Impact of yeast and fungi (1→3)(1→6)-β-glucan concentrates on viscoelastic behavior and bread making performance of gluten-free rice-based doughs.

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Abstract

The addition of bioactive β-glucan to gluten-free breads is of special interest to people suffering from celiac disease. Most of the studies found in literature involve cereal (1 → 3)(1 → 4)-β-glucan, while those from yeast and fungi are still nearly unexplored. This study focuses on the effect of fortifying gluten-free rice-based doughs and breads with (1 → 3)(1 → 6)-β-glucan concentrates derived from yeasts –soluble (SBG) and insoluble (IBG)– and fungi (*Pleurotus Ostreatus*) (FBG). SBG-enriched doughs were less firm and exhibited lower resistance to deformation than doughs with FBG or IBG. In contrast, FBG- and IBG-enriched doughs increased their resistance to deformation as the concentration increased. Doughs with a firmer consistency as determined by a forward extrusion test (FBG- and IBG-enriched doughs) corresponded to those with larger dynamic moduli and lower frequency dependence, lower elastic deformation and higher viscosity at steady state. The physical quality of breads was significantly improved by addition of all types of (1→3)(1→6)-β-glucan at optimized dough hydration. They caused an increase in the specific volume of the breads, a reduction in their hardness and longer shelf-life. Sensory evaluation also demonstrated an improvement in bread organoleptic attributes when SBG was added.

Keywords: (1 → 3)(1 → 6)-β-glucan, rheology, gluten-free, dough, bread

1. Introduction

β-glucan (BG) is a homoglucose polymer widely distributed in the cell walls of microorganisms, particularly of the baker's and brewer's yeast *Saccharomyces cerevisiae*, mushrooms, and cereals (mainly oats and barley) (Kittisuban et al., 2014; Liu et al., 2008). The differences in the macromolecular structure between β-glucans from different sources (yeast, fungal, and cereal β-glucans) have been previously described by Volman et al. (2008). The β-glucans of yeast and fungi consist of 1,3 β-linked glycopyranosyl residues with small numbers of 1,6 β-linked branches. In contrast, oat and barley cell walls contain unbranched β-glucans with 1,3 and 1,4 β-linked glycopyranosyl residues. BG from cereals is classified as dietary fiber that is not hydrolyzed in the human digestive tract, and is a non-calorific ingredient (Borchani et al., 2016). BG obtained from yeast and mushrooms presents positive effects on human health, such as immune-stimulation, anti-inflammatory, antimicrobial, and anti-tumoral responses (Chan et al., 2009; Kim et al., 2011; Rop et al., 2009; Santipanichwong & Suphantharika, 2009; Worrasinchai et al., 2006). (1→3)(1→6)-β-glucans have also demonstrated hypoglycemic activity in animal studies, and are likewise known for their hypocholesterolemic activity and in reducing atherosclerosis (Miranda-Nantes et al. 2011). Certain preparations of yeast-derived (1→3)(1→6)-β-glucan have recently been approved as novel food ingredients by the European Food Safety Authority (EFSA) and given "Generally Recognized as Safe" status by US Food and Drug Administration. So far, the EFSA has not yet approved a health claim on immune function for yeast (1→3)(1→6)-β-glucan preparations (EFSA, 2011; Samuelsen et al., 2014).

The cell wall of yeast (*Saccharomyces cerevisiae*) contains about 50-65% of (1→3)(1→6)-β-glucan (Kogan & Kocher, 2007). The difference in molecular and structural features according to the origin of BG leads to differences in their physical properties and, thereby, different effects on the functionality of food systems (Banchathanakij & Suphantharika, 2009). The potential applications of (1 → 3)(1 → 6)-β-glucan in food stuffs have previously been reported. This includes as a thickening, water-holding, and oil-binding agent and emulsion stabilizer, as a fat replacer in food emulsions (Santipanichwong & Suphantharika, 2009; Worrasinchai et al., 2006), and as a texture modifier in starch gels. (Satrapai & Suphantharika, 2007). However, its application in enrichment of gluten-free products is mostly unexplored, even though it holds special interest to people suffering from celiac disease. These individuals have a significant rate of other associated chronic diseases, such as gastritis, vitamin B deficiency, cardiomyopathy, and skin problems, as well as obesity-metabolic syndrome and diabetes, due to their higher fat and calorie-dense diets compared to the general population (Lerner & Matthias, 2015; Lebwohl et al., 2014; Helert et al., 2009; Cronin & Shanahan, 1997). Given the recognized health benefits of (1→3)(1→6)-β-glucans, fortifying high-consumption foods such as bread is of great importance for the general population but, in particular, for patients with celiac disease (Miranda-Nantes et al. 2011). However, only a few studies have focused on (1 → 3)(1 → 6)-β-glucan enrichment of cakes (Kim et al., 2011), gluten-free rice noodles (Heo et al., 2014), and starch breads (Kittisuban et al., 2014). Kim et al. (2011) used a BG-enriched product (51.4% BG) from *Lentinus edodes* (insoluble fiber) as a high-fiber and low-calorie substitute for wheat flour cakes. The result was increased batter viscosity with more shear-thinning behavior and improved elastic properties. Overall, the cakes containing more BG showed decreased volume and increased hardness, although no significant differences were observed between the control and cakes containing 1 g of yeast β-glucan per serving. Heo et al. (2014) also added *Lentinus* *edodes* powdered extract (40.1% BG) to improve the functional properties of gluten-free rice noodles. They found that the use of such fibrous material improved the thermo-mechanical properties of rice flour in a dough system, leading to rice noodles with greater extensibility and firmness in all three concentrations evaluated (4, 8, and 12%). Kittisuban et al. (2014) used response surface methodology to analyze effects of hydroxypropyl methylcellulose (HPMC), yeast β-glucan extract (insoluble, 72.2% BG), and whey protein isolate (WPI) on physical properties of gluten-free bread baked from formulas based on rice starch. β-glucan affected the specific volume differently depending on the WPI levels, i.e., slightly increased volume with β-glucan levels at low WPI levels. This was explained by the ability of β-glucan to increase dough consistency, which would improve gas retention and dough development. However, they found a decrease in the loaf volume of gluten-free bread, accompanied by crumb hardness, at the highest β-glucan level especially in the presence of the highest WPI content. Authors related it with too rigid dough that could cause a limited and slow expansion of the gas cells during proofing. That result indicated that there was an optimum value for dough consistency. The constant dough hydration applied by these authors could be, at least in part, responsible for results obtained. This is because limiting dough hydration by the addition of ingredients with specific water absorption behavior (such as insoluble-BG-enriched materials) can mask the impact of its presence on dough consistency, which has well known, dramatic effects on dough development ability (Ronda et al., 2015).

The great impact of the solubility of fibers on gluten-free dough rheology and bread making has been concluded from previous studies (Martinez et al., 2014). Soluble fibers (being mainly pure carbohydrate polymers such as inulin and polydextrose) decreased dough consistency, favored volume increase during fermentation, and produced breads with higher specific volumes, lower hardness, darker color, and greater cell density than control breads. In contrast, insoluble fibers from oat, bamboo, potato, and pea, particularly those of coarse particle size, decreased specific volume and increased markedly bread firmness.

The enrichment of gluten-free bread with health promoting ingredients (which can also positively affect the sensorial quality of this product) is worth examining. Consequently, the first aim of this study was to investigate the effect of different (1 → 3)(1 → 6)-β-glucan commercial concentrates of high purity and different declared water solubility, derived from various sources, on rice-based dough rheology at constant water content. The sources were yeasts –soluble powder (SBG) and insoluble powder (IBG)– and fungi –from *Pleurotus Ostreatus* insoluble powder (FBG). In addition, the physical and sensorial properties of BG-enriched gluten-free breads formulated at adapted dough hydration to get similar consistencies were also evaluated.

2. Materials 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 mm, 150 mm>63.2%>100 mm, and 30.8%<100 mm) was supplied by Herba Ricemills S.L.U (Tarragona, Spain). Salt, sugar, and sunflower oil were purchased from the local market. The HPMC 4 KM was a gift from Dow Chemical (Midland, Michigan, USA). (1→3)(1→6)-β-glucans (BG) used in dough and bread formulations were of three types. Two of them were obtained from the yeast *Saccharomyces cerevisiae*: one water insoluble (IBG) (Wellmune WGP® Dispersible Powder) and the other soluble (SBG) (Wellmune Soluble Powder®); Biothera (Eagan, Minnesota, USA) provided both as free samples. Wellmune WGP® consists of the purified yeast beta-glucan from the cell wall of baker's yeast. The natural form is a small spherical particle 2-4 microns in diameter. The second form, Wellmune Soluble Powder®, has a wide range of molecular weights ranging from a few thousand Daltons to several million Daltons, with an average molecular weight of 100 – 150kDa (information provided by the supplier). The third BG, insoluble (FBG), was derived from the fungus *Pleurotus ostreatus* (Pleuran), given as a gift by Pleuran, s.r.o. (Bratislava, Slovak Republic); the average molecular weight of FBG is 760kDa (commercial information). As indicated by the suppliers, the proximate composition of these products was: IBG: 79.25% purity (dry basis), 84.49% carbohydrates, 7.50% fat, 4.51% moisture, 2.86% protein, and 0.77% ash; SBG: 91.35% purity (dry basis), 92.11% carbohydrates, 6.79% moisture, 0.89% ash, 0.68% protein, and <0.01% fat; FBG: 90.62% purity (dry basis), 2.56% moisture, and absence of lipids and proteins.

2.2. Dough preparation

A straight dough process was performed following the formula on a 100 g rice flour basis: 92% water, 6% oil, 5% sucrose, 1.8% salt, 2% HPMC, and BG. The levels of pure BG incorporated into this formulation were 0 (control), 0.5, 1, and 2% in flour basis. Different amounts of commercial BG concentrates were added according to BG purity of each ingredient. The amount of BG added did not alter the amount of the other ingredients in the formulation. The dough was made by first blending the solid ingredients in a kitchen-aid professional mixer (KPM5) for 10 s at speed 2. Then, liquid ingredients (oil and water at 20±2 ºC) were added and mixed for 5 min at speed 6 (Pérez-Quirce et al., 2014). Each dough was made in duplicate.

2.3. Rheological characterization of dough

2.3.1. Small deformation mechanical test. Oscillatory and creep recovery tests

Oscillatory and creep-recovery tests were carried out at 25 °C with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) using 60 mm serrated parallel plate geometry. The GF dough was placed on the rheometer plate using a 3-mm gap and trimmed, and then vaseline oil was applied to the air-exposed surfaces to prevent sample drying during testing. Before the measurement, the dough was allowed to rest for 500 s. A stress sweep from 0.1 to 200 Pa at 1Hz was performed to establish the linear viscoelastic region (LVR). Frequency sweeps were carried out from 10 to 0.1 Hz in the LVR and data were fitted to a power law model as in previous studies (Ronda et al., 2013). The recorded viscoelastic parameters, G_1' and G_1'', and $(tan\ \delta)_1$, represent the elastic and viscous moduli and the loss tangent, respectively, at a frequency of 1Hz. The *a*, *b* and *c* exponents quantify the dependence of the dynamic moduli and the loss tangent on the oscillation frequency. Each test was carried out at least in duplicate.

Creep tests were performed by imposing a sudden step of shear stress at 1.5 Pa in the LVR for 150 s. In the recovery phase, the stress was suddenly removed and the sample was allowed to recover the elastic (instantaneous and retarded) part of the deformation for 300 s. Each test was done in triplicate. Burgers model was fitted to creep and recovery tests data, described in terms of compliance J (strain divided by the stress) (Lazaridou et al., 2007; Ronda et al., 2013). In this model, J_0 represents the instantaneous compliance, related to the instantaneous elastic dough deformation when the sample is submitted to a sudden/constant stress; J_1 is the retarded elastic or viscoelastic compliance related to the retarded dough deformation; and λ_1 is the retardation time required for this deformation, obtained from both the creep and the recovery phases (Steffe, 1996). η_0 is the steady viscosity estimated from the creep phase. J_{max} is the maximum creep compliance related to the maximum dough deformation obtained at the end of the creep step. Similar equations were used for the recovery compliance $J_r(t)$. As there is no viscous flow in the recovery phase, equations consist only of parameters describing the elastic response after removal of the shear stress. Up to a limit, higher compliances generally facilitate higher dough development during proofing and baking (Ronda et al., 2017).

2.3.2. Large deformation mechanical test: forward extrusion test

Forward extrusion assays of formulated rice doughs were done in a TA-XT plus texture analyzer (Stable Micro Systems, Surrey, UK), equipped with a 25-kg load cell and operating at 10 mm/s head speed, following the method described in detail elsewhere (Ronda et al., 2015). Compression force–time curve allowed evaluating the average force obtained from the plateau, representing the force necessary to continue with the extrusion process, and the area under the curve, representing the energy needed for the extrusion. Both magnitudes were used to define the sample consistency. All measurements were performed in triplicate.

2.4. Bread preparation

Breads were made in duplicate according the formulation described in Section 2.2. This included 3 g of dried yeast per 100 g of rice flour and a water amount optimized individually for each type and level of BG to obtain the same force during the extrusion test (see 2.3.2) as with the control dough formulated with 92 g water per 100 g of rice flour. Optimized dough hydration increased with rising amounts of IBG and FBG and decreased with SBG, becoming between 90% and 104% depending on the formulation (Table 1). The baking process, described in detail elsewhere (Pérez-Quirce et al., 2014), was carried out in a Sveba Dahlen oven (Fristad, Sweden) at 170 °C for 20 min with 7s steam at the beginning of the baking. After baking, breads were left for one hour at room temperature before analysis. To study the effect on staling, breads were stored at 4±2 °C in polyethylene bags. An experimental design of 12 elaborations (see Table 1) was carried out.

Table 1: Amounts of β-glucan concentrates obtained from yeast (IBG and SBG) and fungi (FBG) and dough hydration in each enriched gluten-free bread elaboration

BG Type	BG (% rfb*)	WATER (% rfb*)
Control	0	92
SBG	0.5	91.5
SBG	1	91
SBG	2	90
IBG	0.5	95
IBG	1	98
IBG	2	104
FBG	0.5	94
FBG	1	96
FBG	2	100

*rfb: rice flour basis. SBG: soluble (1-3)(1-6)-β-glucan yeast; IBG: insoluble (1-3)(1-6)-β-glucan yeast; FBG: fungal (1-3)(1-6)-β-glucan (FBG).

2.5. Electron microscope photomicrographs of fibers and crumb breads

Fiber and bread crumb photomicrographs were taken with a Quanta 200FEI (Hillsboro, Oregon, USA) environmental scanning electron microscope (ESEM). Fiber photomicrographs were taken in beam deceleration mode (BDM) at 2 keV in low vacuum mode with a backscattered electron detector (BSED). Crumb samples were directly mounted on stubs. Observations were made with an accelerating voltage of 10 keV.

2.6. Evaluation of bread quality

Bread volume was determined in four replicates by a Volscan profiler analyzer (Stable Microsystems, Surrey, UK). Breads were weighed immediately after removal from the pan once cooled. Crumb texture was determined in quadruplicate samples with a TA-XT2 texture analyzer (Stable Microsystems, Surrey, UK) using "Texture Expert" software. A 20-mm diameter aluminum cylindrical probe was employed in a 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. Hardness (N), chewiness (N), cohesiveness, springiness, and resilience were calculated from the TPA graph (Gómez et al., 2007). Analyses were made at 20±3 °C on two central slices (20 mm thickness) from two breads of each dough. Breads were analyzed fresh and after one day of storage at 4 ±2 °C. Moreover, a staling kinetics study was carried out on breads enriched with the maximum addition of BG (2%) by measuring texture at 0, 1, 2, 4, 7, and 9 days after baking and storage at 4±2 °C. The Avrami equation was used for fitting the evolution of crumb firmness with time (Ronda and Roos, 2011). Crumb and crust color was measured with a Minolta spectrophotometer CN-508i (Minolta Co. LTD., Japan) in the CIE L*C*h coordinates using the D65 illuminant, and the 2° standard observer as reported elsewhere (Ronda et al., 2015). Crumb grain characteristics of bread were assessed by using a digital image analysis system using ImageJ software. The crumb grain characteristics studied were the mean cell area (mm^2) and the cell density ($cells/cm^2$). Loaf images were previously acquired at 600 dots per inch with a Hp Scanjet G3110 scanner (Hewlett Packard Enterprise, Palo Alto, CA, USA). The analysis was performed on 30 x 50 mm squares taken from the center of the loaf. Crumb grain parameters were measured in duplicate.

2.7. Sensory analysis

Sensory analysis was conducted on BG-supplemented bread samples using a multisample difference test following the guidelines suggested by Meilgaard et al. (2007). A trained panel of eight panelists rated the intensity of nine attributes on a numerical intensity scale of nine points ranging from 1 (not perceived or very low) to 9 (extremely intense or very high). The control sample was used as a reference and was positioned in the middle of the scale (Ronda et al., 2005). The samples were evaluated in terms of crust uniformity, crumb grain uniformity, odor and flavor intensity, aftertaste persistency, crumb humidity, crumb adhesiveness, crumb softness, and crumb cohesiveness. Each attribute was presented separately.

2.8. Statistical analyses

Statgraphics Centurion v.16 (Bitstream, Cambridge, MN, USA) was used for non-linear regressions to fit creep-recovery data to Burgers model. ANOVA analysis, LSD (Least Significant Difference) test ($p<0.05$), and Pearson correlation analysis were performed using the v.6. Statistica package (Tulsa, OK, USA). The two factors studied in ANOVA analysis were the type of (1 → 3)(1 → 6)-β-glucan added to the dough and its addition level. In the sensory analysis, the effect of panelist was also checked.

3. Results and discussion

3.1. BG ingredient microstructure

Fig. 1 shows the photomicrographs of the different BG-containing ingredients used in this study. BG from yeast exhibited spherical or slightly lenticular morphologies responding to its origin from yeast cells, while SBG showed a smooth surface, and IBG, a grainy surface. The particles of FBG reflected the crushed matrix of mushroom tissue, showing an irregular surface and polyhedral shape, and seemed to be more disintegrable. All particles of BG ingredients showed an average particle size below 50 μm, which means ingredients of fine structure.

3.2. Viscoelastic properties of fiber-enriched doughs at constant dough hydration

Dough prepared with either constant consistency or constant water addition has been used for testing the effect of added substances. The latter, previously used by Krupa-Kozak et al. (2012) and Nunes, Ryan, and Arendt (2009), was selected in this study for dough rheology characterization because recorded differences in dough behavior may be directly related to the added ingredients. Moreover, this approach allows better objectivity in the comparison of the availability of water for starch gelatinization.

The viscoelasticity of gluten-free doughs was examined by oscillatory and creep measurements. Table 2 shows the parameters obtained from fitting frequency sweeps and creep test data to Power law and Burgers model, respectively. The R^2 values of the fitting of power law to frequency sweep results were always above 0.99. The fitting of Burgers model to creep-recovery test data led always to R^2 values above 0.97. All rheological properties of doughs were significantly ($p<0.05$) affected by the type of the ingredient used and the level of addition. The double interaction (type of BG*level) also had a significant effect on all rheological dough properties, except for the exponents a, b and the phase shift tangent; this indicates that the level of addition had a different effect depending on ingredient type in most viscoelastic properties. In all cases the storage modulus (G') was much larger than the loss modulus (G''), implying the prevalence of elastic features over viscous, suggesting a typical weak gel structure, in agreement with previous studies on GF dough enrichment with cereal BG (Hager et al, 2011; Lazaridou et al., 2007; Ronda et al., 2013, 2015). Both moduli showed slight increases with angular frequency as evidenced by the low a and b exponents. The elastic and viscous moduli at 1 Hz, G_1', and G_1'' decreased as soluble product SBG was added, to 30% at the highest addition level, denoting a softer dough consistency. This has been observed by Ziobro et al. (2013) and Peressini et al. (2015) with the addition of inulin to gluten-free and wheat doughs, respectively.

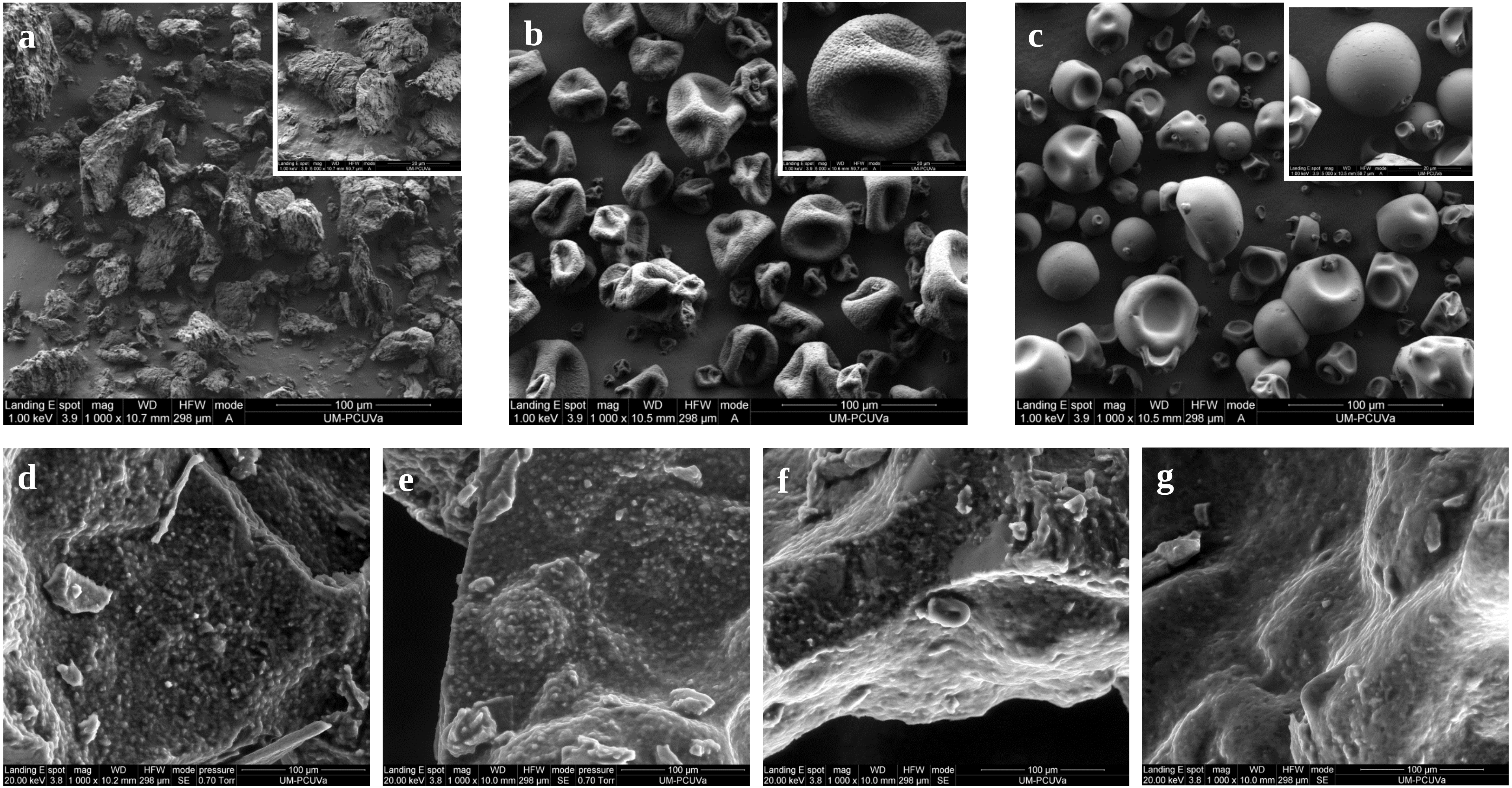

Figure 1. Scanning electron microscope photomicrographs of the commercial (1→3)(1→6)-β-glucan products: (a) FBG. (b) IBG. (c) SBG; and of the gluten-free breads: (d) Control bread (without BG). (e) Bread with 2% FBG. (f) Bread with 2% IBG. (g) Bread with 2% SBG. The inserts included in a), b) and c) show a detail of the BG particulate surface.

Table 2: Viscoelastic properties of gluten-free doughs at constant dough hydration (92% flour basis)

Sample	Dose (%)	Oscillatory tests- Power law model parameters: G'_1 (kPa)	a	G''_1 (kPa)	b	$(\tan \delta)_1$	c	Creep tests- Burgers model parameters: J_0 (10^{-4} Pa^{-1})	J_1 (10^{-4} Pa^{-1})	λ_1 (s)	η_0 (10^4 Pa·s)	J_{max} (10^{-4} Pa^{-1})
Control	0	3.2 bc	0.33 f	1.7 bcd	0.324 e	0.52 e	-0.00 cd	6.2 e	8.9 e	19 a	10 ab	30.8 e
SBG	0.5	2.6 ab	0.33 def	1.4 abc	0.318 de	0.54 e	-0.01 bcd	6.3 e	13.1 f	22 a	8 ab	38.3 f
SBG	1	2.5 ab	0.33 ef	1.3 ab	0.304 cde	0.53 e	-0.03 ab	6.9 e	12.2 f	24 a	9 ab	36.4 f
SBG	2	2.3 a	0.30 cde	1.2 a	0.295 bcd	0.51 de	-0.00 cd	5.7 e	12.5 f	17 a	7 a	40.2 f
IBG	0.5	4.0 d	0.28 abc	1.8 cde	0.301 cd	0.44 abc	0.02 ef	4.5 d	7.4 de	19 a	16 c	21.7 cd
IBG	1	5.2 e	0.26 a	2.1 ef	0.276 ab	0.40 ab	0.02 ef	2.9 bc	4.4 bc	23 a	25 d	13.2 b
IBG	2	12.2 g	0.26 ab	4.8 h	0.264 a	0.39 a	0.00 de	1.4 a	2.2 a	23 a	47 e	6.7 a
FBG	0.5	3.9 cd	0.30 bcde	1.9 def	0.281 abc	0.49 cde	-0.01 bc	3.6 cd	9.0 e	23 a	14 bc	23.1 d
FBG	1	4.8 de	0.29 bcd	2.2 f	0.297 bcd	0.46 bcd	0.00 de	3.1 c	5.8 cd	21 a	19 cd	16.8 bc
FBG	2	7.6 f	0.25 a	3.1 g	0.266 a	0.40 ab	0.01 def	1.8 ab	2.6 ab	21 a	42 e	8.2 a
SE		0.3	0.02	0.1	0.008	0.02	0.01	0.4	0.7	3	3	0.2
Factor I (type)		***	***	***	**	***	*	*	***	ns	***	***
Factor II (level)		***	*	***	**	*	ns	ns	***	ns	***	***
Factor I x Factor II		***	ns	***	ns	ns	ns	ns	**	ns	***	***

Values with a common letter in the same column are not significantly different ($p>0.05$). SE: Standard error obtained from ANOVA analysis. *. **. ***, and ns indicate the level of significance in the effects of type of BG, the level of addition, and their interaction. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and ns= not significant ($p>0.05$).

This effect is probably because a soluble ingredient dissolved in the aqueous solution envelops the starch granules lubricating the dough and decreases the capacity of the starch to absorb water, which leads to a softer and less elastic dough consistency (Martinez et al., 2014). The insoluble products, IBG or FBG, showed the opposite trend, increasing G_1' and G_1'' moduli to 280% and 200% for IBG, and to 140% and 85% for FBG, respectively. The more pronounced effect of yeast IBG than fungi FBG on dough viscoelastic moduli confirms that the more regular the insoluble fiber is (compare the shapes of IBG and FBG in Fig. 1), the greater the impact it has on dough structure. This was demonstrated by Martinez et al. (2014), in agreement with Sabanis et al. (2009), who reported that insoluble fibers led to firmer doughs, requiring an increase of the water content in their formulations. Insoluble fiber remains almost unaltered in the dough, leading to larger, more irregular structures than in the control dough (Martinez et al., 2014). The loss tangent (tan δ_1) was unaffected by SBG and decreased by IBG and FBG, indicating that doughs enriched with insoluble ingredients had a lower viscous to elastic moduli ratio. The a and b exponents, that inform of the degree of dependence of G 'and G'' on the frequency, decreased with the addition of insoluble BGs. The exponent c was always very low, and ranged between -0.03 and 0.02. This denotes the elastic modulus (G') had similar dependence on angular frequency than the viscous modulus (G'').

Burgers model parameters from creep tests are collected in Table 2. Creep/recovery curves of gluten-free doughs (not shown) exhibited typical viscoelastic behavior, combining both viscous fluid and elastic components, similar to the corresponding curves obtained previously for rice flour (Ronda et al., 2013; 2015) and other gluten-free doughs (Lazaridou et al., 2007). Because the tests were performed in the LVR, all creep compliance parameters and the equivalents for the recovery phase followed a strong parallelism and the setting parameters of both segments were equivalent; for this reason, recovery phase data are not shown. The addition of SBG did not affect the instantaneous elastic compliance (J_0) of the dough. However, the retarded (J_1) and the maximum (J_{max}) compliances increased significantly, regardless of the addition level. In contrast, insoluble products (FBG or IBG) decreased J_0, J_1, and J_{max}.. Furthermore, changes were magnified as the addition level increased, but were unaffected by fiber source. Maximum depletion in all compliance values, at 2% addition, were around -75%, indicating stiffer doughs with lower dough deformation submitted to a constant stress. Factors providing a decrease in compliance parameters increased the viscosity at the steady state, (η_0). This agrees with previous studies on GF doughs enriched with cereal BG (Ronda et al., 2013; 2015; Lazaridou et al., 2007) in tests performed both in and outside the LVR. No significant effects were detected on dough steady viscosity as result of SBG presence, while the addition of IBG or FBG increased the viscosity markedly, to +350% at 2% level. Dough viscosity increase might be attributed to the high water retention capacity of insoluble ingredients, as observed by Rosell et al. (2009) for insoluble fibers.

3.3. Optimization of dough hydration to constant consistency by forward extrusion test

There were important differences in rheological behavior of doughs enriched with different ingredients depending on their solubility and source. Knowing this led us to optimize the hydration of doughs used for bread elaboration to a similar consistency to remove the important, well known effect this factor has on bread making. The water content of enriched doughs was adapted to obtain the same consistency as the control dough formulated with 92% water per 100g of rice flour, evaluated from the forward extrusion test. The force and energy required for the control dough during the extrusion test were 7.2 g_f and 598 $g_f{\cdot}s$, respectively. Dough hydration necessities increased with rising amounts of IBG and FBG and decreased with SBG: to 104% and 100% for the insoluble BGs, and to 90% for the soluble SBG at the highest additions (Table 1). The level of dough hydration to similar consistency obtained with this procedure was coherent with the results obtained from fundamental rheological tests: The lower the G', G'', and η_0 and the larger the J_o, J_1, and J_{max} were, the lower the water required for similar dough consistency were.

3.4. Bread properties

The enriched breads characteristics are shown in Table 3 and Fig. 2. All BGs increased the specific volume of breads, the effect being lowest at the highest addition level (2%). Adding FBG led to breads with the highest specific volume. There were no significant differences among breads enriched with SBG or IBG. Other authors found the highest specific bread volumes with soluble fibers (Martinez et al., 2014) when compared with those made with insoluble fibers. These authors concluded that, in general, higher specific volumes were found from doughs with a softer consistency, which could be attributed to the fact that a firmer consistency could limit dough expansion during proofing and baking. The optimization of the water content to similar dough consistency can explain our different results and allows confirming that these ingredients had a beneficial effect on bread volume in spite of their effect on dough consistency. Besides, the fine structure of the $(1 \rightarrow 3)(1 \rightarrow 6)$-β-glucan containing insoluble products used can also facilitate the higher bread volumes obtained, given that fine fibers have been reported to lead to higher bread volumes than coarser ones (Martinez et al., 2014). At 2% fiber addition the specific volume of breads started to decrease, although they still surpassed the control bread volume. Lazaridou et al. (2007), working at adapted dough hydration, also found an increase in the specific volume of gluten-free breads as a result of oat BG (90% purity) enrichment; this increase was higher at 1% than at 2% addition level.

Table 3: Textural and morphogeometrical properties of breads

Sample	Dose (%)	Specific volume (mL/g)	Loss of weight (%)	Hardness (N)	Chewiness (N)	Resilience (N)	Mean cell area (mm^2)	Cell density ($cells/cm^2$)	Crust Color			Crumb Color			Δ Hardness 1 day (N)
									L*	C*	h	L*	C*	h	
Control	0	3.34 a	16.6 a	0.80 d	0.412 d	0.34 de	0.19 a	48 d	58 a	61 a	31 c	75 e	94 de	98 c	1.3 c
SBG	0.5	3.83 d	17.3 b	0.53 bc	0.258 bc	0.34 cde	0.26 bc	49 d	63 de	68 e	34 d	72 bcd	96 ef	97 bc	0.8 ab
SBG	1	3.83 d	17.8 bc	0.60 c	0.282 c	0.34 bcde	0.23 ab	48 d	59 ab	65 d	33 d	71 bc	94 cde	98 c	0.7 ab
SBG	2	3.57 b	16.2 a	0.59 c	0.285 c	0.36 e	0.19 a	47 d	61 cd	63 bc	31 c	77 e	93 bcd	98 c	1.3 bc
IBG	0.5	3.90 d	18.4 cd	0.55 bc	0.266 bc	0.31 a	0.24 ab	46 cd	61 c	62 ab	26 b	71 bc	90 b	97 bc	0.4 a
IBG	1	3.77 cd	18.7 de	0.51 bc	0.246 ac	0.33 abc	0.31 cd	41 b	59 a	65 d	26 b	73 cd	92 bc	98 c	0.7 a
IBG	2	3.68 bc	19.3 e	0.48 abc	0.220 abc	0.33 abc	0.49 e	35 a	60 bc	64 cd	23 a	68 a	86 a	98 bc	1.0 ab
FBG	0.5	4.43 f	19.3 e	0.37 a	0.167 a	0.31 ab	0.25 b	47 cd	64 e	67 e	30 c	75 de	93 cd	96 ab	0.7 a
FBG	1	3.88 d	19.1 de	0.46 ab	0.204 abc	0.32 abc	0.23 ab	48 d	60 bc	68 e	31 c	70 ab	94 cde	97 b	0.7 a
FBG	2	4.07 e	21.5 f	0.37 a	0.181 ab	0.31 a	0.34 d	43 bc	62 d	63 bc	26 b	68 a	95 ef	96 a	0.9 a
SE		0.05	0.3	0.04	0.003	0.01	0.02	1	1	2	1	1	1	1	0.2
Factor I (type)		***	***	***	**	**	***	***	***	***	***	***	***	***	ns
Factor II (level)		***	**	ns	ns	ns	***	**	***	***	***	*	**	**	*
Factor I x Factor II		***	***	ns	ns	ns	***	*	ns	***	*	***	***	ns	ns

Values with a common letter in the same column are not significantly different ($p>0.05$). ΔHardness 1 day: Firmness increase in 1 storage day. SE: Standard error obtained from ANOVA analysis. *. **. ***, and ns indicate the level of significance in the effects of type of BG, the level of addition, and their interaction. * $p<0.05$, ** $p<0.01$, ***$p<0.001$, and ns= not significant ($p>0.05$).

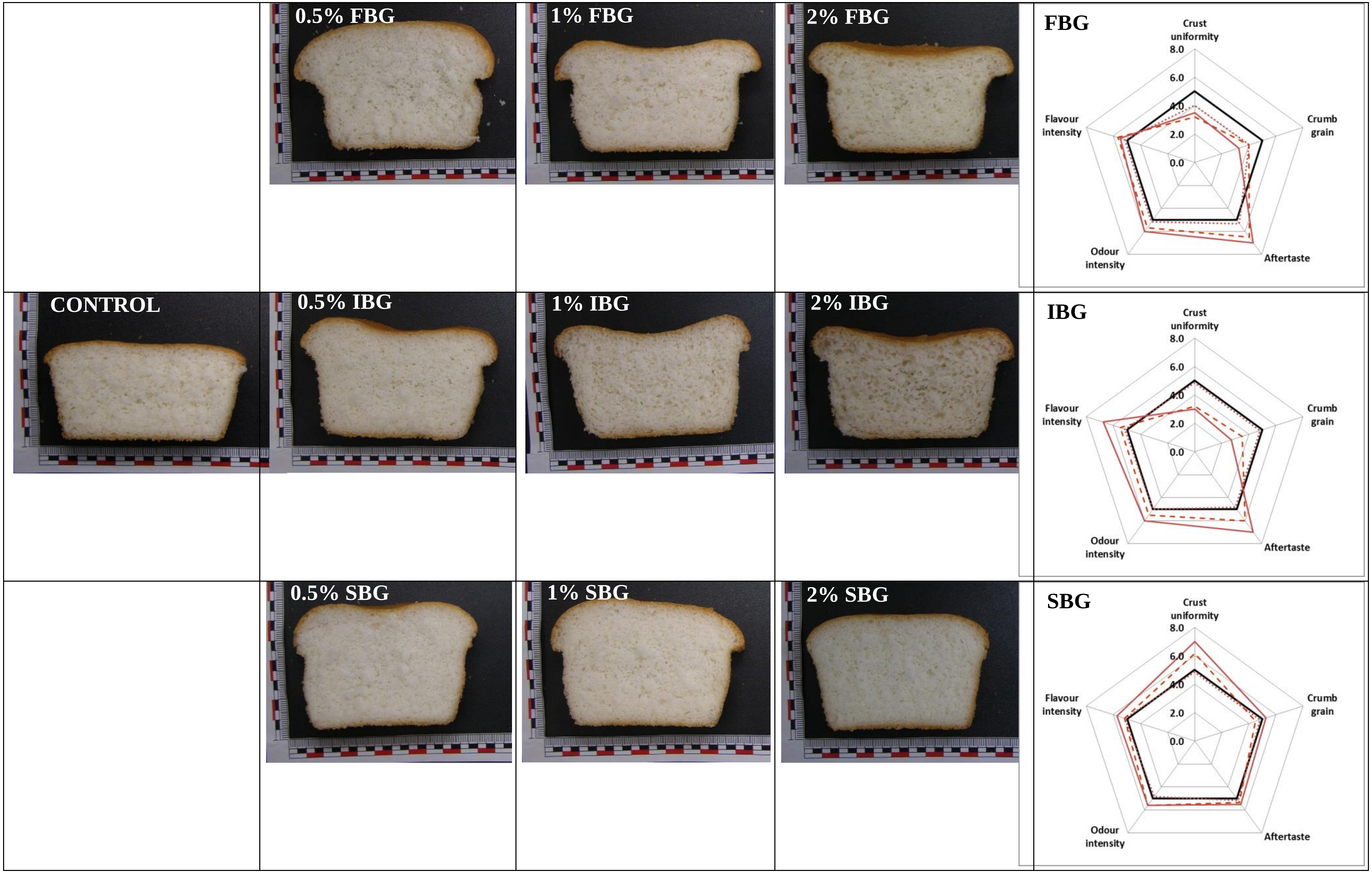

Figure 2. Effect of BG addition on crumb structure and sensory properties of gluten-free breads depending on BG addition level and type.

······ 0.5%BG - - - 1% BG; —— 2% BG; —— Control

Kim et al. (2011) observed that replacing flour with 1 g BG from *Lentinus edodes*/100g flour led to cakes of volume similar to the control, while substituting 3g BG decreased the volume significantly. As for bread texture, all the BG tested products reduced bread crumb hardness and chewiness significantly ($p<0.05$) with respect to the control bread, regardless of the amount added. SBG and IBG showed a similar effect on these textural properties, while FBG led to the lowest values. A reciprocal relationship was found between the specific volume of breads and their hardness. This significant correlation ($p<0.01$; $r=-0.85$) has been reported in previous studies on gluten-free bread, including studies on the effects of adding fiber (Sabanis et al., 2009; Kittisuban et al., 2014; Martinez et al., 2014); it is probably due to the lower resistance of crumbs to deformation with a higher percentage of air content in breads of higher volume. Kim et al. (2011) reported an increase in the hardness of wheat cakes as the amount of BG from *Lentinus edodes* increased. Kittisuban et al. (2014) also found that the crumb hardness increased with BG concentration, and attributed that effect to the water binding capacity of the BGs when they functioned at constant hydration. The different results obtained in our study are surely due to the optimization of dough hydration as function of ingredient presence. Springiness and cohesiveness, whose average values in crumb breads were 0.812 and 0.582, were not significantly affected by ingredient presence. However, as can be seen in Table 3, resilience decreased significantly with insoluble BG, although SBG did not affect it. Resilience is a more sensitive parameter than springiness and cohesiveness. It quantifies the instant recovery capacity after crumb compression, while the latter two parameters evaluate the recovery capacity after a waiting (recovery) time. It should be noted that breads made with a soluble ingredient were more capable of recovering, with less friable crumb, which improved their quality compared with breads enriched with insoluble fibers (see Section 3.5).

By adding insoluble BGs the mean cell area of bread crumb increased to more than double that of the control bread value in the case of 2% IBG (see Table 3 and Fig. 2). This change took place with a simultaneous decrease in cell density, with a significant correlation between them ($p<0.001$; $r=-0.93$). SBG-enriched breads showed lower mean cell area and higher cell density, similar to the control bread. Different effects of ingredients on crumb grain depending on their solubility can be explained in view of the microphotographs that show the structure of 2% BG-enriched bread crumbs (Fig. 1). Soluble BG product helped create a uniform network that helped wrap the bubbles and avoided coalescence phenomena. Fig. 1g shows that the crumb surface of bread with SBG was smoother than the control and insoluble-enriched breads (Figs. 1d, 1e, and 1f). This indicates that adding SBG improved the homogeneity of the crumb structure, leading to a fine structure. Insoluble ingredients, particularly the more rounded ones (such as IBG in Fig. 1f) as demonstrated by Martinez et al. (2014), created points of rupture in the structure; this made it easier for bubbles to associate and led to coarser crumb grain structure. An improved crumb was previously observed with the addition of oat BG to both GF breads (Lazaridou et al., 2007; Ronda et al., 2015) and wheat breads (Wang, Miller, and Hoseney, 1998). This effect of oat BG addition was related to the capacity of this soluble fiber to prevent coalescence of the cells during dough proofing and baking.

The BG-product-enriched breads showed crusts with significantly higher L* and C* coordinates than those of the control sample. This means that BG-fortified breads were lighter and more vividly colored than the control bread. The higher dough water content (in the case of adding an insoluble ingredient) and the addition of a water-retaining ingredient, such as BG, decreased the Maillard reactions rate and yielded a lighter crust (Perez-Quirce et al., 2014). This has previously been noted by Hager et al. (2011) and Ronda et al. (2015) for cereal BG. BG moderately affected the crust hue, h, depending on BG solubility: IBG decreased it, producing a reddish crust; while SBG increased it, leading to yellowish ones. Enriched breads showed slightly darker crumbs (lower L*). Likewise, in the case of insoluble ingredients, enriched breads showed significantly lower Chroma (IBG) and hue (FBG), denoting less vivid and reddish crumbs, respectively. The slight effect of fiber on bread crumb color could be related to the original –although very slight– color of these ingredients.

Figure 3 presents crumb firmness evolution during the storage of BG-added GF breads at 4±2 °C. The correlation coefficients of Avrami model fitting to experimental data, R^2, ranged from 0.96 (for FBG) to >0.99 (for the rest). The values of the Avrami model parameters (F_o, F_∞, k, n) were (0.59N, 2.5N, 0.59 d^{-n}, 0.37), (0.48N, 2.2 N, 0.93 d^{-n}, 0.19), (0.38N, 2.3 N, 0.35 d^{-n}, 0.50), and (0.80N, 3.4 N, 0.39 d^{-n}, 0.69) for SBG-, IBG-, FBG-added breads, and control bread, respectively. The half-life time, $t_{1/2}$, reports the time required to achieve 50% of leveling-off extent of the firmness. The half-life time was 1.5, 0.2, 3.9, and 2.3 days, respectively, meaning faster hardening during storage of yeast BGs-added crumbs. The rate constants of crumb firming found in this study were of the same order of magnitude as that found in other studies for gluten-free breads, 0.44 d^{-n} (Ronda and Roos, 2011), and inulin-enriched wheat breads, 0.35 d^{-n} (Ronda et al., 2014), stored at 4±2 °C. From the first days, FBG-added breads showed the lowest crumb firmness, while breads with yeast BG fiber (IBG and SBG) showed firmness after one day of storage similar to that of the control bread. However, the lower levelling-off firmness values, F_∞, of fiber-added breads (ranging from 2.2 to 2.5N versus 3.5N for the control bread) and the results presented in Fig. 3 show that BG fiber (especially FBG) helped reduce bread hardening after long-term storages. The parameter F_o was similar to values of firmness measured in fresh breads (Table 3), which confirms the good fit of the Avrami model to experimental data. The higher rate constant, k, and the lowest half-life time, $t_{1/2}$, obtained for

IBG-added breads indicate a faster change in firmness (from the initial fresh bread value to the levelling-off one) in comparison with the remaining breads.

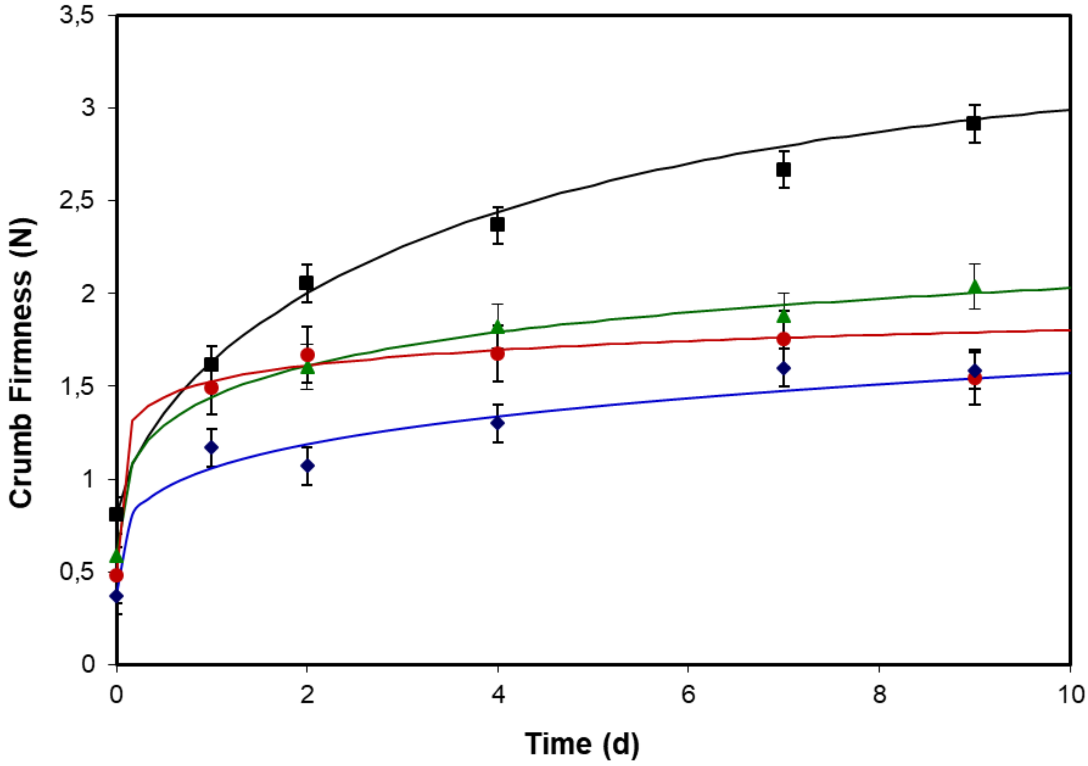

Figure 3: Hardening kinetics in BG-enriched breads stored at 4 ± 2 ºC. The continuous lines resulted from fitting the Avrami equation to experimental data. The error bars represent the standard deviation. ■ Control; ▲ SBG ● IBG ◆ FBG

3.5. Sensory evaluation

The panelist effect on sensory evaluation was previously verified and was not significant ($p>0.05$) for any sensory attributes. Panelists were unable to find significant ($p<0.05$) differences between BG-enriched and control samples in terms of crumb humidity, crumb adhesiveness, crumb softness and crumb cohesiveness (data not shown). Similar results were obtained previously by Martins et al. (2015), who studied BG-bread fortification with dry spent yeast; they reported no significant differences in sensorial attributes of the final product. An analogous behavior was also found in fiber-enriched products such as inulin-fortified snacks (Peressini et al., 2015). Figure 2 shows the sensory evaluation results of BG-supplemented gluten-free breads in terms of crust uniformity, crumb grain uniformity, odor and flavor intensity, and aftertaste persistency, in comparison to the control bread (which was positioned in the middle of the scale). SBG-supplemented bread at a level of 0.5-2% showed a sensorial profile very similar to control bread except for crust uniformity. This parameter significantly

($p<0.05$) increased when SBG was added, probably due to the higher volumes obtained in the fortified breads. Conversely, adding IBG and FBG resulted in lower scores than those of the control sample, denoting that the loaves looked worse and the crumb grain was less uniform. Insoluble-BG-fortification led to higher flavor and odor intensity (mainly for IBG) and aftertaste (for both IBG and FBG) scores than control bread. Panelists indicated that these differences stemmed from a slight strange, undesirable taste and smell.

4. Conclusions

This study demonstrated the feasibility of enriching gluten-free bread with (1 → 3)(1 → 6)-β-glucan enriched products obtained from yeast or fungi at 0.5-2% level. The effect on dough rheological properties was very dependent on BG source and solubility. In general, the soluble enriched product tested decreased G', G'', and steady viscosity and increased compliances. However, doughs with insoluble fibers generally showed the opposite trend, increasing their resistance to deformation as the concentration increased. The physical quality of breads was significantly improved by adding (1→3)(1→6)-β-glucan at optimized dough hydration. The addition of (1→3)(1→6)-β-glucan products caused an increase in the specific volume of the breads and reduced their hardness, while leading to delayed crumb hardening during storage. Sensory evaluation also demonstrated an improvement in bread organoleptic attributes when soluble BG was added. Insoluble BG extracts, both from yeast or fungi, at 1% and 2% levels, gave breads a very slight strange, undesirable taste and smell not found in the control rice flour bread. The use of a more complex, optimized formulation could help overcome this slightly undesirable taste/odor. Additional studies are still pending in this sense.

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