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Guar gum fortified white breads for prospective postprandial glycaemic control – Effects on bread quality and galactomannan molecular weight

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ABSTRACT

Guar galactomannans effectively reduce post-prandial glycaemic responses and can be used to improve the health impact of white bread. Here we compare the impact of four guars containing galactomannans with varying weight-average molecular weight (M_w) at three different wheat flour substitution levels (5, 10 and 15%) on bread quality. The negative impact of galactomannan incorporation on bread quality becomes more pronounced at higher substitution levels and with higher M_w. However, M_w is likely to be an important parameter for the efficacy of such breads. But we observed a degradation of galactomannan during baking, which was likely caused by enzymatic activity in the dough. A decreased contact time between wheat flour and guar gum, along with pre-hydration of the guar, improved sensory attributes such as texture, markedly decreased the presence of large air holes in the crumb and reduced the degree of depolymerisation of galactomannan during baking. The results from this study give an important starting point for future work of optimizing the baking procedure of guar fortified breads keeping in mind not only bread quality but also a retained M_w of the active ingredient.

1. Introduction

Due to their high content of starch, most breads have a high glycaemic index GI (Atkinson, Foster-Powell, & Brand-Miller, 2008). As a diet rich in high-GI foods is associated with increased risk of development of diabetes (Barclay et al., 2008; Ludwig, 2002), reducing the GI of bread, which is a staple food worldwide, could have tremendous health implications.

Viscous dietary fibres such as guar galactomannan (referred to hereafter as just galactomannan) are highly effective at reducing postprandial glycaemic responses when incorporated into foods or drinks containing available carbohydrates such as glucose solutions (Jenkins et al., 1978), mashed potatoes (Fuessl, Adrian, Bacarese-Hamilton, & Bloom, 1986), soup (Wolever, Jenkins, Nineham, & Alberti, 1979), breakfast cereals (Fairchild, Ellis, Byrne, Luzio, & Mir, 1996), crispy bars (Williams et al., 2004) and white bread (Apling, Khan, & Ellis, 1978; Jenkins et al., 1976; Wolever et al., 1979), alone or in combination with other viscous fibres. Effects seem to be higher for liquid compared to solid foods (Wolever et al., 1979) and for foods containing galactomannan instead of a pre-load with galactomannan before the meal (Fuessl et al., 1986). Several studies have shown post-prandial glucose attenuating effects for white bread meals providing 5–16 g guar and 50–106 g available carbohydrate (Boers et al., 2016; Ellis, Apling, Leeds, & Bolster, 1981; Gatenby, Ellis, Morgan, & Judd, 1996; Jenkins et al., 1976; Wolever et al., 1979). The effect has been ascribed to the highly viscous properties of galactomannan and was abolished when hydrolysed non-viscous galactomannan was used in a glucose drink (Jenkins et al., 1978).

However, incorporation of galactomannan into bread at levels necessary to achieve a potential reduction of post-prandial glycaemic response has a negative impact on bread quality. Collapsed walls and large air holes in the crumbs are some of the reported challenges (Apling et al., 1978; Ellis, 1984). In clinical trials, guar breads were judged too moist, with a sticky crumb and hard to swallow by test subjects (Ellis, 1984). Slimy mouthfeel and tooth packing were also reported for other products with high contents of galactomannan (Williams et al., 2004). These negative effects of guar on bread quality were only prominent in breads with high guar content, while lower incorporation levels up to 3% improved bread quality (Apling et al., 1978). Indeed, guar is frequently used as emulsifying, stabilising and thickening agent in the food industry and can significantly improve the shelf life and sensory properties of bakery products. In gluten-free bakery products guar can be used as a stabilizer at concentrations up to 2% (Gambuś, Nowotna, Ziobro, Gumul, & Sikora, 2001) and addition of low levels (e.g. 0.2 g per

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100 g flour) in wheat doughs may improve wheat dough tolerance to frozen storage (Mandala, Kapetanakou, & Kostaropoulos, 2008). Also for fresh breads, incorporation of 0.25–1% guar has been shown to improve dough and bread characteristics of wheat breads (Rodge, Sonkamble, Salve, & Hashmi, 2012).

Some efforts have been made to improve the bread quality of breads containing the high levels of guar gum necessary for a potential reduction of post-prandial glycaemia. Optimization of the baking process and the use of vital gluten, for example, successfully improved the quality of breads with high levels of guar (Apling, Leeds, Wolever, & Jenkins, 1977). Also the use of galactomannan with lower weight-average molecular weight (M_w) resulted in improved bread acceptability (Ellis, Dawoud, & Morris, 1991; Ellis et al., 1981).

Since the ability of galactomannan, and indeed several other soluble dietary fibres with potential to decrease post-prandial glycaemia is linked to their viscous properties, *Mw* and solubility of galactomannan is therefore expected to affect the efficacy of galactomannan containing bread. Viscosity of jejunal digesta of pigs fed guar were negatively correlated with glucose absorption over a 4 h period (Ellis, Roberts, Low, & Morgan, 1995). In a human study with bread, no differences in physiological activity of guar bread prepared with galactomannan of varying M_w was seen (Gatenby et al., 1996). However, although M_w of galactomannan was estimated by intrinsic viscosity measurements in a capillary viscometer, measurement of galactomannan M_w in finished breads or another complex food matrices has never been done. This information is, however, important to fully describe the physicochemical properties of galactomannan in the food as eaten, which may not only help to further explain mechanisms of action but aid the design and development of galactomannan containing foods with optimal physiological effects.

The aim of this study was to increase the knowledge needed for future development of guar breads with optimal bread properties and highest potential efficacy for reduction of glycaemia. Four different guar galactomannan preparations varying in M_w (from 40 to 2200 kDa) were used at three different wheat flour substitution levels (5, 10 and 15%). Galactomannan physicochemical properties such as Mw and solubility were investigated in the final breads and at different stages of the baking process as these parameters are closely related to the potential reduction of glycaemia. Potential strategies to improve both bread quality and galactomannan M_w in final breads were investigated.

2. Materials and methods

2.1. Raw materials

Commercial wheat flour of high protein strength (31.6% wet gluten, dough stability time 16.1 min) was obtained from Lantmännen (Lantmännen Cerealia, Oslo, Norway). Four grades of powdered guar (*Cyamopsis tetragonoloba*) containing galactomannans of different M_w's (M5, M30, M100 and M400) were obtained as a kind gift from Dr. Graham Sworn (DuPont Nutrition & Health, Paris, France).

2.2. Farinograph water absorption

Water absorption was determined in wheat doughs substituted with 0, 5, 10 or 15% (w/w) of four different guars at 14% moisture basis according to ISO 5530–1 (International Organization for Standardization, 2013), using a Perten DoughLab (Perten Instruments, Hägersten, Sweden) equipped with a 300 g bowl. The DoughLab settings were 63 rpm, 30 °C and 500 FU and all measurements were performed in duplicate.

2.3. Standard baking procedure

Doughs containing 300 g composite flour (wheat and 0, 5, 10 or 15% guar), 1% vegetable lipids (A/S Pals, Oslo, Norway), 1.5% NaCl and 1% dry yeast (Idun Industry A/S, Skjetten, Norway) and water according to the determined water absorptions were prepared in the DoughLab. The bowl temperature was set to 22 °C to achieve a final dough temperature of 27 ± 1 °C. After an initial premixing of the dry ingredients at 63 rpm for 1 min, water was added, and the mixing speed was increased to 126 rpm. This speed was kept until the accumulated specific mixing energy reached 11.5 Wh kg^{-1}. All doughs were fermented for 60 min at 27 °C and 70% relative humidity (RH) in a fermentation cabinet (Lillnord A/S, Odder, Denmark). Subsequently, the doughs were divided into three pieces of 150 g and moulded either by hand or in a Dough Rounder R10 (FriulCo Srl, Maniago (PN), Italy) and placed in 8 × 8 cm pans greased with an oil/wax spray (Goldwax, Sonneweld Group, Papendrecht, The Netherlands). The dough pieces were then proved for 45 min at 30 °C and 70% RH in a proving cabinet (Lillnord A/S, Odder, Denmark) and baked in a rotating hearth oven (Revent type 626 G EL IAC, Revent International, Väsby, Sweden) for 20 min. Immediately after the breads were put into the oven, the temperature was reduced from 240 °C to 220 °C and steam (from 2 l of water) was injected. Breads were depanned immediately after baking and cooled for a minimum of 1 h before analysis.

2.4. Mixing energy and dough incubation

To study the effect of energy input during mixing on galactomannan M_w, doughs containing 10% M400 were mixed to a total energy input of 5, 11.5 and 18 Wh kg^{-1} in the DoughLab as described above. The doughs were frozen after mixing. In addition, four doughs containing 10% M5, M30, M100 and M400 respectively were prepared in the DoughLab, as described above, with and without yeast. After mixing, the doughs were divided into 6 equally sized pieces. One piece was frozen immediately in liquid nitrogen. The other five pieces were placed in the fermentation cabinet at 27 °C and 70% RH. One piece was taken out and frozen in liquid nitrogen after 15, 30, 60, 120 and 180 min. Subsequently, all dough pieces were freeze-dried.

2.5. Improvement of baking procedure

Breads with 10% M400 were chosen for improvement of the baking procedure since the reduction of galactomannan M_w was most pronounced for M400 and 10% was the medium substitution level used in this study. Four doughs containing 270 g wheat flour, 1% dry yeast, 1.5% salt and 30 g M400, along with one dough with no added guar, were prepared in duplicate. One of the guar doughs was prepared according to the standard baking procedure. The other three guar doughs were prepared in a two-step procedure. First, doughs without guar were prepared in the DoughLab and fermented for 1 h at 27 °C and 70% relative humidity (RH) in the fermentation cabinet. For one of these doughs, powdered guar was added to the dough after fermentation and mixed into the dough using a Bear Varimixer Teddy (Teddy W5A, A/S Wodschow & Co, Brøndby, Denmark) equipped with a dough hook for 1 min at speed 1. Then, 120 mL water was added followed by mixing for 30 s at speed 1 and 2 min at speed 3. For two doughs, guar was pre-hydrated by mixing with either 120 mL or 200 mL water in the Bear Varimixer Teddy equipped with a whisk for 5 min at speed 4 and left to hydrate for 20 min. The pre-hydrated guar and the doughs were then mixed for 3 min at speed 3 using the dough hook.

Subsequently, all doughs were divided into three pieces of 150 g and moulded either by hand or in a Dough Rounder R10 (FriulCo Srl, Maniago (PN), Italy) and placed in 8 × 8 cm greased pans. Proving and baking procedures were identical to those described in the standard baking procedure.

2.6. Bread characteristics

One hour after baking, weight, volume, and specific volume of three breads from each batch were determined by laser topography using a

BVM 6630 vol meter (Perten Instruments, Hägersten, Sweden). Crumb structure of bread slices (25 mm) was analysed by using a C-cell imaging system (Calibre Control International Ltd., Warrington, UK). This type of image displays the individual cells within the product slice. Each one is colour coded according to its prominence, based on its area and depth, quantified by the "volume" parameter. Bread crumb firmness, expressed as the force at 25% compression, was measured according to the AACC 74-09 method (AACC Approved Methods of Analysis, 1999) using a TA. XT Plus Texture Analyzer (Stable Micro Systems Ltd, Godalming, UK) fitted with a 10 kg load cell and a 35 mm diameter aluminium probe.

2.7. Freeze drying

Breads were freeze dried using a Christ Gamma 1–16 LSCplus (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Weights were recorded before and after freeze dying and the content of free water in the breads was calculated gravimetrically. After freeze-drying the breads were milled on a Retsch ZM100 centrifugal mill with a 0.5 mm mesh (Retsch GmbH, Haan, Germany).

2.8. In vitro digestion

Breads were subjected to a simulated static *in vitro* digestion as described by Brodtkorb et al. (2019). A total amount of 2 g bread crumbs per tube were digested in duplicate as previously described (Rieder, Knutsen, & Ballance, 2017). After digestion, enzymes were inactivated in a boiling water bath for 10 min. Tubes were cooled and centrifuged at 4000 × *g* for 10 min. The supernatants were aliquoted for determination of M_w and galactomannan content.

2.9. Compositional analyses

The content of water and ash (Latimer, 2019), protein (Dumas, 1831; Mariotti, Tome, & Mirand, 2008), lipid (Folch, Lees, & Stanley, 1957) and non-starch polysaccharides (NSP), including galactomannan (Englyst, Quigley, & Hudson, 1994), were analysed using standard methods. Galactomannan contents were estimated by the sum of galactose and mannose content from NSP.

2.10. Determination of weight-average molecular weight of galactomannan

Determination of weight average molecular weight (M_w) of the extracted galactomannans was performed using size-exclusion chromatography with multiangle laser light scattering (SEC-MALLS). To extract galactomannan from the guar powder and freeze-dried breads, approximately 0.5 g samples were boiled with 70% ethanol for 10 min for inhibition of endogenous enzymatic activity. The samples were then centrifuged (1500 × *g*, 10 min) and supernatants were discarded. For removal of starch, the pellets were boiled with thermostable α-amylase (Novozymes A/S, Bagsværd, Denmark) for 10 min. Following centrifugation (1500 × *g*, 10 min), supernatants were transferred to new tubes and polysaccharides were precipitated by adding 1 × volume of 100% ethanol. Polysaccharides in supernatants after *in vitro* digestion were directly precipitated with 1 × volume of 100% ethanol without further extraction. Precipitated samples were centrifuged (1500 × *g*, 10 min) and the supernatants were removed. Arabinoxylans were removed by treating the pellets with 5 U β-xylanase M6 (Megazyme Ltd., Bray, Ireland), and thereafter the pellets were washed twice with 30 mL 50% ethanol and once with 100% ethanol, before drying overnight at 60 °C. Approximately 2 mg of the dried extracted material was wetted with 20 μL 80% ethanol and incubated for 1 h at room temperature, before addition of 1.5 mL 0.1 M $NaNO_3$ containing 0.02 M NaN_3. The samples were boiled in a water bath for 5 min and shaken in a Retsch MM 400 oscillating mill for 5 min at 25 Hz (Retsch GmbH, Haan, Germany). This procedure was repeated until the solutions were clear. The samples were then filtered through a 0.8 μm syringe filter and transferred to HPLC vials.

An amount of 0.1 mL filtered sample was injected on a Shimadzu LC-20 HPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a Tosch PWXL pre column (Tosch Bioscience LLC, King of Prussia, PA, USA) and two size exclusion chromatography (SEC) columns (Tosch Gel G6000 PWXL and G5000 PWXL). The system was running at ambient temperature, using 0.1 M $NaNO_3$ containing 0.02% NaN_3 as eluent at a flow rate of 0.5 mL min^{-1}. Following separation, signals were detected on a system consisting of a Dawn Heleos +8 eight angle laser light scattering photometer (MALLS, $\lambda_0 = 633$ nm), a Viscostar II viscometer and an Optilab T-rEX refractive index detector (dRI) and processed with Astra version 6 software (Wyatt Technology Corporation, Santa Barbara, CA, USA). A first order ($R_2 = 0{,}99$) 'Zimm fit' to Kc/R_θ vs. $\sin^2(\theta/2)$ was used to construct a Debye plot to compute molecular weight (M) for each assumed dilute and monodisperse 'slice' across the chromatogram. A plot of M vs. elution time was then fitted to a first order exponential within the region where detector sensitivity is high and then forward and back extrapolated to higher and lower mass regions where the signal intensity of MALS and the RI detector is respectively low. From this M_w was computed.

The refractive index increment (dn/dc) was set to 0.146 ml/g and the second virial coefficient (A_2, in mol mL g^{-2}) was set to 0. Pullulan (Polymer Standards Service GmbH, Mainz, Germany) with a M_w of 429 kDa was used as control.

2.11. Statistics

Statistical analysis was performed using Minitab 19 (Minitab Inc., PA, USA). Tests of normality (Ryan Joiner test) and homogeneity of variance (Bartlett's test) were performed. Normally distributed data were thereafter analysed using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. Non-normal distributed data were analysed using Kruskal-Wallis non-parametric test, followed by pairwise Mann Whitney tests for identification of differences. Means were considered significantly different at $p < 0.05$.

3. Results and discussion

3.1. Raw materials

Except for M_w of galactomannan, the different guars did not vary much in composition. The galactomannan content ranged between 73

Table 1
Nutrient composition of guars (n = 3). Values are reported as mean ± SD and in g/100 g dry weight, except for weight average molecular weight (M_w) of galactomannan which is reported in kilodaltons (kDa). Different letters in the same row indicate significant differences ($p < 0.05$) between the different guar grades.

	Guar M5	Guar M30	Guar M100	Guar M400
Galactomannan M_w (kDa)	39.3 ± 0.2d	303.3 ± 13.9c	1347 ± 2.4b	2194 ± 44a
Galactomannan	73.2 ± 1.0c	79.0 ± 0.1b	79.1 ± 0.2b	80.7 ± 0.3a
Other NSP	3.3 ± 0.1	3.5 ± 0.2	3.3 ± 0.2	3.4 ± 0.1
Ratio Mannose: Galactose	1.9 ± 0.01	1.8 ± 0.02	1.8 ± 0.02	1.8 ± 0.01
Water	9.4 ± 0.05a	7.3 ± 0.04c	7.5 ± 0.03b	9.4 ± 0.05a
Minerals (Ash)	3.8 ± 0.09a	1.8 ± 0.04b	1.1 ± 0.03c	0.5 ± 0.12d
Lipids	0.2 ± 0.02d	0.5 ± 0.01a	0.5 ± 0.02b	0.4 ± 0.02c
Protein*	3.0 ± 0.05c	3.2 ± 0.03b	3.4 ± 0.05a	2.9 ± 0.05c

* Protein calculated from total nitrogen content, conversion factor 5.4 (Mariotti et al., 2008).

and 81% (Table 1), which is also in accordance with previous studies (Grundy, McClements, Ballance, & Wilde, 2018; Mudgil, Barak, & Khatkar, 2014; Wang, Ellis, & Ross-Murphy, 2002). Water and lipid contents were between 7.2 and 9.5% and 0.2–0.5%, respectively. Protein content was around 3% for all guars, somewhat lower than previously reported, which most likely is due to differences in the nitrogen-to-protein conversion factors applied (Mudgil et al., 2014). The mineral (ash) content increased with decreasing galactomannan M_w, which is in accordance with earlier reports and related to the introduction of salt during hydrolysis treatment to produce low molecular weight samples (Dawoud, 1989; Wang, Ellis, & Ross-Murphy, 2003). The ratios between mannose and galactose, which are a measure of degree of branching, ranged between 1.76 for M30 and 1.86 for M5, which is also in accordance with what has been presented earlier. Non-starch polysaccharide (NSP) contents, other than galactomannan, were the same for all guar gums.

3.2. Water absorption and bread quality

One of the major characteristics of guar is its very high water-absorption capacity. The Farinograph water absorption, reflecting the amount of water needed to achieve a set dough consistency was thus higher in all composite flours than in the pure wheat flour (Fig. 1) and increased both with replacement level and molecular weight of the guar. This resulted in a higher moisture content in the guar doughs, and although some of this extra water evaporated during baking, all guar breads had higher moisture content compared to the control bread (data not shown). These findings are in accordance with earlier studies (Ellis, 1984; Ellis et al., 1991). The higher water content of the guar containing doughs resulted in a slight increase in baking loss compared to the control bread and thus a lower weight of the baked breads. But this cannot explain the observed differences in specific volume (vol/wt), which were instead related to differences in volume of the small breads (150 g dough pieces).

Both replacement level of guar and M_w of galactomannan affected the specific volume. Replacement levels of 5% and 10% resulted in increased specific volumes compared to the control bread for M30, M100 and M400. Replacing wheat flour in a dough inevitably leads to a reduced amount of gluten and reduced development of gluten network enabling entrapment of gas formed during fermentation. Adding hydrocolloids may to a certain extent counteract the reduction of gluten by increasing the viscosity of the water phase in the dough. Higher viscosity of the water phase may contribute to stabilisation of gas bubbles formed during fermentation and thus, improve the gas retention capacity of the dough (Courtin & Delcour, 2002; Rieder, Holtekjolen, Sahlstrom, & Moldestad, 2012). Hydrocolloids with higher M_w increase the viscosity to a higher extent than those with lower M_w. A replacement level of 15% guar resulted in decreased specific volume of all guar breads compared with the control. This may be explained by the higher reduction in gluten proteins due to higher replacement levels, but also by an impaired gluten network development since galactomannan will compete with gluten proteins for water. At 15% replacement level, the gas stabilisation due to increased viscosity of the water phase seems to be unable to counteract the negative effects on the gluten network. It is also possible that the viscosity becomes too high to aid proper gas cell expansion, which could also result in decreased loaf volume. In previous studies a higher water addition than Farinograph water absorption of the wheat flour-guar mixture was found to give better baking results (Apling et al., 1978; Ellis, 1984) but the authors acknowledged difficulties to balance the need for sufficient moisture to avoid stiff doughs (low rise) and too much moisture resulting in sticky doughs (difficulties with moulding). For breads baked with M5 guar, the specific volume was lower than in the control bread for all replacement levels. Due to the low molecular weight of M5, it is not able to generate a high viscosity of the water phase and will therefore not contribute much to improved gas retention, while at the same time wheat flour replacement inevitably results in gluten dilution.

All guar substituted breads had softer crumbs than the control bread. At the 5% wheat flour replacement level, the decrease in crumb firmness was more prominent with increasing M_w, while at 10 and 15% replacement levels differences were mainly between M5 and the other guar grades. The addition of guar to bread has, also in previous studies, been shown to soften the texture (Ashraf et al., 2020; Dawoud, 1989; Ellis, 1984; Ribotta, Perez, Leon, & Anon, 2004). The firmness of the crumb is influenced by a range of factors, such as moisture contents, starch/gluten network, baking time, bulk density of the bread, loaf volume and gas cell structures (Ashraf et al., 2020). In regular wheat bread (control bread in Fig. 2) the small air bubbles formed during fermentation are retained within the gluten network resulting in a bread crumb with evenly distributed small cells. In guar substituted breads, however, air holes had developed in the crumb (Fig. 2). Visually, both size and number of holes seemed to increase with increasing M_w of the

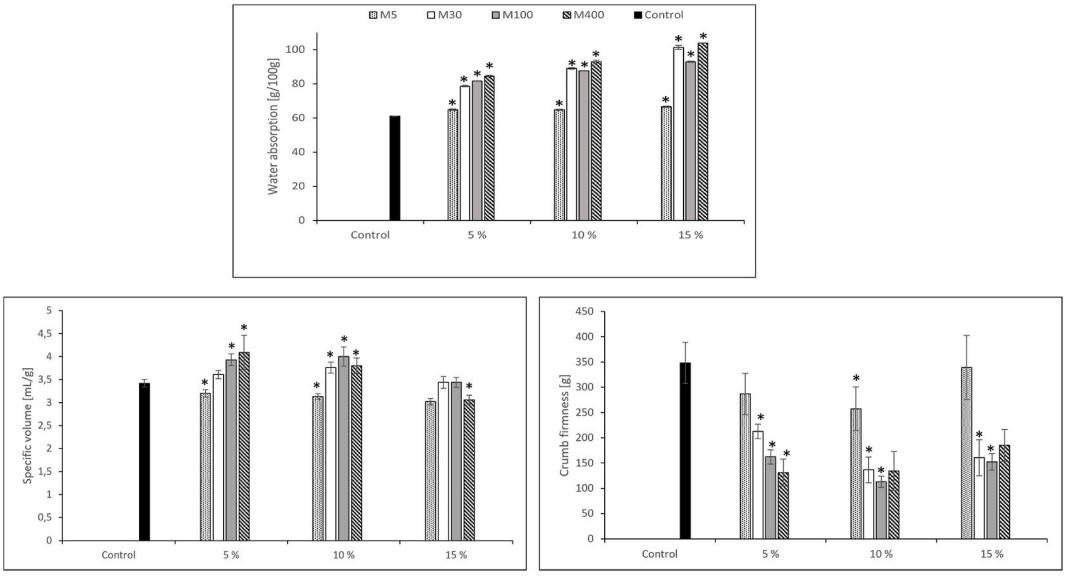

Fig. 1. Water absorption, specific volume and crumb firmness in control bread and breads with wheat flour replacement levels of 5, 10 and 15% of the guars M5, M30, M100 and M400. Values are given as mean ± SD (n = 6). * indicate significant difference ($p < 0.05$) between guar breads and control.

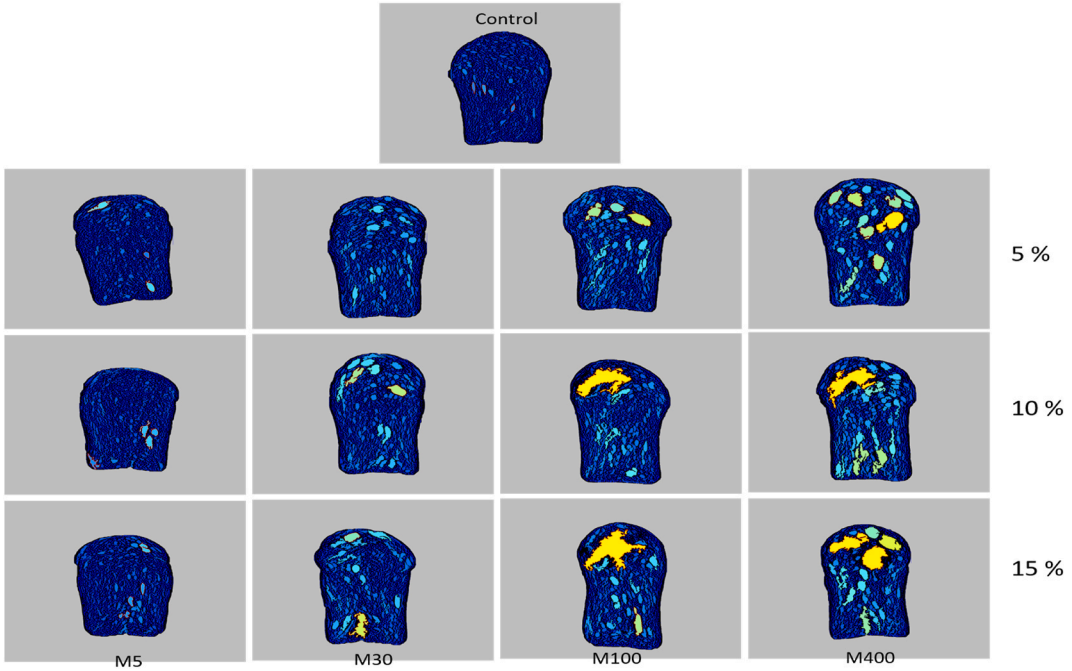

Fig. 2. Images of representative bread slices from control bread and breads with wheat flour replacement levels of 5, 10 and 15% of the guars M5, M30, M100 and M400. The images are adapted from the c-cell software and illustrate cell size and distribution. Small cells are coloured in dark blue and larger ones are shown in lighter shades of blue, green and yellow. Cells large enough to be classified as holes are outlined in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

galactomannan and wheat replacement level. Similar effects have been shown previously (Ellis, 1984; Jenkins et al., 1976). The increased specific volumes were, at least partly, caused by these holes and they may also have affected the texture measurements. Unlike previous studies, no clear problems with collapsed side walls of the loaves were observed, which may be explained by the smaller dough and bread size (150 g vs 470 g dough) in our study but may also be related to differences in both flour and guar quality (Apling et al., 1978; Ellis, 1984).

The M_w of galactomannan was significantly reduced during baking (Table 2). The changes were less pronounced for the low M_w galactomannans in guars (M5 and M30) than in the varieties with higher galactomannan M_w's and reductions were larger for breads with lower addition levels. *In vitro* digestion of the breads did not seem to increase galactomannan depolymerisation further.

Table 2
Weight average molecular weight (M_w) of galactomannan extracted from guar, breads and *in vitro* digesta of breads with wheat replacement levels of 5, 10 and 15% of the guars M5, M30, M100 and M400, along with galactomannan solubility in *in vitro* digesta of breads. Results are given as mean ± SD (n = 2).

		M_w (kDa)			Galactomannan solubility
		Guar	Bread	*In vitro* digesta	
M5	5%	39.3 ± 0.2	37.8 ± 0.1	36.4 ± 0.1	78.3 ± 0.6
	10%		38.2 ± 2.7	37.7 ± 0.5	84.4 ± 2.5
	15%		37.6 ± 0.6	37.0 ± 0.1	86.0 ± 4.1
M30	5%	303.3 ± 13.9	152.3 ± 26.7	189.7 ± 1.7	86.9 ± 0.9
	10%		212.5 ± 1.5	224.3 ± 3.1	87.2 ± 0.9
	15%		231.4 ± 15.5	231.6 ± 7.5	88.2 ± 1.5
M100	5%	1347 ± 2.4	486.8 ± 52.5	519.1 ± 0.1	87.0 ± 6.2
	10%		516.8 ± 23.0	609.0 ± 14.1	86.1 ± 0.9
	15%		571.9 ± 22.7	645.2 ± 2.5	72.1 ± 9.6
M400	5%	2194 ± 44	576.3 ± 43.1	598.2 ± 7.9	89.8 ± 4.6
	10%		593.0 ± 1.3	711.8 ± 21.0	82.3 ± 18.1
	15%		604.9 ± 18.1	736.5 ± 18.2	82.1 ± 15.0

Since previous studies have not measured the galactomannan M_w in the finished food product, such a decrease in galactomannan M_w during baking has never been reported before. It is, however, extremely important as it may have a huge impact on the efficacy of guar breads. The ability of galactomannan to attenuate post-prandial glycaemic responses has been linked to its viscous properties (Ellis et al., 1995; Jenkins et al., 1978). A high M_w and high solubility are required to achieve viscous solutions. While the solubility of galactomannan during simulated digestion was high for all breads (Table 2), the M_w of M100 and M400 was dramatically reduced. Such a reduction of M_w of a viscous dietary fiber during processing has received much attention in the last decades in cereal beta-glucans (Kivela, Henniges, Sontag-Strohm, & Potthast, 2012; Rieder, Ballance, Lovaas, & Knutsen, 2015; Rieder et al., 2017). Cereal beta-glucans are known for their ability to reduce post-prandial glycaemic responses and LDL-cholesterol levels and the EFSA has authorized corresponding health claims for cereal beta-glucans. However, effects of cereal beta-glucans have been clearly linked to M and solubility under physiological conditions in various clinical trials (Tosh, 2013; Wolever et al., 2010). Efforts have therefore been made to minimize beta-glucan degradation during processing (Rieder et al., 2015), which could possibly be transferred to galactomannan. The observed marked degradation of the high, but not the low M_w galactomannans may also help to understand previous results. In a previous study with breads baked with guar gums varying in M_w, no difference in post-prandial blood glucose rise between the breads was noticed (Gatenby et al., 1996). However, since M_w was only measured in the ingredients and not the final bread, this lack of difference may have arisen from a difference in galactomannan degradation during baking as we have seen here (Table 2). Earlier results showing a greater blood glucose attenuating effect of guar in a liquid food (soup) as opposed to a solid food (bread) might indeed also be related to a partial

depolymerisation of galactomannan during bread making (Wolever et al., 1979).

3.3. Mixing energy and dough incubation

The first step to potentially prevent or significantly reduce the degradation of galactomannan during food processing is to understand the mechanism by which it gets degraded. Even though the stability of pure galactomannan solutions has been extensively investigated with respect to thermal, mechanic and enzymatic degradation (Kök, Hill, & Mitchell, 1999; Mudgil, Barak, & Khatkar, 2012; Prajapat, Subhedar, & Gogate, 2016; Wang, Ellis, & Ross-Murphy, 2000), the stability of galactomannan in complex matrices such as bread has never been investigated.

As galactomannan can be susceptible to mechanical energy, the effect of dough mixing on galactomannan M_w was studied by varying the total energy input during mixing from 5 to 18 Wh kg^{-1}, in addition to 11.5 Wh kg^{-1} used in the original protocol. However, this had no impact on galactomannan depolymerisation (data not shown).

Varying the fermentation time, however, resulted in a significant time-dependent degradation of M_w for the high M_w guars (Fig. 3). The changes were, however, only minor for the low M_w grades. However, as the addition of guar in all the doughs is weight based, the observed differences might be related to different enzyme to substrate ratios. Yeast did not contribute to this degradation as the experiment was performed on doughs with (results not shown) and without (Fig. 3) addition of yeast with no differences in degradation.

The pattern of depolymerisation and subsequent broadening of the molecular weight distribution (increase in polydispersity index) for the high M_w guar grades (Fig. 3) is similar to that described for initial (up to 20 h) depolymerisation of pure galactomannan solutions at concentrations of 0.5% (w/w) by β-mannanase (Mahammad, Prud'homme, Roberts, & Khan, 2006). An increase in polydispersity index, in particular, points to enzymatic activity as the cause for depolymerisation of galactomannan in dough rather than mechanical or chemical hydrolysis where polydispersity of galactomannan would be expected to decrease (Mahammad et al., 2006).

3.4. Improvement of baking procedure

Previously it has been shown that a reduction of the degradation of β-glucan, caused by endogenous β-glucanase activity in wheat flour, can be obtained by reducing the contact time between wheat and barley β-glucan during baking by adding the barley flour to the wheat dough after fermentation (Rieder et al., 2015). Since the results in the dough incubation experiment indicated that the depolymerisation of galactomannan might be caused by enzymatic activity, a similar procedure was adapted in this study. Breads containing 10% M400 guar were chosen as model breads in this experiment as depolymerisation of galactomannan seemed to increase with increasing M_w. Since this experiment was performed with a new batch of wheat flour, the control bread (without guar) and the standard baking procedure for 10% M400 (mixing all ingredients prior to fermentation) were repeated. One recipe was prepared by adding dry guar powder with the amount of extra water determined in the WA experiment in the dough after fermentation. In the last two recipes the guar powder was pre-hydrated for 20 min before addition to the doughs, using either 120 mL or 200 mL water in the pre-hydrating process. Optimal hydration of the guar may improve bread quality (Ellis, 1984) but has also been linked to higher efficacy (Wolever et al., 1979).

In Table 3 the bread quality parameters are presented, while Fig. 4 presents images of the breads and the internal structure of the breads. In bread 1, where guar was added before fermentation, most of the parameters were comparable to the corresponding bread from the standard baking (10% M400 in Fig. 1 and Table 2). Minor differences such as a slightly lower crumb firmness and a slightly higher M_w can probably be ascribed to the difference in wheat flour. Bread 1 had the highest specific volume. In addition, the crumb had large air holes, and the mean value of crumb firmness was lower than for all the other breads. The attempt to add guar powder, along with extra water to the fermented dough (bread 2), resulted in a compact, small bread with a hard, "lumpy" texture. This was probably a result of non-efficient hydration of the guar giving a non-uniform distribution of guar particles in the bread crumb. Pre-hydration of guar, regardless of amount of extra water, gave lower specific volume than for the control bread, most likely due to disturbance of the gluten network and loss of air when incorporating guar to the already developed dough by kneading. Both of these breads had a texture quite similar to the control bread and the amount of large air holes was reduced compared to the bread where the guar was added prior to fermentation. Proper hydration of guar through pre-hydration seems to have contributed to a better bread structure compared to bread 2 and helps to avoid the large air holes in bread 1.

The degradation of galactomannan was lower in all of the breads where guar was added after fermentation compared to the one where it was added prior to fermentation. The degradation was lowest for the bread in which guar had not been pre-hydrated. This may be related to incomplete hydration of some of the guar particles and/or non-uniform distribution in the bread crumb, which may have resulted in decreased

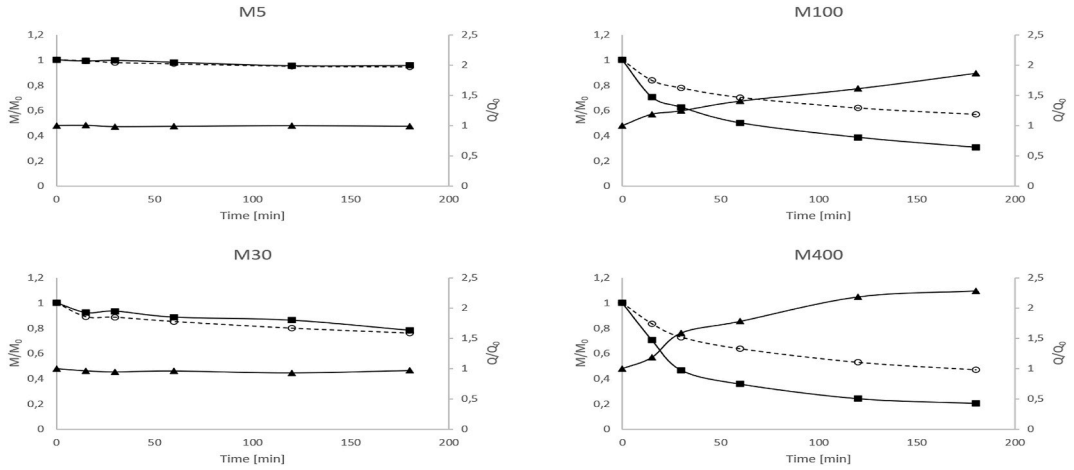

Fig. 3. Relative changes in number (M_n) and weight average molecular weights (M_w) and polydispersity indices (Q) of guar galactomannan as a function of fermenting time in doughs containing 10% of the different guars. Filled squares: M_w, non-filled circles: M_n, filled triangles: Q. M_0 and Q_o are the initial molecular weight averages and polydispersity index, respectively.

Table 3
Specific volume, crumb firmness and M_w in control bread and breads with 10% wheat flour substitution by M400 guar gum with different baking procedures. Values are given as mean ± SD (n = 6) from two replicate doughs. Different letters indicate significant difference ($p < 0.05$) between baking procedures.

	Pre-hydrated	Amount extra water [mL]	Guar added before or after fermentation	Specific volume [mL g^{-1}]	Crumb firmness [g]	M_w [kDa]
Control	n.a.	n.a.	n.a.	3.59 ± 0.09^{b}	155.7 ± 22^{b}	n.a.
1	No	120	Before	3.97 ± 0.14^{a}	93.9 ± 43.4^{b}	704 ± 17^{c}
2	No	120	After	2.02 ± 0.10^{e}	463.6 ± 99.4^{a}	1243 ± 29^{a}
3	Yes	120	After	3.28 ± 0.07^{c}	157.3 ± 43.8^{b}	880 ± 16^{b}
4	Yes	200	After	2.85 ± 0.22^{d}	171.0 ± 35.7^{b}	893 ± 50^{b}

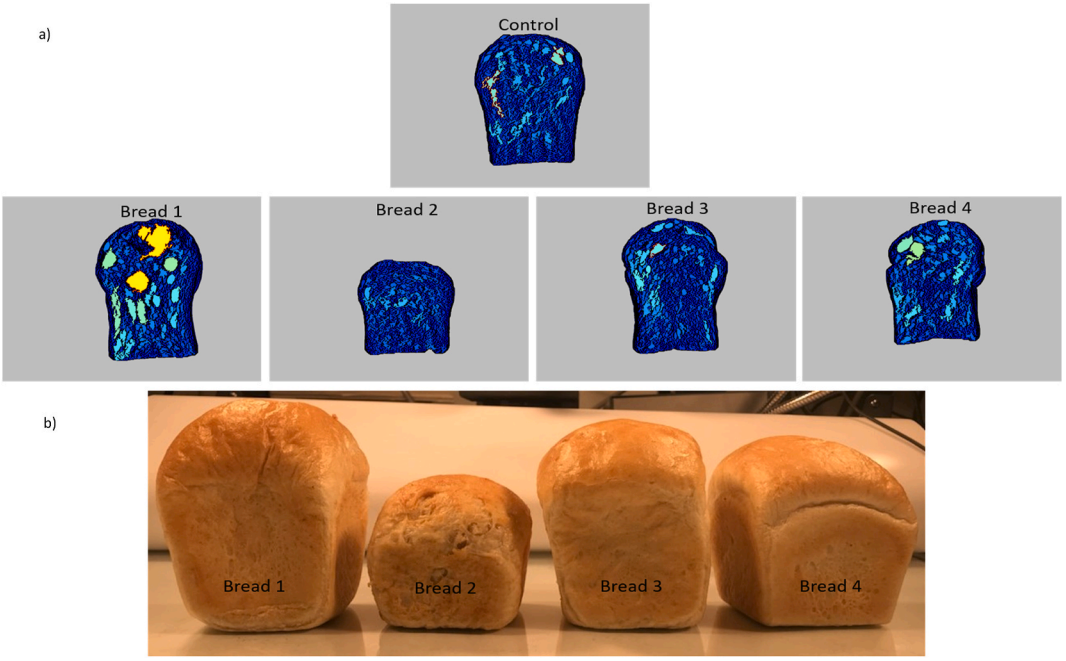

Fig. 4. a) Images of representative bread slices of control breads and breads with 10% wheat flour substitution by M400 guar with different baking procedure. The images are adapted from the c-cell software and illustrate cell size and distribution. Small cells are coloured in dark blue and larger ones are shown in lighter shades of blue, green, and yellow. Cells large enough to be classified as holes are outlined in red. b) Image of breads with 10% wheat flour substitution by M400 guar with different baking procedures. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

degradation of a part of the galactomannan due to decreased accessibility for enzymes associated with the wheat flour. Similar results have been reported for barley bread, where a pre-hydration of barley (scalding) increased the accessibility of β-glucan to degradation by wheat flour enzymes (Rieder et al., 2015). Galactomannan degradation was similar in the two breads with pre-hydrated guar, and lower than in the bread where guar had been added prior to fermentation. Compared to the M_w in the galactomannan directly extracted from guar (Table 2), the reduction of M_w in the pre-hydrated guar breads was 59–60%, while it was 68% in the bread baked using standard baking procedure. Thus, no marked improvement of degradation of galactomannan could be achieved, but the change of the baking procedure improved the structure of the guar breads and the formation of large air holes could be avoided.

4. Conclusions

The development of guar fortified breads with a potential impact on blood glucose regulation is challenging. The negative impact of guar incorporation on bread quality becomes more pronounced at higher substitution levels and with higher M_w of the galactomannan in the guar. However, molecular weight is likely to be an important parameter for the efficacy of such breads. The observed degradation of galactomannan during baking is therefore another challenge, which needs to be overcome. The depolymerisation of galactomannan during baking was shown to likely be caused by enzymatic activity. Decreasing the contact time between wheat flour and guar, along with pre-hydrating the guar, improved sensory attributes such as texture, markedly decreased the presence of large air holes in the crumb and reduced the degree of depolymerisation of galactomannan during baking. However, further research is needed to further optimize the baking procedure with regards to both bread quality and galactomannan M_w. The impact of M_w, solubility and viscosity of galactomannan in guar fortified breads on post-prandial glycaemic regulation should be investigated *in vivo*.

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CRediT authorship contribution statement

Hanne K. Mæhre: Conceptualization, Formal analysis, Data curation, Writing – original draft, Conceived and designed the analysis, Collected the data, Contributed data or analysis tools, Performed the analysis, Wrote the paper, Revision and approval of the manuscript to be published. **Sophia Weisensee:** Formal analysis, Data curation,

Collected the data, Contributed data or analysis tools, Performed the analysis, Revision and approval of the manuscript to be published. **Simon Ballance:** Conceptualization, Formal analysis, Data curation, Writing – original draft, Conceived and designed the analysis, Contributed data or analysis tools, Wrote the paper, Revision and approval of the manuscript to be published. **Anne Rieder:** Conceptualization, Formal analysis, Data curation, Writing – original draft, Conceived and designed the analysis, Contributed data or analysis tools, Wrote the paper, Revision and approval of the manuscript to be published.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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