Effect of β-glucans on water redistribution and gluten structure in a model dough during the mixing process

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Abstract. Farinographic and FT-IR analysis were used to determine water redistribution and structural changes in the gluten network during dough mixing as a result of model bread dough supplementation with two types of β-glucans. The β-glucans were obtained from barley and beer yeast. Both polysaccharides were added to the dough in the amounts of 3, 6 and 9%. The farinographic studies show that both β-glucans have a similar effect on the course of chemically induced gluten dehydration and mechanical destruction. The application of a water redistribution model shows that barley β-glucan caused higher physical dehydration of the gluten network in comparison with yeast β-glucan. Additionally, both β-glucans did not differ significantly in their chemical reactivity to gluten. This finding was confirmed by the FT-IR results. Both β-glucans caused similar structural changes in the gluten network during dough mixing. An analysis of the spectral region connected with water populations indicates that water molecules form hydrogen bonds with β-glucans rather than with the gluten network during dough mixing.

Keywords: gluten network, β-glucan, farinogram, gluten dehydration, FT-IR, secondary structure

INTRODUCTION

β-glucans are among the most widespread polysaccharides in nature i.e. glucans. Glucans are divided into three groups: α-glucans, β-glucans, and mixed α, β-glucans. α-glucans act as an energetic source for metabolism, while β-glucans are components of plant cell walls (Synytsya and Novak, 2014). In the food industry, β-glucans with both cereal and fungal origins have become the subject of research since they can act as food thickeners, fat replacers, viscosity imparting agents, emulsifiers, a source of fibre etc. (Lee et al., 2009). Additionally, they are characterized by antioxidative, immunostimulatory and antitumor properties (Nakashima et al., 2018). Thus β-glucans like other dietary fibre polysaccharides (e.g. inulin, pectins) can be used as a bread supplement.

The supplementation of bread dough with dietary fibre polysaccharides induces changes in the dough quality and hence in the structure of the gluten network. These changes may be related to the competition for water between the gluten network and the polysaccharides (Bock and Damodaran, 2013). This hypothesis was confirmed through spectroscopic (Nawrocka et al., 2017a, 2018a) and farinographic and modelling studies (Miś et al., 2020). The spectroscopic studies showed the formation of random coils and aggregated β structures i.e. β-sheets and β-turns connected by intermolecular hydrogen bonds. Tryptophan and tyrosine residues buried within the hydrophobic environment of the protein complex indicated the folding of the polypeptide chains. Moreover, the dependence between the behaviour of tyrosine residues and the water-holding capacity (WHC) of the polysaccharides was observed. The larger the WHC value of the polysaccharide used, the more hydrogen bonds

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were formed by the tyrosine residues (values of the tyrosine doublet decrease). Spectroscopic studies have also shown that the structural changes depended on the water-solubility of the fibre polysaccharides. The farinographic results fitted by a regression model showed how the water is redistributed between individual components of the model bread dough during the mixing process. The objects of the studies were one-component (gluten), two-component (starch-gluten), and three-component (starch-gluten-fibre) doughs. The results demonstrated that two types of water redistribution during dough mixing may be distinguished: physical and chemical redistribution connected with fibre-hydration capacity and chemical interactions between the gluten proteins and fibre, respectively.

β-glucans may be obtained from different sources such as cereals, yeast, algae etc. Their chemical structure and properties depend on the source from which they were isolated (Sandula et al., 1999). Previous studies concerning different fibre polysaccharides has shown that different chemical structures and molecular sizes can affect the mechanism of interaction between gluten proteins and fibre polysaccharides (Nawrocka et al., 2018a, b). Thus, the aim of the present study was to determine how β-glucans of different biological origin affect the process of water migration between gluten and polysaccharide and the structure of the gluten network during dough mixing.

MATERIALS AND METHODS

Wheat gluten and sodium chloride were purchased from Sigma-Aldrich (Poland) and used as received. Wheat starch was purchased from Cargill (The Netherlands). β-glucan from barley was obtained from the Institute of Human Nutrition Sciences, Warsaw University of Life Sciences – SGGW (Warsaw, Poland). β-glucan from beer yeast (Leiber Beta Saccharomyces cerevisiae) was purchased from Leiber GmbH (Germany). According to the manufacturer, β-glucan from beer yeast is a mixture of 1,3 and 1,6 – β-D-glucans. Double-distilled water was used in all experiments.

Barley β-D-glucan was extracted from a semi-concentrated barley preparation that had a concentration of 25.6% of β-D-glucan (DKSH, Switzerland). The method of β-D-glucan extraction was based on an enzymatic treatment. 3 g of barley preparation were placed in a 50 mL falcon tube with 30 mL of phosphate buffer at pH 9.5. The samples were mixed on a rotator with shaking for 1 h (70 rpm) (IntelliMixer RM-2, Elmi Ltd., Latvia). Then the samples were centrifuged at 8000g (Hettich Universal 320R, Germany) for 10 min and the supernatant was collected. All of the samples were then treated with thermostable α-amylase at 80°C (Termamyl SC, Novozyymes, Denmark) after adjusting the pH to the optimal value (pH 7.0). Digestion was stopped after a negative iodine test was obtained. In the next step, the solutions were cooled down, and the pH was decreased to 3.5 by the addition of 2 M acetic acid to reduce protein solubility, the solutions were placed in a 95°C water bath to continue protein denaturation and precipitation. The proteins were collected in pellet form during centrifugation and the supernatant was added to ethanol in the ratio of 1:2. During storage for 24 h at 4°C the β-glucan precipitated. It was collected using centrifugation and washed with 96% ethanol to obtain a clean gum. Then, the gum was freeze-dried. The purity of the β-D-glucan was determined using a Megazyme International Kit for mixed linkage β-D-glucan (Megazyme, Ireland) and was assessed to be equal to 85.9% and a 60 000-65 000 g mol⁻¹ molecular weight was determined using size-exclusion chromatography with calcofluor detection (Rieder et al., 2015).

The model dough – β-glucan samples were prepared in a Farinograph – E (Brabender, Germany) according to the method described by Nawrocka et al. (2017a). Briefly, model flour reconstituted from wheat starch and wheat gluten in a constant weight proportion (80:15 w/w) was used to obtain model dough. The model dough was supplemented with two types of β-glucan (β-glucan from barley (BAR), and beer yeast (BY)) in the amounts of 3, 6 and 9%. The supplemented dough samples were prepared according to modified standard procedure ICC 115/1. The samples for structural analysis were taken after 20 min of dough mixing. The dough samples were prepared at 65% water absorption. The model dough – β-glucan samples were taken at the end of the mixing process for spectroscopic study.

The dough mixing tests were performed with the use of a Farinograph-E equipped with an S 50 mixer with sigma blades for 50 g of flour (model 810114, Brabender, Duisburg, Germany). For the preparation of the free-component blend, wheat starch, wheat gluten, and beta-glucan supplements were used in the form of air-dried powders, with each of them being at the same hydration level ($H_s=H_g=H_f=4/86$). There were onstant weight proportions of starch to gluten ($\phi_s/\phi_f=80/15$), which is characteristic for native bread wheat flour. The doses of the beta-glucan supplements ($\phi_b$) were 0.03 (3%), 0.06 (6%), and 0.09 (9%). One hydration level of the starch-gluten-beta-glucan dough was used, i.e. $H_{s+g+f}=79/86$. The mixing time was prolonged to 90 minutes in order to obtain an appropriate regression model. Dough consistency measurements were recorded at a frequency of 30 min⁻¹.

The obtained farinograms were analysed using a regression model developed by Miś et al. (2020). This allowed of to link changes in the consistency of the starch-gluten-beta-glucan dough ($C_{s+g+f}$) as a function of the hydration level of the gluten component ($H_g$) and mixing time ($t$) in the course of processes and phenomena, such as: the redistribution of water between the gluten and starch components ($H_{w,s}$), the physical dehydration of gluten by beta-glucans (PhD), the chemical modification of gluten by the beta-glucans (chemical dehydration of gluten – ChD$_{g}$, and ChD$_{g,p}$, as represented by the second and third farinograph peaks), the
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sigmoideal mechanical destruction of the gluten network – SD and the destructive effect of mixing to soften the dough consistency (flattening time of the farinogram – \( t_f \)), using the following formula:

\[
C_{r,T}(H_d(t)) = (3884.19 \exp(4.21881(1−H_d)) + (\exp(2.5\cdot0.70755−H_d))−\exp(3.49108(2.34331−H_d))) t \exp(−t/F)) \frac{(1+SD(1+exp(b_3(\phi)(c_3(\phi)−t))))}{b_3(\phi)·b_3(\phi)·b_3(\phi)}·
\]

where: \( H_d(t) = H_d(t=0) + H_{ma} + PhD + ChD, \)

\[
H_{ma} = (\exp(b_1(\phi)(c_1(\phi)−t)) \exp(b_1(\phi)(c_1(\phi)−t)))+(ChD/1+exp(b_1(\phi)(c_1(\phi)−t)))
\]

and \( ChD = ChD_t/1+exp(b_1(\phi)(c_1(\phi)−t))+ChD_{f}/(1+exp(b_1(\phi)(c_1(\phi)−t)))). \)

The gluten samples were prepared for FT-IR measurement according to Nawrocka et al. (2017b). Briefly, the gluten was washed out from the unmodified and modified dough samples using a 10% aqueous solution of D₂O according to Nawrocka et al. (2017b). The samples were moisturized in order to eliminate water oscillations from the amide I band.

The FT-IR spectra were acquired using a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, Madison, WI, USA) equipped with a diamond attenuated total reflectance (ATR) attachment according to Nawrocka et al. (2017b).

The preparation of the spectra to analysis and spectra analyses were conducted using ORIGIN (v.9.0 PRO, OriginLab Corporation, USA). First, all spectra were normalized at 2485 cm⁻¹ (characteristic band of D₂O) and a spectrum of the 10% aqueous solution of D₂O was subtracted from all of the sample spectra to obtain difference spectra in the OH stretching region (2500-4000 cm⁻¹). In order to determine changes in the secondary structure of the gluten network, the difference spectra were calculated in the amide I (1570-1720 cm⁻¹) and amide III (1200-1350 cm⁻¹) bands. A spectrum of the control sample (gluten washed out from the unmodified model dough) was subtracted from the spectra of the supplemented samples. All spectra were area – normalized in both the amide I and amide III band. The secondary structures from the amide I and amide III bands were assigned according to Barth (2007) and Cai and Singh (1999), respectively.

RESULTS AND DISCUSSION

The effect of β-glucan supplementation on the model starch-gluten behaviour during mixing is shown in Fig. 1a and b. Increasing the dose of these supplements from 0.03 to 0.09 resulted in a more than twofold increase in the consistency of the dough, which indicates the extremely high thickening capacity of β-glucans. At the same time, the shape of the farinograms underwent a significant evolution. The movement of the third dehydration peaks towards shorter mixing times was observed with an increase in the level of β-glucan supplementation. Similar effects, but to a much narrower extent, were reported in a previous article for a model dough supplemented with commercial dietary fibre preparations (Miš et al., 2020). The main factor for the displacement of the dehydration peaks is the thickening properties of β-glucans. The thickened consistency, which is due to the more efficient energy transfer from the mixer to the dough, accelerates the development of the gluten network (Isaak et al., 2019; Mani et al., 1992), as well as its chemical modification which is induced by the presence of the supplement and mechanical destruction under the influence of mixing.

β-glucan supplementation is distinguished by the overlapping of the second dehydration peak (\( p_2 \)) with the first peak of dough development (\( p_1 \)). As a result of this phenomenon (Fig. 1a), farinograms representing barley β-glucan are characterized by only two peaks (\( p_2 \) and \( p_1 \)), although a barely marked peak of dough development may be observed at \( \phi_f = 0.03 \). Stages of the overlapping of the
p₁ and p₂ peaks with the increase in the supplement dose (ϕ₁) are well reflected in the course displayed within yeast β-glucan farinograms (Fig. 1b). The results of a more detailed analysis of the farinograms was carried out using the water redistribution model which is shown in Table 1. The barley and beer yeast β-glucans showed very high water-binding capacities, estimated using the redistribution index (Rᵢ), they were 5.9 and 5.3, respectively. For comparison, the Rᵢ range for the six dietary fibre preparations reported by Miś et al. (2020) occurred at significantly lower levels, from 1.5 (chokeberry) to 3.3 (carrot fibre). In addition to the higher Rᵢ index, the presence of barley β-glucan in the dough increased the rate of gluten hydration (bᵢ), but at the same time it contributed to a higher level of gluten physical dehydration (PhD), up to 0.04, and 2.5 times faster over its course (bᵢ) as compared to the yeast β-glucan effect. However, both kinds of β-glucans did not differ significantly in their chemical reactivity to gluten, as reflected in the size of the p₁ and p₂ dehydration peaks (ChD_p₁ and ChD_p₂). Also, no significant differences were noted when analysing the total effect of the physical and chemical dehydration of gluten (PhD + ChD_p₁ + ChD_p₂), which is indicated by similar levels of total dehydration caused by barley and yeast β-glucans, and at the highest β-glucan dose they amounted to −0.89 and −0.88, respectively.

It should be emphasized that the levels of chemical dehydration of gluten did not completely depend on the dose of beta glucans used. This reaction was different from that observed for most fibre preparations (Miś et al., 2020). Only the carrot fibre with the highest Rᵢ showed a reaction similar to that of β-glucans. Nevertheless, beta-glucan doses had a significant effect on the rate of chemical dehydration (bᵢ) and mechanical destruction (bᵢ) and also on the location of their maxima (cᵢ) and cᵢ). With the increase in the beta-glucan dose, the values of bᵢ, cᵢ and cᵢ gradually decreased. On the other hand, the opposite relationship was observed for the parameters of bᵢ, SD and bᵢ, where the increase in the dose generally influenced the increase in the dynamics of dehydration and destruction as well as the level of destruction.

### Table 1. Characteristics of the physical and chemical redistribution of water between model dough components, wheat gluten and barley (BAR) or beer yeast (BY) β-glucans, during mixing

<table>
<thead>
<tr>
<th>β-glucan dose-independent parameters</th>
<th>β-glucan dose-dependent parameters</th>
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<tbody>
<tr>
<td>Name</td>
<td>BAR</td>
</tr>
<tr>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>ρ</td>
<td>0.99967b</td>
</tr>
<tr>
<td>±0.00002</td>
<td>±0.00023</td>
</tr>
<tr>
<td>RMSE</td>
<td>2.80a</td>
</tr>
<tr>
<td>(FU)</td>
<td>±0.12</td>
</tr>
</tbody>
</table>

Parameter values in the same row followed by different superscript letters are significantly different from each other (α≤0.05). *Constants fixed arbitrarily, in case of yeast β-glucan combinations: cᵢ = −8.68 + 3.89ϕᵢ(−1/3).
The differences in the dynamics of dehydration and destruction between the barley and yeast β-glucans were subtle. Compared to barley β-glucans, the yeast ones were characterized by more labile parameters as a function of the dose amount ($\phi_f$). Statistically significant differences were revealed mainly at the highest dose of β-glucans.

The above-mentioned phenomenon of the overlapping of the $p_1$ and $p_2$ peaks made it difficult to precisely locate the $p_2$ peak ($c_{p_2}$) and, in the case of yeast β-glucan, also the maximum rate of mechanical destruction ($c_D$). Only after introducing arbitrarily fixed $c_{p_2}$ and $c_D$ values into the model, was there a marked improvement in the reproducibility of the results of the regression analysis. However, these difficulties were probably the reason for the worse fit of the model to the farinograms of the dough supplemented with yeast β-glucan, and resulted in almost twice the estimation error (RMSE).

The presented results of the analysis of the farinograms show a high similarity of barley and yeast β-glucans concerning their influence on the course of chemically induced gluten dehydration and mechanical destruction. Moreover, the greatest differences were revealed in their hydration properties affecting the physical redistribution of water between the components of the dough.

The structure of the β-glucans was studied using FT-IR spectroscopy. FT-IR spectra of both β-glucans in the spectral region 800-1800 cm$^{-1}$ are presented in Fig. 2. Both spectra show a few similar bands that can be located in the anomic region (896 cm$^{-1}$), polysaccharide region (994, 1014, and 1154 cm$^{-1}$), and protein region (1639, 1200-1340 cm$^{-1}$). According to Synytsya and Novak (2014), the anomic region contains bands assigned to complex skeletal vibrations sensitive to the anomic structure of glucose, whereas the polysaccharide region is dominated by intense bands of CC and CO stretching vibrations originating from the glycosidic bonds and pyranoid ring. Moreover, the BY spectrum shows additional bands at 1590 and 1351 cm$^{-1}$. The first band may be assigned to the assymetrical stretching vibrations of COO$^-$ and may indicate the presence of carboxymethyl β-glucan in the BY preparation (Sandula et al., 1999). On the other hand, this band can also be assigned to NO$_2$ antisymmetric stretching in aliphatic nitro compounds because a band at 1351 cm$^{-1}$ is simultaneously observed. This band may also be connected with the same kind of oscillations (Shurvell, 2006). The results indicate that the bands at 1590 and 1351 cm$^{-1}$ are related to the vibrations of the NO$_2$ groups rather than to the presence of carboxymethyl β-glucan because the FT-IR spectrum of the BY preparation does not show a strong band at ca. 1420 cm$^{-1}$. This band indicates the carboxymethylation of β-glucan (Sandula et al., 1999).

The amide I (1570-1720 cm$^{-1}$) and amide III (1200-1340 cm$^{-1}$) bands can be used to determine the secondary structure of proteins. Figure 3 presents difference spectra in the above-mentioned bands. The difference spectra show which secondary structures appear/disappear as a result of model dough supplementation with β-glucans. In the case of the amide I band, the spectra can be divided into two spectral regions: positive (1590-1640 cm$^{-1}$) and negative (1640-1690 cm$^{-1}$). The positive region contains bands assigned to pseudo-β-sheets (1611, 1616 cm$^{-1}$), and...
parallel β-sheets (1630 cm⁻¹). While characteristic bands for hydrogen bonded β-turns (1645, 1660 cm⁻¹), β-turns (1677 cm⁻¹), and α-helix (1654 cm⁻¹) have a negative orientation (Barth, 2007; Nawrocka et al., 2018a). Regardless of the type of β-glucan used, the observed structural changes in the amide I band are similar. α-helices, β-turns with and without hydrogen bonds participate in the formation of aggregated β structures (strong bands associated with pseudo-β-sheets).

An analysis of the difference spectra in the amide III band also shows similar changes in the secondary structure of the gluten network regardless of the β-glucan used. The β-sheets and β-turn regions contain negative bands at ca. 1228 and 1280 cm⁻¹, respectively. A strong positive band at ca. 1251 cm⁻¹ is observed in the spectral region connected with random coils. Both positive and negative bands are present in the α-helix region. A negative band at ca. 1228 cm⁻¹ was observed after model dough supplementation with microcrystalline cellulose and inulin (Nawrocka et al., 2018a). The authors claimed that this band may be connected with the formation of hydrated β-sheets and/or hydrated extended chains because a relationship between the bands at 1226 and 1593 cm⁻¹ was found during analysis. However, the amide I band from the present study does not contain a band at 1593 cm⁻¹. Moreover, β-glucans adsorb more water than microcrystalline cellulose and inulin because the water retention capacity (WRC) of β-glucan is two times higher (ca. 13 (Oliveira et al., 2012)) than that of cellulose and inulin (ca. 5-6 (Nawrocka et al., 2017a)). For the above-mentioned reasons, the band at 1228 cm⁻¹ cannot be connected with hydrated secondary structures. Anderle and Mendelsohn (1987) assigned this band to β-sheets with inter- and intrachain H-bonds. Nawrocka et al. (2018b) assigned this band to type I hydrogen bonds (–HN···O=C–), which are formed between polypeptide chains of gluten proteins as intermolecular bonds leading to gluten aggregation. A negative orientation of this band indicates the cleavage of intermolecular H-bonds during mixing and the formation of random coils, which is observed as a strong positive band in the spectral region of 1250-1270 cm⁻¹ (Fig. 3c, d). The α-helix region contains a positive band at 1324 cm⁻¹. A similar band was observed after model dough supplementation with microcrystalline cellulose and inulin (Nawrocka et al., 2018a). This band may also be connected with structural changes in the polysaccharide molecule that appeared as a result of chemical interactions during the dough mixing process. However, this band has not been observed in the FT-IR spectra of

![Fig. 3. FT-IR difference spectra calculated in the amide I (a, b) and III bands (c, d) for β-glucans obtained from barley (BAR) and beer yeast (BY).](image-url)
pure β-glucans (Fig. 1). Additionally, such a band has been detected in the analysis of polysaccharides containing β-glycosidic bonds. For pectins which have α-glycosidic bonds in their structure, such a band was not observed (Nawrocka et al., 2018a).

The structural changes in the gluten network modified by two types of β-glucan are similar although the rheological behaviour of the model dough observed at the farinograms (Fig. 1) depends on the type of β-glucan used. However, a detailed analysis of the farinograms with the application of a regression model shows that the β-glucans did not differ significantly in their chemical reactivity, dehydration and destruction effects.

The water populations present in the gluten network may be determined by analysis of the OH stretching region (2800-4000 cm\(^{-1}\)) in the FT-IR spectra. Difference spectra in the OH stretching region for model dough supplemented with β-glucans as well as for the control sample are presented in Fig. 4. The spectrum of the control sample contains three positive and two negative bands that may be assigned to water molecules bonded to the gluten network by strong hydrogen bonds (3 052 cm\(^{-1}\)) and weak hydrogen bonds (3 190 cm\(^{-1}\)) (Bock and Damodaran, 2013), water molecules participating in two hydrogen bonds (3 275 cm\(^{-1}\)) (Cotugno et al., 2001), small hydrogen bonded water clusters (3 381 cm\(^{-1}\)), and free water (ca. 3 631 cm\(^{-1}\)) (Bock and Damodaran, 2013). Similar bands in the spectrum of the gluten network washed out from the model dough were observed by Nawrocka et al. (2018a). The spectra of the supplemented gluten samples show similar bands with a higher intensity as compared to the control sample. The higher band intensity may indicate the formation of a higher number of hydrogen bonded water molecules. However, the same kinds of hydrogen bonds can be formed between water molecules and β-glucan since the formation or cleavage of H bonds between water and gluten polypeptide chains are observed as a shift in the band rather than a band intensity increase (Liu et al., 2002; Nawrocka et al., 2018a). The biggest changes in the OH stretching region are observed after model dough supplementation with 9% BAR. A negative band at 3 240 cm\(^{-1}\) appears instead of two positive bands at 3 190 and 3 275 cm\(^{-1}\). According to Nawrocka et al. (2017b), the band at 3 240 cm\(^{-1}\) can be related to the formation of weak hydrogen bonds between carbonyl groups in the gluten network and/or polysaccharides with water. Moreover, the authors claimed that the presence of this band confirmed the competition for water between wheat dough components and polysaccharides.

In general, β-glucans did not affect the types of water molecules present in the gluten network. An increase in band intensity after β-glucan supplementation suggests that water molecules form hydrogen bonds with β-glucans during dough mixing. This statement is in agreement with the farinographic results.

**CONCLUSIONS**

1. Analysis of the farinograms shows that both β-glucans have a similar effect on the course of chemically induced gluten dehydration and mechanical destruction.
2. The water-binding capacities obtained from the water redistribution model of both β-glucans are very high.
3. Barley β-glucan caused higher physical dehydration of the gluten network in comparison with yeast β-glucan.
4. The water redistribution model shows that both β-glucans did not differ significantly in their chemical reactivity to gluten. This statement was confirmed by the FT-IR results. Both β-glucans caused similar structural changes in the gluten network during mixing.
5. An analysis of the spectral region connected with water populations indicates that water molecules form hydrogen bonds with β-glucans rather than with the gluten network during dough mixing.
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