Effect of different beta-glucan preparation pretreatments on fortified bread quality

Marcin Andrzej KUREK1*, Jarosław WYRWOSZ1, Magdalena BRZESKA1, Małgorzata MOCZKOWSKA1, Sabina KARP1, Agnieszka WIERZBICKA1

Abstract
This research explores the different beta-glucan preparation pretreatments prior to fortifying the bread (hydrating, freezing, boiling, blending and their combination). Breads containing hydrated or dry beta-glucan preparations demonstrated lower values of specific volume than the control. On the other hand, the beta-glucan preparation, which was boiled or blended after freezing showed higher specific volume. The firmest bread was achieved by adding beta-glucan preparation which was hydrated and blended (significant at p ≤ 0.05) and the least firm was after application of a frozen blended preparation. In our study, we researched the influence of pretreatment the beta-glucan preparation on the concentration of this compound. Our breads contain from 1.62 to 1.99 g beta-glucan/100 g of product. It seems that the best way of pretreatment is drying, freezing or boiling after freezing the beta-glucan preparation (significant at p ≤ 0.05). In general, the best performing method was boiling the beta-glucan preparation following dough addition.

Keywords: beta-glucan; pretreatment; bread; quality.

Practical Application: Research helps to provide most appropriate beta-glucan preparation prior to baking.

1 Introduction
The increased awareness of consumers regarding health issues has evoked food producers to address the need to supply the market with functional products. Bakery products are one of the most widely eaten products all over the world. During the last decade, these products have been explored for development of their functionality for human health (Mudgil et al., 2016).

One of the substances that could enhance bakery products in their functionality is beta-glucan. This is a polysaccharide that can be found in cereals, like oats and barley (Wang et al., 2016). Beta-glucan’s health benefits were proved by the European Food Safety Authority (2011) with claims that beta-glucan is recognized as an agent that could lower the blood cholesterol and postprandial glucose response. Moreover, the lowering of LDL cholesterol levels was documented as well.

Generally, the fortification of bakery products with dietary fiber could lower its quality. Although beta-glucan is a valuable nutrient, its application in bakery products is difficult due to its viscosity and water-holding capacity (Harasym et al., 2015). There are some techniques of pretreatment of beta-glucan preparations or other cereal raw materials following bread making and their consist of high-pressure homogenization, by adding antioxidants, enzymatic hydrolysis or thermo-mechanical degradation (Harasym et al., 2015; Taghinia et al., 2016; Tosh et al., 2010). Some of them are effective but they have drawbacks like high cost, low yield and long processing times which makes them inapplicable for the industry (Sibakov et al., 2011). Some of the mechanical treatments of polysaccharide raw material was explored previously and thermal treatment like freezing or freeze milling were also applied to spelt grains or beta-glucans from barleys (Benito-Román et al., 2013; Niemi et al., 2012). However, there is no a comprehensive approach towards using different pretreatments of beta-glucan preparations on the quality of fortified wheat bread. Therefore, we decided to present in this research the application of freezing, blending, boiling and mixing these treatments with each other in the pretreatment of the beta-glucan preparation prior to fortifying wheat bread.

2 Materials and methods
2.1 Raw materials
Commercial wheat flour was provided by a local supplier and consisted of 13.70% moisture content, 10.86% proteins, 0.49% ash and 27.4% gluten. The basic component levels in flour were measured with near-infrared spectroscopy (NIRFlex N-500, Buchi, Switzerland). The oat beta-glucan preparation consisted of 44% dietary fibre (23% of insoluble fractions, 21% of soluble fractions-containing 16% beta-glucan) (Microstructure Inc., Poland). Other constituents of dough like salt and fresh yeast water were bought in a local supermarket.

2.2 Beta-glucan preparation pretreatment
The beta-glucan preparations were done as presented on the scheme (Figure 1). The control sample was the bread without any beta-glucan preparation addition. Dry sample was the
beta-glucan preparation with a moisture content 12% in the form of bright yellow powder. The hydrated sample was examined to find out whether hydration of beta-glucan and colloid forming could influence the consistency of dough and final bread quality. Boiling of the beta-glucan preparation was done to eliminate the interference of starch gelatinization from the preparation during baking and was done prior to mixing. Freezing was conducted to find out whether gentle sublimation of water while thawing from the hydrated preparation influences the quality of bread. The blendered samples were done to decrease the particle size of beta-glucan. The ratio between water and beta-glucan dry preparation in all samples was 6:1 (v/w).

2.3 Bread preparation

The bread was prepared as in a previous study (Kurek et al., 2015). Briefly, to generate the dough (500 g of flour, 9 g of vital gluten, and 8 g of salt and 8 g of salt), 12 g of the fiber preparation was used per 100 g of the flour mixture to achieve a final amount of 2 g of beta-glucan per 100 g. The ingredients were mixed for 6 min at 200 rpm in a TRQ-42, RM Spiral mixer (Gastro, Poland). Water was added based on rheological measurements to achieve 500 BU (Brabender Units). Preliminary fermentation was conducted at 37 °C and 80% relative humidity for 60 min. The mixer bowl was turned once after 30 min, and the dough was divided into 350 g pieces. Each piece was inserted into forms for the proofing process until reaching oven maturity (37 °C, 80% relative humidity). The direct, single-phase method was used to bake the bread. Baking was conducted at 220 °C for 25 minutes in a convection oven (Convection oven CPE 110, Kuppersbush, Germany). There was a batch of 6 loaves baked from each beta-glucan preparation.

2.4 Rheology

Rheological measurements were conducted with a Mars III rheometer (Thermo Haake). The frequency sweep test was conducted in the plate-cone geometry (60 mm) with a 2-mm gap. The frequency of oscillation to compare the samples was 1 Hz, shear stress was 60 Pa, angle rotation sensor was at 2°, and temperature of the measurement was 25 °C. Measurements were performed in triplicate and the elastic modulus $G'$ was the measured parameter.

2.5 Specific volume

The volume of baked bread rolls during storage was determined according to the rapeseed displacement method (López, 2014). The results are the average of four replicates and presented as volume in mL/g of product.

2.6 Bread texture

Mechanical characteristics of bread in a double compression cycle were recorded in the Instron 5965 Universal Testing Machine (Instron, USA) with a maximal load of 500 N, 50% penetration depth with a 40 mm diameter probe and a 20 s gap between cycles on $20 \times 20 \times 20$ mm crumb cubes. The results were expressed as selected parameters of TPA, the maximum level of firmness, springiness and cohesiveness. The parameters analyzed were firmness (the peak force during first compression; N), springiness (the ratio between the recovered height after the first compression and the original sample height), and cohesiveness (area 2/area 1; -). Measurements were taken 24 h after baking. The texture studies were conducted in triplicate for each sample.

2.7 Porosity

Computer image analysis of TIFF images were used to estimate porosity, as previously described (Kurek et al., 2016). The loaf was cut into 2.5-cm-thick slices. The slices were digitally photographed using lightening from lamps, with the color temperature at 5400 K. The measurement of porosity involved archiving the images of the sample by a digital camera (Micro Publisher 5.0 RTV, QImaging, BC, Canada) equipped with a linear polarizing filter of 46 mm (Keiser, CA, USA), and a lighting system (RB-5004-HF, Kaiser, CA, USA) with 4 fluorescent lamps (Dulux L 36W/954, AC 230V/50Hz, Osram GmbH, Munich, Germany) and a color temperature of 5400 K. Pictures were
taken at a resolution of 2560 × 1920 pixels. The digital camera was connected to a PC computer using the Image Pro Plus 7.0 software (Media Cybernetics Inc., MD, USA). The software was used to calculate: the pore size using the calibration ruler, presented in mm²; the porosity, the area covered by pores on the whole area of the examined slice (in %).

2.8 Crumb color

Color determinations were carried out on bread crumbs 24 h after baking using the Minolta CR-400 colorimeter (Konica Minolta Inc., Japan) with illuminant D65, and a measurement area of ø=8mm, standard observers 2°. Three different slices were analyzed for color 10 times and the results were expressed in accordance to the CIExLab color space. Determined parameters were L (L = 0 (black) and L = 100 (white)), a (- a = greenness and +a = redness), b (-b = blueness and +b = yellowness). Then, the difference of color, ΔE, was calculated taking the control sample as the reference material with the following Equation 1:

$$\Delta E = \sqrt{(L_0 - L_1)^2 + ((a_0 - a_1)^2 + (b_0 - b_1)^2}$$

(1)

2.9 Water content

The water activity was measured by the dew point methodology using a Water Activity Meter 4TE (Aqualab, Pullman, USA).

2.10 Beta-glucan

The beta-glucan content in bread was determined by employing the AOAC method (Method 995.16), with modifications (beta-glucan Assay Kit - Mixed Linkage, Megazyme, Ireland) (Association of Official Analytical Chemists, 2010).

2.11 Statistics

The two sample comparison procedure and the ANOVA were performed to determine whether or not there were significant differences in the comparison of two or more than two samples. This was achieved using Statistica 10 (StatSoft, Tulsa, USA), considering p-values of less than 0.05 if operating at the 5% significance level. The correlations were estimated using the Pearson's test to assess whether the beta-glucan content is connected with other measured parameters.

3 Results and discussion

3.1 Rheology

The rheological measurements were conducted on the beta-glucan preparations. Significant differences were observed between the samples. In general, the G’ showed the tendency to be lower parallel to the heaviness of treatments (Figure 2). The highest G’ values were recorded in the sample of hydrated (1222 Pa), meaning the preparation that was allowed to hydrate without any force or thermal treatment had the stiffest consistency. In all samples, the blended preparations had lower values than the non-blendered. This could be caused by the different water migration in the beta-glucan when the particle size is lower (Liu et al., 2015).

3.2 Volume measurement

Specific volume is a significant parameter when studying the quality of bread and involves the volume and weight of bread. Figure 3 depicts the changes in the specific volume of bread crumb prepared with a beta-glucan preparation after different pretreatments. The results showed that pretreatment of the beta-glucan preparation influenced specific volume. Breads containing hydrated or dry beta-glucan preparations demonstrated lower values of specific volume than control (significant at p ≤ 0.05). On the other hand, beta-glucan preparations that were boiled or blended after freezing showed higher levels.

Only bread prepared from the beta-glucan preparation after freezing has a similar specific volume to control. Significant differences between the mean values in control and analyzed breads (p ≤ 0.05) occurred for products containing blended or boiled beta-glucan preparations (after boiled, frozen or hydrated). Generally, beta-glucan addition caused a lowering of the specific volume of bread, which is reported by other authors (Mudgil et al., 2016). In our study, the increase of bread loaf volume could be caused by blending the samples because this decreases the particles of beta-glucan preparations. Moreover, the boiled sample had the highest specific volume due to the prior gelatinization of starch (Aghamirzaei et al., 2015). The
heat treatment is responsible for changing the viscosity of beta-glucan which is caused by decreasing the molecular weight of beta-glucan (Ames et al., 2015).

3.3 Texture profile analysis and porosity

The effects of different beta-glucan preparation pretreatments on bread firmness, springiness, cohesiveness and porosity is shown in Table 1. The firmest bread was achieved by adding a beta-glucan preparation which was hydrated and blended (significant at p ≤ 0.05) and the least firm was after application of the frozen blended preparation (significant at p ≤ 0.05).

The hydrated and blended beta-glucan sample could lead to higher absorption of water due to decreasing particle size and a higher surface development. Excess water that is absorbed during mixing is prone to evaporate during baking, so the structure loses its softness (Kurek et al., 2017). All breads containing the beta-glucan preparation had higher springiness than the control, but only the addition of dry or hydrated blended beta-glucan preparations showed significant differences (p ≤ 0.05). The preparation of beta-glucan prior to mixing of dough could hold more water than the constituents of the flour so the springiness is higher (Martínez et al., 2014). Bread's cohesiveness was demonstrated between 0.57 and 0.67, but there were no significant differences between all groups. Different pretreatments of the beta-glucan preparation influenced the porosity and mean pore size which could be caused by the depolymerization of the constituents of bread (starch-gluten network) which leads to a different formation of the bread's structure. Beta-glucan is a hydrocolloid which could interfere during baking due to gelatinization at nearly the same temperature as starch (Symons & Brennan, 2004). However, the techno-functional properties of bread could be preserved after adding beta-glucan (Collar & Angioloni, 2014).

3.4 Color changes

For all pretreated beta-glucan preparations, ΔE is over 3.5 (Table 1) and even an untrained observer can notice the difference between the color of the bread prepared with and without a beta-glucan preparation (Robertson, 1990). The most noticeable difference is in case of the preparation which was boiled (ΔE = 6.12). In general, the lightness (L*) of bread prepared with beta-glucan is lower than the control sample. This could be caused by the addition of beta-glucan (L* = 62.15, a* = 0.12 and b* = 13.18) and the new bindings of beta-glucan, as the polysaccharide and proteins which are the normal constituent of dough will lead to the caramelization and increased concentration of Maillard reactions products (Erbas et al., 2012).

3.5 Water activity

The next parameter measured was water activity (Figure 4). The higher value of aw in food, the easier microorganisms growth occurs (Altamirano-Fortoul & Rosell, 2011). Bacteria usually require at least aw = 0.91, which all breads had in our study. The lowest value of water activity after baking occurred with beta-glucan in the dry preparation or after freezing and blending. Freezing or boiling preparations provided the highest aw in bread crumb (significant at p ≤ 0.05). Similar water activity to control was observed in the bread with hydrated and hydrated blended preparation. The increase of water addition to obtain the optimal rheological properties of dough could lead to the impression that water is more accessible for bacteria and molds (Kariluoto et al., 2014).

Table 1. The texture profile analysis (TPA), porosity and color parameters of bread crumb prepared with beta-glucan preparation after different pretreatment.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Firmness (N)</th>
<th>Springness</th>
<th>Cohesiveness</th>
<th>Porosity (%)</th>
<th>Pore mean size (mm²)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.80 ± 0.37ab</td>
<td>0.59 ± 0.03a</td>
<td>0.57 ± 0.04d</td>
<td>0.35 ± 0.01cd</td>
<td>0.44 ± 0.01a</td>
<td>70.75 ± 1.85</td>
<td>-0.93 ± 0.04f</td>
<td>11.06 ± 0.53a</td>
<td>-</td>
</tr>
<tr>
<td>Dry</td>
<td>3.74 ± 1.11ab</td>
<td>0.79 ± 0.04b</td>
<td>0.66 ± 0.05f</td>
<td>0.38 ± 0.01ce</td>
<td>0.43 ± 0.01e</td>
<td>67.23 ± 2.03</td>
<td>-0.19 ± 0.23e</td>
<td>14.26 ± 0.78c</td>
<td>4.81</td>
</tr>
<tr>
<td>Hydrated</td>
<td>3.73 ± 0.97ab</td>
<td>0.75 ± 0.07ab</td>
<td>0.63 ± 0.03f</td>
<td>0.41 ± 0.01de</td>
<td>0.49 ± 0.01de</td>
<td>66.73 ± 3.28</td>
<td>-0.30 ± 0.20cb</td>
<td>14.64 ± 1.14b</td>
<td>5.41</td>
</tr>
<tr>
<td>Frozen</td>
<td>4.15 ± 0.60ab</td>
<td>0.66 ± 0.09a</td>
<td>0.67 ± 0.08de</td>
<td>0.41 ± 0.05cd</td>
<td>0.55 ± 0.05cd</td>
<td>67.66 ± 1.76</td>
<td>-0.21 ± 0.46f</td>
<td>15.66 ± 1.57b</td>
<td>5.59</td>
</tr>
<tr>
<td>Boiled</td>
<td>3.14 ± 1.05ab</td>
<td>0.71 ± 0.09b</td>
<td>0.57 ± 0.05f</td>
<td>0.45 ± 0.01fe</td>
<td>0.56 ± 0.01fe</td>
<td>65.48 ± 3.92</td>
<td>-0.17 ± 0.10a</td>
<td>14.09 ± 1.09a</td>
<td>6.12</td>
</tr>
<tr>
<td>Hydrated blended</td>
<td>4.21 ± 1.02ab</td>
<td>0.78 ± 0.04a</td>
<td>0.64 ± 0.03e</td>
<td>0.32 ± 0.02ce</td>
<td>0.49 ± 0.05ce</td>
<td>69.60 ± 1.37</td>
<td>-0.28 ± 0.10a</td>
<td>14.75 ± 1.04b</td>
<td>3.92</td>
</tr>
<tr>
<td>Frozen boiled</td>
<td>3.41 ± 0.92ab</td>
<td>0.71 ± 0.05ab</td>
<td>0.64 ± 0.02f</td>
<td>0.24 ± 0.01de</td>
<td>0.52 ± 0.01de</td>
<td>67.70 ± 1.83</td>
<td>-0.18 ± 0.28ae</td>
<td>15.36 ± 0.90c</td>
<td>5.95</td>
</tr>
<tr>
<td>Frozen blended</td>
<td>2.25 ± 0.34bc</td>
<td>0.68 ± 0.13a</td>
<td>0.60 ± 0.04d</td>
<td>0.22 ± 0.01c</td>
<td>0.54 ± 0.01d</td>
<td>67.63 ± 3.04</td>
<td>-0.31 ± 0.12d</td>
<td>15.08 ± 0.45d</td>
<td>5.72</td>
</tr>
<tr>
<td>Boiled blended</td>
<td>2.57 ± 0.21ab</td>
<td>0.71 ± 0.08a</td>
<td>0.57 ± 0.03f</td>
<td>0.27 ± 0.02de</td>
<td>0.55 ± 0.01de</td>
<td>68.19 ± 1.71</td>
<td>-0.09 ± 0.21b</td>
<td>15.14 ± 1.35b</td>
<td>4.89</td>
</tr>
</tbody>
</table>

Letters a, b, c indicate the significant differences between the mean values in columns (p ≤ 0.05). L* = (0 (black) and 100 (white)); a* = (-a (greenness) and +a (redness)); b* = (-b (blueness) and +b (yellowness)); ΔE = difference of color.

Figure 4. The water activity of bread crumb prepared with β-glucan preparation after different pretreatment.
boiling the beta-glucan preparation prior to use in bread making as this method increases the fortified bread quality and has a higher content of beta-glucan in final product.

**References**


**Figure 5.** Content of β-glucan in bread crumb prepared with beta-glucan preparation after different pretreatment. Letters a, b, c indicate the significant differences between the mean values in columns (p ≤ 0.05).

The different values of water activity could be explained by water crystal formation during freezing and sublimation (Carrillo-Meza et al., 2016).

**3.6 Beta-glucan content**

The content of beta-glucan is considered one of the most important parameters in the addition of fiber to bakery products. In our study, we researched the influence of pretreating beta-glucan preparations on the concentration of this compound. Bread content ranged from 1.62 to 1.99 g beta-glucan / 100 g of product (Figure 5). It seems that the best way of pretreatment in terms of beta-glucan preservation is drying, freezing or boiling after freezing the beta-glucan preparation (significant at p ≤ 0.05). The mechanism of this phenomenon could consist of a higher particle size which stabilizes the longer polymeric chains of beta-glucan more so than the blended samples. The least effective process is blending after freezing. The beta-glucan presence in a control sample resulted from the addition of baker’s yeast to the product which contains a small amount of this compound. We also correlated the concentration of beta-glucan with other parameters that we measured. It turns out that a higher level of beta-glucan in bread significantly influenced the color (L* : -0.711, a* : 0.742 and b* : 0.757). Other parameters that were significantly influenced by beta-glucan addition but with lower Pearson coefficients were springiness (0.477) and cohesiveness (0.349) as well as mean pore size (0.410). Other research revealed that the beta-glucan addition stabilized the air cells in bread and therefore caused the coalescence of the bread’s structure (Jalil et al., 2015).

**4 Conclusions**

Beta-glucan is a widely recognized health-beneficial food ingredient which could result in a good physiological response in the human organism. Our study reveals that there could be several pretreatments of beta-glucan preparations which could cause better technological properties of bread with beta-glucan addition. In general, the best performing method was boiling the beta-glucan preparation following dough addition. This had highest quality properties compared to others for most measured traits. Therefore, we recommend the industrial application of


