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The behaviour and susceptibility to degradation of high and low molecular weight barley β -glucan in wheat bread during baking and in vitro digestion

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Abstract

The behaviour and susceptibility to degradation of a high molecular weight (HMW) and low molecular weight (LMW) barley b-glucan in white bread during manufacture and in vitro digestion were investigated. The incorporation of both HMW and LMW barley b-glucan resulted in stiffer dough, lowered loaf volume and height compared to the control. The HMW barley b-glucan caused the greatest loss of dough and bread quality. Breads with HMW and LMW barley β -glucan exhibited attenuated reducing sugars release (RSR) over a 300 min in vitro digestion compared to the control, with generally no difference between the breads. HMW barley b-glucan was degraded during bread manufacture; however, degradation of the LMW barley b-glucan was not apparent. An in vitro digestion did not result in any further degradation of the molecular weight (MW) of either barley β -glucan. © 2006 Elsevier Ltd. All rights reserved.

Keywords: Barley; b-glucan; Dietary fibre; Glycaemic; Bread; Starch

1. Introduction

Bread is a popular and convenient cereal commodity; however, in recent years, the nutritional contribution of bread to the diet has been scrutinised in the light of lowcarbohydrate diets (Atkins) and glycaemic index (GI) scoring, where white wheat bread, the most popular form in many Western diets, is considered to be a high GI food ([Foster-Powell & Brand Miller, 1995](#page-8-0)). White bread is also a poor source of dietary fibre, containing typically less than 2.5% [\(Anon, 2003](#page-8-0)).

In recent years, improving the nutritional profile of bread has been of much interest. One possible method is enrichment with soluble fibres, including guar and $(1 \rightarrow 3)(1 \rightarrow 4)$ - β -D-glucan (hereafter referred to as β -glucan). The most widely known nutritional benefits of b-glucans (from both oat and barley grains) are the attenuation of blood glucose and insulin ([Wood, Braaten, Fra](#page-8-0)[ser, Riedel, & Poste, 1990; Wood et al., 1994\)](#page-8-0), and hypocholestrolemic effects ([Beer, Arrigoni, & Amado,](#page-8-0) [1995; Braaten, Wood, & Scott, 1994](#page-8-0)). A strong correlation between soluble dietary fibre addition to bread and improved glycaemic control has been found. [Pick, Haw](#page-8-0)[rysh, Gee, and Toth \(1998\) Cavallero, Empilli, Brighenti,](#page-8-0) and Stanca (2002) found that barley β -glucan rich breads elicited lower glycaemic responses than did a reference white wheat bread.

Incorporation of β -glucan in the form of native cereal flours or extracts into breads can often result in undesirable physicochemical changes, including reduced dough extensibility, reductions in loaf height and volume and changes to crumb structure ([Cavallero et al., 2002; Gill, Vasanthan,](#page-8-0) [Ooraikul, & Rossnagel, 2002; Knuckles, Hudson, & Chiu,](#page-8-0)

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[1997; Symons & Brennan, 2004](#page-8-0)), which ultimately may result in reduced consumer acceptance. It is likely that this loss of dough and bread quality is in part related to increased water-binding capacities and viscosity of the b-glucan which, in turn, maybe related to molecular weight (MW), although few studies have examined this. Limiting negative changes in bread quality, maybe achieved by incorporating reduced or lower MW β -glucan extracts. However, with a reduction in MW, a reduction in physiological response is anticipated. Among other factors, the viscosity of b-glucan will depend upon concentration in solution and MW distribution ([Beer, Wood, & Weisz,](#page-8-0) [1997\)](#page-8-0). The high viscosity of β -glucan is widely accepted to be necessary for the attenuation of blood glucose and insulin levels ([Wood et al., 1994](#page-8-0)). Increased luminal viscosity is also believed to be important in lowering serum cholesterol ([Jenkins et al., 1987](#page-8-0)). The studies of [Wood et al.](#page-8-0) [\(1994\)](#page-8-0) revealed that reductions in viscosity of an oat gum (by acid hydrolysis) reduced or eliminated the capacity to attenuate postprandial glucose absorbance. Similar studies with solid foods, such as bread, are limited.

The MW of β -glucan may be further lowered by the conditions of bread making, which are known to lead to MW degradation of b-glucans ([Andersson et al., 2004\)](#page-8-0), the implication of this being the possible loss of physiological activity. [Frank, Sundberg, Kamal-Eldin, Vessby, and](#page-8-0) \AA [man \(2004\)](#page-8-0) reported that oat breads containing high or low MW b-glucan did not differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. The conditions of the gastrointestinal tract have also been reported to favour the degradation of β -glucan [\(Johansen,](#page-8-0) [Bach Knudsen, Wood, & Fulcher, 1997](#page-8-0)).

The aim of this paper is to explore and compare the behaviour of a high and low molecular weight barley β -glucan (hereafter referred to as HMW and LMW) in bread, systematically examining physicochemical changes in the dough and bread, the capacity of the β -glucans to reduce in vitro digestion of available carbohydrates, changes to bread and in vitro digest micro-structure and finally the susceptibility of the β -glucans to MW degradation during bread processing and in vitro digestion.

2. Materials and methods

2.1. Materials

HMW and LMW barley β -glucan extracts were purchased from Megazyme™ International Ireland Ltd. MW

Table 1 HMW and LMW barley β -glucan composition

$%$ Component (dwb)	HMW extract	LMW extract		
β -glucan	\sim 95	\sim 95		
Starch	0.21	< 0.12		
Protein	1.6			
Moisture	3.6			

of the barley β -glucans were 510,000 and 160,000 Da, respectively. The composition of the extracts is illustrated in Table 1. Commercial bread wheat flour was supplied by Shipton Mill, Stroud, UK. Vegetable fat, dried yeast, salt and sugar were purchased from a local store.

2.2. Methods

2.2.1. General analysis

Moisture was determined according to Approved Method 44-15A ([AACC, 2000](#page-8-0)). Total starch, β -glucan and total dietary fibre were determined using the total starch assay kit (Approved Method 76.13, [AACC,](#page-8-0) [2000\)](#page-8-0), b-glucan enzymatic assay kit (Approved Method 32-23 [AACC, 2000](#page-8-0)) and dietary fibre assay kit (Approved Method 32–07 ([AACC, 2000\)](#page-8-0)), respectively. All assay kits were supplied by Megazyme™ International Ireland Ltd. Nitrogen was determined using a nitrogen analyser (Model FP-2000, Leco Instruments Ltd., St Joseph, MI), and protein content was estimated by using a conversion factor of 6.25. Results are reported on a dry weight basis (dwb).

2.2.2. Bread making

White wheat breads containing approximately 4.5 g β -glucan/100 g bread (dwb) in the form of HMW or LMW barley β -glucan were manufactured using a straight dough, long fermentation bread-making process. An additional sample with no β -glucan was also prepared as a control. Basic dough formula, on a 250 g flour basis, consisted of dried yeast (6 g), salt (6 g), sugar (2 g) and 5% HMW or LMW barley β -glucan (when added). For the control bread 140 ml of water was used, whilst an additional 10 ml of water was incorporated into the HMW and LMW barley β -glucan mixes in order to allow the formation of a workable dough. To prepare the doughs, all dry ingredients were mixed in a food processor for 10 s to ensure complete homogeneity. Water was added and the mixture was processed for 45 s to allow formation of a dough. Doughs were fermented for 2 h at $40 °C$, after which they were kneaded and divided into 70 g portions and proofed in miniature tins with the following dimensions: top; 85 mm (l) by 50 mm (w); bottom; 75 mm (l) by 40 mm (w) for a further 55 min. Breads were baked at $220 °C$ for 25 min. Following baking, breads were cooled for 1 h before subsequent analyses. Proximate composition of the breads was determined and is presented in Table 2.

Table 2 Control, HMW and LMW barley β -glucan bread composition^a

Bread	% Available starch	$%$ Protein	$% \beta$ -glucan	% Total dietary fibre
Control	$69.5 + 0.25$	$16.1 + 0.02$	$0.14 + 0.00$	$5.88 + 0.78$
HMW	$64.8 + 1.31$	$15.1 + 0.06$	$4.30 + 0.10$	$10.6 + 0.51$
LMW	$64.6 + 0.26$	$15.3 + 0.01$	$4.47 + 0.04$	$11.4 + 0.29$

^a All measurements are mean values \pm SD of duplicate determinations.

2.2.3. Dough rheology

Resistance to extension (mean max force g) and extensibility (mean distance at max force mm) of the doughs were measured using a texture analyser (TA-XT2) (Stable Micro Systems, Surrey, England) equipped with a Kiefer dough and extensibility rig (A/KIE) using a 5 kg load cell ([Symons & Brennan, 2004](#page-8-0)).

2.2.4. Bread quality evaluation

Loaf height was determined using calibrated callipers and reported in centimetres. Loaf volume was measured using Approved Method 10–05 [\(AACC, 2000\)](#page-8-0) guidelines for measurement of volume by rapeseed displacement. A texture analyser (TA-XT2) (Stable Micro Systems, Surrey, England) was used to measure bread firmness. An AACC 36 mm cylinder probe with radius (P/36 R) and 5 kg load cell was used [\(Symons & Brennan, 2004](#page-8-0)).

2.2.5. In vitro digestion of breads

Bread samples were subjected to an in vitro digestion based on the method of [Brighenti, Pellegrini, Casiraghi,](#page-8-0) [and Testolin \(1995\),](#page-8-0) slightly modified. Samples of bread (equivalent to 2 g available starch) were reduced to a size of approx 1 cm^3 , diluted with sodium phosphate buffer (pH 6.9), reduced to pH 1.5 (HCl acid) and digested with pepsin (from porcine stomach mucosa) (115 U/g starch) (Sigma–Aldrich, UK) for 30 min at 37 °C. The pH of the mixtures was re-adjusted to pH 6.9 (NaOH), diluted to 50 ml (sodium phosphate buffer) and porcine pancreatic a-amylase (110 U/g starch) (Sigma–Aldrich, UK) was added. A sample blank (with deactivated enzyme) was also prepared. The mixtures were transferred to prepared dialysis tubing (Medicell International Ltd., UK) and placed in 450 ml of sodium phosphate buffer for 5 h at 37° C. Tubes were agitated every 15 min to simulate gut movements. Duplicate aliquots (1 ml) were taken every 30 min, replacing the volume each time with 1 ml of fresh buffer. Dialysate was analysed for total dialysable sugars by the 3,5-dinitrosalicylic acid method [\(James, 1999](#page-8-0)).

Reducing sugars released (RSR), consisting of the dialysed fragments of digested starch, was expressed in maltose equivalents as a percentage of available carbohydrate present in the sample using the following calculation:

$$
RSR = (A_{\text{sample}} \times 500 \times 0.95 / A_{\text{maltose}} \times SS) \times 100,
$$

where A_{sample} was the value of absorbance at 540 nm, A_{maltose} was the value of absorbance of a solution containing 1 mg of pure maltose per ml/phosphate buffer, SS was the amount of starch (in mg) contained within the sample, 500 was the total volume, and 0.95 was the conversion from maltose to starch.

2.2.6. Micro-structure of baked breads and in vitro digests

Baked and in vitro digested breads (samples taken at 300 min) were frozen in liquid nitrogen and freeze-dried. The freeze-dried samples were transversely fractured to expose interior surfaces and 1 mm sections were mounted onto pre-glued stubs. All prepared specimens were sputter coated with gold (Emitech K550 Sputter Coater, Ashford, UK) and examined by a scanning electron microscope (SEM JEOL JSM6100, Oxford, UK).

2.2.7. Extraction and analysis of β -glucan Calcofluor average MW (M_{cf}) and MW distribution

Wheat flour, HMW and LMW barley β -glucan, baked breads and in vitro digests (samples taken at 30, 150 and 300 min) were selected for MW analysis. Samples with a moisture content of $>10\%$ were freeze-dried prior to analysis. Enzymes in samples were inactivated by boiling in 50% ethanol for 15 min. b-glucan in products (100 mg) was extracted with hot deionised water (20 ml) with added CaCl₂ (0.28 mg/ml of water) and thermostable α -amylase (50 μ l, Megazyme, Wicklow, Ireland) following the method of Rimsten, Stenberg, Andersson, Andersson, and Aman [\(2003\)](#page-8-0). The mixtures were immediately placed in a boiling water bath for 90 min, with occasional mixing by vortex. After cooling to room temperature, tubes were centrifuged (1500g for 15 min), and supernatants were filtered $(0.45 \mu m)$ before injecting into a high performance size exclusion chromatograph with fluoresence detection (HPSEC-FD) system (set up according to [Wood, Weisz,](#page-8-0) [& Mahn, 1991](#page-8-0) and [Wood, Weisz, & Blackwell, 1991](#page-8-0) with some modifications).

The HPSEC-FD system consisted of two pumps (LC-10AD, Shimadzu, Miniato, Japan) coupled to a degasser (SDU 2006, Prolab, Reinach, Switzerland), one delivering the eluent $(0.1 \text{ M } \text{NaNO}_3 \text{ with } 0.02\% \text{ NaNO}_3)$ at a flow rate of 0.5 ml/min and the other one delivering Calcofluor solution (0.05% fluorescent brightner 28 (Sigma–Aldrich, Sweden) in 0.1 M tris(hydroxymethyl)-(aminomethane) (Tris) adjusted to pH 8) at a flow rate of 0.5 ml/min though a pulse reducer. An injector (Midas type 830, Spark, Emmen, Holland) was coupled to the system before a guard column (OHpak SB-G, Shodex, Showa Denko KK, Kawasaki, Japan) and two columns in series (OHpak SB-806HQ and SB-804HQ, Shodex, Showa Denko KK, Kawasaki, Japan). Calcofluor was delivered postcolumn by a mixing loop placed together with the columns in an oven maintained at 60° C. For detection, a fluorescent detector (1100 series G1321A, Agilent Technologies, Waldbroon, Germany) was used with the wave-lengths $\lambda_{\rm ex} = 415$ nm and $\lambda_{\rm em} = 445$ nm according to [Suortti](#page-8-0) [\(1993\)](#page-8-0) at a gain setting of 8.

The system was calibrated using β -glucan fractions with narrow MW ranges. By using the regression line of the calibration curve, Calcofluor average MW (M_{cf}) could be calculated. The M_{cf} over the distribution divided into *n* slices was defined as:

$$
M_{\rm cf} = \frac{\sum_{i=1}^{n} (w_i c_i)}{\sum_{i=1}^{n} c_i}
$$

where w_i was the MW at a slice i given by the calibration and c_i was the corresponding concentration, expressed as Calcofluor response. This average includes only β -glucan

molecules large enough to be detected with Calcofluor (β -glucan molecules with a MW below 10⁴ are excluded [\(Munck, 1989](#page-8-0))). Percentiles were also calculated, describing the MW at which 10%, 50%, and 90% of the distribution fall below that value. Results are means of duplicate analyses.

2.2.8. Statistical analysis

All determinations were made at least in duplicate and mean values \pm standard deviation (SD) are presented. Data were statistically evaluated by analysis of variance (ANOVA), performed using the Minitab 13 statistical software package (Minitab Inc., State College, PA, USA), followed by Tukey's test. Significance was defined as $P \leq 0.05$.

3. Results and discussion

3.1. Effects of HMW and LMW barley β -glucan inclusion on the rheological properties of bread dough

Table 3 illustrates the effects of HMW and LMW barley b-glucan inclusion on the rheological properties of bread dough. The resistance to extension of bread dough, containing both types of barley β -glucan, was significantly higher than that of the control bread dough ($P < 0.05$). The extensibility of the doughs containing the HMW and LMW barley β -glucan was also significantly reduced compared to the control dough. In particular, the HMW barley β -glucan inclusion yielded the greatest resistance ($P \le 0.05$) and also the lowest extensibility of the samples.

The production of doughs with high resistance to extension may be attributed to the viscous influence of β -glucans, and, since increasing MW is related to higher viscosity, this would explain the highest resistance to extension exhibited by the HMW barley β -glucan-containing dough. Loss of extensibility may be caused by excessive binding of water by the β -glucans which, in turn, leads to an impaired gluten network, again the HMW β -glucan causing the greatest loss in extensibility, suggesting a greater water-binding capacity of the HMW β -glucan. [Symons and Brennan \(2004\)](#page-8-0) reported stiffer and less elastic doughs with barley β -glucan incorporation in bread doughs. In practical terms, the stiffness of the doughs may be overcome by the addition of water, which allows for the increased water absorption of the doughs as a result of barley β -glucan inclusion; however, the loss of the elasticity of the doughs is not so easily rectified.

3.2. Effects of HMW and LMW barley β -glucan inclusion on bread quality evaluation

Inclusion of HMW and LMW barley β -glucan in bread resulted in a significant decrease in loaf volume and height (Table 3), the reduction in height and volume being greatest in the HMW bread. In comparison to the control sample, breads containing HMW and LMW barley β -glucan gave higher values in compression force measurements; however, the difference was only significant between the control and LMW barley β -glucan bread ($P \le 0.05$).

Many authors have reported loss of height, volume and increased firmness as a result of β -glucan addition ([Caval](#page-8-0)[lero et al., 2002; Gill et al., 2002; Knuckles, Hudson, et al.,](#page-8-0) [1997; Symons & Brennan, 2004\)](#page-8-0). It is possible that, when added to wheat flour during bread making, β -glucan could tightly bind appreciable amounts of water, making it less available for the development of the gluten network, which results in an underdeveloped gluten network and hence reduced loaf volume. Additionally or alternatively, the decreased volume may be attributed to a reduction in steam production as a result of water-binding by the β -glucans. Our results indicate that the HMW barley β -glucan may have the greatest water-binding capacity, resulting in greatest loss of bread quality. The higher compression value exhibited by the LMW barley β -glucan bread is difficult to explain but may be related to the differing viscoelastic characteristics of the b-glucan ([Vaikousi, Biliaderis, &](#page-8-0) [Izydorczyk, 2004](#page-8-0)).

3.3. Effects of HMW and LMW barley β -glucan inclusion on the in vitro digestibility of breads

[Fig. 1](#page-4-0) illustrates the effect of HMW and LMW barley b-glucan inclusion on the RSR from the bread matrix during an in vitro digestion process. The results reveal a significant decrease in RSR from both breads (LMW 90– 300 min digestion and HMW 120–300 min compared to the control) ($P \le 0.05$). Generally there was no significant difference between the RSR values of the HMW and LMW barley β -glucan breads ($P > 0.05$).

The general similarity of RSR between the HMW and LMW barley β -glucan breads is surprising, since the relationship between MW, viscosity and reductions in sugar diffusion has been illustrated by other workers [\(Wood](#page-8-0) [et al., 1994; Wood, Beer, & Butler, 2000\)](#page-8-0); however, the majority of these studies are with homogeneous solutions

Table 3

Control, HMW and LMW barley β -glucan dough rheology and baked bread evaluation^a

Height (cm) Volume (ml) Distance (mm) Firmness (g) Extension (g) $5.40^{\rm b} + 0.02$ $212^a + 2.0$ $6.18^a + 0.08$ $33.34^{\circ} + 0.65$ $-29.83^{\rm a}+0.47$ $5.44^{a,b} + 0.21$ $100^{\circ} + 0.0$ $3.65^{\circ} + 0.11$ $74.28^{\rm a} + 1.03$ $-22.30^{\circ}+0.69$ $118^b + 2.89$ $4.03^b + 0.13$ $-23.75^{\rm b}+0.32$ $49.18^{b} + 2.98$ $6.07^{\rm a} + 0.40^{\rm b}$				
	Bread			
	Control HMW LMW			

Values in the same column followed by the same letter are not significantly different ($P > 0.05$).
^a All measurements are mean values \pm SD of triplicate determinations.

Fig. 1. Reducing sugars released (RSR) (expressed as maltose equivalents, as a percentage of total available carbohydrates) from wheat breads substituted with HMW and LMW barley β -glucan. Values are means of triplicate determinations. Standard deviations are represented as error bars.

of soluble fibre and glucose as opposed to foods with a solid matrix. The similarity and ability of both the HMW and LMW barley β -glucan to reduce RSR might be explained by a mechanism of action of β -glucan in solid foods different from that in liquids, with MW and viscosity having a lesser role in a solid matrix. [Brennan, Blake, Ellis,](#page-8-0) [and Schofield \(1996\)](#page-8-0) illustrated that guar gum (physicochemically similar to β -glucan) had the ability to modify the micro-structure of wheat breads, resulting in a significant reduction in starch hydrolysis compared with the control. This effect was independent of the MW of guar gum contained in the wheat bread. The ability of soluble fibres to reduce starch granule hydrolysis, regardless of MW, may partly explain why wheat bread containing guar gum of low MW reduced postprandial glycaemia and plasma insulin concentrations in diabetic ([Gatenby, Ellis,](#page-8-0) [Morgan, Dawoud, & Judd, 1996](#page-8-0)) and non diabetic [\(Ellis,](#page-8-0)

Percentiles describing MW $(x10^{-4})$ at which 10%, 50%, and 90% of the distribution fall below that value^A

a-e Means values in the same column followed by the same letter are not significantly different ($P > 0.05$).

^A All measurements are mean values of duplicate determinations.

^B Coefficient of variation (%) for (M_{cf}) .

[Dawoud, & Morris, 1991](#page-8-0)) human subjects. Few studies have examined the effect of β -glucan addition on the micro-structure of cereal foods; in this current study, scanning electron microscopy was used to investigate the microstructure of the breads and in vitro digests.

It is also possible that the degradation of the HMW barley b-glucan (as illustrated in [Fig. 3](#page-6-0) and [Table 4](#page-4-0) and discussed in Section [3.5\)](#page-6-0) may have resulted in a reduction in its ability to attenuate RSR, resulting in behaviour more like the LMW barley β -glucan.

c) LMW bread baked

f) LMW bread 300 minute in vitro digest

Fig. 2. Scanning electron micrographs of baked and digested breads: (a) control baked; (b) HMW baked; (c) LMW baked; (d) control digest; (e) HMW digest; (f) LMW digest.

3.4. Effects of HMW and LMW barley β -glucan inclusion on the micro-structure of baked and in vitro digested breads

[Fig. 2](#page-5-0) contains scanning electron micrographs of control, HMW and LMW barley b-glucan-enriched baked

Table 5 Calcofluor average MW (M_{cf}) and MW distribution of β -glucans from in vitro bread digests (30, 150 and 300 min)

Sample	$(M_{\rm cf})$ (×10 ⁻⁴ g/mol)	CV^B	Distribution		
			10%	50%	90%
30 min					
Control	13 ^e	6.8	4.1°	9.3°	25°
HMW	$26^{\rm b}$	4.2	5.4^{b}	17 ^b	58 ^a
LMW	21°	1.6	6.8 ^a	16 ^b	$40^{b,c}$
150 min					
Control	15 ^e	5.5	3.5°	8.9°	31 ^d
HMW	30 ^a	0.8	6.9 ^a	21 ^a	62 ^a
LMW	$20^{\text{c,d}}$	0.0	6.6^{a}	16 ^b	$38^{b,c}$
300 min					
Control	18 ^d	4.3	3.3°	9.2°	$42^{\rm b}$
HMW	30 ^a	1.0	71 ^a	21 ^a	61 ^a
LMW	$20^{c,d}$	2.3	6.6 ^a	16 ^b	37 ^c

Percentiles describing MW $(\times 10^{-4})$ at which 10%, 50% and 90% of the distribution fall below that value^A.
 $a-e$ Means values in the same column followed by the same letter are not

significantly different ($P > 0.05$).

Control

^A All measurements are mean values of duplicate determinations.
^B Coefficient of variation $\binom{0}{0}$ for (M_1)

Coefficient of variation (%) for (M_{cf}) .

breads and in vitro digests (300 min). [Fig. 2](#page-5-0)(a), the control baked bread, has an even structure with the presence of relatively exposed large and small starch granules. The HMW and LMW barley β -glucan breads ([Fig. 2](#page-5-0)(b) and (c)) have a more compact and uneven structure with fewer starch granules exposed. [Fig. 2\(](#page-5-0)d), the control in vitro digest (300 min), has a very porous appearance with relatively few undigested starch granules. The HMW and LMW barley β -glucan bread in vitro digests ([Fig. 2](#page-5-0)(e) and (f), respectively) have a more compact appearance with retention of undigested starch granules.

These images clearly illustrate that the inclusion of barley b-glucan within bread has an impact upon structure which, in turn, may change the physicochemical properties of breads and the rate of amylolytic activity and starch hydrolysis. This observation is consistent with those of [Brennan et al. \(1996\) and Tudorica et al. \(2002\).](#page-8-0) The change in bread structure does not appear to vary with the MW of the barley β -glucan.

3.5. Effects of baking and in vitro digestion on the Calcofluor average MW (M_{cf}) and MW distribution of HMW and LMW barley β -glucan

Calcofluor average MW (M_{cf}) and distribution of the b-glucans, within the wheat flour, HMW and LMW barley b-glucan breads and in vitro digests, were determined after

LMW

HMW

Fig. 3. MW (g/mol) distribution of β -glucan in flour, extracts and baked breads. Dotted lines represent 10%, 50%, and 90% percentiles and dashed line represents Calcofluor average MW (M_{cf}) . Values are means of duplicate determinations.

Fig. 4. MW (g/mol) distribution of β -glucan in in vitro bread digests. Dotted lines represent 10%, 50%, and 90% percentiles and dashed line represents Calcofluor average MW (M_{cf}) . Values are means of duplicate determinations.

extraction with boiling water and hydrolysis of starch with a thermostable α -amylase from *Bacillus licheniformis*. This method is illustrated to extract from 7% to 75% of the b-glucan in cereal samples with no apparent depolymerisa-tion [\(Rimsten et al., 2003\)](#page-8-0). The M_{cf} at which 10%, 50%, and 90% of the distribution fall below are illustrated in [Tables 4 and 5.](#page-4-0)

The effects of bread manufacture on the MW distributions differ between the HMW and LMW barley β -glucan [\(Fig. 3\)](#page-6-0). The M_{cf} of HMW barley β -glucan decreased from 64×10^4 to 31×10^4 during bread manufacture. The fact that the LMW barley β -glucan does not change at all is interesting but difficult to explain. The distributions in the control β -glucan are of less importance because of the very low β -glucan content compared to the breads with added β -glucan. The degradation of barley β -glucan during bread processing has been illustrated by [Knuckles, Yokoy](#page-8-0)[ama, and Chiu \(1997\), Andersson et al. \(2004\)](#page-8-0) and [Trogh](#page-8-0) [et al. \(2004\).](#page-8-0) These studies have clearly demonstrated an enzymatic hydrolysis of the β -glucan, most likely from enzymes present in the flour or in added yeast.

The distributions in the in vitro digests do not change drastically during treatment (Fig. 4); this is probably because the enzymes responsible for β -glucan degradation were inactivated during baking. The slight increase in M_{cf} [\(Table 5\)](#page-6-0) might be explained by an increase in extractability.

4. Conclusion

This study clearly illustrates the potential of both HMW and LMW barley β -glucans to improve the nutritional quality of white wheat breads by reducing starch digestibility. This, in turn, may have a potential to regulate in vivo sugar release from white wheat bread, a traditionally high glycaemic food. The results illustrate that loss of dough and bread quality are related to MW, with the HMW barley β-glucan resulting in the greatest changes to dough and bread characteristics. Barley β -glucan of a higher MW appears to be more susceptible to degradation during bread processing than is lower MW β -glucan. Collectively, these results suggest that it may be technologically easier to incorporate LMW barley β -glucan fractions into breads, which may make barley β -glucan more appealing to the food industry.

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References

- AACC. (2000). Approved methods. 10th ed. St Paul, MN: The American Association of Cereal Chemists.
- Andersson, A. A. M., Armo, E., Grangeon, E., Fredriksson, H., Andersson, R., & Aman, P. (2004). Molecular weight and structure of $(1 \rightarrow 3, 1 \rightarrow 4)$ - β -glucans in dough and bread from hull-less barley milling fractions. Journal of Cereal Science, 40, 195–204.
- Anon (2003). Perception and understanding of health components of bread. London, UK: Federation of Bakers.
- Beer, M. U., Arrigoni, E., & Amado, R. (1995). Effect of oat gum on blood cholesterol levels in healthy-young men. European Journal of Clinical Nutrition, 49, 517–522.
- Beer, M. U., Wood, P. J., & Weisz, J. (1997). Molecular weight distribution and $(1 \rightarrow 3)$ $(1 \rightarrow 4)$ - β -D-glucan content of consecutive extracts of various oat and barley cultivars. Cereal Chemistry, 74, 476–480.
- Braaten, J. T., Wood, P. J., & Scott, F. W. (1994). Oat β -glucan reduces serum cholesterol concentration in hypercholestrolemic subjects. European Journal of Clinical Nutrition, 48, 465–474.
- Brennan, C. S., Blake, D. E., Ellis, P. R., & Schofield, J. D. (1996). Effects of guar galactomannan on wheat bread microstructure and on the in vitro and in vivo digestibility of starch in bread. Journal of Cereal Science, 24, 151–160.
- Brighenti, F., Pellegrini, N., Casiraghi, M. C., & Testolin, G. (1995). Effects of guar galactomannan on wheat bread microstructure and on the in vitro and in vivo digestibility of starch in bread. European Journal of Clinical Nutrition, 49, S81–S88.
- Cavallero, A., Empilli, S., Brighenti, F., & Stanca, A. M. (2002). High $(1 \rightarrow 3, 1 \rightarrow 4)$ - β -glucan fractions in bread making and their effect on human glycemic response. Journal of Cereal Science, 36, 59-66.
- Ellis, P. R., Dawoud, F. M., & Morris, E. R. (1991). Blood glucose, plasma insulin and sensory responses to guar containing wheat breads: effect of molecular weight and particle size of guar gum. British Journal of Nutrition, 66, 363–379.
- Foster-Powell, K., & Brand Miller, J. (1995). International tables of glycaemic index. American Journal of Clinical Nutrition, 62, 871S–893S.
- Frank, J., Sundberg, B., Kamal-Eldin, A., Vessby, B., & Åman, P. (2004). Yeast leavened oat breads with high or low molecular β -glucan do not differ in the effects on blood concentrations of lipid, insulin, or glucose in humans. Journal of Nutrition, 134, 1384–1388.
- Gatenby, S. J., Ellis, P. R., Morgan, L. M., Dawoud, F. M., & Judd, P. A. (1996). Effect of depolymerised guar gum on acute metabolic variables in patients with non-insulin-dependent diabetes. Diabetic Medicine, 13, 358–364.
- Gill, S., Vasanthan, T., Ooraikul, B., & Rossnagel, B. (2002). Wheat bread quality as influenced by the substitution of waxy and regular barley flours in their native and extruded forms. Journal of Cereal Science, 36, 219–237.
- James, C. S. (1999). Analytical chemistry of foods pp. 123–125. USA: Aspen Publishers.
- Jenkins, D. J., Jenkins, A. L., Wolever, T. M., Collier, G. R., Rao, A. V., & Thompson, L. V. (1987). Starchy foods and fiber: reduced rate of digestion and improved carbohydrate metabolism. Scandinavian Journal of Gastroenterology, 129, 132–141.
- Johansen, H. N., Bach Knudsen, K. E., Wood, P. J., & Fulcher, R. G. (1997). Physico-chemical properties and the degradation of oat bran

polysaccharides in the gut of pigs. Journal of the Science of Food and Agriculture, 73, 81–92.

- Knuckles, B. E., Hudson, C. A., & Chiu, M. M. (1997). Effect of B-glucan barley fractions in high-fiber bread and pasta. Cereal Food World, 42, 94–99.
- Knuckles, B. E., Yokoyama, W. H., & Chiu, M. M. (1997). Molecular characterization of barley β -glucans by size exclusion chromatography with multiple-angle laser light scattering and other detectors. Cereal Chemistry, 74, 599–604.
- Munck, L. (1989). Practical experiences in development of fluorescence analyses in an applied food research laboratory. In L. Munck (Ed.), Fluorescence analyses in foods (pp. 1–32). Essex, United Kingdom: Longman Scientific and Technical.
- Pick, M. E., Hawrysh, Z. J., Gee, M. I., & Toth, E. (1998). Barley bread products improve glycemic control of type 2 diabetic subjects. International Journal of Food Science and Nutrition, 49, 71–78.
- Rimsten, L., Stenberg, T., Andersson, R., Andersson, A., & Åman, P. (2003). Determination of β -glucan molecular weight using SEC with Calcofluor detection in cereal extracts. Cereal Chemistry, 80, 485–490.
- Suortti, T. (1993). Size-exclusion chromatographic determination of bglucan with postcolumn reaction detection. Journal of Chromatography, 632, 105–110.
- Symons, L. J., & Brennan, C. S. (2004). The influence of a $(1 \rightarrow 3) (1 \rightarrow 4)$ b-D-glucan rich fraction on the physico-chemical properties and in vitro reducing sugar release of white wheat breads. Journal of Food Science, 69, 463–467.
- Trogh, I., Courtin, C. M., Andersson, A. A. M., Aman, P., Sorensen, J. F., & Delcour, J. A. (2004). The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and $(1 \rightarrow 3)(1 \rightarrow 4)$ -β-D-glucan levels. *Journal* of Cereal Science, 40, 257–267.
- Tudorica, C. M., Kuri, V., & Brennan, C. S. (2002). Nutritional and physicochemical characteristics of dietary fiber enriched pasta. Journal of Agricultural and Food Chemistry, 50, 347–356.
- Vaikousi, H., Biliaderis, C. G., & Izydorczyk, M. S. (2004). Solution flow behaviour and gelling properties of water soluble barley $(1 \rightarrow 3, 1 \rightarrow 4)$ b-glucans varying in molecular size. Journal of Cereal Science, 39, 119–137.
- Wood, P. J., Beer, M. U., & Butler, G. (2000). Evaluation of role of concentration and molecular weight of oat β -glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. British Journal of Nutrition, 84, 19–23.
- Wood, P. J., Braaten, J. T., Fraser, W. S., Riedel, D., & Poste, L. M. (1990). Solution flow behaviour and gelling properties of water soluble barley $(1 \rightarrow 3, 1 \rightarrow 4)$ - β -glucans varying in molecular size. *Journal of* Agricultural and Food Chemistry, 38, 753–757.
- Wood, P. J., Braaten, J. T., Scott, F. W., Riedel, K. D., Wolynetz, M. S., & Collins, M. W. (1994). Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. British Journal of Nutrition, 72, 731–743.
- Wood, P. J., Weisz, J., & Blackwell, B. A. (1991). Molecular characterisation of cereal β -D-glucans: Structural analysis of oat β -D-glucan and rapid structural evaluation of β -D-glucan from different sources by high-performance liquid chromatography of oligosaccharides released by lichenase. Cereal Chemistry, 68, 31–39.
- Wood, P. J., Weisz, J., & Mahn, W. S. (1991). Molecular characterisation of cereal b-glucans. II. Size-exclusion chromatography for comparison of molecular weight. Cereal Chemistry, 68, 530–536.