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# Effects of two barley β-glucan isolates on wheat flour dough and bread properties

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ABSTRACT

The effects of wheat flour fortification with two different molecular weight barley β-glucan isolates ($1.00 \times 10^5$, BG-100 and $2.03 \times 10^5$, BG-200) on the rheological properties of dough and bread characteristics, using flours from two wheat cultivars that differ in their breadmaking quality, have been examined. The farinograph water absorption of doughs and the moisture content and water activity of the breads increased with increasing β-glucan content; the β-glucan isolate with the higher molecular weight (BG-200) exerted a greater effect than did BG-100. The addition of β-glucans to the dough formula increased the development time, the stability, the resistance to deformation and the extensibility of the poor breadmaking quality doughs, as well as the specific volumes of the respective breads, exceeding even that of the good breadmaking cultivar. Furthermore, the colour of the bread crumbs got darker and their structure became coarser, whereas the bread crumb firmness decreased with increasing level of β-glucan addition. Generally, the BG-200 was more effective in increasing the specific bread volume and reducing the crumb firmness, especially when used to fortify the poor breadmaking quality flour. The results further indicate a requirement for optimisation of the fortified doughs (level and molecular size of the β-glucan) to maximise bread quality attributes (loaf volume, texture, and staling events).



## 1. Introduction

Consumer awareness about the health benefits of high-fibre diets has shifted food manufacturing towards fibre-enriched products, including high fibre baked goods (Bhatty, 1986; Newman, Ore, Abbott, & Newman, 1998). The increasing interest in barley as a food ingredient during recent years followed several literature reports that associate its consumption with lower serum cholesterol levels (Bourdon et al., 1999), as well as moderate postprandial insulin responses and blood glucose levels (Cavallero, Empilli, Brighenti, & Stanca, 2002). A mixed-linkage β-glucan, present in high levels in barley and known to impart high viscosity to solutions, was recognised as responsible for such effects (Wood, Weisz, & Blackwell, 1991). The molecular size and the concentration of β-glucans, as well as their molecular features (e.g., $DP_3/DP_4$ ratio) are important variables in determining the physical properties of these polymers, such as solubility, viscosity and gel-forming ability (Skendi, Biliaderis, Lazaridou, & Izydorczyk, 2003; Vaikousi, Biliaderis, & Izydorczyk, 2004). Although a strong link between β-glucan consumption and improved physiological effects has emerged, the β-glucan intake from baked products is still relatively low. In this context, the development of breads enriched in β-glucans, either by incorporating β-glucan-rich grain fractions or β-glucan concentrates has become a challenging issue for the baking industry, in an effort to provide nutritionally improved bakery items that also maintain acceptable sensorial quality.

Traditionally, barley has not been used in bakery products because it lacks gluten proteins and the end-products have poor sensory qualities (Bhatty, 1999). Furthermore, earlier studies by Pomeranz, Shogren, Finney, and Bechtel (1977) showed that addition of fibrous materials to wheat flour weakens the crumb cell structure, due to the dilution/weakening of the wheat gluten protein network whereas Dubois (1978) emphasised that, especially the water-insoluble fractions, impair the gas retention of the dough and thereby change the texture and appearance of the baked product.

More recent studies have demonstrated that β-glucan-enriched barley fractions, blended with wheat flour, can produce bread with acceptable sensory properties (Cavallero et al., 2002; Knuckles, Hudson, Chiu, & Sayre, 1997). Jacobs, Izydorczyk, Preston, and Dexter (2008) reported that the method of bread production strongly influences the quality of barley-supplemented bread. Wang, Miller, and Hoseney (1998) showed that β-glucan fractions may improve crumb structure of the wheat breads and do not largely affect the loaf volume. However, Gill, Vasanthan, Ooraikul, and Rossnagel (2002) and Izydorczyk and Dexter (2008) reported reduction of the loaf volume upon addition of barley fractions. Such studies,

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conducted on wheat flours enriched with barley or oat flour/fibre fractions, demonstrate the difficulties in assessing the real impact of the β-glucans, due to the complex molecular constitution and physical form of all flour fractions included in the dough formulation.

To our knowledge, no systematic studies on the effect of supplementation of wheat flour with purified barley β-glucan preparations on wheat bread have yet been reported. The effects described above with barley and oat flour fractions, along with the health benefits of the fortified products, suggested the need to assess the functional potential of cereal β-glucans for use in improving wheat flour properties, including dough handling characteristics and baking performance. The present investigation was therefore undertaken to study the dough rheological properties and bread quality attributes (volume, colour, crumb structure, firmness, and staling processes) of β-glucan-supplemented wheat bread. Such study may broaden our understanding of how the molecular size and the concentration of water-soluble β-glucans affect the quality characteristics of the dough and bread made from two different baking quality wheat flours.

## 2. Materials and methods

### 2.1. Materials

Whole barley kernels from a mixture of two Greek barley cultivars, Kos and Persephoni, and a commercial concentrate, were used as a source of barley β-glucans; the barley grains were provided by the National Agricultural Research Foundation, Cereal Research Institute, Thessaloniki, Greece, whereas the β-glucan concentrate was provided by CEBA Ltd (Lund, Sweden). Whole wheat seeds from two *Triticum aestivum* cultivars, Dion of poor breadmaking quality and Yekora of good breadmaking quality, were provided by the National Agricultural Research Foundation, Cereal Research Institute, Thessaloniki, Greece. Termamyl (L120) was a product of Novozymes A/S, (Bagsvaerd, Denmark) and Pancreatin was obtained from Sigma–Aldrich Co. (Gillingham, Dorset). All other reagents and chemicals used were of analytical reagent grade.

### 2.2. Methods

#### 2.2.1. Milling process

A cleaner-separator (Labofix, Emceka, Germany) removed the impurities from wheat grain seeds that were subsequently conditioned to the appropriate moisture content 18 h prior to milling with a Quadrumat Senior (Brabender, Duisburg, Germany) mill. The characteristics of these flours were as follows: Dion (protein content 12.4%, ash 0.483%, moisture 10.7%, gluten index 26.2%); Yekora (protein content 17.0%, ash 0.643%, moisture 10.7%, gluten index 67.2%). The methods used to determine flour quality characteristics were: AACC-Method 08-01 (1983) for ash, the micro-Kjeldahl method of AACC-Method 46-13 (1986) for crude protein, the ICC-Standard 110/1 (1976) for moisture, and the ICC-Standard 155 (1994) for Gluten Index.

#### 2.2.2. Isolation and purification of β-glucan preparations

Whole barley seeds from the cultivars Persefoni and Kos were milled in a CAMAS mill, using a 1 mm sieve, and the flour obtained (~100 g) was boiled in 1 l of aqueous ethanol (82% v/v), for 2 h at 85 °C to inactivate the endogenous barley β-glucanases. The residue was washed carefully with ethanol and dried at 40 °C overnight before β-glucan extraction. This material was used to obtain the β-glucan preparation named BG-200 (higher molecular weight), whereas the isolate obtained from the commercial concentrate was named BG-100 (lower molecular weight). The extraction and the purification procedures were explained in detail elsewhere (Skendi, Papageorgiou, & Biliaderis, 2009).

The isolated preparations were analysed to obtain information about the molecular size, the cellotriosyl/cellotetraosyl ($DP_3/DP_4$) ratio and the protein level, according to procedures described elsewhere (Skendi et al., 2003). The BG-100 β-glucan preparation had a molecular size of $10^5$ Da, a $DP_3/DP_4$ ratio of 2.6 and a protein content of 0.5%, whereas the BG-200 isolate had a molecular weight of $2.03 \times 10^5$ Da, a $DP_3/DP_4$ ratio of 2.1 and a protein content of 5%. Determination of the β-glucan content of the two isolates by the method of McCleary and Glennie-Holmes (1985), using the Megazyme® mixed linkage β-glucan assay kit, gave values of 84.5% and 81.3% for the BG-100 and BG-200 preparations, respectively.

#### 2.2.3. Farinograph tests

Doughs fortified with β-glucans at levels of 0%, 0.2%, 0.6%, 1.0%, and 1.4% w/w, calculated on a flour dry weight basis, were tested according to the ICC-standard method 115/1 (1992). The β-glucan isolates in a dry powder form were first mixed well with the wheat flour into the mixing bowl (300 g) of the farinograph (Brabender, Duisburg, Germany) that was connected with a circulating water pump and a thermostat which operated at 30 ± 0.2 °C; farinograph water absorption, dough development time, and dough stability were thus determined.

#### 2.2.4. Extensograph tests

The control (unfortified) and the β-glucan-enriched doughs were prepared in the 300 g mixing bowl of the Farinograph (Brabender, Duisburg, Germany). The wheat flour was first mixed well with the β-glucan at different concentration levels, before salt and water addition, to produce the dough samples. Water was then added to produce dough with a consistency of 500 BU (Brabender Units), followed by 5 min of mixing. A test piece (150 g) was rounded into a ball, shaped into a cylinder and clamped into the holder. After 45, 90, and 135 min resting times in the fermenting cabinet at 30–32 °C, each dough piece was stretched in the Brabender Extensograph by a hook until rupture, as described in the ICC-Standard 114/1 method (1992). The stretching force was thus recorded as a function of time, and the resistance to constant deformation after 50 mm stretching ($R_{50}$) and the extensibility ($E$) were obtained.

#### 2.2.5. Breadmaking

The basic dough recipe for bread production (300 g flour basis) consisted of: salt (6 g), yeast "Giotis" (a commercial brand from Giotis, SA, Greece) (5 g), and the required water to reach, at maximum development time, 500 BU. Breads were fortified at different levels with the β-glucan preparations (0, 0.2, 0.6, 1.0%, and 1.4% w/w based on flour dry weight). Bread doughs were prepared by mixing all ingredients in a 300 g farinograph bowl until they reached maximum development. Fermented doughs were obtained after a two-step bulk fermentation and proofing up to the optimum volume increase. After the first step, the fermented doughs were divided into four 100 g pieces, hand-moulded and put into tin pans for proofing at 32 °C and then baked at 210 °C for 23 min. Following baking, the bread loaves were cooled at room temperature and weighed after one hour. The loaf volume was determined by the rapeseed replacement method and the specific loaf volume (ml/g) was calculated, based on the average of 12 breads. Subsequently, breads were sealed in polyethylene bags to monitor changes in bread characteristics upon storage (4 °C).

#### 2.2.6. Image acquisition and analysis

Sliced breads were scanned full scale in 256 grey levels at 150 dpi (dots per inch) with a scanner (Hewlett Packard Scan Jet 6100C) and a supporting software DeskScan II, Version 2.7, Hew-

lett–Packard, Co. Brightness was adjusted to 150 units and contrast to 170 units. Image analysis was performed using the UTHSCSA ImageTool programme (Version 2.0, University of Texas Health Science Centre, San Antonio, Texas). The threshold method used for differentiating gas cells and non-cells, was determined as described by Crowley, Grau, and Arendt (2000). This analysis was performed independently for each image, and three slices per loaf were analysed in each case. Care was taken to obtain a subimage area of 30 × 30 mm from the same position (loaf centre) on each slice. The crumb features chosen were total number of cells, number of cells smaller than 4mm$^2$, total cell area, mean cell area, mean cell area of cells greater than 4mm$^2$, and cell to total area ratio. Cell shape analysis for the different bread types was performed by counting the percentage of cells that fell into a particular shape factor category: $0.00 < X \leqslant 0.20$, $0.20 < X \leqslant 0.50$, $0.50 < X \leqslant 0.80$, and $0.80 < X \leqslant 1.00$.

#### 2.2.7. Colorimetry

Crumb colour was measured using a Hunter Lab, MiniScanXE Plus, (Hunter Lab, MiniScanXE Plus, Reston, Virginia, USA). Colour values, $L^*$, $a^*$ and $b^*$, were recorded, each value being the average of four measurements at different points of the bread crumb. $L^*$ value is the lightness variable from 100 for perfect white to zero for black, whilst $a^*$ and $b^*$ values are the chromaticity values, +redness/−greenness and + yellowness/−blueness, respectively. The colour was measured 1 h after baking. Mean values of four observations were recorded from each loaf and the means of at least 4 breads were taken for comparison purposes.

#### 2.2.8. Moisture content and water activity ($a_w$) determination

The moisture content of bread crumbs stored for 1, 4, and 8 days (at 4 °C) in hermetically sealed polyethylene bags was determined according to the ICC-Standard method 110/1 (1976). Water activity ($a_w$) in all previously mentioned bread crumbs was measured with an Aqua Lab apparatus (Aqua Lab, Model 3TE, Decagon Devices, Inc., Pullman, Washington, USA).

#### 2.2.9. Bread firmness

Bread firmness testing of controls and β-glucan-fortified bread crumbs stored at 4 °C, to accelerate the bread staling events, for 1, 4, and 8 days was performed with a TA-XT2i® instrument (Stable Micro Systems, Godalming, Surrey, UK) at room temperature, using a cylindrical probe of 20 mm diameter, according to the AACC-method 74-09 (1988). All samples were allowed to rest at room temperature after removing them from the refrigerator until the breads reached the desired temperature. Cutting of slices was performed immediately after removing them from the plastic bags in order to avoid moisture loss. Compression tests were recorded on two slices from the centre of each loaf, each with an average thickness of 25 mm, cut with a commercial electric knife (Rohnson, model Ak-8, P.R.C.). Measurements from at least three bread loaves were taken for each formulation and sampling time.

#### 2.2.10. Differential scanning calorimetry of bread crumb

The staling of breads was also assessed by DSC following storage of the bread crumbs at 4 °C as specified above. The bread crumbs were then chopped, frozen with liquid nitrogen and subsequently freeze-dried. The freeze-dried bread crumbs were very well pulverised to facilitate the DSC measurements. Modulated DSC experiments were carried out using a MDSC 2920 (TA Instruments, New Castle, DE). Lyophilized bread samples were weighed into aluminium pans and hydrated (40% w/w solids). The aqueous powdered dispersions were hermetically sealed and allowed to hydrate for 2 h, and subsequently analysed under a dry nitrogen purge using the modulated mode. Heating rate was 5 °C per minute at a modulation of +/−0.75 °C every 60 s. Data processing was done using the Thermal Analysis Software provided by the TA Instruments. The instrument was calibrated with indium and an empty aluminium pan was used as a reference.

### 2.3. Statistical analyses

All data were analysed with the SPSS 11.0 software package. Comparisons amongst means of samples were carried by ANOVA (Analysis of Variance) using the Duncan's multiple range test. Significant differences amongst mean values were considered at $p < 0.05$.

## 3. Results and discussion

### 3.1. Effect of β-glucans on farinograph parameters

The farinograph properties of wheat flours are strongly dependent on protein content and quality (Khatkar, Bell, & Schofield, 1996). The results for control Dion and Yekora doughs (without addition of β-glucan) confirm this view (Table 1). Generally, the farinograph properties of β-glucan-enriched doughs differed from those of control doughs (Table 1). Addition of β-glucans to the dough formula increased the development time and improved the stability of the poor breadmaking quality doughs (Table 1). Furthermore, the addition of the BG-100 to the good breadmaking flour (Yekora) increased the dough development time but simultaneously decreased its stability (except for doughs with 0.2% β-glucan). In contrast, the addition of the BG-200 above 0.2% resulted in a progressive decrease of the dough development time for the Yekora flour and a large decline in the dough stability with increasing level of the added polysaccharide, confirming the findings of Cavallero et al. (2002). In contrast, Mohamed, Rayas-Duarte, Xu, Palmquist, and Inglett (2005) showed that, with increased amounts of β-glucan, there was no large reduction in dough stability. Discrepancies related to the influence of the β-glucans on the bread quality characteristics may arise from differences in the molecular size, solubility and the concentration range of the polysaccharide, as well as the flour types used for fortification amongst the various studies. Overall, the magnitude of the changes in development time of doughs fortified with BG-100 was greater than those of BG-200 for each flour used.

The addition of β-glucans also caused an increase in the farinograph water absorption of all doughs tested (Table 1). The same behaviour has been observed when purified arabinoxylans (Biliaderis, Izydorczyk, & Rattan, 1995), β-glucan enriched flour fractions from different sources (Cavallero et al., 2002; Knuckles et al., 1997; Mohamed et al., 2005) or isolated and purified β-glucans (Skendi et al., 2009) have been added to wheat flours; such effects have been attributed to the high water absorbing capacity of these non-starch polysaccharides and their ability to compete for water with other constituents in the dough system. Generally, the BG-200 sample produced a larger increase in the farinograph water absorption than did its lower molecular weight counterpart. Significant positive correlations ($p < 0.01$) between farinograph water absorption and the amount of the β-glucan added were noted; $r^2 = 0.99$ and $r^2 = 0.92$ for BG-200 and BG-100 preparations, respectively.

### 3.2. Effects of β-glucan on extensograph parameters

Desirable dough properties are usually associated with good dough resistance and extensibility values. The effects of β-glucan addition on the extensograph parameters throughout 135 min of proofing time are shown in Table 2. At high proofing times, all Dion doughs enriched with BG-100 exhibited higher $R_{50}$ values than did

**Table 1**
Farinograph data of doughs and specific volumes of breads from Dion and Yekora flours with β-glucans added at different levels of concentration.

| Sample | Water absorption (expressed in % per 100 g flour of 14.0% moisture)[a] | Dough development time (min)[a] | Stability (min)[a] | Specific volume (ml/g)[b] |
|---|---|---|---|---|
| *DION* | | | | |
| Control | 50.3 $^{1}$ ± 0.40 | 1.8 $^{1}$ ± 0.0 | 1.5 $^{1}$ ± 0.1 | 2.46 $^{2}$ ± 0.12 |
| 0.2% (BG-100) | 52.6 $^{2}$ ± 0.42 | 4.5 $^{2}$ ± 0.1 | 3.7 $^{2}$ ± 0.0 | 2.69 $^{3}$ ± 0.12 |
| 0.6% (BG-100) | 52.8 $^{2}$ ± 0.57 | 4.8 $^{2}$ ± 0.1 | 4.0 $^{3}$ ± 0.1 | 2.88 $^{4}$ ± 0.18 |
| 1.0% (BG-100) | 52.8 $^{2}$ ± 0.28 | 4.7 $^{2}$ ± 0.2 | 4.3 $^{4}$ ± 0.0 | 2.29 $^{1,2}$ ± 0.18 |
| 1.4% (BG-100) | 53.0 $^{2}$ ± 0.43 | 5.3 $^{3}$ ± 0.1 | 4.8 $^{5}$ ± 0.1 | 2.26 $^{1}$ ± 0.16 |
| Control | 50.3 $^{1}$ ± 0.40 | 1.8 $^{1}$ ± 0.0 | 1.5 $^{1}$ ± 0.1 | 2.46 $^{1}$ ± 0.12 |
| 0.2% (BG-200) | 51.0 $^{2}$ ± 0.14 | 3.1 $^{2}$ ± 0.1 | 2.8 $^{3}$ ± 0.0 | 2.93 $^{2}$ ± 0.23 |
| 0.6% (BG-200) | 52.8 $^{2}$ ± 0.13 | 3.3 $^{3}$ ± 0.0 | 3.5 $^{4}$ ± 0.0 | 3.37 $^{3}$ ± 0.20 |
| 1.0% (BG-200) | 54.4 $^{3}$ ± 0.31 | 3.5 $^{4}$ ± 0.1 | 3.0 $^{3}$ ± 0.3 | 3.21 $^{3}$ ± 0.20 |
| 1.4% (BG-200) | 56.2 $^{3}$ ± 0.14 | 3.7 $^{4}$ ± 0.0 | 2.0 $^{2}$ ± 0.2 | 2.55 $^{1}$ ± 0.18 |
| *YEKORA* | | | | |
| Control | 55.0 $^{1}$ ± 0.38 | 6.9 $^{1}$ ± 0.1 | 9.0 $^{2}$ ± 0.0 | 2.55 $^{2}$ ± 0.16 |
| 0.2% (BG-100) | 57.1 $^{2}$ ± 0.41 | 8.5 $^{2}$ ± 0.2 | 10.5 $^{3}$ ± 0.1 | 2.54 $^{2}$ ± 0.18 |
| 0.6% (BG-100) | 57.3 $^{3}$ ± 0.13 | 10.2 $^{3}$ ± 0.0 | 8.2 $^{1}$ ± 0.1 | 2.60 $^{2}$ ± 0.28 |
| 1.0% (BG-100) | 59.1 $^{4}$ ± 0.10 | 10.7 $^{3,4}$ ± 0.3 | 9.0 $^{2}$ ± 0.1 | 2.30 $^{1}$ ± 0.09 |
| 1.4% (BG-100) | 59.0 $^{5}$ ± 0.30 | 11.0 $^{4}$ ± 0.3 | 8.0 $^{1}$ ± 0.3 | 2.26 $^{1}$ ± 0.10 |
| Control | 55.0 $^{1}$ ± 0.38 | 6.9 $^{3}$ ± 0.1 | 9.0 $^{5}$ ± 0.0 | 2.55 $^{2}$ ± 0.16 |
| 0.2% (BG-200) | 55.7 $^{2}$ ± 0.20 | 7.5 $^{4}$ ± 0.2 | 8.3 $^{4}$ ± 0.0 | 2.58 $^{2}$ ± 0.27 |
| 0.6% (BG-200) | 58.1 $^{3}$ ± 0.27 | 6.7 $^{3}$ ± 0.1 | 5.3 $^{3}$ ± 0.0 | 2.66 $^{2,3}$ ± 0.17 |
| 1.0% (BG-200) | 60.1 $^{4}$ ± 0.30 | 6.3 $^{2}$ ± 0.1 | 4.0 $^{2}$ ± 0.1 | 2.80 $^{3}$ ± 0.13 |
| 1.4% (BG-200) | 62.3 $^{5}$ ± 0.03 | 5.5 $^{1}$ ± 0.2 | 3.7 $^{1}$ ± 0.1 | 2.29 $^{1}$ ± 0.09 |

[a] Values are means of triplicate measurements; means with any similar superscripts within each group (combination of flour type and β-glucan isolate) are not significantly different ($p = 0.05$) by the Duncan's multiple range test.
[b] Mean values of at least four measurements; means with any similar superscripts within each group (combination of flour type and β -glucan isolate) are not significantly different ($p = 0.05$) by the Duncan's multiple range test.

**Table 2**
Extensional properties of doughs made from Dion and Yekora flours enriched with two β-glucan isolates at different levels of concentration.

| Sample | $R_{50}$ (BU) | | | $E$ (mm) | | |
|---|---|---|---|---|---|---|
| | 45 min | 90 min | 135 min | 45 min | 90 min | 135 min |
| *DION* | | | | | | |
| Control | 85 $^{1a}$ | 93 $^{1a}$ | 103 $^{1b}$ | 165 $^{1a}$ | 214 $^{2a}$ | 241 $^{2a}$ |
| 0.2% (BG-100) | 123 $^{3a}$ | 136 $^{2a}$ | 148 $^{2,3a}$ | 198 $^{1,2a}$ | 193 $^{1,2a}$ | 202 $^{1a}$ |
| 0.6% (BG-100) | 103 $^{2a}$ | 115 $^{1,2a}$ | 118 $^{1,2a}$ | 230 $^{2b}$ | 205 $^{1,2a}$ | 215 $^{1,2a,b}$ |
| 1.0% (BG-100) | 93 $^{1a}$ | 95 $^{1a}$ | 95 $^{1a}$ | 235 $^{2a}$ | 220 $^{2a}$ | 222 $^{1,2a}$ |
| 1.4% (BG-100) | 143 $^{4a}$ | 183 $^{3b}$ | 188 $^{3b}$ | 194 $^{1,2a}$ | 185 $^{1a}$ | 195 $^{1a}$ |
| Control | 85 $^{1a}$ | 93 $^{1a}$ | 103 $^{1b}$ | 165 $^{1a}$ | 214 $^{3a}$ | 241 $^{3a}$ |
| 0.2% (BG-200) | 138 $^{3a}$ | 155 $^{1b}$ | 178 $^{2c}$ | 238 $^{2b}$ | 214 $^{2a}$ | 204 $^{3a}$ |
| 0.6% (BG-200) | 185 $^{4a}$ | 303 $^{3b}$ | 360 $^{4c}$ | 194 $^{1,2b}$ | 161 $^{1a,b}$ | 150 $^{1a}$ |
| 1.0% (BG-200) | 125 $^{2a}$ | 210 $^{2b}$ | 210 $^{2b}$ | 222 $^{1,2a}$ | 176 $^{1a}$ | 178 $^{2a}$ |
| 1.4% (BG-200) | 200 $^{5a}$ | 265 $^{3a}$ | 273 $^{3a}$ | 199 $^{1,2a}$ | 177 $^{1a}$ | 182 $^{2a}$ |
| *YEKORA* | | | | | | |
| Control | 285 $^{3a}$ | 290 $^{1a}$ | 338 $^{2b}$ | 235 $^{3b}$ | 216 $^{1b}$ | 175 $^{1a}$ |
| 0.2% (BG-100) | 223 $^{1a}$ | 238 $^{1b}$ | 268 $^{1c}$ | 231 $^{2,3b}$ | 204 $^{1a}$ | 208 $^{2a}$ |
| 0.6% (BG-100) | 245 $^{1,2a}$ | 280 $^{1b}$ | 295 $^{1b}$ | 207 $^{1a}$ | 189 $^{1a}$ | 189 $^{1,2a}$ |
| 1.0% (BG-100) | 255 $^{2a}$ | 368 $^{2b}$ | 398 $^{3b}$ | 211 $^{1,2a}$ | 159 $^{1a}$ | 188 $^{1,2a}$ |
| 1.4% (BG-100) | 235 $^{1,2a}$ | 398 $^{2b}$ | 420 $^{3b}$ | 233 $^{2,3b}$ | 179 $^{1a}$ | 178 $^{1a}$ |
| Control | 285 $^{1a}$ | 290 $^{1a}$ | 338 $^{1b}$ | 235 $^{4b}$ | 216 $^{3b}$ | 175 $^{2a}$ |
| 0.2% (BG-200) | 310 $^{1a}$ | 355 $^{2a}$ | 560 $^{2b}$ | 194 $^{3b}$ | 189 $^{2b}$ | 155 $^{1a}$ |
| 0.6% (BG-200) | 580 $^{2a}$ | 600 $^{3a}$ | n.d. | 157 $^{2}$ | 110 $^{1}$ | n.d. |
| 1.0% (BG-200) | 675 $^{3}$ | n.d. | n.d. | 139 $^{1,2}$ | n.d. | n.d. |
| 1.4% (BG-200) | 735 $^{4}$ | n.d. | n.d. | 129 $^{1}$ | n.d. | n.d. |

$R_{50}$, resistance (Brabender unit) and $E$, extensibility (mm) mean values of duplicate data. Different numbers in the same column in each group (combination of flour type and β-glucan isolate) or letters within the same line used as superscripts indicate differences ($p = 0.05$) amongst the means, as determined by the Duncan's multiple range test. n.d.: measurement out of maximum value of extensograph paper.

the control (except for 1%). In Yekora doughs, the higher than control $R_{50}$ values were observed only for doughs supplemented with BG-100 at 1.0% and 1.4%. On the other hand, addition of the BG-200 increased the $R_{50}$ values of all doughs, regardless of the type of flour used.

The extensibility of all β-glucan-enriched doughs from the Dion flour, after 135 min of proofing time, remained similar or had decreased compared to the controls (Table 2); the higher molecular weight β-glucan produced a greater decrease than did the BG-100. Overall, the β-glucan addition to the Dion flour seemed to result in extensibility and $R_{50}$ values similar to the Yekora control dough, thus leading to enhancement of its gas retention properties. On the contrary, the increase of the $R_{50}$ values of the Yekora (good breadmaking quality flour) β-glucan-enriched doughs (especially at high addition levels) could result in a reduction of gas retention capacity and possible deterioration of the gluten network structure during proofing.

### 3.3. Effects of β-glucan on the specific loaf volume

Addition of β-glucans to wheat flours caused variable effects on the specific loaf volume (Table 1); the results depended on the molecular size and concentration of β-glucans, as well as on the quality of base wheat flour. Incorporation of β-glucans, up to the 0.6% level of addition, into the Dion flour increased the specific loaf volume of the respective bread loaves. However, further supplementation reduced the specific loaf volume. Similar behaviour was observed by Gill et al. (2002) for wheat flour substituted with a barley flour fraction rich in β-glucans. Addition of β-glucans to the good breadmaking quality (Yekora) flour reduced the specific volume of the bread compared to the control only at the highest addition levels (1.4%). Izydorczyk and Dexter (2008) reported a reduction in loaf volume of wheat breads fortified with 2% of barley β-glucan preparations with molecular weights of $8.00 \times 10^5$ and $2.00 \times 10^5$. Newman et al. (1998) also observed a decrease in bread volume in waxy barley breads, and Cavallero et al. (2002) reported similar responses with breads made from β-glucan-enriched flour fractions. It is generally agreed that addition of β-glucan-enriched barley fractions to wheat flour reduces loaf volume, due to the combined effects of gluten dilution and disruption of the gluten network structure (Bhatty, 1986; Dubois, 1978; Pomeranz et al., 1977). It is also postulated that β-glucans, due to their strong water absorbing properties, could suppress the amount of steam generated, resulting in reduced loaf volume and greater crumb firmness (Gill et al., 2002). Andersson et al. (2004) also observed that inclusion of barley β-glucans flours into bread was accompanied by an increase in mixing and fermentation time, resulting in lowering of their molecular size; however, the molecular structure of β-glucans was unaffected by these processes. It was also noted that the baking process itself did not seem to affect the molecular weight of the β-glucans.

The results of this study demonstrated that the BG-200-enriched breads had greater specific loaf volumes than had loaves supplemented with BG-100 at the corresponding level of β-glucan addition (Table 1); moreover, the improvement was more pronounced when the poor breadmaking quality wheat flour was used. The molecular size of β-glucan preparations, the properties of wheat flour, as well as the level of β-glucan addition, all seem to affect the rheological properties of the dough and consequently influence the specific loaf volume of the fortified bread loaves.

### 3.4. Influence of the β-glucan addition on moisture content and water activity of stored breads

With increasing level of β-glucan addition, there was a continuous increase in the moisture content of bread (Table 3). Significant differences in the moisture content of breads ($p < 0.05$) were also related to the molecular weight of β-glucan preparations used in this study; BG-200 had a much greater effect on the moisture content of bread than had BG-100.

The crumb moisture decreases during storage (as the moisture migrates from crumb towards crust), accelerating starch-gluten interactions and bread firming (He & Hoseney, 1990). On the other hand, the water retention capacity of the fortified bread crumbs increases with increasing amount of β-glucans added in the bread recipe (Table 3). Compared to the moisture content of baked bread stored for one day, no significant decrease in moisture content occurred up to the fourth day of storage. The moisture content of breads stored for 8 days generally decreased; however, the effects were variable. Generally, β-glucan addition did not seem to largely alter the water activity of the bread crumbs measured after 24 h of storage compared to that of the control (without addition of β-glucan), except for highly fortified products with the BG-200 that exhibited greater water activity than did the respective controls (Table 3). After four days of storage, all β-glucan-enriched breads showed higher $a_w$ values than did the respective control breads whereas, after eight days, only those breads made from Yekora flour differed from their control.

### 3.5. Image analysis

Breads fortified with high levels of β-glucan, particularly those fortified with BG-200, exhibited more irregular gas cells than did the controls. Image analysis was performed on each scanned bread

**Table 3**
Water activity ($a_w$) and moisture content of bread loaves stored for 1, 4, and 8 days at 4 °C.[A]

| Sample | $a_w$ | | | Moisture content (%) | | |
|---|---|---|---|---|---|---|
| | 1 day | 4 day | 8 day | 1 day | 4 day | 8 day |
| *DION* | | | | | | |
| Control | 0.963 $^{1,2b}$ | 0.951 $^{1a}$ | 0.946 $^{1a}$ | 40.2 $^{1b}$ | 39.7 $^{1b}$ | 35.9 $^{1a}$ |
| 0.2% (BG-100) | 0.962 $^{1b}$ | 0.952 $^{1a}$ | 0.950 $^{1a}$ | 41.5 $^{2c}$ | 40.4 $^{1b}$ | 36.7 $^{1,2a}$ |
| 0.6% (BG-100) | 0.965 $^{1,2b}$ | 0.960 $^{2b}$ | 0.943 $^{1a}$ | 42.1 $^{3b}$ | 41.7 $^{2b}$ | 37.1 $^{2,3a}$ |
| 1.0% (BG-100) | 0.966 $^{2b}$ | 0.963 $^{2b}$ | 0.943 $^{1a}$ | 42.2 $^{3b}$ | 41.8 $^{2b}$ | 38.0 $^{3a}$ |
| 1.4% (BG-100) | 0.966 $^{2b}$ | 0.965 $^{2b}$ | 0.944 $^{1a}$ | 42.0 $^{3b}$ | 41.8 $^{2b}$ | 37.8 $^{2,3a}$ |
| Control | 0.963 $^{1b}$ | 0.951 $^{1a}$ | 0.946 $^{1a}$ | 40.2 $^{1b}$ | 39.7 $^{1b}$ | 35.9 $^{1a}$ |
| 0.2% (BG-200) | 0.963 $^{1b}$ | 0.960 $^{2b}$ | 0.946 $^{1a}$ | 41.5 $^{2b}$ | 41.2 $^{2b}$ | 37.5 $^{2a}$ |
| 0.6% (BG-200) | 0.964 $^{1b}$ | 0.965 $^{3b}$ | 0.952 $^{1a}$ | 41.9 $^{2b}$ | 41.6 $^{2b}$ | 38.6 $^{3a}$ |
| 1.0% (BG-200) | 0.967 $^{2b}$ | 0.970 $^{3,4b}$ | 0.946 $^{1a}$ | 42.8 $^{3b}$ | 42.6 $^{3b}$ | 39.1 $^{3a}$ |
| 1.4% (BG-200) | 0.968 $^{2b}$ | 0.971 $^{4c}$ | 0.954 $^{1a}$ | 42.7 $^{3b}$ | 42.4 $^{3b}$ | 40.4 $^{4a}$ |
| *YEKORA* | | | | | | |
| Control | 0.967 $^{1,2c}$ | 0.957 $^{1b}$ | 0.950 $^{1a}$ | 42.8 $^{1b}$ | 42.0 $^{1b}$ | 39.2 $^{1a}$ |
| 0.2% (BG-100) | 0.965 $^{1b}$ | 0.959 $^{1,2a}$ | 0.957 $^{1,2a}$ | 43.8 $^{2c}$ | 42.9 $^{2b}$ | 39.9 $^{1,2a}$ |
| 0.6% (BG-100) | 0.967 $^{1,2b}$ | 0.966 $^{2,3b}$ | 0.952 $^{1,2a}$ | 43.7 $^{2c}$ | 43.1 $^{2b}$ | 40.2 $^{2a}$ |
| 1.0% (BG-100) | 0.968 $^{1,2a}$ | 0.966 $^{3a}$ | 0.960 $^{2a}$ | 43.8 $^{2b}$ | 43.6 $^{3b}$ | 41.6 $^{3a}$ |
| 1.4% (BG-100) | 0.969 $^{2a}$ | 0.972 $^{3a}$ | 0.960 $^{2a}$ | 44.1 $^{2b}$ | 43.8 $^{3b}$ | 41.1 $^{3a}$ |
| Control | 0.967 $^{1c}$ | 0.957 $^{1b}$ | 0.950 $^{1a}$ | 42.8 $^{1b}$ | 42.0 $^{1b}$ | 39.2 $^{1a}$ |
| 0.2% (BG-200) | 0.968 $^{1b}$ | 0.970 $^{2b}$ | 0.964 $^{2a}$ | 44.2 $^{2c}$ | 43.3 $^{2b}$ | 40.0 $^{2a}$ |
| 0.6% (BG-200) | 0.970 $^{2b}$ | 0.972 $^{2,3b}$ | 0.962 $^{2a}$ | 44.3 $^{2b}$ | 43.7 $^{2,3b}$ | 40.5 $^{2a}$ |
| 1.0% (BG-200) | 0.973 $^{3b}$ | 0.972 $^{2,3b}$ | 0.963 $^{2a}$ | 45.5 $^{3c}$ | 44.3 $^{3b}$ | 42.2 $^{3a}$ |
| 1.4% (BG-200) | 0.971 $^{2a,b}$ | 0.975 $^{3b}$ | 0.967 $^{2a}$ | 45.2 $^{3b}$ | 45.0 $^{4b}$ | 43.4 $^{4a}$ |

[A] Mean values of triplicates. Different numbers in the same column in each group (combination of flour type and β-glucan isolate) or letters within the same line used as superscripts indicate differences ($p = 0.05$) amongst the means, as determined by the Duncan's multiple range test.

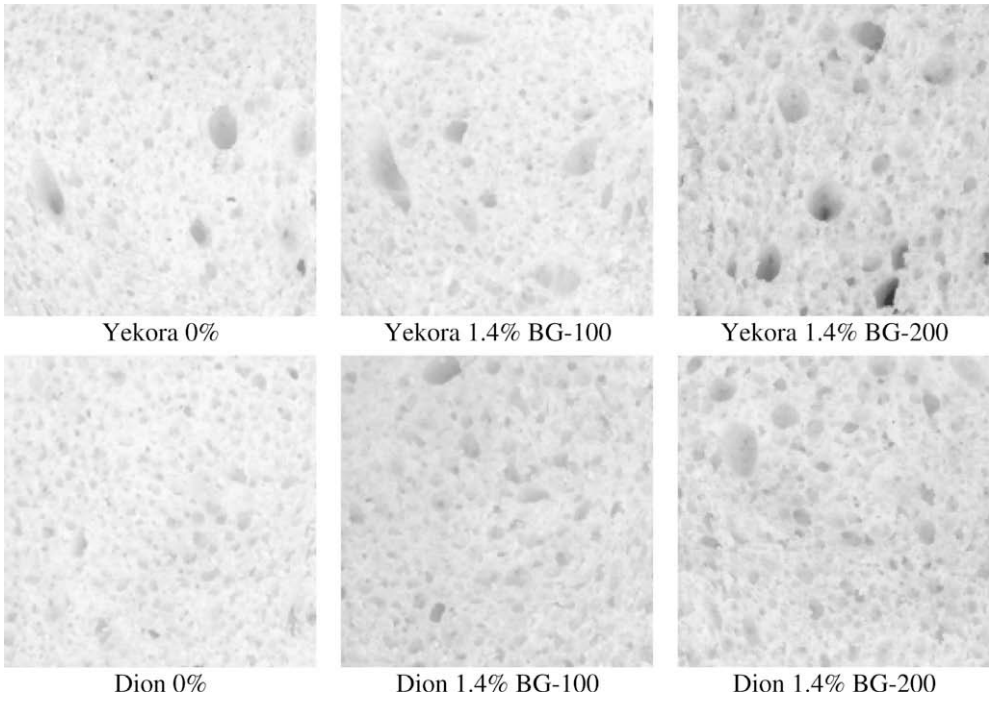


**Fig. 1.** Digital images (30 × 30 mm crumb area) of control and β-glucan-enriched breads from Yekora and Dion wheat flours.

slice to provide a more detailed view of the bread texture (Fig. 1). It is a common perception that the cell size of bread crumb has a significant effect on its texture and mouthfeel perception. Day and Rogers (1996) showed that the fineness score, computed using appropriate software, was correlated with the human perception of fineness. In this study, there were no statistical differences in crumb features between the two control (no added β-glucan) bread samples (Table 4). The total number of cells detected in the two control breads was similar to values reported by Crowley et al. (2000). The gas cells occupied 17.2% and 17.1% of the cross-sectional area of Yekora and Dion control breads, respectively. These values are smaller than those reported by Crowley et al. (2000), but comparable to the results reported by Sapirstein, Roller, and Bushuk (1994).

Statistical analysis showed significant differences in the image analysis parameters for all breads supplemented with different levels of β-glucans (Table 4). Addition of β-glucans to the standard bread formula increased the total number of gas cells detected,

**Table 4**
Gas cell characteristics of control and β-glucan-enriched bread crumbs from Dion and Yekora wheat flours.[a]

| Sample | Total number of cells/9 $cm^2$ | Number of cells < 4 $mm^2$ | Total cell area ($mm^2$) | Mean cell area ($mm^2$) | Mean area of cells > 4 $mm^2$ | Cell to total area ratio |
|---|---|---|---|---|---|---|
| *DION* | | | | | | |
| Control | 338 $^{1}$ | 336 $^{1}$ | 154.2 $^{1}$ | 0.37 $^{1}$ | 2.50 $^{1}$ | 0.17 $^{1}$ |
| 0.2% (BG-100) | 449 $^{2}$ | 441 $^{2}$ | 245.3 $^{3}$ | 0.44 $^{1}$ | 2.92 $^{1,2}$ | 0.27 $^{3}$ |
| 0.6% (BG-100) | 560 $^{3}$ | 550 $^{3}$ | 262.3 $^{3}$ | 0.47 $^{1}$ | 3.25 $^{2}$ | 0.29 $^{3}$ |
| 1.0% (BG-100) | 449 $^{2}$ | 448 $^{2}$ | 108.8 $^{1}$ | 0.24 $^{1}$ | 2.74 $^{1,2}$ | 0.12 $^{1}$ |
| 1.4% (BG-100) | 438 $^{2}$ | 434 $^{2}$ | 164.9 $^{2}$ | 0.38 $^{1}$ | 2.84 $^{1,2}$ | 0.18 $^{2}$ |
| Control | 338 $^{1}$ | 336 $^{1}$ | 154.2 $^{1,2}$ | 0.37 $^{1}$ | 2.50 $^{1}$ | 2.50 $^{1,2}$ |
| 0.2% (BG-200) | 432 $^{2}$ | 426 $^{2}$ | 201.3 $^{2}$ | 0.46 $^{1,2}$ | 3.48 $^{2}$ | 0.22 $^{2}$ |
| 0.6% (BG-200) | 448 $^{2}$ | 441 $^{2}$ | 260.3 $^{3}$ | 0.56 $^{2}$ | 3.19 $^{2}$ | 0.29 $^{3}$ |
| 1.0% (BG-200) | 443 $^{2}$ | 438 $^{2}$ | 180.6 $^{1,2}$ | 0.41 $^{1,2}$ | 3.38 $^{2}$ | 0.20 $^{1,2}$ |
| 1.4% (BG-200) | 416 $^{2}$ | 409 $^{2}$ | 184.5 $^{2}$ | 0.44 $^{1,2}$ | 2.55 $^{1}$ | 0.21 $^{2}$ |
| *YEKORA* | | | | | | |
| Control | 352 $^{1}$ | 347 $^{1}$ | 155.5 $^{1}$ | 0.44 $^{1}$ | 3.89 $^{1,2}$ | 0.17 $^{1}$ |
| 0.2% (BG-100) | 462 $^{2}$ | 454 $^{2}$ | 299.2 $^{3}$ | 0.64 $^{2}$ | 4.77 $^{2}$ | 0.33 $^{3}$ |
| 0.6% (BG-100) | 642 $^{4}$ | 632 $^{4}$ | 256.5 $^{2,3}$ | 0.42 $^{1,2}$ | 3.51 $^{1}$ | 0.29 $^{2,3}$ |
| 1.0% (BG-100) | 480 $^{2}$ | 475 $^{2}$ | 217.4 $^{2}$ | 0.38 $^{1,2}$ | 3.50 $^{1}$ | 0.24 $^{2}$ |
| 1.4% (BG-100) | 547 $^{3}$ | 542 $^{3}$ | 215.2 $^{2}$ | 0.35 $^{1}$ | 3.76 $^{1,2}$ | 0.24 $^{2}$ |
| Control | 352 $^{1}$ | 347 $^{1}$ | 155.5 $^{1}$ | 0.44 $^{1,2}$ | 3.89 $^{1,2}$ | 0.17 $^{1}$ |
| 0.2% (BG-200) | 522 $^{3}$ | 510 $^{3}$ | 337.8 $^{2}$ | 0.69 $^{2}$ | 3.78 $^{1,2}$ | 0.38 $^{2}$ |
| 0.6% (BG-200) | 549 $^{3}$ | 543 $^{3}$ | 173.6 $^{1}$ | 0.33 $^{1}$ | 2.95 $^{1}$ | 0.19 $^{1}$ |
| 1.0% (BG-200) | 430 $^{2}$ | 417 $^{2}$ | 500.6 $^{3}$ | 1.15 $^{3}$ | 4.34 $^{2}$ | 0.55 $^{3}$ |
| 1.4% (BG-200) | 453 $^{2}$ | 439 $^{2}$ | 345.4 $^{2}$ | 0.80 $^{2}$ | 3.90 $^{1,2}$ | 0.38 $^{2}$ |

[a] Mean values of triplicates. Means with any similar superscripts within each group (combination of flour type and β-glucan isolate) are not significantly different ($p = 0.05$) by the Duncan's multiple range test.

with a more pronounced effect for BG-100 than for BG-200. Furthermore, the number of cells smaller than 4 $mm^2$ (considered as small cells) increased with increasing β-glucan concentration up to a certain level of addition and then decreased; the effects exerted by BG-100 were greater than those by BG-200.

Generally, the mean gas cell area for BG-100-supplemented Dion bread crumbs did not differ significantly from the control, whereas the BG-200-supplemented Dion breads exhibited a larger mean gas cell area than did the control (Table 4). Furthermore, the addition β-glucans increased the number of large cells (>4 $mm^2$), implying that formation of larger gas cells occurs during baking; this in turn might be responsible for the observed decrease in bread crumb uniformity. Although incorporation of β-glucans seems to increase the gas retention capacity of the gluten network, the integrity of gas cells cannot be maintained throughout the course of the breadmaking process and, consequently, small gas cells coalesce into larger ones; the greater the degree of gas cells coalescence during breadmaking, the coarser is the crumb structure of the resulting bread. Breads made with β-glucan-enriched Yekora flour generally showed coarser crumb structure than did the respective loaves with the Dion flour (Fig. 1).

The degree of cell elongation is a measure of the strength of the dough and a direct measure of bread chewiness (Hoseney, 1994). Breads supplemented with β-glucans showed fewer round gas cells than did their respective controls (data not shown). Similar effects have been reported by Crowley et al. (2000) when fat and emulsifiers were incorporated in the bread recipe. The decrease of gas cell roundness was especially evident when the Dion wheat flour was supplemented with BG-100 and the Yekora flour with BG-200.

Since the quantity of dough used to produce bread loaves was the same in all baked products, theoretically the higher values of the total cell area imply higher bread volumes. The experimental data indeed showed that the total cell area reached its maximal value at the same β-glucan concentration level (0.6%) when a maximum specific volume of the breads was noticed.

### 3.6. Effect of β-glucan on bread colour

The brightness ($L^*$) of bread crumb was greatly influenced by the level of β-glucan addition; generally, the $L^*$ values of bread crumbs decreased significantly ($p < 0.05$) with increasing β-glucan level, except for Dion breads supplemented with BG-100 at low addition levels that showed $L^*$ values similar to the respective control (Table 5). The addition of BG-200 decreased the $L^*$ values to a greater extent than did BG-100. Gill et al. (2002) also observed a decrease of bread crumb brightness upon addition of barley flours containing β-glucans.

The $a^*$ values of breads fortified with the BG-200 were higher than were those of the control breads, whereas the addition of the BG-100 did not have any effect on the $a^*$ values (Table 5). The $b^*$ crumb colour values of breads fortified with BG-100 did not change with increasing fortification level, whilst BG-200 slightly decreased the $b^*$ of Dion and Yekora bread crumbs.

### 3.7. Firmness and staling events

Crumb firmness of the breads, measured after 24 h of storage, decreased with increasing β-glucan level, reaching a minimum, and thereafter the trend was reversed, but always the values were smaller than were their respective controls (Table 5). Only the Yekora breads fortified with the highest level of β-glucan concentration (1.4%) exhibited higher firmness than the control. The increase in hardness of the bread crumb fortified with β-glucans, at a high level of addition, may be a consequence of the thickening of the walls surrounding gas cells, as proposed by Rosell, Rojas, and de Barber (2001). Generally, water promotes starch recrystallization and indeed the water content of β-glucan-enriched breads was significantly higher than that of control Yekora and Dion breads. However, the DSC measurements did not show an increase of starch retrogradation. Furthermore, β-glucan-enriched breads exhibited softer breadcrumbs than the controls measured after

**Table 5**
Colour of bread crumbs measured 1 h after baking, and crumb firmness and amylopectin chain ordering in staling endotherm (J/g) of breads stored for 1, 4, and 8 days at 4 °C.

| | Crumb Colour[A,B] | | | Crumb Firmness (N)[C,D] | | | Staling endotherm (J/g)[E] | |
|---|---|---|---|---|---|---|---|---|
| | $L^*$ | $a^*$ | $b^*$ | 1 day | 4 day | 8 day | 4 day | 8 day |
| *DION* | | | | | | | | |
| Control | 79.40 $^{7}$ | 0.80 $^{1}$ | 21.96 $^{3}$ | 29.0 $^{6a}$ | 27.7 $^{3,4a}$ | 45.2 $^{4,5b}$ | 3.94 $^{1a}$ | 4.53 $^{1b}$ |
| 0.2% (BG-100) | 78.55 $^{5,6,7}$ | 0.79 $^{1}$ | 22.19 $^{3}$ | 13.7 $^{2,3a}$ | 25.6 $^{3b}$ | 36.7 $^{3c}$ | n.d. | n.d. |
| 0.6% (BG-100) | 79.26 $^{6,7}$ | 0.81 $^{1}$ | 21.62 $^{2,3}$ | 12.6 $^{2a}$ | 27.4 $^{4b}$ | 45.4 $^{5c}$ | n.d. | n.d. |
| 1.0% (BG-100) | 78.27 $^{4,5}$ | 0.90 $^{1}$ | 21.34 $^{2,3}$ | 17.0 $^{4a}$ | 27.2 $^{3,4b}$ | 43.8 $^{5c}$ | n.d. | n.d. |
| 1.4% (BG-100) | 78.40 $^{5\ 6}$ | 0.95 $^{1}$ | 20.02 $^{1}$ | 21.6 $^{5a}$ | 30.3 $^{4b}$ | 39.4 $^{3,4c}$ | 4.11 $^{1a}$ | 4.79 $^{1a}$ |
| 0.2% (BG-200) | 77.31 $^{3}$ | 1.00 $^{1,2}$ | 21.42 $^{2,3}$ | 14.9 $^{3a}$ | 21.9 $^{2b}$ | 26.5 $^{2c}$ | n.d. | n.d. |
| 0.6% (BG-200) | 77.44 $^{3\ 4}$ | 1.23 $^{2}$ | 20.26 $^{1}$ | 10.9 $^{1a}$ | 16.7$^{1b}$ | 19.1 $^{1c}$ | n.d. | n.d. |
| 1.0% (BG-200) | 76.18$^{2}$ | 1.58$^{3}$ | 20.66 $^{1,2}$ | 9.5 $^{1a}$ | 17.4$^{1b}$ | 27.4 $^{2c}$ | n.d. | n.d. |
| 1.4% (BG-200) | 74.62 $^{1}$ | 2.01 $^{4}$ | 20.90 $^{1,2}$ | 22.3 $^{5a}$ | 21.8 $^{2a}$ | 22.0 $^{1a}$ | 5.53 $^{1a}$ | 5.24 $^{1a}$ |
| *YEKORA* | | | | | | | | |
| Control | 80.41 $^{6}$ | 0.74 $^{1}$ | 18.14 $^{1,2}$ | 15.0 $^{4a}$ | 20.7 $^{3,4b}$ | 22.7 $^{3,4b}$ | 4.23 $^{1a}$ | 4.93 $^{1a}$ |
| 0.2% (BG-100) | 79.06 $^{5}$ | 0.75 $^{1}$ | 18.41 $^{2,3}$ | 10.8 $^{1a}$ | 20.8 $^{3,4b}$ | 21.4 $^{2,3b}$ | n.d. | n.d. |
| 0.6% (BG-100) | 79.07 $^{5}$ | 0.81 $^{1}$ | 18.40 $^{2,3}$ | 10.6 $^{1a}$ | 18.0 $^{1,2b}$ | 24.4 $^{4c}$ | n.d. | n.d. |
| 1.0% (BG-100) | 79.26 $^{5}$ | 0.79 $^{1}$ | 18.06 $^{1,2}$ | 11.1 $^{1,2a}$ | 21.0 $^{3,4b}$ | 21.0 $^{4c}$ | n.d. | n.d. |
| 1.4% (BG-100) | 77.04 $^{3}$ | 0.77 $^{1}$ | 17.37 $^{1}$ | 29.1 $^{6c}$ | 20.6 $^{3,4b}$ | 18.8 $^{1a}$ | 4.90 $^{1a}$ | 4.97 $^{1a}$ |
| 0.2% (BG-200) | 78.29 $^{4\ 5}$ | 1.04 $^{2}$ | 18.52 $^{2,3}$ | 12.3 $^{2,3a}$ | 22.3 $^{4c}$ | 17.9 $^{1b}$ | n.d. | n.d. |
| 0.6% (BG-200) | 77.75 $^{3\ 4}$ | 1.18$^{2}$ | 18.12 $^{1,2}$ | 9.9 $^{1a}$ | 16.1 $^{1b}$ | 19.1 $^{1,2c}$ | n.d. | n.d. |
| 1.0% (BG-200) | 76.06 $^{2}$ | 1.58$^{3}$ | 18.23 $^{2,3}$ | 13.7 $^{3,4a}$ | 19.4 $^{2,3b}$ | 17.7 $^{1b}$ | n.d. | n.d. |
| 1.4% (BG-200) | 73.55 $^{1}$ | 2.11 $^{4}$ | 19.05 $^{3}$ | 23.4 $^{5b}$ | 26.6 $^{5c}$ | 17.8 $^{1a}$ | 4.68 $^{1a}$ | 4.69 $^{1a}$ |

n.d.: Measurement was not performed.

[A] $L^*$ = lightness, higher values indicate lighter colour; $a^*$ = redness, and $b^*$ = yellowness; higher values indicate higher colour intensity.

[B] Mean values of four loaves; means with any similar superscripts within each group (with the same flour type) are not significantly different ($p = 0.05$) by the Duncan's multiple range test.

[C] Crumb firmness was measured as CFV (compression force value, N).

[D] Mean values of six measurements; different numbers in the same column in each group (with the same flour type) or letters within the same line used as superscripts indicate differences ($p = 0.05$) amongst the means, as determined by the Duncan's multiple range test.

[E] Mean values of triplicates; different numbers in the same column in each group (with the same flour type) or letters within the same line used as superscripts indicate differences ($p = 0.05$) amongst the means, as determined by the Duncan's multiple range test.

four and eight days of storage (except for Yekora bread enriched with 1.4% of BG-200 after four days storage).

The decrease in bread firmness was more pronounced when the β-glucan isolates were added to the poor breadmaking quality flour (Dion) than to the good breadmaking flour (Yekora). These results suggest that incorporation of β-glucans into low quality wheat flour seems to be more effective in reducing bread firmness than when it is added to good breadmaking flour. Gill et al. (2002) suggested that, in addition to a plasticization effect brought about in the composite gluten-starch matrix (higher hydration leads to softening), β-glucans added to wheat flour would compete for water with native wheat starch granules in the dough. This, in turn, might restrict swelling and solubilisation of the starch during baking, and thereby reduce firmness (Gill et al., 2002).

The texture results showed that the firmness values of all β-glucan-supplemented breadcrumbs, after eight days of storage, were similar to or significantly lower than those of the respective controls after storage (Table 5). The BG-200-enriched breads presented a much softer crumb than did the control bread, implying an antistaling action, whereas the respective breads with BG-100 exhibited an increase in hardness during storage, especially those of the poor quality flour. Nevertheless, the enthalpy values of β-glucan-supplemented breads during storage did not show any significant changes in starch retrogradation amongst the samples.

Bread firmness is broadly used as a tool to measure bread staling; however, staling is a very complex phenomenon that cannot be explained by a single variant. The softening effect of β-glucans could be attributed to their higher water retention capacity, and a possible inhibition of the amylopectin retrogradation (Biliaderis et al., 1995). Furthermore, the decrease in the crumb firmness observed when β-glucan is added to the bread formula may be a consequence of an increase of the total area of gas cells; the lowest bread firmness was indeed recorded for the β-glucan concentration at which gas cell area was maximised. Schiraldi, Piazza, and Riva (1996) also suggested that bread firmness depends largely on the formation of a cross-linked network rather than the development of amylopectin crystallites in the ageing gluten-starch composite matrix when there are no changes in the moisture content. In the present study, no significant losses of moisture in breads after 4 days of storage were noted, whereas the firmness of the bread crumbs increased significantly.

Finally, it is possible that water migration that occurs amongst bread components, leads to formation of a stiffer network involving β-glucans. The significant moisture loss observed upon longer storage may also bring about structural changes in the macromolecules (Martin, Zeleznak, & Hoseney, 1991). The moisture loss, starch recrystallization and increase in β-glucan concentration in the composite network may all contribute to the localised molecular aggregation and/or gelation events that would further increase bread crumb firmness after eight days of storage.

## 4. Conclusion

The aim of this study was to explore the impact of molecular size and concentration of barley β-glucans on wheat flour dough and bread characteristics. Two wheat flours of different breadmaking quality were enriched with β-glucans which varied in their molecular size. It appears that the effects of β-glucans on dough rheology and bread characteristics strongly depend on the molecular size of the polysaccharide, the supplementation level and the quality of the base flour used. A continuous increase in the farinograph water absorption of the doughs was observed with increasing fortification level; the high molecular weight preparation (BG-200) showed a greater impact than did its low molecular weight counterpart, presumably due to a higher water binding capacity of the former. Generally, breads containing barley β-glucans showed lower moisture loss during storage than did the respective controls. Moreover, a reduction in moisture loss was evident with increasing concentration of β-glucans.

The BG-100 preparation was more effective in increasing dough stability of the Dion doughs, and improving the dough development time than was BG-200. The β-glucan addition increased the $R_{50}$ and E values of the poor breadmaking quality dough, strengthened the gluten matrix and increased the gas retention capacity of the dough. Generally, β-glucan-enriched doughs yielded breads with specific volumes that initially increased with increasing polysaccharide concentration, reached a maximum, and thereafter decreased. Increasing the β-glucan content also increased the number of gas cells but it produced a coarser and darker crumb structure with less rounded cells. The total cell area of the bread crumbs reached maximum values at the same concentration of β-glucans at which a maximum specific volume of bread loaf was achieved. In general, the addition of β-glucans, up to a certain level, decreased the firmness of bread during storage; the BG-200 was more effective in improving crumb softness than was BG-100, for both types of flour. Overall, the results of this work indicate that, in order to enhance the quality attributes of β-glucan-supplemented wheat breads, optimisation trials are required, taking into consideration the quality of base flour, as well as the level and molecular features of β-glucans to be used for fortification.

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