteristic. In general, insoluble large polymer networks are rather unfermentable in the colon and mainly regulate the colon transit time, while water-soluble polymers and oligomers can be fermented by the colon microbiota. The latter can be correlated to additional physiological benefits (Salamone et al., 2021; Verspreet et al., 2016).

Cellulose has a strong intrinsic potential as a dietary fibre additive since it is a natural, abundant, odourless and colourless polymer that can be produced economically feasible and sustainably (Klemm et al., 2005). Due to its crystallinity, the high molecular weight of its individual polymers and their assembly in strong fibre bundles, cellulose is, however, primarily insoluble and unfermentable, limiting its physiological benefits.

In a recent study, we demonstrated that we could turn microcrystalline cellulose (MC) into amorphised depolymerised cellulose (ADC) with an average degree of polymerisation of below 40 glucose monomers and a crystallinity index below 0.3, using a planetary ball mill and acid hydrolysis. Furthermore, we demonstrated that reducing the cellulose chain length and crystallinity drastically enhanced the

fermentability of the cellulose by colon microbiota on several occasions (Thielemans et al., 2023).

While such an ADC fibre could be an interesting ingredient for fibre fortification of the diet, its use is only feasible if its incorporation in food systems does not result in extensive quality losses.

The incorporation of various types of dietary fibre in bread has been studied extensively. Most fibre types cause organoleptic or techno-functional drawbacks (Khalid et al., 2017; Wang et al., 2002). Flour substitution by cereal bran, water-unextractable arabinoxylan or inulin typically reduces the bread loaf volume, crumb texture quality and sensory acceptance (Chen et al., 1988; Hemdane, Jacobs, et al., 2016; Morris & Morris, 2012; Pomeranz et al., 1977). For example, substituting 3% of wheat flour with inulin caused a loaf volume loss of 19% (Wang et al., 2002), while an 11% addition of broad bean hull caused a 12% loaf volume reduction and a crumb quality loss (Ni et al., 2020). When incorporating insoluble fibre components, these loaf volume reductions can be linked to suboptimal dough development and rheological properties, preventing proper dough rise during fermentation and baking (Gómez et al., 2003). The suboptimal dough development has several causes. First, insoluble fibres typically have a high water absorption capacity since they consist of hydrophilic carbohydrate polymers and are often organised in supramolecular structures that can hold water. This leads to competition for water between the gluten and the incorporated fibre, disturbed water distribution in the dough and a weakened gluten network (Bock & Damodaran, 2013; Courtin & Delcour, 2002; Hemdane, Langenaeken, et al., 2016; Nawrocka et al., 2017). Next, the incorporation of insoluble fibre results in the dilution of gluten and physical disturbances in the starch-gluten network (Gan et al., 1989; Hemdane, Jacobs, et al., 2016; Rubel et al., 2015). Several

attempts were made to engineer fibre properties towards optimal bread quality. Controlling the hydration behaviour and particle size of the fibre ingredients turned out to be essential, but these material properties are hard to control for complex materials such as cereal bran (De Bondt et al., 2021; de Erive et al., 2020; Hemdane, Langenaeken, et al., 2016; Noort et al., 2010).

Compared to other dietary fibres, cellulose incorporation in bread recipes has scarcely been investigated. Correa and Ferrero observed no volume loss upon 1.5% flour substitution by MC (Correa & Ferrero, 2015), while Gómez et al. (2003) observed significantly decreased bread loaf volume and sensory scores with 2% MC. The latter researchers attributed these volume losses mainly to the water and volume competition between the cellulose and dough constituents, in analogy to the general effect of insoluble fibre in bread. Ren et al. (2020) observed a significant volume loss at 10% incorporation with MC as well and concluded that the morphology of the cellulose might impact the dough and bread quality.

Against this background, the objective of this study was to gain a mechanistic understanding of how amorphisation and chain length reduction of cellulose affect its impact on fibre-enriched bread-making. Different modified cellulose samples were used, of which several were previously described by Thielemans et al. (2022). Samples were compared with a focus on aggregate properties and water binding capacity, as these were hypothesised to affect the bread-making process. Bread loaf volume was chosen as the primary end-product quality parameter as it is a good indicator of how the viscoelastic properties of bread dough and the resulting gas-retention capacity are affected. More mechanistic insights into the impact of the physicochemical properties of cellulose on bread-making were obtained using ^{1}H-NMR, DSC and rheological measurements on the dough. With this approach, we

investigated the potential of modifying cellulose for desired functionality in bread and generated structural insights about incorporating water-insoluble dietary fibre in bread recipes.

2. Material and methods

2.1 Materials

Microcrystalline cellulose (MC; Avicel PH-101, with a moisture content of 3.7 wt%) and citric acid (99%) were from Sigma-Aldrich (Deurne, Belgium). Bread loaves were made with Bilux wheat flour from Dossche Mills (Deinze, Belgium). Sugar, salt and fresh compressed baker's yeast (Koningsgist, AB Mauri, Dordrecht, The Netherlands) were bought at a local supermarket.

2.2. Modification of cellulose

Starting from MC, three types of cellulose samples with different properties were produced. The production of the first two samples and their characteristics are as described by Thielemans et al. (2022). In short, MC was milled in batches of 20 g in a planetary ball mill (PM 100, Retch GmbH, Germany) for 260 min at 400 rpm in a sample holder with a zirconium oxide coating, with six zirconium oxide balls (Ø 10 mm), to obtain amorphised cellulose (AC; sample BM in Thielemans et al., 2022; Table 1). Depolymerised cellulose (DC; sample SMCC 29 in Thielemans et al., 2022; Table 1) was made by hydrolysing this AC with an aqueous 10% citric acid solution. Batches of 20 g AC were hydrolysed for 16 h at 130 °C in a closed container with a solid-to-liquid ratio of 4.8 wt%. After hydrolysis, the insoluble material was washed with demineralised water until neutral pH and dried in an oven for 45 h at 60 °C. Amorphised depolymerised cellulose (ADC) was produced by performing an additional treatment of the DC fibres in the planetary ball mill (30 min at 500 rpm).

2.3 Characterisation of modified cellulose

The **average degree of polymerisation (avDP)** of the amorphised depolymerised cellulose (ADC) was determined viscometrically in triplicate, based on the method of

the French Institute for Normalisation (Afnor, 1981). A sample (0.075 g) was dissolved in an aqueous 0.5 M bis(ethylenediamine)copper(II)hydroxide solution (15 mL), and the viscosity of this solution at 25.0°C was measured with a capillary viscometer, type nr. 509 04 (Schott Geräte, Jena, Germany). The avDP was calculated from the boundary viscosity of the solution (η), based on the empirical relation: Average DP^α=η/K, with α and K empirical constants equal to 1 and $7.5x10^{-3}$, respectively, as defined by Afnor (1981). The boundary viscosity η was determined from $\eta_a = \eta.C.10^{((0.14.\eta.C))}$, with η_a the specific viscosity of the solution, and C the cellulose concentration (g/mL).

The **crystallinity index** of ADC was determined by X-ray powder diffraction (XRD) on a high-throughput STOE STADI P Combi diffractometer (STOE & Cie GmbH, Darmstadt, Germany) in transition mode with Ge(111) monochromatic X-ray inlet beams (λ = 1.5406 Å, Cu Kα source). Crystallinity indexes were determined by the peak-height method of Segal and coworkers (1959), using the following formula:

$$Crystallinity\ index = \frac{I_{002} - I_{am}}{I_{002}}$$

Here, I_{002} represents the highest measured intensity of the 002 interference (in arbitrary units), and I_{am} the minimal intensity between the (10$\bar{1}$) and the (021) reflection.

The **aggregate size** of ADC in water was determined in triplicate with an LS 13 320 laser diffraction particle size analyser equipped with a wet module (Beckman Coulter Inc, Indianapolis, IN, USA). A He-Ne laser beam was emitted on a 5% suspension of cellulose in water, and the volumetric particle size distribution was calculated from the intensity profile of the scattered light with the Mie theory using the instrument's software.

The **aggregate shape** of all samples was visualised using a JEOL JSM- 6010 LV scanning electron microscope (SEM) at an acceleration voltage of 10 kV after coating the cellulose with an Au/Pd alloy using a JEOL JSC-1300 sputter coater.

The **BET specific surface area** of ADC was measured by nitrogen physisorption (Tristar II 3020, Micromeritics) at -196 °C on samples dried for 16 h at 80 °C. The relative nitrogen pressure was varied between 0.01 and 0.99 (p/p_0), and the t-plot method (Harkins & Jura, 1944) on the adsorption branch was used to determine the specific surface area.

The **strong water retention capacity (SWRC)** of ADC was determined in triplicate based on the method described by De Bondt et al. (2020). Cellulose (50 mg) was added in the upper part of a QIAprep Spin Miniprep Column (Qiagen) together with 700 µl deionised water. After 1 h hydration, the Miniprep was centrifuged for 10 min at 4000 g, and the upper part (with hydrated sample) was weighed (m_{centr}). The SWRC was calculated with the following equation:

$$Strong\ water\ retention\ capacity\ \left(\frac{mL}{g}\right) = \frac{m_{centr} - m_{blanc} - m_{DM}}{m_{DM}}$$

with m_{blanc} the mass of the upper part after a blanc measurement (without sample), and m_{DM} the mass of dry matter of cellulose in the column.

2.4 Bread making

Bread was made in triplicate on a 10-gram scale according to the straight dough procedure of Shogren and Finney (Shogren & Finney, 1984). In a 10 g pin bowl mixer (National Manufacturing, Lincoln, NE, USA), 10.0 g of a flour-cellulose mixture (14.0% moisture content) was mixed at 23 °C for 240 s with 5.3% compressed baker's yeast, 6.0% sucrose, 1.5% salt and water. Cellulose incorporation was performed at a 5%,

10%, 15% and 20% flour substitution level. The water absorption (WA), referring to water content in a standard recipe with wheat flour with 14.0% moisture, was varied between 56% and 76%. Based on the maximal volume of cellulose-enriched bread loaves, the optimal WA was determined for each recipe as well. The mixed doughs were fermented for 90 minutes in a fermentation cabinet (National Manufacturing) at 30°C and relative humidity of 90%. Punching steps were performed with a dough sheeter (National Manufacturing) after 52, 77 and 90 min of fermentation, as described by Shogren & Finney (1984). After the last punching, the dough was moulded and proofed for another 36 min in a lightly greased baking tin (25 mm x 25 mm x 50 mm). Doughs were baked for 13 min at 232 °C in a rotary oven (National Manufacturing). After cooling, the bread loaves were weighed, and loaf volumes were determined with a Volscan Profiler (Stable Micro Systems, UK). The loaves were photographed in their entirety and after being cut in half, for visual inspection of the crumb and crust colour.

2.5 Proton nuclear magnetic resonance measurements

The mobility of water in interaction with each of the cellulose samples was analysed by analysis of the proton distributions in a 40% cellulose-in-water mixture. Furthermore, proton distributions in unfermented bread doughs with 20% cellulose incorporation and a control dough without cellulose addition were analysed. The bread doughs were made with the WA that gave the highest bread loaf volume. This was 56.0% for control bread, 76.0% for the bread with MC incorporation and 64.0% for AC, DC and ADC incorporation. The doughs were prepared as described in section 2.4, and three aliquots from the dough were analysed 30 minutes after mixing.

Low field ^{1}H-NMR measurements were performed at 25 °C with a Minispec mq 20 TD NMR spectrometer (Bruker, Rheinstetten, Germany) at an operating resonance

frequency of 20 MHz for 1H (magnetic field strength of 0.47 T, recycle delay of 2 s). A Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, consisting of a 90° pulse followed by 180° pulses after 0.1 ms, was used to obtain T_2 relaxation signals of the protons. Afterwards, these relaxation signals were converted into a continuous distribution with an inverse Laplace transformation using the CONTIN software, as described by Nivelle *et al*. (2017). The different proton populations were characterised by their area and mean T_2 relaxation time.

2.6 Differential scanning calorimetry analyses

The impact of the cellulose samples on starch gelatinisation was analysed with differential scanning calorimetry with a Q2000 DSC (TA Instruments, New Castle, DE, USA). Flour or a flour-cellulose mixture was accurately weighed (2.50 to 4.00 mg) in an aluminium pan (Hitachi High-Technologies, Tokyo, Japan). Deionised water was added in excess (1:3 w/w), the pans were sealed hermetically and heated from 0 to 130 °C at 4 °C/min together with an empty reference pan. The DSC system was calibrated with indium. DSC thermograms were analysed for temperatures associated with starch gelatinisation, using TA Universal Analysis software. All measurements were done in triplicate.

2.7 Dough rheology analysis

Dough rheology was measured using the method of Meerts et al. (2017a) with some adaptations. Doughs with 20% cellulose sample incorporation and an optimal WA were mixed and pressed between two plates at a 4.0 mm distance for 1 min. A dough without cellulose addition served as a control. After 30 min rest, uniform dough strands (12.5 mm – 4.0 mm – 4.0 mm) were prepared using a hollow stamp. These dough strands (7 per dough) were attached with two clamps to an extensional viscosity fixture

231 (EVF), mounted on an ARES-G2 rheometer (TA Instruments, New Castle, USA).
232 Extension occurred at a nominal extension rate of 12 s^{-1} until a Hencky strain of 2.89.
233 Resulting profiles are characterised by the maximal transient extensional viscosity
234 (η_{max}), the extrapolated transient extensional viscosity at maximal strain (η_{0max}), and
235 the strain hardening index (SHI). The η_{max} represents the average extensional
236 viscosity of the dough at strain 2.89, while the η_{0max} is the extrapolated viscosity value
237 at strain 2.89 from the fitted curve of the linear envelope. The SHI is calculated by
238 dividing the η_{max} by η_{0max}, and reflects the visco-elastic property of the dough (Meerts
239 et al., 2017a).

3. Results

3.1 Mapping the physicochemical properties of modified cellulose

MC was modified through a combination of planetary ball mill treatments and acid hydrolysis. The sequential modifications are presented schematically in Figure 1. Next to differences in fibre properties, the treatments altered the structure of the supramolecular aggregates in which the cellulose fibres are assembled (Figure 1). MC is composed of irregular rod-like aggregates with large cavities, most probably originating from the botanical structure in the plant. This irregular rod-like shape disappears upon ball milling and further modification (Figure 1). AC fibres were organised in spherical aggregates with a relatively regular and dense surface. During hydrolysis and drying, the spherical character of the aggregates remained (Figure 1).

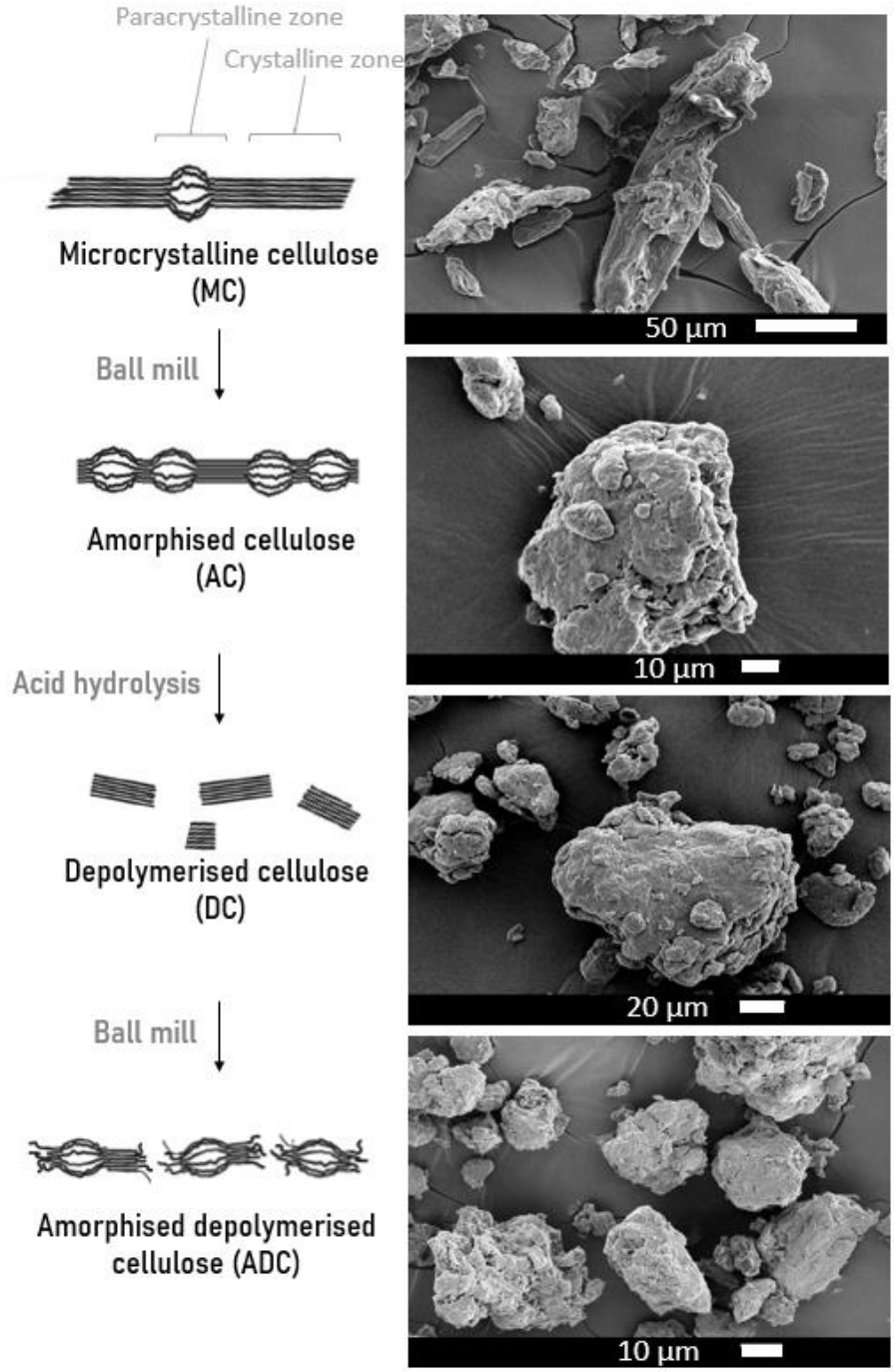


Figure 1: The sequence of processing steps used for the conversion of microcrystalline cellulose (MC) into amorphised cellulose (AC), depolymerised cellulose (DC) and amorphised depolymerised cellulose (ADC), and their aggregational changes. Aggregates are visualised with scanning electron microscopy (magnifications of 500, 1100, 600 and 1100 times for MC, AC, DC and ADC, respectively).

In Table 1, the sample properties (avDP and crystallinity index), median particle size (d50) measured by laser diffraction, BET specific surface area and strong water

retention capacity (SWRC) of ADC are summarised. The physicochemical properties of MC, AC and DC were as reported in Thielemans et al. (2022).

Table 1: Physicochemical parameters of amorphised depolymerised cellulose (ADC).

Average degree of polymerisation	29 ± 1
Crystallinity index (%)	<30
Median Particle size (µm)	47.1 ± 5.5
Specific surface area (m^2/g)	0.33 ± 0.06
Strong water retention capacity (g H_2O/g DM)	0.57 ± 0.01

~~Ball-milling of MC into AC decreased the crystallinity index from 72 to less than 30, while the avDP decreased from 147 ± 0 to 124 ± 0. The acid hydrolysis of AC into DC resulted in an efficient depolymerisation as the avDP decreased to 28 ± 1. The crystallinity index again increased to 62 (Thielemans et al., 2022). A short ball mill treatment of 30 min after hydrolysis sufficed to produce ADC fibres with a crystallinity below 30, while the avDP was not affected (Table 1).~~

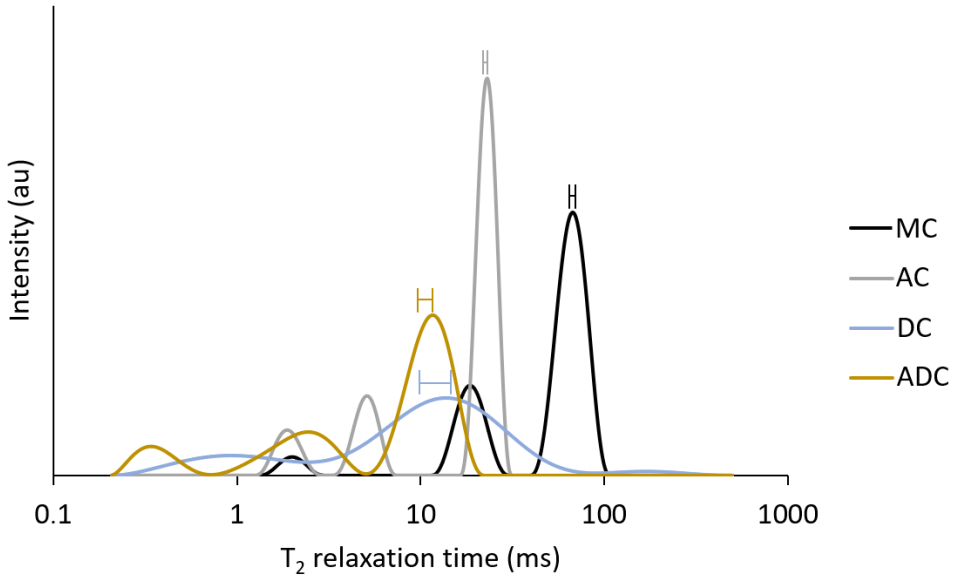
~~The median particle size of the ADC fibres was slightly higher than the DC fibres (29.1 ± 1.4 µm) but lower than the AC fibres (60.7 ± 17.4) (Thielemans et al., 2022). Thielemans et al. (2022) reported that the specific surface area was not impacted by ball-milling of MC (0.93 ± 0.08 m^2/g) into AC (0.91 ± 0.01 m^2/g). The acid hydrolysis decreased the specific surface area down to 0.63 ± 0.29 m^2/g, and the second ball mill treatment further decreased the specific surface area to 0.33 ± 0.06 m^2/g (Table 1).~~

~~The SWRC is an important measure for the amount of water that can be withheld by a fibre when subjected to strong centrifugal forces. It is a relevant characteristic for incorporation in bread doughs, in which the water distribution is known to define the~~

~~quality of the end product (De Bondt et al., 2020). The SWRC of ADC was higher than this of AC (0.50 ± 0.04 g/g DM) and DC (0.44 ± 0.01 g/g DM), but lower than MC (0.65 ± 0.02 g/g DM) (Thielemans et al., 2022).~~

To investigate the strength and type of water binding to the cellulosic surface, time domain proton nuclear magnetic resonance (^{1}H-NMR) was performed on a 40% cellulose sample-water mixture. The continuous proton distributions in function of T_2 relaxation time after a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence are given in Figure 2. The area of a particular peak relates to the amount of protons in the specific population, while the mean T_2 relaxation time relates to the proton mobility within that population. This proton mobility is associated with the cellulose-water interaction and the distance of the water to the cellulosic surface (De Wever et al., 2021). The biggest proton population in an MC-water mixture, representing water protons in interaction with the cellulose, had a mobility of 65.4 ± 2.7 ms, which was significantly higher ($p < 0.05$) than the biggest proton populations in mixtures with the modified cellulose samples (21.8 ± 0.5 ms for AC and below 15.0 ms for DC and ADC).

The differences observed in strength and type of water binding of the different cellulose samples did not affect the starch gelatinisation. The onset, peak end conclusion temperature of starch gelatinisation was not significantly different between flour or mixtures of 80% flour and 20% MC, AC, DC or ADC (Figure A2).



298

299 *Figure 2: The proton mobility distributions after a Carr-Purcell-Meiboom-Gill (CPMG) pulse*
300 *sequence in a 40% cellulose-in-water mixture of microcrystalline cellulose (MC), amorphised*
301 *cellulose (AC), depolymerised cellulose (DC) and amorphised depolymerised cellulose (ADC).*
302 *The T_2 relaxation time represents the mobility of the proton population. The average T_2*
303 *relaxation time and standard deviation of the most mobile proton population are indicated in*
304 *the figure.*

305 ### *3.2 Impact of incorporation of modified cellulose on bread properties*

306 In Figure 3, the volumes of bread loaves with 5% (Figure 3a) and 20% (Figure 3b)
307 substitution of flour with (modified) cellulose samples, relative to a control bread
308 without cellulose addition, are given. At a 5% substitution level, a water absorption
309 (WA) of 56%, i.e. the farinograph WA for a dough without cellulose incorporation, was
310 used in first instance. At this WA, bread loaves with AC, DC or ADC incorporated
311 showed a slightly lower loaf volume than the control bread, while the MC lowered the
312 bread loaf volume by 13 ± 2% (Figure 3a). In the second instance, the WA was
313 optimised for each dough with cellulose incorporation to ensure sufficient hydration of
314 the flour constituents and achieve the highest specific bread volume. The optimal WA
315 was 65% for the dough with MC and 63% for the dough with DC and ADC
316 incorporation. Since the specific loaf volume of bread with AC incorporation only
317 decreased upon adding more water, the optimal WA was the same as the standard

WA (56%). This optimisation of the WA significantly improved ($p < 0.05$) the relative loaf volume for bread with MC (from 0.87 ± 0.02 to 0.95 ± 0.00).

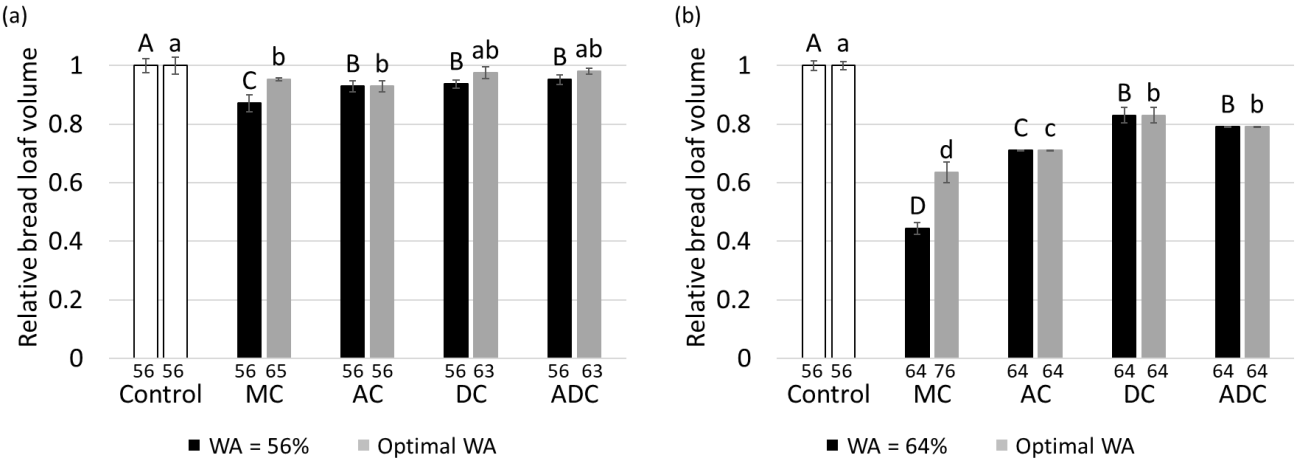


Figure 3: Loaf volumes of bread with (a) 5% and (b) 20% cellulose incorporation, expressed relative to a control bread without cellulose addition baked on the same day. Bread loaves are baked using a standard water absorption (WA) of 56% (a) and 64% (b)(black) and optimal WA (grey). Control breads were always baked at 56%. Statistical differences ($p<0.05$) are indicated with capital (for standard WA) or small (for optimal WA) letters. The average absolute volume of the control bread was 51.5 mL.

At a substitution level of 20%, bread loaf volumes were lower for all (modified) cellulose fibres (Figure 3b). As shown in Figure A1, increasing the cellulose content to 10% caused a first part of this loaf volume drop for all cellulose samples. The additional loaf volume drop between 10% and 20% incorporation level depended on the cellulose sample (Figure A1). At a WA of 64%, the optimal WA for dough with AC incorporation, the loaf volume reduction for bread with 20% MC incorporation was 65.6 ± 1.4%. This was only 16.2 ± 1.9% and 20.9 ± 0.1% when incorporating DC and ADC, respectively. The loaf volumes of bread with ADC and DC did not significantly differ ($p<0.05$). Increasing the water content in the recipe resulted in increased loaf volumes only for the bread loaves with 20% MC incorporation. When working with a WA of 76% instead of 64% for MC, loaf volume losses were limited to 36.4 ± 2.9%. However, the bread

with MC incorporation still had a significantly lower loaf volume ($p<0.05$) when using this optimal WA than the bread loaves with the modified celluloses incorporated.

In Figure 4, the effect of 20% cellulose incorporation (MC, DC, and ADC) on the crumb and crust colour of bread loaves with optimal WA is shown. Next to volume loss, visual inspection showed that incorporating the cellulose samples made the crust colour lighter while the crumb colour remained unchanged. MC incorporation had the most significant effect on crust colour.

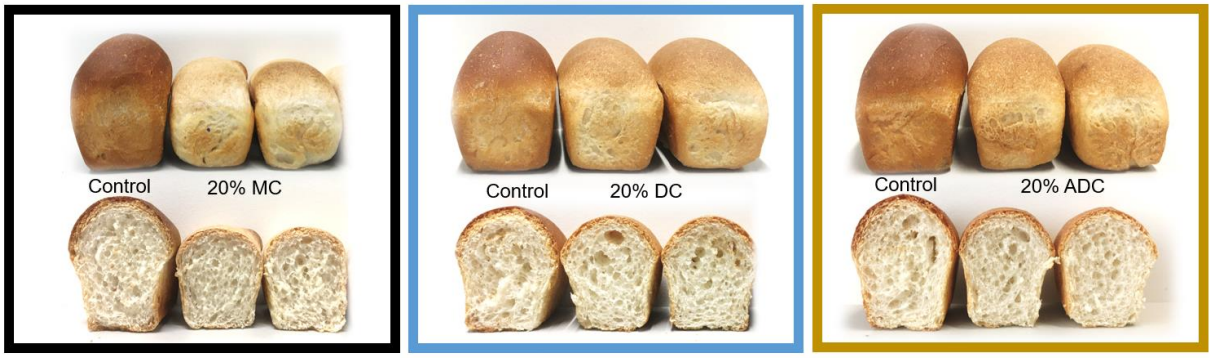
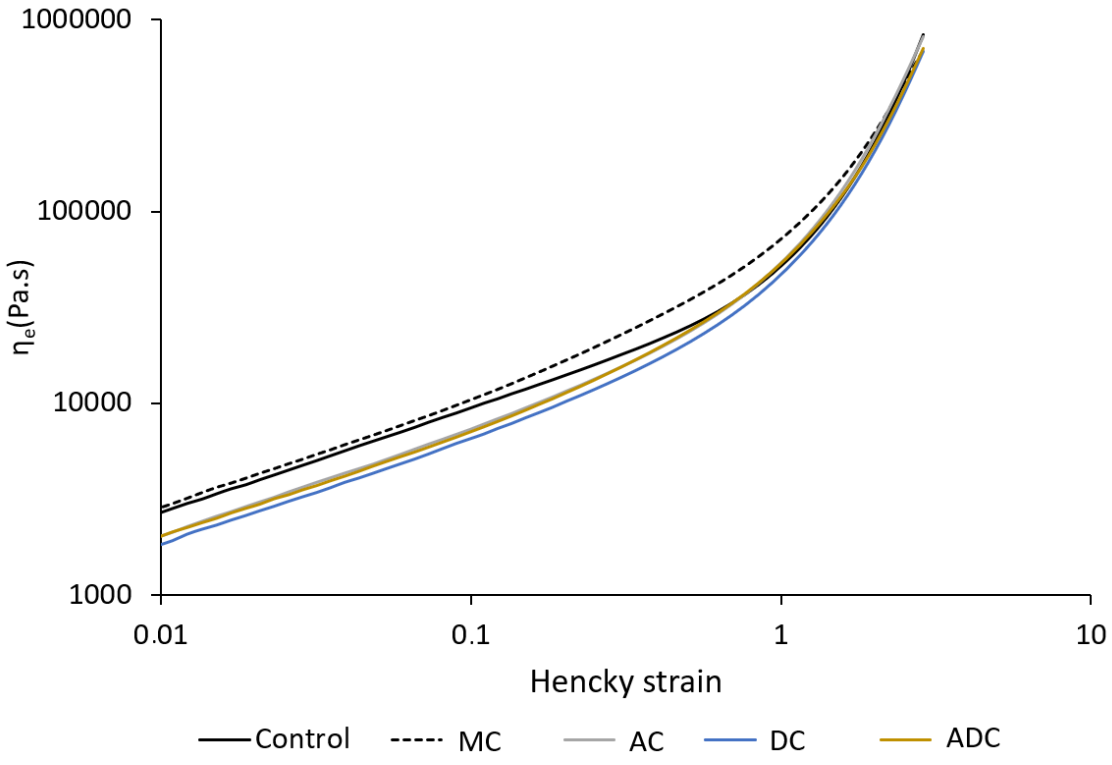


Figure 4: Crumb and crust colour of breads with 20% unmodified MC, depolymerised cellulose (DC) or amorphised depolymerised cellulose (ADC) addition, compared to a bread without cellulose addition. Breads were made at optimal water absorption, which is 56% (control), 76% (MC) and 66% (DC and ADC).

3.3 Dough properties with 20% cellulose at optimal WA

A significantly lower loaf volume loss was observed for loaves with 20% DC compared to 20% MC ($p<0.05$), even when made using their optimal WA. To understand the reason for this, the rheological behaviour of doughs with 20% cellulose incorporation at optimal WA was studied using elongational viscosity measurements (Figure 5). The incorporation of AC, DC and ADC shifted the viscosity in the linear envelope downwards, resulting in extrapolated linear viscosities at a strain of 2.89 that were comparable to that of control bread dough. On the contrary, MC incorporation caused a slight increase in extrapolated viscosity at maximal strain because of a higher slope

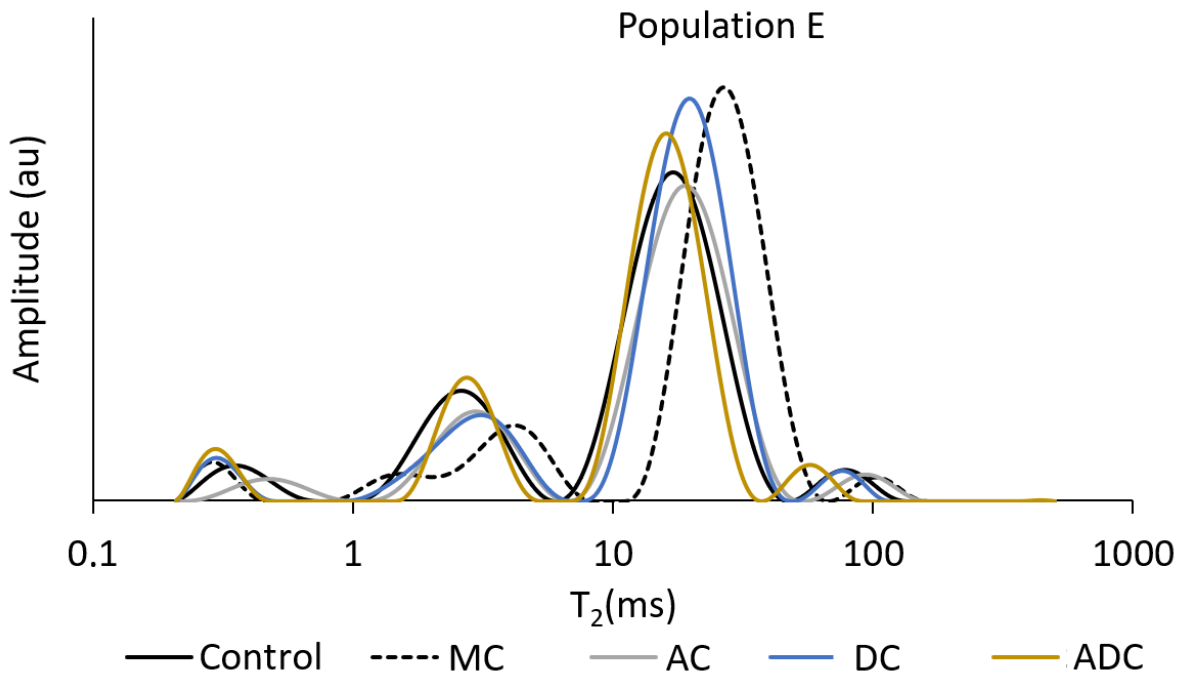
in the linear envelope. As a result, the strain hardening index (SHI) of these doughs was significantly lower than the SHI of the control dough ($p<0.05$). The incorporation of 20% AC and DC fibres into the dough did not decrease the SHI of the dough.



	Maximal viscosity (10^5 Pa.s)	Linear viscosity at strain 2.89 (10^5 Pa.s)	Strain hardening index (SHI)
Control	131.0^{a}	6.4^{ab}	20.6^{a}
MC	86.6^{c}	8.0^{a}	11.2^{b}
AC	115.9^{ab}	5.4^{ab}	21.7^{a}
DC	99.3^{bc}	4.7^{b}	21.2^{a}
ADC	103.0^{bc}	5.3^{ab}	19.8^{a}

Figure 5: Extensional viscosity curves, and derived rheological parameters, for doughs with 20% cellulose sample incorporation and a control dough without it. Doughs were prepared at their optimal water absorption, which was 56%, 76%, 64%, 66% and 66% for the control dough and doughs with microcrystalline cellulose (MC), amorphised cellulose (AC), depolymerised cellulose (DC) and amorphised depolymerised cellulose (ADC) incorporation, respectively.

Proton mobility distribution curves of bread doughs, measured by ^{1}H-NMR relaxometry, are generally used to gain insights into the water distribution, starch-water interactions or gluten conformation in the dough (Hemdane et al., 2017; Nivelle et al., 2017). In Figure 6, the proton mobility curves of doughs with 20% cellulose sample addition (at optimal WA), generated by a CPMG pulse sequence, are shown. As Bosmans et al. (2012) described, such a distribution curve is characterised by several proton populations, with each a different mobility. Population E mainly represents the mobile and exchangeable OH protons of bulk water, starch and gluten (Bosmans et al., 2012). Furthermore, we can hypothesise that this population also contains exchangeable protons close to the cellulose. Since this population E includes most of the water in the dough, the mobility and peak area of this population are summarised in Figure 6 as well. Incorporation of 20% MC or ADC (at optimal WA) causes a significant increase in the area of this proton population peak ($p<0.05$) while incorporating AC or DC does not result in an altered peak area. The mobility of this proton population slightly increases upon the incorporation of AC and DC, while bigger mobility shifts are detected upon the incorporation of MC (from 15.7 ms to 25.9 ms).



	Area pop. E	T_2 relaxation time Pop. E (ms)
Control	3.05[a]	15.7[a]
MC	3.34[b]	25.9[d]
AC	2.95[a]	17.8[b]
DC	3.11[a]	18.5[c]
ADC	3.29[b]	15.8[a]

Figure 6: Proton mobility distribution curves, and derived characteristics, after a CPMG pulse sequence of doughs without (control) and with 20% cellulose sample addition. Doughs were prepared at their optimal water absorption, which is 56%, 76%, 64%, 66% and 66% for the control dough and dough with microcrystalline cellulose (MC), amorphised cellulose (AC), depolymerised cellulose (DC) and amorphised depolymerised cellulose (ADC) incorporation, respectively.

4. Discussion

4.1 Physicochemical changes in cellulose resulting from modification

We modified MC into amorphised cellulose (AC), depolymerised cellulose (DC) and amorphised depolymerised cellulose (ADC) because each of these modified samples showed enhanced fermentability by human colon microbiota in an in vitro set-up (Thielemans et al., 2023). Next to altering the chain length and crystallinity, the process used to obtain these samples also changed their aggregate structure and hydration properties.

Microcrystalline cellulose consists of irregular rod-like aggregates with large cavities (Figure 1). MC has a strong water retention capacity (SWRC) of 0.65 ± 0.02 g water per g dry matter (DM) (Thielemans et al., 2022). The SWRC is an important measure for the amount of water that can be withheld by a fibre when subjected to strong centrifugal forces. It is a relevant characteristic for incorporation in bread doughs, in which the water distribution is known to define the quality of the end product (De Bondt et al., 2020). Low field ^{1}H-NMR measurements on an MC-water mixture demonstrated that the water is mainly entrapped within larger capillaries and cavities in the aggregates (Figure 2).

When MC is milled in a **planetary ball mill** to form AC, the large rods are converted into spherical aggregates with a relatively regular and dense surface (Figure 1). Thielemans et al. (2022) reported that ball-milling was shown to reduce the crystallinity index from 72 to less than 30, while the avDP decreased from 147 ± 0 to 124 ± 0. The specific surface area was not impacted by ball-milling of MC (0.93 ± 0.08 m²/g) into AC (0.91 ± 0.01 m²/g), but the SWRC decreased to 0.50 ± 0.04 g water/g DM (Thielemans et al., 2022). This decrease can be explained by compaction of the

material (Lee et al., 2017; Vaidya et al., 2016). Nevertheless, the bound water at these modified celluloses is closer to the cellulosic surface than water in the neighbourhood of MC (Figure 2). This relates to the presence of water in small micropores in the aggregate or close to the external surface.

Acid hydrolysis and oven-drying of AC to form DC did not affect the spherical character of the aggregates (Figure 1). Thielemans et al. (2022) did report a decrease in median particle size from AC (60.7 ± 17.4 µm) to DC (29.1 ± 1.4 µm) and showed that the acid hydrolysis resulted in an efficient depolymerisation as the avDP decreased to 28 ± 1, while the crystallinity index again increased to 62 (Thielemans et al., 2022). The specific surface area was shown to decrease down to 0.63 ± 0.29 m^2/g (Thielemans et al., 2022). This decreased specific surface area was caused by the loss of para-crystalline zones during hydrolysis and hornification during oven drying (Fan et al., 1980; Salmén & Stevanic, 2018). Only a small effect was observed in the SWRC (0.44 ± 0.01 g/g DM) (Thielemans et al., 2022).

A short **ball mill treatment** of 30 min after hydrolysis sufficed to produce ADC fibres with a crystallinity below 30, while the avDP was not affected (Table 1, Thielemans et al., 2022). The spherical shape of the aggregates (Figure 1) was not affected, but the median particle size increased in comparison to DC (Table 1, Thielemans et al., 2022). Furthermore, the SWRC increased due to this ball mill step to a value higher than for the AC sample (Table 1, Thielemans et al. 2022). This higher SWRC might be caused by the creation of micropores in the aggregates during ball milling or by the creation of para-crystalline zones, which can act as water binding sites as well (Khazraji & Robert, 2013).

4.2 Implication of physicochemical changes on bread properties

From the state of the art, we know that adding insoluble (cellulose) aggregates to a bread recipe reduces the final bread loaf volume. In analogy with the addition of wheat bran to bread, it can be expected that this loaf volume reduction was not caused by a reduced gas production during fermentation but rather by a decreased gas retention capacity of the dough, due to changes in rheological behaviour of the dough (Bock & Damodaran, 2013; De Bondt et al., 2021). This is because the aggregates hindered the formation of the gluten network sterically, competed for water with the gluten, and caused a dilution of the functional starch and gluten in the dough (Gómez et al., 2003; Hemdane, Langenaeken, et al., 2016). Therefore, we can hypothesise that the differences in aggregate structure and hydration play a prominent role in the behaviour of insoluble cellulose in dough and bread.

Incorporating the rod-like aggregates of **MC** with a relatively high SWRC in a standard bread dough recipe at a 5% substitution level disturbed the water distribution balance in the dough, resulting in a reduction of the loaf volume with 12.8 ± 2.3%. At a 5% substitution level, this volume reduction could be counteracted by adding more water to the recipe (Figure 3a). At higher substitution levels (20%), the loaf volume loss increased to 65.6 ± 1.4% at a standardised WA. Adding water to the limit of dough manageability did not result in acceptable loaf volumes (Figure 3b). Furthermore, the crust colour and crumb structure were negatively affected in all cases. The crust colour differences reflect the loaf volume differences, with high volumes leading to darker crusts and low volumes associated with less crust colouration. A high water holding capacity and low loaf volume can be hypothesised to reduce the surface temperature during baking.

In doughs enriched with 20% MC at optimal WA, the water availability for gluten was high enough, as evidenced by the fact that the area or mobility of proton population E

(representing the easily exchangeable water in the dough) was not lowered (Figure 6). However, the quality of the gluten network in this dough did not reach that of the control dough without cellulose addition anymore (Figure 5), which probably caused this loaf volume loss. Meerts *et al.* (2017a) observed that the SHI of dough is associated with the gas retention capacity of the dough during the fermentation phase. Adding MC decreased the SHI, indeed, and a voluminous crumb could not be formed. This decreased SHI value resulted from a higher extrapolated linear viscosity at maximal strain (Figure 5). Therefore, we can state that improper gluten hydration caused this suboptimal network development (Meerts et al., 2017b), despite the high water availability for the gluten, which was detected in Figure 6. The additional water that was incorporated in the MC-enriched bread recipe might have been trapped in the cavities of the MC, preventing proper water distribution and gluten hydration during mixing. As a result, the incorporation of 20% MC minimally led to a bread loaf volume decrease of 36.4 ± 2.9% compared to the control at optimal WA. The decreased bread loaf volume could not be attributed to an effect of the MC on starch gelatinisation during breadmaking (Figure A2). In comparison, the addition of 15% wheat bran to starch did result in a significant increase of more than 2°C in onset, peak and conclusion temperature in excess water in another study (De Bondt et al., 2020).

Incorporating **AC, DC or ADC** in a bread recipe had less impact on the bread loaf properties. At a 5% substitution degree, incorporating these samples with spherical aggregates and a reduced SWRC did not reduce the final bread loaf volume or require additional water in the recipe (Figure 3a). At a substitution degree of 20%, the bread loaf volumes decreased by 29.0 ± 0.1%, 16.2 ± 1.9% and 20.9 ± 0.1% upon AC, DC and ADC incorporation, respectively. However, these bread loaves required less addition of water in the recipe than MC-enriched bread and had significantly higher

loaf volumes ($p<0.05$; Figure 3b). Dough assessment demonstrated that incorporating these cellulose samples had less impact on the water distribution and dough rheology. The SHI's of these doughs were similar to that of a control dough, and the mobility of exchangeable proton population E increased only slightly. The water attraction of the modified fibres in the dough did not seem to hamper the hydration of the gluten to the same extent as MC (with capillary-bound water). Therefore, at a 20% substitution level, AC, DC and ADC-enriched doughs with a balanced water distribution could be obtained at an only slightly increased WA. These resulted in decent bread as well.

Among the three modified cellulose samples, DC incorporation resulted in the highest bread loaf volume. This could be explained by the fact that DC fibres had the lowest SWRC of all cellulose samples. This association between SWRC of a fibre and loaf volume of the fibre-enriched bread was previously observed for wheat bran addition as well (De Bondt et al., 2021). Nevertheless, the difference in loaf volume between MC- and ADC-enriched bread could not be predicted based on the SWRC only. Therefore, the data in this work demonstrates that also the type and strength of water binding by fibres play a key role in the water distribution in the dough, and the resulting end-product quality.

5. Conclusions

In this work, we demonstrated that modifying the hydration behaviour of microcrystalline cellulose (MC) is a valuable strategy for improving bread volume and quality of cellulose-enriched breads. Techno-functional drawbacks of incorporating insoluble cellulose into a bread recipe can be linked to a complex interplay between competition for water and volume with the functional ingredients in the dough. By treating MC in a ball mill and with acid hydrolysis, these drawbacks could be

minimised. Incorporation of processed cellulose samples (depolymerised cellulose (DC) and amorphised depolymerised cellulose (ADC)) with unique chain length, crystallinity and aggregates size and shape, in a bread dough has fewer adverse effects on the water balance within the dough than the incorporation of MC. As a result, the gluten network in these doughs is stronger, and bread loaf volume losses can be limited to less than 21% upon the incorporation of 20% of cellulose. To the contrary, the incorporation of 20% MC resulted in higher loaf volume losses and unacceptable bread. Given that the ADC and DC fibres are partially fermentable in the colon (Thielemans et al., 2023), these results are very promising for producing dietary fibre-enriched wheat flour bread with high amounts of fermentable dietary fibre. Although the current production process of the fibres might not be feasible on a commercial scale, the obtained fundamental insights linking cellulose properties to its functionality in dough and bread can help develop new fibres with improved functionality and fermentability.

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Appendix A

Figure A1: Relative loaf volumes of bread loaves with 5, 10, 15 and 20% cellulose incorporation levels, baked on water absorption levels of 56%, 58%, 64% and 64%, respectively. Bread loaves were enriched with microcrystalline cellulose (MC), amorphised cellulose (AC), depolymerised cellulose (DC) and amorphised depolymerised cellulose (ADC).

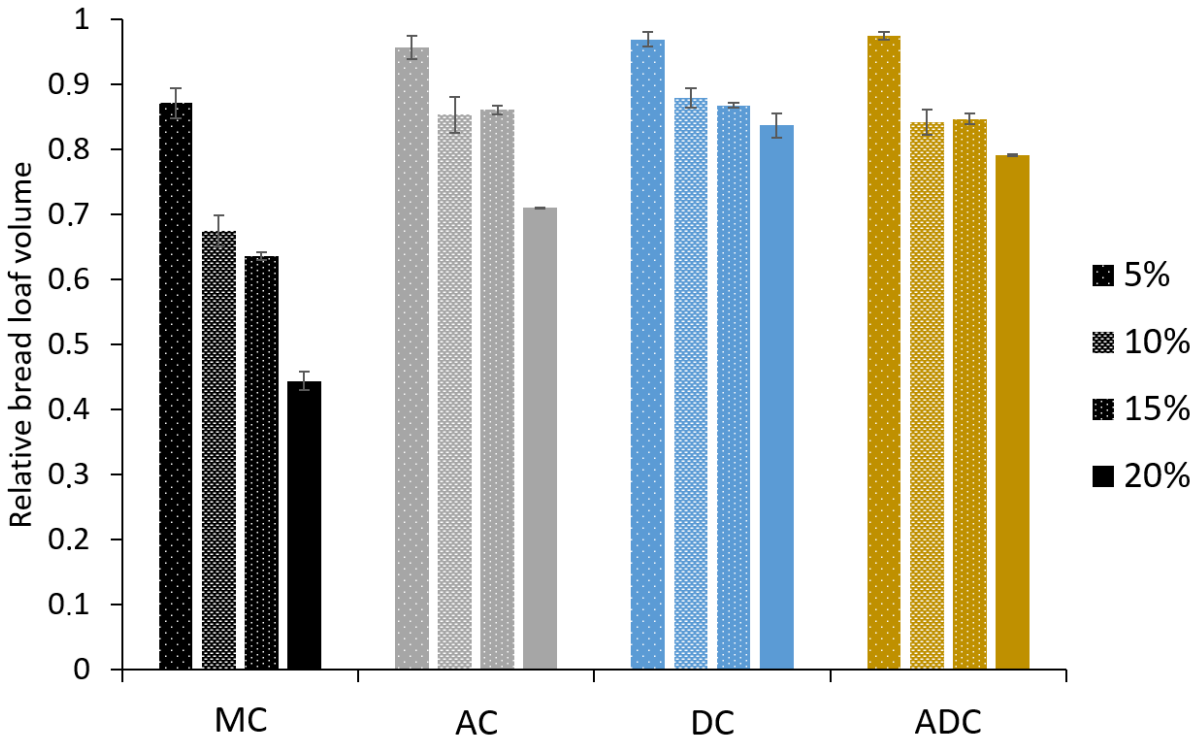
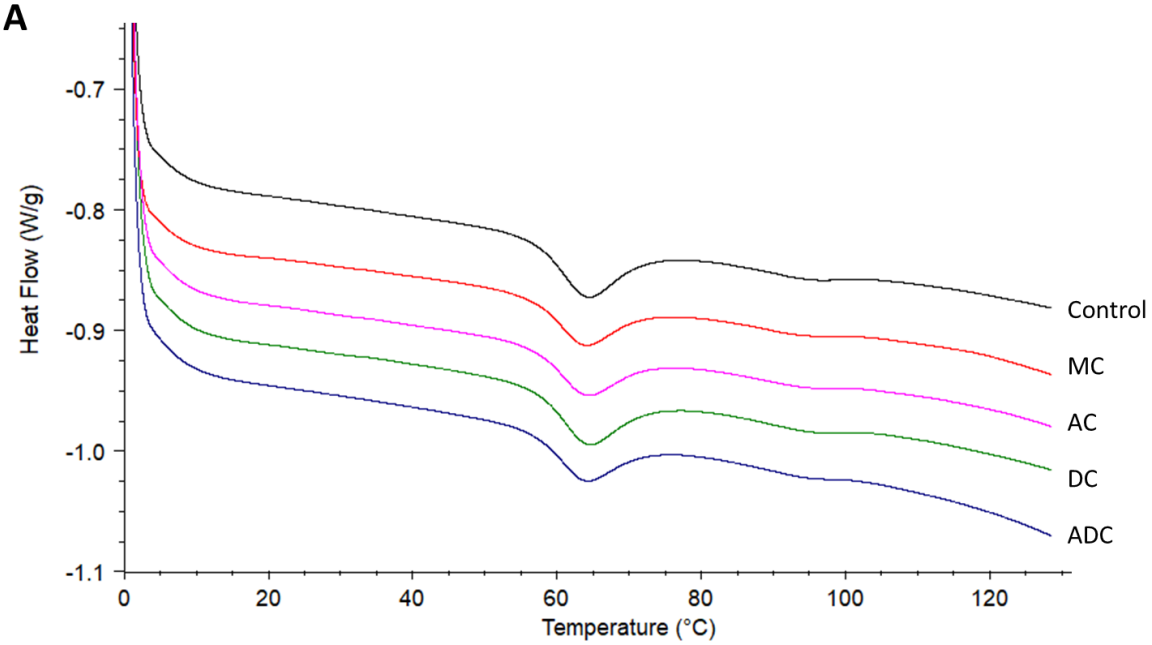


Figure A2: (A) Differential scanning calorimetry profiles of flour (Control) and mixtures of 80% flour with 20% microcrystalline cellulose (MC), amorphised cellulose (AC), depolymerised cellulose (DC) or amorphised depolymerised cellulose (ADC). Mixtures were analysed in excess water (1:3 w/w). (B) Onset, peak and conclusion starch gelatinisation temperatures. No significant differences (p <0.05) were observed.

A



B

	Onset temperature (°C)	Peak temperature (°C)	Conclusion temperature (°C)
Control	57.23 ± 0.43	64.24 ± 0.22	73.04 ± 0.23
MC	56.84 ± 0.04	63.83 ± 0.05	72.56 ± 0.17
AC	56.88 ± 0.14	63.86 ± 0.24	72.75 ± 0.40
DC	56.95 ± 0.33	63.97 ± 0.41	73.22 ± 0.69
ADC	57.01 ± 0.25	64.02 ± 0.25	73.24 ± 0.63