Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads.

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Abstract

The impact of associated viscous dietary fibers (hydroxypropylmethylcellulose semi-firm –SFE- and weak –NE- gel forming, and barley ß-glucan, BBG) incorporated at different amounts (1.6–7.5%, flour basis) into gluten-free rice-based dough formulations, on the breadmaking performance and staling behaviour of hydrated (70-110%, flour basis) fibre-flour composite blends has been investigated. Single BBG addition fails to mimic gluten visco-elasticity properly, but simultaneous incorporation of either SFE or NE contribute to bread improvement in terms of bigger volume and smoother crumb. 3.3 g of BBG (70% purity) and 104 mL of water addition to 100 g rice flour provided sensorially accepted breads (7.6/10) with a theoretical ß-glucan content of 1.24 g/100 g GF bread that would allow a daily ß-glucan intake of 3 g provided a bread consumption of 240 g/day. A daily intake of 240 g of BBG-enriched GF bread (four servings) is high enough to meet the requirements of the EFSA health claim (3 g/day), contributing a reduced blood cholesterol level. Complementary tests should be carried out to test the amount and molecular weight of ß-glucan in the final bread before assuring the nutritional benefit of this addition.

Keywords: Gluten-free; Hydrocolloids; ß-glucan; bread quality
1. Introduction

The increased consumer demand for healthy foods has driven to address concerted efforts from both research and industry to develop breads that combine properly health benefits with good physico-chemical and sensory properties. This goal is specially challenging in gluten-free (GF) breadmaking where the lack of gluten biopolymer seriously constrains dough visco-elastic character, leads to a failure in carbon dioxide entrapment, and hence deteriorates the techno-functional quality of resulting breads. In addition, a poor nutritional balance often characterises the multi-ingredient GF matrices (Thomson 2009). GF baked goods are often low in fibre, both soluble and insoluble; consequently its enrichment with dietary fibre seems to be necessary (Sabanis et al. 2009).

The natural, synthetic and biotechnological hydrocolloids, because of their high water-binding capacity and their structure-creating behaviour, are mostly used in the different recipes for replacing the gluten network and its functionality (Houben, Hoechstoetter, and Becker 2012). Water availability plays a crucial role in the functionality of hydrocolloids by binding to the macromolecules in three different ways: via hydrogen bounds, embedded in inter- or intramolecular openings or immobilized by structuring (Anton 2008). The modified cellulose derivative hydroxypropyl-methyl-cellulose - HPMC- (linear and neutral polymer) has, because of its hydrophilic character, a high water-binding capacity and also has, in its structure, hydrophobic methyl and hydrophilic hydroxypropyl groups located, which makes HPMC an interface activity in the dough system during the resting period promoting dispersion and preventing coalescence of the gas bubbles. HPMC can create a reversible, heat-set gel network (Haque et al 1993) that leads to an increase in dough viscosity and stabilization of the
boundaries of the expanding gas cells. During baking, the gas-binding capacity is increased and higher volume can be achieved (Bell 1990; Collar et al 1999).

The positive effects of isolates of cereal β-glucans have been recently reviewed by Wood (2010), with most of the data deriving from studies with oat β-glucans, followed by barley and rye (Kinner et al 2011). The European Food Safety Authority (EFSA) has recently approved health claims for foods that contribute to the diet 3 g per day of β-glucan (BG) from oat or barley for its ability to reduce the LDL-cholesterol level in blood, and for foods that provide at least 4 g / 30 g carbohydrate, for reducing the postprandial glycaemic response (EFSA 2011). Isolates of cereal β-glucan are hydrocolloids with thickening properties (Lazaridou et al 2007) that could replace or supplement the action of HPMC when added in appropriate amounts, besides increasing nutritional value of GF bread in terms of dietary fiber content with proven health promoting effects. It is also stressed that high concentration of β-glucan decreases the water availability for the protein network and thus impairs the baking properties of wheat breads (Gill et al 2002). Molecular mass and structure, chain length, bonds and chemical modification, added doses, raw materials and process parameters used -pH value, temperature, shearing, ionic bonds and the attendance of ions- account for some factors determining the significance of associated hydrocolloids in bread performance (Houben, Hoechstoetter, and Becker 2012).

Previous studies have shown a great difference in the effect of β-glucan and HPMC of different gel strengths on gluten-free rice dough viscoelastic behaviour (Collar et al 1999; Collar, Santos, and Rosell 2005; Collar, Santos, and Rosell 2007; Ronda et al 2013). The water competition of the fibre macromolecules expliciting different water binding and gelling abilities resulted in additive, synergistic and/or antagonistic effects on major rheological features. The present study aims to establish the effect of -viscous
dietary fibres blends at different hydration levels on GF bread quality and its staling keepability. A correlation study between dough and bread properties was carried out to know the relationships between rheological performance and physico-chemical and sensory pattern of β-glucan-enriched GF breads.

2. Material and methods

2.1 Materials

Rice flour (12.5% moisture, 0.46 % ash, 7.5% protein, 0.49 % fat and 79.1 % starch, particle size distribution: 6% > 150 µm, 150 µm > 63.2% > 100 µm, 30.8% < 100 µm) was supplied by Herba Ricemills S.L.U (Tarragona, Spain). Salt, sugar, and sunflower oil were purchased from the local market. Two types of hydroxypropylmetylcellulose (HPMC) (E464) from Shin-Etsu Chemical Co Ltd. (Japan) were used: HPMC SFE4000 (27 – 30 % methoxyl content, 4.0 – 7.5 % hydroxypropoxyl content) coded SFE and HPMC NE4000 (19 – 24 % methoxyl content, 4.0 – 12 % hydroxypropoxyl content) coded NE. According to manufacturer’s application notes, both types of HPMC (10 % moisture content) develop the same apparent viscosity (4300 ± 1300 cP) in 2% aqueous solution at 20°C, but different gel strength after thermal treatment. HPMC-SFE forms a semi-firm gel (gelation temperature 58-63°C) while HPMC-NE 4000 forms a weak and sticky gel (gelation temperature 61-65°C). (1-3)(1-4) β-glucan (BBG) was obtained from barley, Barliv™, and supplied as free sample by Cargill (Barcelona, Spain). The characteristics of BBG were: 6 % moisture, 2.2 % soluble protein, 2.6 % ash and 0.9 % fat (commercial data); 70% purity (Megazyme® kit); 5.6 % starch (Megazyme® kit); 140 kDa molecular weight (size exclusion HPLC). BBG extract was analysed for gluten content and a concentration under the detection limit was obtained (< 6.2 mg/kg of gGluten) using the ELISA test based on the R5 antibody.

2.2. Dough preparation and breadmaking
A straight dough process was performed using the following formula on a 100 g rice flour basis: 6% oil, 5% sucrose, 2% salt, 3% dried yeast, and 70% water. Combinations of fibres according to a Draper-Lin small composite design for sampling (Draper and Lin 1990) were added to the basic formula at different hydration levels (Table 1).

Design factors (quantitative independent factors) tested at five levels (-1.4142, -1, 0, 1, 1.4142), included SFE (from 0.10 to 2.50 g/100 g flour basis), NE (from 0.10 to 2.50 g/100 g flour basis), BBG (from 0.10 to 3.90 g/100 g flour basis), and WATER (from 0 to 40 mL extra water with respect to 70 mL/100 g flour basis, that was the minimum amount added). The model resulted in 19 different combinations of fibre-enriched hydrated rice-based doughs, including three central point replicates. These replicates were made in order to know the repeatability and accuracy of results. GF dough-making was achieved by blending first solid ingredients in a kitchen-aid professional mixer (KPM5) for 10 s at speed 2. Then, liquid ingredients (oil and water at 20 ± 2°C) were added and mixed for 5 min at speed 6. The dough, 200 g, was placed into an aluminium oil coated pan and was proofed at 30°C and (90 ± 5)% relative moisture for 40 min. Subsequently, baking was carried out in a Salva oven (Lezo, Spain) at 190°C for 40 min. After baking, breads were removed from the pan and left for one hour at room temperature before analysis. To study the effect on staling, breads were stored for 0, 1, 2, 5, 7 and 9 days at (4 ± 2) °C. To study the effect on staling, breads were stored in hermetic polypropylene bags for 0, 1, 2, 5, 7 and 9 days at (4 ± 2) °C. Seven breads were made in eachper run (one batch).

2.3. Evaluation of bread quality
Bread volume was determined in duplicate using a volume analyzer BVM-L370 TexVol Instruments (Viken, Sweden). The bread was weighed immediately after removal from the pan once cooled. A digital calibre was used to measure bread height and width.

Crumb texture was determined in triplicate with a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) provided with the software “Texture Expert”. An Aluminium 20 mm diameter cylindrical probe was used in a “Texture Profile Analysis” double compression test (TPA) to penetrate to 50% depth, at 1 mm/s speed test, with a 30 s delay between first and second compression (Collar, Bollain, and Angioloni 2005). Hardness (N), chewiness (N), cohesiveness, springiness and resilience were calculated from the TPA graphic. Analysis was carried out at (20 ± 2) ºC for bread slices of 20 mm thickness taken from the centre of the loaf.

Colour was measured with a Minolta spectrophotometer CN-508i (Minolta, Co.LTD, Japan). Results were obtained in the CIE L*a*b* coordinates using the D65 standard illuminant, and the 2º standard observer (CIE 1931). The hue (h) and the chroma (C*) were calculated from them with the equations 

\[ h = \text{atan}(b*/a*) \]

\[ C* = ((a*)^2 + (b*)^2)^{1/2} \]

(Ronda et al 2005). L* ranges from 0 (black) to 100 (white). The hue scale extends from 0º (red), 90º (yellow), 180º (green) to 270º (blue). The chroma informs about the purity of the colour: a near zero C* value corresponds to a colour of low purity, near grey. On the opposite high C* values mean colours of high purity near the pure spectral colours.

Colour determinations were made 5x5 times: bread crumb and crust colours were checked at five different points on each bread and every point was measured five times.

2.4 Sensory analysis

Sensory analysis was performed by a panel of ten semi-trained judges (four males and six females aged 25–52), who scored the size and the uniformity of cells (crumb grain), the softness and chewiness of the crumb, the crumbliness of the crust, the taste and...
Flavour (intensity and type), and the overall acceptability. Ratings were given to breads wrapped in plastic bags and stored at room temperature for 24 hours. A semi-structured scale from 1 (very little) to 10 (very much) was used. The attributes tested were: crumb grain (1=very large and inhomogeneous cells; 10= very small and homogeneous cells), crumb softness (1= very hard; 10= very soft), crumb chewiness (1=very rubbery, requiring many bites to swallow; 10= very little rubbery), crust crumbliness (1= very soft and annealed crust; 10=very crunchy crust) taste and flavour intensity (1: very little; 10: very much). Additionally, an hedonic, overall acceptability test was also included in the sensory evaluation with an scale from 1 (dislike very much) to 10 (like very much).

2.5. Statistical analyses

Multivariate statistical analysis of data (non-linear regression, stepwise regression analysis, and Pearson correlation analysis) was performed by using Statgraphics Centurion v.6 program (Bitstream, Cambridge, MN, USA).

3 Results and discussion

Morpho-geometrical features, texture characteristics and sensory scores were measured to assess the quality and staling behaviour of fibre-enriched GF breads made according to a Draper-Lin design. Retrieved instrumental physical parameters and sensory results were analysed for dependence on dough hydration and on viscous dietary fibres, and for correlations between dough and bread parameters (Table 5 and 6).

3.1 Effect on physical properties of fresh breads

Analytical data on bread characteristics (Table 2) were fitted to multiple regression equations using added principles (SFE, NE, BBG, WATER) as independent factors in order to estimate response surfaces of dependent analytical variables. Significant
coefficients (95% confidence interval) obtained from the stepwise regression fitting model are included in Table 32.

Specific volume and height/width rate

Loaf specific volume of the breads, that varied between 1.4 and 5.1 mL/g, and the loaf height/width ratio, that ranged 0.28 – 0.87, exhibited similar trends, as could be expected in pan breads where the width comes mainly determined by the mould size.

The multiple regression equations obtained for height/width and specific volume explained the 99% and 91% of their variability, respectively (Table 42). Specific volume increased with WATER, SFE and BBG addition until a maximum (Fig.1a and Fig.1b). The positive coefficients of the linear terms and the negative coefficients of the quadratic ones of these three factors account for this evolution (Table 24). It should be noticed the non significant individual effect of NE (p>0.05) on both the height and the specific volume of breads, were probably masked by the greater effect of the remaining design factors. The comparison by pairs of the experimental results obtained for each run (runs 1, 5, 10), allowed to confirm a significant increase (p<0.05) in the specific volume for increasing SFE amounts, provided remaining factors keep constant (data not shown). A significant positive interaction SFE*NE was observed, which means that NE enhanced the individual SFE effect on bread size. The effect of SFE on specific volume did not showed a significant dependence on dough water content, being 1.6% the dose that led to the maximum size of the bread (Fig 1.a). From the multivariate regression equation the individual addition of 1.6% SFE to the dough with the minimum water content tested (70%, equivalent to 0 level in the design) would nearly double the initial volume of the bread, passing from 1.4 mL/g to 2.7 mL/g. For this SFE dose, a water increase from 70% to 90% would lead to an additional specific volume increase by 37%. 90% of dough hydration (equivalent to 20 level in the design) led to the
maximum bread specific volume, only dependent on BBG dose. The positive interaction 

BBG*WATER (Table 42) indicates that water favors the effect of BBG on specific 

volume. However, optimal water content of dough was not significantly dependent on 

SFE and NE. This fact establishes an important difference between the HPMC and BBG 

action on GF breads: BBG requires an important additional amount of water to show a 

beneficial effect on bread volume while HPMC acts even in adverse conditions of low 

dough hydration showing an effect little dependent on dough water content. The effect 

of BBG on the bread specific volume is shown in Fig 1b. The individual addition of 

BBG to dough (70% water, flour basis) decreased markedly the height and the volume 

of GF breads (see also the negative coefficients of the quadratic terms). The same effect 

was observed in wheat breads by Brennan & Cleary (2007) when β-glucan content 

varied from 2.5% to 5%. Some authors (Cavallero et al 2002; Gill et al 2002) relate the 

above mentioned effect to the high water binding capacity of β-glucan that restricts 

available water for the development of gluten network. The same reason might explain 

BBG effect on GF breads (Ronda et al 2013). Doughs with too low disposable water 

are probably too consistent to get a certain development during proofing and subsequent 

baking (Ronda et al. 2013). BBG added to reduced-water doughs is unable of 

establishing cross-links or entanglements in the dough and, thus, dough structure cannot 

be developed. It was previously shown that BBG was unable to decrease the elastic 

compliance, $J_0$, at dough water content of 70% as HPMC did (Ronda et al 2013). But, in 

these conditions, it conferred the major effects on dough $G'$ modulus. So that, BBG 

addition to doughs only increased dough consistency, which made dough development 

even more difficult, leading finally to a lower bread volume. Simultaneous addition of 

water and BBG counteracted the single BBG effect through the significant positive 

efficient of the interaction BBG*Water (Table 4), leading to an increase of 34% in
bread volume compared to the maximum value obtained in BBG absence. In a previous work (Ronda et al. 2013), a significant BBG*Water negative interaction on the elastic modulus \( G' \) was found - for the same GF doughs, - allowing to relate the increase in bread volume with a decrease in dough consistency. The additional amount of water required to get the maximum volume in bread increased at a rate of 5 - 6 % per 1% increase of BBG in dough formulation (flour basis in both cases): the dough hydration needed for 2% BBG would be 101%, and for 3.5% BBG it would be 109 %. However, the model predicts that without HPMC, even with the optimal dose of water, BBG addition above 2.5% would start to decrease specific volume attaining a reduction of 24% at the maximum dose tested (3.9%). Lazaridou et al. (2007) also observed a maximum on bread specific volume with water, and obtained an additional increase in bread volume by 1% - 2% \( \beta \)-glucan addition to dough concluded the same results in GF doughs when water and \( \beta \)-glucan varied concomitantly - (Lazaridou et al 2007). Significant bread volume increases were also obtained when oat and barley dietary fibres were added to GF doughs of adapted hydration - (Sabanis et al. 2009). They also observed a maximum on bread specific volume with water, and obtained an additional increase in bread volume by 1% - 2% \( \beta \)-glucan addition to dough. Hydrocolloids can improve dough development and gas retention by increasing dough viscosity (Houben, Hoechstoetter, and Becker 2012), but, there is an optimum value for the resistance to deformation. Too high values can cause a limited and slow expansion of the gas cells during proofing (Van Vliet et al 1992). From the negative sign of the quadratic coefficients of BBG and SFE, a dose leading to a maximum in bread height and specific volume may be retrieved. For HPMC, besides water retention properties associated to hydrophilic nature, hydrophobic groups induce additional properties including increased interfacial activity within the dough system during proofing, and leading to gel network
formation on heating over the breadmaking process. Such network structures serve to increase viscosity and to further strengthen the boundaries of the expanding cells in the dough, thus increase gas retention through baking, and consequently lead to a better loaf volume (Bell 1990).

It is noteworthy that formulations yielding higher specific volume and loaf height in breads correspond to high doses of both SFE and water that gave poor quality breads with large gas cells (pictures not shown), in agreement with previous works (Haque and Morris 1994; McCarthy et al 2005; Nishita et al 1976) on high water GF breads. Authors related pocket formation to a poor dough consistency due to an excess of water. Up to a certain level, a soft consistency, as promoted by high water addition and limited amounts of hydrocolloids, seems to be advantageous, allowing a larger increase in volume. However, the excessive low consistency seems to cause the bubbles to become unstable, coalescing and resulting in large holes, after the crust has formed. In preliminary tests, with individual additions of either SFE or NE, it was found that the occurrence of grain defect took place mainly for SFE breads and for water doses above 90%, flour basis. Owing to the differences between the two types of HPMC used in the present work, defects could be related to the facility of crust forming that could be higher in the case of SFE (a semi-firm gel forming) than in NE (a weak gel forming) helping retain the large bubbles formed inside the crumb. With no exception, defects were only observed at water amounts above 90%.

**Loss of weight**

The loss of weight during baking varied between 26 g and 41 g, equivalent to 13% and 21% with respect to the initial amount of the baked dough (Table 2). Table 42 shows that the loss of weight depended mainly on the water content of the dough. The significant \( p<0.05 \) coefficients of the regression equation
quadratic coefficients) obtained for water indicate that water loss increased up to a maximum with increased dough water content (Table 42). When water content is low, increasing water results in an increase of the rate of water lost by evaporation. However, when the water content is high enough, above a critical amount an additional water increase did not lead to an additional increase in the loss of weight, since the amount of water lost during baking is determined by baking time that remained constant in this study. In absence of hydrocolloids the maximum loss of weight increase was 20% and took place at a dough hydration of 94%. The marked effect of water content on loss of weight during baking probably masked the effect of the remaining design factors.

Although no significant coefficients were obtained for SFE in the multifactor regression, the comparison by pairs of the experimental results obtained for each run (see runs 3, 7 and 9), allowed to confirm a significant increase (p<0.05) in the loss of water during baking for increasing SFE amounts, provided remaining factors keep constant (data not shown). Similar results were previously obtained in hydrocolloid-added cake systems where HPMC was the only tested hydrocolloid that led to losses of weight in baking above the control cake (without hydrocolloid) (Gómez et al. 2007). The individual addition of BBG to dough decreased the loss of weight during baking as indicated by the negative linear significant coefficient. At a flour hydration level of 70%, the individual addition of 3.9% BBG led to a 27% decrease in the loss of weight. This was probably due to the high water binding capacity of β-glucans in line with previous results (Brennan and Cleary 2007). However, the positive sign of the BBG*WATER interaction coefficient anticipates that an increase in dough water content decreased the individual ability of BBG to reduce the loss of weight during baking. Accordingly, the amount of water that led to the maximum loss of weight in BBG absence, 94%, increased from 94% to 104% for 2% BBG and to 112% for 3.9%
BBG. The mentioned dough hydration values that maximized the loss of weight during baking also maximized the bread specific volume at each BBG addition.

Texture of the bread

Fresh bread crumb firmness varied from 1 N to 17 N (Table 4), which means a very wide range of crumb hardness among tested breads. From the multiple regression study, firmness was only significantly affected (p < 0.05) by both water and BBG (Table 4). These only two factors could explain 91% of the variability of fresh bread firmness, as indicates the R² value included in Table 4. The effect of cellulose derivatives, which was detected by the analysis and comparison of individual experiences (Table 2), was notably smaller than that associated to the other two ingredients. The individual addition of SFE led to a decrease in firmness (runs 3, 7 and 9) that did not reach a significant level, while NE hardly showed any effect (runs 1, 5 and 10). In absence of hydrocolloids, a significantly softer crumb was observed when the water content was increased from 70% to 90% (Fig. 1c), as it was observed earlier (McCarthy et al 2005). However, as can be predicted from the positive quadratic coefficient, additional amounts of water made crumb firmer again. Firmness evolution could be explained in parallel to specific volume evolution. In fact, both properties showed a significant (p<0.001) negative correlation (see section 3.3). Water dosage that led to a minimum crumb firmness led to a concomitant maximum specific volume. The same could be reported from the BBG effect. Additional amounts of water in absence of hydrocolloids, probably decreased dough consistency too much and hindered dough gas retention ability during proofing and baking. The fact would explain the decrease in bread volume and the consequent increase in bread firmness.

The individual effect of BBG can be concluded from the high positive coefficient of the linear term of the regression equation (Table 4). Accordingly, the individual
addition of BBG to the lowest hydration dough (70% flour basis, 0 level of water),
increased bread firmness more than seven times the initial firmness of bread (runs 2 and 8). Other authors observed that BBG increased firmness and decreased loaf volume of wheat breads (Brennan and Cleary 2007; Cavallero et al 2002). These effects were attributed to gluten dilution and to the BBG ability to binding appreciable amounts of water, making it less available for the development of gluten network. Similar results were obtained in GF bread related to the high water binding of BBG. However, the significant negative interaction between water and BBG explains that the simultaneous addition of BBG and water counteracted the individual effect of BBG. From the predictive model, addition of 4% BBG with water content above 95 % would lead to crumbs with a similar firmness to those obtained with doses of cellulose derivative around 1.5 %. Fig. 1 shows the firmness of fresh breads and their evolution during nine days of storage. It allows the comparison of runs where only varies a factor while the rest are constant in the intermediate level of the range studied. The comparison of runs 7 and 11 shows the importance of dough hydration on BBG-enriched GF bread development (Table 2). Chewiness of breads ranged 0.3 – 5.8 N and varied in parallel as hardness did (Table 4). This could be expected as both textural properties are directly related. Cohesiveness (varied between 0.4 and 0.6) and springiness (ranged 0.6 – 0.9), also involved in chewiness equation, showed minor effects. The resilience variation could not be correlated to the studied factors, probably due to the low values and the low variations observed between runs (0.19 – 0.29). Springiness - or the after stress retarded recovery capacity, increased with single addition of BBG and/or water (see significant quadratic terms in Table 2). From the multivariate regression equation it could be predicted a 21% increase in crumb springiness obtained from low hydration dough.
(70% water) with 3% BBG and of 39% for 3.9% BBG. In absence of hydrocolloids the
increase of dough hydration from 80% to 110% led to springiness increases around a
10% per each 10 % increase in dough water content. The negative interaction
WATER*BBG (Table 4)-explains that the simultaneous increase of water and BBG in
dough did not lead to the expected springiness as result of the sum of the individual
effect of Water and BBG.

**Crumb and crust colour**

Bread crust colour features showed bigger dependence on both hydrocolloid and water
contents, than bread crumb. Bread crusts showed lightness values that ranged 60-74%.
The a* and b* coordinates varied between 2–13 and 19–30 respectively (see Fig 2)
leading to hue values that ranged of 64 – 84 degrees, and chromas between of 19 – 32
(Table 2-data not shown). Crust colours had higher L* and b* coordinates and lower a*
one than other authors found (Lazaridou et al, 2007) probably due to the use of corn as
starch source in that work. This could be due to the presence of corn starch and
caseinate in the formula of the previous work. In our case, the matrix was rice flour, of a
very white colour, that hardly contributed to the bread colour. Attending theFrom
multivariate regression equation (, from the positive quadratic term in Table 4 Table 2) it
would be predictable a 10% increase in bread crust Lightness for the maximum dough
hydration of 110% (40 level) with respect to the lowest one (70%). This was probably
due to the reduction of Maillard reaction progress, main responsible for crust
browning during baking, dueas consequence of to this reaction precursor’s dilution.

Single SFE incorporation addition to a dough with the minimum water content, 70%, to
dough would lead to a 12% of maximum decrease in bread crust Lightness L* at a dose
of 2%. However, as it can be concluded from the negative regression coefficient.
Decrease of available water in crust due to the SFE water binding in a matrix with
limited amount of water (70% water, flour basis) may affect the Maillard reaction. An additional dose of SFE did not show additional effect (Table 2), as predicted by the positive coefficient of the quadratic term shown in Table 4. Probably, above 2%, SFE hardly hydrated, and did not contribute to Maillard reaction notably. Single BBG increased crust Lightness until 14% for the maximum dose tested, 3.9%, in accordance with the positive quadratic coefficient in the regression equation. The effect, also observed in previous works (Lazaridou et al 2007), could be explained by the already mentioned BBG water retention capacity during baking, that led to breads with significantly lower weight loss. Simultaneous increase of BBG and Water led to darker bread crusts than expected for the individual action of BBG, as indicated by the negative sign of the interaction (significant BBG*Water coefficient), concomitant with the increase in baking water loss already mentioned in the previous section.

Bread crust hues significantly affected by all the factors studied (Table 2) corresponded to more yellowish (h=90) than reddish (h=0) colours. Bread crust hue was significantly affected by all the factors studied (Table 4). It was observed that, in general, breads with lower crust hues (more reddish) had lower Lightness values. This is probably due to a common origin of both crust colour attributes. The circumstances that favoured Maillard reaction and decreased Lightness (SFE increase and the water/-BBG decrease) led to reddish and darker bread crust. Lazaridou et al (2007) also observed a tendency to yellowish in 1% oat β-glucan-added GF-bread crusts (Lazaridou et al 2007). The crust hue was also significantly decreased by NE, although in less extent than SFE, as it can be concluded from the lower linear and quadratic regression coefficients (Table 4). Crumb colour, that mainly depends on the own ingredient colours, was not very affected by the design factors due to the white-slight cream colour of added fibers. Only the
chroma showed significant linear dependence on dough hydration and on viscous
dietary fibres with a $R^2$ of 81.4%. The $a^*$ and $b^*$ coordinates of all bread crumbs ranged
from -0.23 to +0.44 and from +7 to +12 respectively. These values corresponded to
crumb hues ($h$) from 87 to 91 degrees, which correspond to yellow colours. The chroma
($C^*$) values ranged 7 to 12. Dough hydration had the highest effect on crumb chroma.
With no added hydrocolloids the maximum water content increase (40%) led to 59% increase in the crumb chroma (leading to the highest crumb chroma values (more vivid colours)). The presence of both types of HPMC counteracted the individual effect of water.

**Bread staling**

Bread staling was assessed by means of hardness evolution over 9 days (Figure 13). Experimental data were fitted to the Avrami equation (Armero and Collar 1998; Ronda and Roos 2011), and adjusted values were plotted leading to the continuous lines. Hardening during storage is expected as a result of moisture loss and starch retrogradation (Ronda et al 2011). Water was the factor with more prominent effect on GF bread staling, as shown. Figure 3c shows that the bread made with the lowest water content, 70% in flour basis reached, had after one storage day, a hardness ten times that of the bread with a 90% water content. After nine days of storage hardness was 80 N, which represents an extremely high value. It should be noted that the curves in Figure 3c correspond to constant concentrations of SFE, NE and BBG of 1.3%, 1.3% and 2% respectively. The bread made with a water content of 70% reached, after one storage day, a hardness ten times that of the bread with a 90% and the same hydrocolloid contents. After nine days of storage hardness was 84 N, which represents an extremely high value. This demonstrates that hydrocolloids, with water restrictions, lead to unsuitable bread characteristics. The different effect of SFE and NE
on bread hardening was also remarkable. Figure 13b shows that SFE increase from 0.1% to 2.5% led to significant depletion in bread hardness during storage, in spite of 1.3% NE presence in bread formulation. On the contrary, same changes in the concentration of NE to a fixed concentration of 1.3% SFE hardly changed hardness curves. SFE, therefore, showed a clearly better ability than NE to increase bread keepability. In previous studies it has been found that carboxymethylcellulose (CMC), added to GF doughs, controlled crumb hardening after three days storage (Lazaridou et al 2007).

As it can be seen in Fig. 1a, higher contents of BBG accelerated the aging of breads with 90% of water and 2.6% of HPMC (1.3% SFE + 1.3% NE). As it can be seen in Figure 3a, higher contents of BBG accelerated the aging of breads with 90% of water and 2.6% of HPMC (1.3% SFE + 1.3% NE). Other authors also observed this effect in breads added with β-glucan (Lazaridou et al 2007). Bread crumbs with 3.9% of BBG were since the second day of storage significant firmer than those with lower BBG addition (0.1%, 2%). This could be due to the This high dose of BBG would have required attending its already commented higher water necessities of this high BBG dose (3.9%), a dough hydration near 110% as was shown before, instead of 90% to minimize crumb firmness. It is also noteworthy that the firming curves of breads with 2% and 3.9% of BBG did not reach a constant value in nine days, unlike the curve for breads with 0.1% BBG, which achieved in five days a stable value. 3.9% BBG delayed the achievement of the maximum hardness during storage at 4ºC, generally attained in 6 – 8 days (Ronda and Roos 2011), probably due to the great water binding ability of BBG, controlling the loss of water during aging (Ronda et al 2011).

Table 2 shows the significant coefficients of the fitting model for the crumb hardening after one (short-term) and nine (long-term) days of storage at 4ºC. The
regression study confirmed the individual effects of HPMC, BBG and Water on the already mentioned crumb hardening. In short-term storage (one day), SFE effect on crumb hardness reduction was much more markedly than NE. Attending the regression equation a dose of 2% SFE would lead to a 40% reduction in the increment of firmness after one storage day. Individual effect of BBG was the opposite: Doses of 2% and 3% led to an increase in the hardness after one day of 17% and 38% respectively. In long-term storage, water content interactions with hydrocolloids exerted the most marked effects on crumb hardness. BBG was responsible for a significant crumb hardening after nine days of storage, but the provision of additional water content to the formula reduced significantly the effect.

### 3.2 Effect on Bread sensory quality

The coefficients of the regression fitting models for the sensory attributes (0.76 ≤ R² ≤ 0.90) are compiled in Table 4. Panelists scored crumb grain for both cell size and distribution, giving low ratings to big and heterogeneously distributed alveoli and high ratings to small and homogeneous gas cells (Table 3). SFE, BBG and water markedly affected crumb grain scores that varied between 2.4±0.5 and 9.7±0.5 in the scale 1-10. Negative coefficients of single BBG and/or Water (Table 4) suggest both factors increased alveoli size and crumb grain heterogeneity, although BBG quadratic coefficient supports that from 1.7% BBG addition crumb grain ratings became higher. SFE and Water showed a significant negative interaction on crumb grain scores, so that, high SFE dosages to softer doughs led to a prominent decrease in crumb grain ratings (Table 3). Lower crumb grain scores were concomitant with the presence of big holes (pockets) in bread crumb, and unaccepted by panelists. Ratings for crumb softness and smoothness (Table 3) were parallel and dependent on Water, BBG and SFE (Table 24). Increased water amounts up to 95%-100% hydration in dough
promoted significantly sensory ratings for crumb smoothness. Higher hydration levels did not provide any additional sensory smoothness perception. BBG increasing single addition decreased smoothness regardless dough water content. Simultaneous SFE presence helped BBG increase crumb smoothness when added up to 2%, flour basis. In fact, SFE counteracted BBG deleterious effect on crumb smoothness, so that SFE appears as a key ingredient in BBG-enriched GF-breads.

Overall acceptance of GF-breads, that varied between 2.2±0.4, for Run 13, and 7.6±0.4, for Run 12, was only significantly affected (p<0.05) by water and BBG dosages. According to panelits’ scores, overall acceptance was mainly/significantly dependent on crumb grain, and softness and smoothness (Table 4). Breads with water formulation greater/equal than 90% (flour basis) deserved acceptance (≥ 5/9), with the exception of Run 13, including 3.9 % BBG. It should be noticed that the bread of Run 12 (3.34% BBG, 0.45% SFE, 2.15% NE, 104% WATER), was individually preferred (7.6±0.4/9). The ratio BBG/Water of this formulation coincided with that predicted from the regression equations as a ratio capable of optimizing the bread quality leading to minimum bread firmness and maximum specific volume.

3.23 Correlation between variables.

Multivariate data handling of dough visco-elastic and bread quality parameters supplied useful information on the significantly correlated dough and bread characteristics. Fundamental and empirical dough rheological properties were reported in a previous work (Ronda et al 2013). Using Pearson correlation analysis, a range of correlation coefficients (r) (from 0.46 to 0.90) was obtained for the relationships between dough viscoelastic parameters and bread properties of fibre-supplemented rice-based matrices (Table 3). Table 6 reports Pearson correlation coefficients for the relationships between bread properties. Bread specific volume strongly correlated with
dough viscosity at the steady state, $\eta_0$, obtained from creep tests ($p<0.001$, $r = -0.76$).

Specific volume explicated a negative correlation with the elastic modulus at 1 Hz, $G_1'$, ($r = -0.64$) and positive with loss tangent, $\tan \delta$, and the exponents “a” and “b” ($r=0.56$, $r= 0.53$, $r=0.56$). The exponents, resulting from fitting power law to oscillatory frequency sweep data, quantify the dependence of dough elastic and viscous moduli on frequency. The higher the dependence of dynamic moduli on the frequency is, the higher $\tan \delta$ and the greater the bread volume. This means that lower dough consistency and less marked solid-like behaviour (within the range $0.19<\tan \delta<0.48$) favoured dough development and bread volume. Baking weight loss was strongly correlated with dough visco-elastic moduli, $G_1'$ and $G_1''$, ($p<0.001$; $r =-0.86$; $r =-0.78$), the viscosity at the steady state, $\eta_0$, ($p<0.01$; $r =-0.68$) and the dough consistency or the force obtained in a back extrusion test ($p<0.001$; $r=-0.82$). The greater the dough consistency means the higher the baking yields. This is probably due to the fact that fibres, substances responsible for the dough consistency increase, were also good water-binding macromolecules. Fresh bread crumb firmness and one- and nine-days stored bread crumb hardening were strongly correlated to all visco-elastic dough properties. The strongest positive correlation was obtained for fresh bread crumb firmness and the elastic modulus, $G_1'$ ($p<0.001$; $r = 0.79$), and the viscosity at steady state, $\eta_0$ ($p<0.001$; $r =0.88$). The short- and long-term bread hardening exhibited similar relationships with dough properties than initial firmness. Sensory scores related to crumb grain, crumb softness and overall acceptance were strongly correlated ($p<0.001$) with fundamental and empirical dough rheological properties.

4. Conclusions
Draper-Lin small composite design for sampling allow to obtain useful information on the significance of different hydrocolloid and water contents on breadmaking performance and staling behaviour of GF rice-based complex/heterogeneous bread matrices. Dough and bread characteristics strongly correlated supporting the key influence of the restoration of dough visco-elasticity on the physico-chemical and sensory achievement of GF final baked goods.

Single BBG by itself fails to mimic gluten visco-elasticity properly, but simultaneous addition of either SFE or NE, in doses around 1.6%, contribute to bread improvement in terms of bigger volume and smoother crumb. The extent of methoxyl and hydroxypropyl substitution of HPMC markedly affects the gel strength. Weak gel forming NE led to harder and lower volume breads than semi-firm gel forming SFE did, probably ascribed to the ability of hydroxypropyl groups to form a stable solvate shell in water restricting available water for starch to swell. It should be noticed that the surplus of water needed to incorporate large amounts of BBG into bread favoured the formation of big holes (pockets) in bread crumb particularly when SFE was included into formulation. Visible holes could be probably related to the easier crust forming ability of SFE than NE helping retain the large bubbles formed inside the crumb. A dough hydration of 90% would be recommended to get the maximum volume of no BBG added breads. This optimal amount of water would increase at a rate of 5-6% per 1% increase in BBG.

3.3 g of BBG (70% purity) and 104mL of water addition to 100 g rice flour provided sensorially accepted breads (7.6±0.4/9 over 10) with a theoretical β-glucan content of 1.24 g/100 g GF bread. It would allow a daily intake of 3 β-glucan provided with a bread consumption of 240 g/day. A daily intake of 240 g of BBG enriched GF bread (four servings), in good accordance with the nutritional
recommendation for bread consumption set between 200 and 300 g of bread per day (Zessner et al. 2011), is high enough to meet the requirements of the EFSA health claim (3 g/day), contributing a reduced blood cholesterol level.

An additional refined optimization of both water and qualitative and quantitative HPMC addition for BBG concentrations from 3 % to 4 %, is still recommended. Complementary tests aimed at establishing the real content and molecular weight of \( \beta \)-glucan in final bread are still pending to assure the expected nutritional benefit of this BBG enrichment to obtain healthy GF breads with improved organoleptic performance.

Acknowledgement

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References


EFSA. (2011). Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and “digestive function” (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal*, 9, 2207.


**Figure 13**: Crumb Firmness evolution during 9 days of storage at 4°C of breads with different amounts of BBG (runs 5, 7 and 13) (a), HPMC (runs 1, 3, 7, 9 and 10) (b) and Water (runs 7, 11 and 14) (c). Intermediate doses of dietary fibres (1.3% SFE; 1.3% NE; 2% BBG) and water (90% WATER) were used as constant factors. Values of firmness are the average of four replicates and error bars represent standard deviation.
Design factors are: hydroxypropylmethylcellulose semi-firm gel forming HPMC SFE 4000 (SFE), hydroxypropylmethylcellulose weak gel forming HPMC NE 4000 (NE), Barley Beta-Glucan (BBG), and water addition (WATER). -1.4142, -1, 0, 1, and 1.4142 indicate coded levels of design factors; axial distance from the center points: 1. 1.4142.

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Table 2: Significant coefficients (95% confidence interval) of design factors (independent variables) of the stepwise regression fitting model for bread characteristics (dependent analytical variables).

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Independent variables: SFE= HPMC SFE 4000; NE= HPMC NE 4000; BBG: Barley Beta-Glucan; WATER= water content above 70%. Blanks correspond to no significant effects at level of 5%; R-SQ adjusted square coefficient of the fitting model.
Table 3: Pearson product moment correlations between pair of variables measured in dough and bread. These correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between the variables. The rheological properties correspond to the fitting of experimental oscillatory measurements to power law model (G' = G'₁·ωᵃ; G'' = G''₁·ωᵇ; tan δ = (tan δ₁)·ωᶜ). Creep test results to the 6-parameter Burgers model (where J₀ and µ₀ are the instantaneous compliance and the steady state viscosity respectively); Consistency is the firmness measured in an extrusion empirical test (Ronda et al. 2013).

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<td>-0.62**</td>
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<td>ns</td>
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<td>ns</td>
<td>-0.52*</td>
<td>ns</td>
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</table>

Asterisks indicate the P-value which tests the statistical significance of the estimated correlations. *p<0.05 (statistically significant non-zero correlations at the 95.0% confidence level); ** p<0.01 (at the 99.0% confidence level); *** p<0.001 (at the 99.9% confidence level). ns: Not significant (p > 0.05)
Table 4: Pearson product moment correlations between pair of variables measured in bread. These correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between the variables.

<table>
<thead>
<tr>
<th></th>
<th>Loss of weight</th>
<th>Firmness 1 day</th>
<th>Firmness 9 days</th>
<th>Chewiness</th>
<th>Crust Lightness</th>
<th>Crust hue</th>
<th>Crust Chroma</th>
<th>Crumb grain</th>
<th>Crumb softness</th>
<th>Overall Acceptance</th>
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<td>-0.80***</td>
<td>-0.72***</td>
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</table>

Asterisks indicate the P-value which tests the statistical significance of the estimated correlations. *p<0.05 (statistically significant non-zero correlations at the 95.0% confidence level); ** p<0.01 (at the 99.0% confidence level); *** p<0.001 (at the 99.9% confidence level). ns: Not significant (p > 0.05)