Accepted Manuscript

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PII: S0023-6438(19)30639-5

DOI: https://doi.org/10.1016/j.lwt.2019.108299

Article Number: 108299

Reference: YFSTL 108299

To appear in: LWT - Food Science and Technology

Received Date: 15 February 2019

Revised Date: 30 May 2019

Accepted Date: 21 June 2019

Please cite this article as: Cajas Locke, J.E., González, L.C., Loubes, M.A., Tolaba, M.P., Optimization of rice bread formulation by mixture design and relationship of bread quality to flour and dough attributes, *LWT - Food Science and Technology* (2019), doi: https://doi.org/10.1016/j.lwt.2019.108299.

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Gluten-free bread

Rice flour selection



Gluten substitutes selection



Mixture design



1	Optimization of rice bread formulation by mixture design and relationship of
2	bread quality to flour and dough attributes
3	
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16	

Abbreviations

GS: gluten substitutes, XG: xanthan gum, GG: guar gum, SA: sodium alginate, HPMC: hydroxypropyl methylcellulose, ECG: espina corona gum, SM: Santa María flour, K: Kapac flour, SP: Señor de Sipan flour, PG: partially gelatinized flour, TG: totally gelatinized flour, D50: median diameter, DI: dispersion index, Δ H: gelatinization enthalpy, WAI: water absorption index, WSI: water solubility index, SWP: swelling power, BV: bread volume, CAF: cell area fraction, MCA: mean cell area, CD: cell density, TPA: texture profile analysis, LVR: linear viscoelastic region.

17 Abstract

The bread making aptitude of five rice flours (native and gelatinized) and five gluten 18 19 substitutes (GS) were prior tested and the best ingredients for mixture design were set. 20 Native flour with a wide distribution of particle size, xanthan gum (XG), guar gum 21 (GG) and sodium alginate (SA) were selected due to their good performance. The effect 22 of formulation on bread volume (BV), cell area fraction (CAF) of breadcrumb and 23 dough rheology was determined by using a simplex centroid mixture design with 24 constrain (2.1 g of GS/100 g of flour). A significant effect of formulation on 25 viscoelasticity of dough was observed. A non-linear relationship between BV and 26 dough viscosity was found with maximum BV at 60000 Pass. The optimum formulation, 27 from XG to GG mass ratio of 0.71, yields maximum values of BV (4.07 ml/g of flour) 28 and CAF (29%); optimum bread presented good textural attributes and a slightly toasted 29 crust.

30

31 Keywords: gluten substitute, image analysis, gas cells, baking quality

32

33 **1. Introduction**

34

The increasing demand of gluten free food has favored the development of rice-based products. Rice is preferred by its hypoallergenic character, high content of easily digestible carbohydrates and low sodium and prolamin contents (Gujral & Rosell, 2004).

In contrast with wheat bread, rice bread production requires the addition of
hydrocolloids with ability to form viscoelastic dough to emulate the gluten functionality
(Kohlwey, Kendall, & Mohindra, 1995). Hydrocolloids contribute with gas retention

42 during fermentation and cooking, playing a key role to set dough structure and bread43 texture.

Gluten has been replaced by gums, hydrocolloids (agar; CMC; HPMC; among others)
and enzymes such as amylases, proteases or hemicellulases (Molina-Rosell, 2013;
Gujral & Rosell, 2004). For rice bread, XG and HPMC have been proposed as GS
because of their beneficial effect on BV although its use makes the product more
expensive (Rosell & Marco, 2008).

49 Mixture design is frequently applied to optimize bread formulation (Yilmaz, Yildiz, 50 Yurt, Toker, & Baştürk, 2015). The influence of flour particle size, on rice bread quality (de la Hera, Martinez, & Gómez, 2013; Sánchez, González, Osella, Torres, & de la 51 52 Torre, 2008) as well as the effect of bread formulation on bread quality and dough 53 rheology (Tao, Xiao, Wu, & Xu, 2018; Witczak, Korus, Ziobro, & Juszczak, 2019) have 54 been studied. Oscillatory tests such as creep-recovery essays have been successfully applied to investigate the rheological behavior of dough and its relationship with quality 55 56 attributes of bread. However, the information concerning to the viscoelastic properties 57 of gluten free dough and the correlations between bread quality and rheological 58 parameters is quite limited (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007). 59 Several studies of gluten free bread using combinations of corn, rice, buckwheat or

Andean cereals flours have been published (Hager & Arendt, 2013; Machado Alencar,
Steel, Alvim, de Morais & Bolini, 2015). However, the present study is focused on rice
based bread from Argentinean rice varieties. Several local rice flours were evaluated in
order to select the most suitable one.

64 The aim of the present work was first to evaluate the bread making aptitude of five rice 65 flours (native and gelatinized) and five GS in order to select the best ingredients (flour 66 and three GS) for mixture design. BV and quality of breadcrumb were evaluated. In a

67	second step the effect of the ratio of three GS on BV, CAF, cell density and viscoelastic
68	behavior of dough were determined by means of simplex centroid mixture design with
69	constrain (2.1 g of GS/100 g of flour). Bread crust color, moisture content and texture
70	profile analysis (TPA) of breadcrumb were measured to characterize the optimal bread.
71	Relationship among viscoelastic parameters of dough and bread quality was also
72	investigated.

- 73
- 74 **2. Materials and methods**
- 75

76 2.1. Materials

77

Three Argentinean commercial rice flours were tested: SM flour (Santa María, Ana Hernández Productos Alimenticios SRL), K flour (Kapac, Alimentos Específicos S.A.) and SP flour (Señor de Sipan, Productos libres de gluten SRL). Partially gelatinized flour (PG) was obtained by high impact milling (Loubes & Tolaba, 2014). Parboiled rice (Molinos Río de la Plata, Argentina) was milling 1 min using a knife mill to obtain totally gelatinized flour (TG). Table 1 summarizes the proximate composition of flours (AOAC, 2000) including amylose content (Morrison & Laignelet, 1983).

SA and HPMC (Sigma-Aldrich, USA), XG and GG (Doña Clara, Argentina), espina
corona gum (Ideasupply Argentina S.A., Argentina) were used as GS. Bread ingredients
(cassava starch, milk powder, egg powder, salt, sugar, dry yeast, sunflower oil) were
purchased at a local market (Doña Clara, Argentina).

89

90 **2.2. Flour characterization**

92	Flour sample (200 g) was sieved through a set of sieves (ASTM N°: 35, 40, 45, 60, 80,
93	100, 120, 140, 200, 270, 325, ASTM Standard, USA). Median diameter (D50) and
94	dispersion index (DI=(D90-D10)/D50) based on mass fractions were reported.
95	Gelatinization temperature and enthalpy (Δ H) were measured in a calorimeter (DSC 822
96	Mettler Toledo, Switzerland) following the procedure of Loubes & Tolaba (2014).
97	Hydration properties were determined at 30°C: WAI (water absorption index) by the
98	method of Chiang & Yeh (2002), SWP (swelling power) and WSI (water solubility
99	index) following the procedure of Loubes & Tolaba (2014). All reported values were
100	the average from three replicates.

101

102 **2.3. Bread making**

103

Bread formulation involved: rice flour (384 g), cassava starch (16 g), milk powder (84 g), sugar (12 g), egg powder (10 g), salt (8 g), dry yeast (8 g), GS (8 g), bi-distilled water (448 g) and sunflower oil (40 ml). Production steps using an electric bread oven (ATMA HP 4040, Argentina) were: mixing (31 rpm, 25 min), fermentation (25 °C-20 min, 32 °C-25 min and 38 °C-45 min) and cooking (121 °C-65 min). Bread was then cooled (60 min, room temperature) and stored (4°C) in plastic bags until further analysis.

- 111
- 112 2.4. Bread quality

113

BV was determined by seed displacement (Sánchez et al., 2008). Breadcrumble quality
was set by image analysis (Pongjaruvat Methacanon, Seetapan, Fuongfuchat, &
Gamonpilas, 2014). Digital images acquired with a multifunction printer (HP PSC

- 117 1610, Brazil) were analyzed by using ImageJ software (v. 1.42q, National Institutes of
 118 Health, USA). The average values of BV, cell area fraction (CAF), mean cell area
 119 (MCA) and cell density (CD) from at least five replicates were reported.
- 120

121 **2.5. Mixture design**

122

123 A simplex centroid design with constrain was applied to analyze the effect GS (2.1 g of 124 GS/100 g of flour) on bread attributes. Each response (Y), was modeled as function of 125 codified factors (x_i , x_j , x_k) by means of the equation (Scheffé, 1958):

126

$$Y = \sum_{i=1}^{3} \beta_i x_i + \sum_{i< j} \sum_{k< j}^{3} \beta_{ij} x_i x_j + \sum_{i< j< k} \sum_{k< j< k} \beta_{ijk} x_i x_j x_k$$
(1)

127

128 where β_i represents the effect of each component, β_{ij} and β_{ijk} the interaction effects 129 between components *i* and *j* and *k*. A linear relationship between the amount of GS and 130 each coded factor was adopted.

131

132 **2.6. Dough rheology**

133

Oscillatory tests were performed at 25° C by duplicate in a controlled-stress rheometer Paar Physica, model MCR 300 (Anton Paar, Graz, Austria) which is provided with two parallel plates (3 cm of diameter, gap = 1 cm) and a Peltier cell. Dough sample (10 g), without yeast, was placed between the plates, then the edges of the sample were sealed using silicone oil (viscosity: 290-310 mPa·s).

139 Linear viscoelastic region (LVR) was set from deformation sweep (0.1-100%) at 140 constant frequency (1 Hz). Frequency sweep (1-100 Hz) was performed at 1% 141 deformation within LVR. Viscoelastic modulus (G' and G", Pa) and damping factor 142 $(\tan \delta = G''/G')$ were recorded as function of frequency. Creep-recovery test involved a 143 first step at constant stress (10 Pa) within the LVR during 60 s to complete the creep 144 stage. Then the stress was removed and the recovery step began and continued for 100 s while the compliance (J, Pa^{-1}) was recorded as function of time (t). J was modeled 145 146 during creep (Eq. 2) and recovery (Eq. 3) phases by Burger's model (Burger, 1935):

147

148
$$J_c(t) = J_0 + J_m \left[1 - \exp(-t/\lambda)\right] + t/\mu_0$$
(2)

149

 $J_r(t) = J_{max} - J_0 - J_m [1 - \exp(-t/\lambda)]$ (3)

150

151 Where J_c and J_r are compliances during creep and recovery, respectively; J_0 , J_m and J_{max} 152 represent the instantaneous, viscoelastic, and maximum creep compliance, respectively; 153 λ is the retardation time and μ_0 is the steady state viscosity.

154

155 2.7. Optimal bread characterization

156

Bread crust color was measured with a Minolta photo-colorimeter (Minolta CM-508d, Tokyo, Japan) using the D illuminant. CIE L*a*b* and CIE L*C*h coordinates were measurements in eight random positions and their average value were reported. Moisture content of breadcrumb was determined by triplicate using 44-15 AACC method (AACC, 2000). TPA of breadcrumb was performed in a universal texture machine (Instron 3345, USA). A cylinder of breadcrumb (diameter: 30 mm; thickness: 20 mm) was compressed at 1 mm/s up to 50% using a cylindrical probe (diameter: 3.5

164 mm). After 10 s the procedure was repeated. Hardness, adhesiveness, cohesiveness and
165 elasticity were obtained through the device's software and the average of 25 replicates
166 was reported.

167

168 **2.8. Statistical analysis**

169

Statistical analyses of single ANOVA and Fisher test (LSD) were performed with a
confidence level of 95%. Multivariate analysis (Pearson's matrix) was applied to detect
correlations between product attributes. Statistical tests and mixture design were made
by using Statgraphics software Centurion version XVI (Statistical graphics Corporation,
USA).

175

176 **3. Results and Discussion**

177

178 **3.1. Flour properties**

179 Particle size, gelatinization and hydration properties are shown in Table 2. Particle size 180 of K and SP flours were similar with peaks at 105 µm, 150 µm, 250 µm and 420 µm 181 (data in Supplementary Material 1). Gelatinized flour (TG) presented peaks at 250 µm, 182 420 µm, and 125 µm. In contrast, flour SM presented a mono modal distribution (peak 183 at 125 µm) while PG flour had a bimodal distribution (peaks at 149 µm and 53 µm). SM and PG presented lower granulometry and DI in comparison with the others. As the 184 185 particle size distribution strongly influences the behavior of dough and sets the bread 186 quality (Sánchez et al., 2008), these evaluations are relevant, and they showed the 187 diversity of rice flour granulometry in the local market.

188 In accordance with the study of Iturriaga, Lopez, & Añon (2004) for long grain rice 189 varieties cultivated in Argentina, peak temperatures between 65 °C and 69 °C were here 190 obtained. Different gelatinization degrees were observed: 78% (PG), 100% (TG) and 191 0% (SM, SP, K). Gelatinized flours showed higher values of hydration properties in 192 comparison with native flours (Table 2). Particularly, PG doubled the values of SWP respect to K and SP native flours. In general, hydration increased as particle size 193 194 decreased; a similar correspondence was also observed by de la Hera et al. (2013). 195 It must be noted that significant correlations were found: D50-WAI (r = -0.70, p < -0.70

196 0.05), D50-WSI (r = -0.82, p < 0.01), WAI- Δ H (r = -0.83, p < 0.01) and WSI- Δ H (r = -197 0.68, p < 0.05). These results evidenced an increase of WAI and WSI as well as a 198 decrease of Δ H with decreasing values of D50. In other words, rice flours with fine 199 granulometry exhibit pre-gelatinized character, high hydration capacity and high 200 solubility.

201

202 **3.2. Flour selection**

203

The selection was based on the effect of flour particle size on bread quality. The aim 204 was to maximize BV and CAF. Breads were made with XG (procedure in section 2.3). 205 206 The highest volume was obtained with K flour (1304 \pm 48 ml) followed by SP flour $(1332 \pm 67 \text{ ml})$. BV of $1098 \pm 55 \text{ ml}$ (SM flour) and $1037 \pm 52 \text{ ml}$ (PG flour) were 207 obtained while TG flour produced the lowest BV (unacceptable). A significant 208 209 correlation BV-DI (r = 0.98; p < 0.05) was found; high values of DI favored BV. The 210 same conclusion was also obtained by Sánchez, González, Osella, Torres, & de la Torre, 211 (1999) and Martínez (2012) who produced rice-based bread.

212	Bread slices used to determine CAF by image analysis are shown in Fig. 1. K flour
213	exhibited the highest CAF (26 \pm 1.5%) followed by SP (19.4 \pm 1.5%), SM (8.1 \pm 0.4%)
214	and PG (8.1 \pm 1.3%). A significant correlation D50-CAF (r = 0.95, p < 0.05) was found.
215	As K flour (DI=1.37 and a multimodal particle size distribution: 105-420 μ m) provided
216	the best performance, it was selected for the optimization of bread formulation (section
217	3.4).

218

219 **3.3. Selection of gluten substitutes**

The effects of GS on BV and breadcrumb quality were determined (Table 3). Breads
were made with K flour and a fixed amount (8 g) of each GS (section 2.3).

222 GG produced the highest volume followed by XG. The difference between them can be 223 attributed to the higher dough viscosity obtained with XG, due to its molecular structure 224 and higher molecular weight (2000 kDa). XG is a polysaccharide containing d-glucose, 225 d-mannose, and d-glucuronic acid as building blocks in a molecular ratio of 3:3:2 with a 226 high number of trisaccharide side chains. On the other hand, GG is a galactomannan 227 with a lower molecular weight of 300 kDa, it has a mannose backbone with galactose 228 side chains (mannose to galactose molar ratio of 1.8). Breads elaborated with SA or 229 HPMC presented, in contrast, the lowest BV.

XG produced the highest CAF followed by SA and GG. Particularly, SA produced the highest mean CA (1.63 mm²/cell) with a CD similar to that of GG. The presence of gas cell uniformly distributed has been associated to the structure stability after shaking, which could be favored by a high viscosity of the mixture (Hager & Arendt, 2013). XG and GG provided good volume while SA produced well-sized cell. Therefore, they were chosen to optimize bread formulations (section 3.4).

237 **3.4. Bread formulations**

238

239 The effects of bread formulation (based on K flour, XG, GG and SA), on bread 240 attributes were determined (Table 4). The combination of gums presented the highest 241 BV but similar values were also obtained with XG-GG-SA and XG-SA formulations. In contrast. SA provoked a significant reduction (p < 0.05) in BV (28%) in comparison 242 with XG-GG combination. The specific volume of bread here obtained (1.3-1.8 ml/g) 243 244 was lower than that reported (3.6-4.3 ml/g) by Aoki, Umemoto, Okamoto, Suzuki, & 245 Tanaka, (2015), who used twice the amount of GS in comparison with the present work. All bread slices presented an acceptable appearance (Figure 2). Wide ranges of CD (6.8-246 21.7 cells/cm²), MCA (1.2-2.79 mm²/cell) and CAF (18.4-29.7%) were obtained (Table 247 4). XG produced, in comparison with others substitutes, higher CD which means a more 248 249 compact breadcrumb (Fig. 2). In contrast, XG-GG mixture and ternary mixture 250 provided the highest values of MCA favoring a greater porosity of breadcrumb (Fig. 2). The CD obtained with GG was similar to that informed by Ziobro, Korus, Witczak, & 251 Juszczak (2012) for bread based on corn and potato starches. CD here obtained were 252 lower than those reported (42-61 cells/cm²) by Machado et al. (2015) for rice bread 253 254 enriched with 20% of quinoa or amaranth flour. The interaction between mixture 255 components is enhanced by adding a protein flour obtaining a more compact porosity.

- 256
- 257 **3.5. Mixture design analysis**
- 258

The effect of GS on BV and CAF was satisfactorily simulated by means of Eq. 1. All GS had significant effects (p < 0.05) on bread quality; the interaction effect between gums was significant. A quadratic model was adequate ($R^2 = 0.945$) to simulate BV as

function of GS (Fig. 3.a) while a cubic expression was required to satisfactorily ($R^2 =$ 262 0.997) model CAF (Fig. 3.b). A synergic effect among gums in the absence of SA was 263 264 detected from the optimization analysis. Maximum values of BV (1645 ml) and CAF (30.1%) were obtained by using XG to GG mass ratios of 0.71 and 1.7, respectively. In 265 266 contrast, the lowest BV (1169 ml) was obtained in the absence of gums, with the 267 maximum concentration of SA. Synergistic interaction between polysaccharides has 268 been attributed to cross linking capacity of polymer's chains, which has a favorable 269 impact on textural properties of bread (Wang, Wang, & Sun, 2002). The synergistic 270 effect among gums has been explained based on the interaction of the exposed mannose 271 segments in the backbone of the guar gum macromolecule with single-helical portions 272 of xanthan molecules to form a complex which yield a three-dimensional network and 273 gel.

Bread quality obtained in the present work is comparable to that informed by Lazaridou
et al. (2007), who used a combination of several functional ingredients (XG, pectin,
agarose, β-glucan, CMC) to reach good BV and texture in rice bread.

Multiple optimization analysis was also performed to maximize BV and CAF simultaneously. It was found a desirability of 0.95 for XG to GG mass ratios of 0.713 (without SA). To validate the model, bread was elaborated with this optimum mixture obtaining 1563 ml of BV and 29% of CAF, in accordance with the predicted values (Eq. 1).

- 282
- 283 **3.5.1. Characterization of optimum bread**
- 284

Optimum bread presented a slight toasted crust (L*: 24.53 ± 0.78 ; a*: 17.72 ± 0.25 ; b*: 286 25.51 ± 0.71) and a breadcrumb with a moisture content of 53.25 ± 1.04% (db).

- 289 breads which present higher luminosity in comparison with wheat breads (Gallagher &
- 290 Gormley, 2002). Optimum bread had values of chroma ($C^* = 31.07 \pm 0.69$), hue (h =
- 291 55.16 ± 0.51) and moisture content similar to those reported in the literature for gluten
- 292 free breads (Martínez, 2012; Ronda, Perez-Quirce, Lazaridou, & Biliaderis, 2015).
- 293 Hardness (20.96 \pm 0.85 N), elasticity (0.81 \pm 0.01), cohesiveness (0.29 \pm 0.01),
- gumminess (6.01 \pm 0.28), chewiness (4.89 \pm 0.24) and adhesiveness (not detected) of
- 295 the optimal bread, reflect a good quality bread considering literature reports (Ronda et
- 296 al., 2015; Machado et al., 2015; Ziobro et al., 2012).
- 297

287

288

- 298 **3.6. Rheological behavior of dough**
- 299
- 300 **3.6.1. Frequency sweep tests**
- 301

LVR was prior tested. XG presented a more extended LVR (0.1 to 8.3%) in comparison
with GG (1-5.2%) or SA (1-4.7%). The highest LVR were obtained for binary (0.18.0%) and ternary (1-17.8%) mixtures due to synergic effect among GS. These results
were similar to those reported by Lazaridou et al. (2007) and Sivaramakrishnan, Senge,
& Chattopadhyay (2004) for dough to make gluten-free bread.

307 Frequency sweep is shown in Fig. 4. G' was independent of frequency within 1 to 50 308 Hz. All mechanical spectra revealed that the elastic character (G' > G") prevails up to a 309 frequency value which was dependent of bread formulation. For XG and GG it was up 310 to 70 Hz while for XG-GG was up to 92 Hz. These results are in accordance with 311 literature reports (Sivaramakrishnan et al., 2004; Gujral & Rosell, 2004). SA produced

312 in contrast a viscous character from 26 Hz with a significant reduction of viscoelastic 313 modulus (788 Pa), at tan $\delta = 1$, in comparison with those of gums (G' = G'' = 6840-314 8890 Pa). The distinctive rheological behavior of different formulations reflects the specific interactions rice flour-GS and GS-GS. As regard the interactions between 315 316 hydrocolloids, it must be mentioned the synergic effect among XG and GG (which was 317 previously explained) and the ternary interaction between SA and the complex formed 318 by GG and XG. Harding, Smith, Lawson, Gahler, & Wood (2011) who studied the 319 macromolecular interactions in ternary mixtures of hydrocolloids, have hypothesized 320 that a complex of two polysaccharides was required to promote non-covalent 321 interactions between SA and the complex of gums.

- 322
- 323 **3.6.2.** Creep-recovery tests
- 324

The significant effect of bread formulation on creep-recovery tests and the viscoelastic character of dough can be appreciated in Fig. 5. XG showed the highest resistance to deformation followed by XG-GG. In contrast, SA and SA-GG produced the maximum values of dough strain and residual deformation at the end of creep and recovery phases. Residual deformation reflects the magnitude of the viscous component. High values are associated to low capacity of gas retention and its unfavorable effect on BV (Table 4).

331 XG due to its rigid, ordered chain conformation, shows high viscosity values at low
332 shear rates favoring dough elasticity and the increase of BV (Lazaridou et al., 2007).

Compliance was satisfactorily simulated ($\mathbb{R}^2 > 0.99$) by Burger's model (Eq. 2 and 3). As shows in Table 5, all compliance values in creep phase (J_0 , J_m) were higher in comparison with those of XG while viscosities (μ_0) were lower than that of XG. These results were comparable to those reported by Lazaridou et al. (2007) for dough to

produce gluten-free bread. Dough involved in pasta production shows in contrast lower
values of compliances (Sozer, 2009). This is due to the higher value of shear stress (
750 Pa) applied in pasta evaluation in comparison with the present work (10 Pa).

For creep phase the relative contributions of instantaneous compliance $(J_0/(J_0+J_m))$ and 340 average compliance $(J_m/(J_{0+} J_m))$ were within 16-29% and 71-84% respectively. Values 341 of mean retardation time (λ) were, in general, slightly higher than that of XG 342 343 formulation. The differences in the viscoelastic behavior can be interpreted in terms of 344 the differences in the stretching of the associative network, which is set by non-covalent 345 intermolecular bonds between starch and GS (Edwards, Peressini, Dexter, & Mulvaney, 346 2001). For recovery phase, the relative elastic contribution $((J_0+J_m)/J_{max})$ was maximum 347 for XG formulation (43.4%) while for GG-SA formulation was minimum (30.1%). 348 Viscous character prevailed at the end of recovery phase for all samples tested. In 349 addition, the retardation times increased significantly (between 18.3 s and 21.9 s) in 350 comparison with those of the creep phase, due to the molecular stretching to which the 351 components of the mixture are subjected during creep. An increase of retardation time 352 during recovery phase, but of smaller magnitude (7 s), was also observed by Hernández-353 Estrada, Rayas-Duarte, Figueroa, & Morales-Sánchez (2014) in wheat-based dough. The influence of GS is related to its molecular structure and the conformation of the 354 355 polysaccharide chains, which determine the possible cross-linking between polymer 356 chains and mixture components.

Among all the substitutes studied, the XG exhibits the lowest compliance (J_m) and the highest steady state viscosity (μ_0). Viscoelastic behavior of dough from XG formulation is explained by the well-known ability of this gum to form a weak gel, as well as to provide high viscosity at low shear rate, due to the rigid conformation of its chains (Doublier & Cuvelier, 1996).

363 **3.7. Relationship between dough and bread attributes**

364

The non-linear relationship between BV and dough viscosity (Fig. 6) was satisfactorily 365 simulated ($R^2 = 0.87$) by a quadratic equation. BV increased with the increase of dough 366 367 viscosity up to 60000 Pa.s (critical value); a further increase of viscosity had a negative 368 effect on volume. Gas retention capacity of dough was optimum at critical viscosity, 369 favoring the production of high values of CD and MCA during baking. Similar 370 relationship BV - dough viscosity were found by Ronda et al. (2015) who elaborated 371 gluten free rice bread with the addition of β -glucans from oat and barley. The 372 relationship here presented can be useful to predict the aptitude of dough to produce 373 good quality bread.

374

375 **4. Conclusions**

376

377 The effect of flour granulometry on bread quality was significant. Multimodal particle 378 size distribution and high dispersion index led to good values of bread volume and gas 379 cells. Due to its positive effect on bread quality: XG, GG and SA were selected to 380 perform the mixture design. A differentiated viscoelastic behavior of dough could be 381 observed from different formulations. Rheological tests evidenced the synergic effects 382 between gums. SA produced low gas retention capacity of dough. In contrast, dough 383 samples elaborated with xanthan gum or combination of gums showed the highest 384 volume and dough resistance. The rheological behavior during creep-recovery test was 385 satisfactorily modeled by Burger's model. The steady state viscosity of dough was

386	related to bread quality. Maximum bread volume was obtained from dough with 60000
387	Pa·s of viscosity.
388	Bread formulation was satisfactorily optimized by mixture design. Bread volume and
389	CAF were significantly enhanced by addition of guar gum and xanthan gum to the
390	formulation. Optimum bread presented desirable attributes in terms of crust color,
391	texture profile and moisture content of the breadcrumb.
392 393	5. Acknowledgments
394	
395	The authors acknowledge the financial support from Buenos Aires University and
396	National Council of Scientific Research of Argentina. This work has been funded by
397	PME-2006-01685 and UBACYT (Project UBACYT 20020170100367BA).
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536 Figure captions

537

538 Fig. 1. Cross-section of pan breads made from different rice flours. SM: Santa María

539 flour, K: Kapac flour, SP: Señor de Sipan flour, PG: Partially gelatinized flour.

540

- 541 Fig. 2. Cross-section of pan breads as function of mixture design. XG: xanthan gum,
- 542 SA: sodium alginate, GG: guar gum.

543

544 Fig. 3. Predicted surfaces of bread quality as function of codified factors a) Bread

545 volume (BV), b) Cell area fraction (CAF) of breadcrumb.

546

Fig. 4. a) Elastic modulus (G') and b) damping factor (tan δ), of dough as function of bread formulation during frequency sweep at 25 °C with constant deformation of 1%.

- 549 XG: xanthan gum (\blacktriangle), SA: sodium alginate (\Box), GG: guar gum (\triangle), XG-GG (\blacklozenge),
- 550 XG-SA-GG (\blacksquare), SA-GG (\blacklozenge), XG-SA (\diamondsuit).

- 552 Fig. 5. Creep-recovery tests as function of bread formulation. XG: xanthan gum (----),
- 553 SA: sodium alginate (----), GG: guar gum (----), XG-GG (---), XG-SA-GG (---), SA-
- 554 GG (—), XG-SA (—).
- 555
- 556 **Fig. 6.** Relationship between bread volume (BV) and steady state viscosity of dough 557 (μ_0) .

(g/100 g, db)SMKSPPGTGMoisture 11.16 ± 0.09^{bc} 11.63 ± 0.11^{c} 11.12 ± 0.17^{b} 10.62 ± 0.03^{a} 12.13 ± 0.21^{c} Carbohydrate 78.99 ± 1.07^{a} 79.80 ± 0.60^{a} 79.20 ± 0.84^{a} 79.5 ± 1.39^{a} 78.10 ± 0.82^{c} Protein 5.92 ± 0.05^{b} 4.18 ± 0.08^{a} 6.89 ± 0.11^{c} 7.04 ± 0.09^{c} 7.22 ± 0.14^{c} Lipid 1.18 ± 0.02^{c} 1.59 ± 0.02^{d} 0.32 ± 0.00^{b} 0.10 ± 0.00^{a} 0.10 ± 0.00^{c} Fiber 2.37 ± 0.09^{d} 1.59 ± 0.03^{b} 0.53 ± 0.01^{a} 1.45 ± 0.04^{b} 2.06 ± 0.03^{c}	Composition			Flours		
Moisture 11.16 ± 0.09^{bc} 11.63 ± 0.11^{c} 11.12 ± 0.17^{b} 10.62 ± 0.03^{a} 12.13 ± 0.21^{c} Carbohydrate 78.99 ± 1.07^{a} 79.80 ± 0.60^{a} 79.20 ± 0.84^{a} 79.5 ± 1.39^{a} 78.10 ± 0.82^{c} Protein 5.92 ± 0.05^{b} 4.18 ± 0.08^{a} 6.89 ± 0.11^{c} 7.04 ± 0.09^{c} 7.22 ± 0.14^{c} Lipid 1.18 ± 0.02^{c} 1.59 ± 0.02^{d} 0.32 ± 0.00^{b} 0.10 ± 0.00^{a} 0.10 ± 0.00^{c} Fiber 2.37 ± 0.09^{d} 1.59 ± 0.03^{b} 0.53 ± 0.01^{a} 1.45 ± 0.04^{b} 2.06 ± 0.03^{c}	(g/100 g, db)	SM	K	SP	PG	TG
Carbohydrate 78.99 ± 1.07^{a} 79.80 ± 0.60^{a} 79.20 ± 0.84^{a} 79.5 ± 1.39^{a} 78.10 ± 0.82^{a} Protein 5.92 ± 0.05^{b} 4.18 ± 0.08^{a} 6.89 ± 0.11^{c} 7.04 ± 0.09^{c} 7.22 ± 0.14^{c} Lipid 1.18 ± 0.02^{c} 1.59 ± 0.02^{d} 0.32 ± 0.00^{b} 0.10 ± 0.00^{a} 0.10 ± 0.00^{c} Fiber 2.37 ± 0.09^{d} 1.59 ± 0.03^{b} 0.53 ± 0.01^{a} 1.45 ± 0.04^{b} 2.06 ± 0.03^{c}	Moisture	11.16 ± 0.09^{bc}	$11.63 \pm 0.11^{\circ}$	11.12 ± 0.17^{b}	10.62 ± 0.03^{a}	12.13 ± 0.21^{d}
Protein 5.92 ± 0.05^{b} 4.18 ± 0.08^{a} 6.89 ± 0.11^{c} 7.04 ± 0.09^{c} 7.22 ± 0.14^{c} Lipid 1.18 ± 0.02^{c} 1.59 ± 0.02^{d} 0.32 ± 0.00^{b} 0.10 ± 0.00^{a} 0.10 ± 0.00^{c} Fiber 2.37 ± 0.09^{d} 1.59 ± 0.03^{b} 0.53 ± 0.01^{a} 1.45 ± 0.04^{b} 2.06 ± 0.03^{c}	Carbohydrate	78.99 ± 1.07^{a}	79.80 ± 0.60^{a}	79.20 ± 0.84^{a}	79.5 ± 1.39^{a}	78.10 ± 0.82^{a}
Lipid 1.18 ± 0.02^{c} 1.59 ± 0.02^{d} 0.32 ± 0.00^{b} 0.10 ± 0.00^{a} 0.10 ± 0.00^{a} Fiber 2.37 ± 0.09^{d} 1.59 ± 0.03^{b} 0.53 ± 0.01^{a} 1.45 ± 0.04^{b} 2.06 ± 0.03^{c}	Protein	$5.92\pm0.05^{\text{b}}$	4.18 ± 0.08^{a}	$6.89 \pm 0.11^{\circ}$	$7.04 \pm 0.09^{\circ}$	$7.22 \pm 0.14^{\circ}$
Fiber 2.37 ± 0.09^{d} 1.59 ± 0.03^{b} 0.53 ± 0.01^{a} 1.45 ± 0.04^{b} 2.06 ± 0.03^{c}	Lipid	$1.18 \pm 0.02^{\circ}$	1.59 ± 0.02^{d}	0.32 ± 0.00^{b}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}
	Fiber	2.37 ± 0.09^{d}	1.59 ± 0.03^{b}	0.53 ± 0.01^{a}	$1.45\pm0.04^{\rm b}$	$2.06 \pm 0.03^{\circ}$
Ash $0.50 \pm 0.00^{\text{b}}$ $1.21 \pm 0.03^{\text{c}}$ $1.95 \pm 0.05^{\text{d}}$ $1.29 \pm 0.04^{\text{c}}$ $0.38 \pm 0.01^{\text{c}}$	Ash	0.50 ± 0.00^{b}	$1.21 \pm 0.03^{\circ}$	1.95 ± 0.05^{d}	$1.29 \pm 0.04^{\circ}$	0.38 ± 0.01^{a}

2 Proximate chemical composition of tested rice flours¹.

3 $\overline{}$ Amylose content was within 18 – 22 g/100 g, db (dry basis).

- 4 SM: Santa María flour, K: Kapac flour, SP: Señor de Sipan flour, PG: Partially
- 5 gelatinized flour, TG: Totally gelatinized flour.

2 Characteristic parameters of particle size distribution, and thermal and hydration

3 properties of tested rice flours.

	D50		Тр	ΔΗ	WAI	WSI	SWP
Flou	rs (µm)	DI	(°C)	(J/g, db)	(g/g, db)	(%)	(g/g, db)
SM	135 ± 2^{a}	0.40 ± 0.03^{a}	64.8 ± 0.1^{a}	4.8 ± 0.2^{b}	$2.99 \pm 0.02^{\circ}$	2.54 ± 0.04^{b}	3.42 ± 0.03^{b}
K	220 ± 22^{b}	1.37 ± 0.2^{bc}	67.6 ± 0.4^{b}	6.2 ± 0.3^{c}	2.43 ± 0.02^a	1.21 ± 0.05^a	2.89 ± 0.06^a
SP	177 ± 9^a	1.64 ± 0.07^{c}	66.4 ± 0.2^{b}	4.0 ± 0.1^{b}	2.52 ± 0.02^{b}	1.34 ± 0.03^a	2.72 ± 0.03^a
PG	156 ± 1^a	0.43 ± 0.02^a	69.4 ± 0.5^{c}	1.5 ± 0.3^{a}	3.39 ± 0.01^{d}	2.80 ± 0.04^{c}	4.62 ± 0.15^{c}
TG	$313 \pm 11^{\circ}$	1.08 ± 0.06^{b}	n.d.	n.d.	4.39 ± 0.03^{e}	$2.75 \pm 0.02^{\circ}$	5.42 ± 0.02^{d}
4	D50: Particle	e sizes corre	sponding to	50% cur	nulative unde	ersize mass ((median
5	diameter), D	I: Dispersion	n index, T	Tp: Peak	gelatinization	temperature	s, ΔH:
6	Gelatinization	enthalpy, W	AI: Water a	bsorption in	ndex, WSI: W	ater solubility	y index,
7	SWP: Swellin	g power.					
8	SM: Santa M	Iaría flour, K	: Kapac flo	our, SP: Se	ñor de Sipan	flour, PG: I	Partially
9	gelatinized flo	our, TG: Totall	y gelatinized	l flour.			
10	n.d.: not detec	ted.					

2 Effect of gluten substitute on bread quality.

Gluten	Bread	Cell area	Mean cell	Cell density	
	volume	fraction	area		
substitute	(ml)	(%)	(mm ² /cell)	(cells/cm)	
XG	1304 ± 48^{ab}	26.0 ± 1.5^{c}	1.20 ± 0.08^{a}	21.7 ± 2.1^{b}	
GG	1474 ± 47^{b}	21.5 ± 2.7^{bc}	1.41 ± 0.07^{ab}	$15.2\pm0.6^{\mathrm{a}}$	
SA	1173 ± 42^a	23.1 ± 0.1^{c}	1.63 ± 0.05^{b}	$14.2\pm0.5^{\rm a}$	
ECG	1312 ± 49^{ab}	13.0 ± 0.7^{a}	1.17 ± 0.07^{a}	11.1 ± 1.1^{a}	
HPMC	1294 ± 48^a	16.4 ± 0.4^{ab}	1.36 ± 0.09^{a}	12.1 ± 0.9^{a}	

3 XG: Xanthan gum, GG: Guar gum, SA: Sodium alginate, ECG: Espina corona gum,

4 HPMC: Hydroxypropyl methylcellulose.

	Glu	ten substitut	t e (g)	Bread	Cell area	Mean cell	Cell
tures	XG	SA	GG	volume	fraction	area	density
Mix	(Coded ¹)	(Coded ¹)	(Coded ¹)	(ml)	(%)	(mm ² /cell)	(cells/cm ²)
1	8 (1)	0 (0)	0 (0)	1304 ± 48^{ab}	26.0 ± 1.5^{bc}	1.20 ± 0.08^{a}	21.7 ± 2.1^{d}
2	0 (0)	0 (0)	8 (1)	1474 ± 47^{bc}	21.5 ± 2.7^{ab}	1.41 ± 0.07^{ab}	$15.2 \pm 0.6^{\circ}$
3	0 (0)	8 (1)	0 (0)	1173 ± 42^{a}	23.1 ± 0.1^{ab}	1.63 ± 0.05^{b}	$14.2\pm0.5^{\rm c}$
4	4 (0.5)	0 (0)	4 (0.5)	1622 ± 81^{c}	$29.7 \pm 3.1^{\circ}$	$2.79\pm0.23^{\rm c}$	10.3 ± 0.4^{b}
5*	2.67 (0.33)	2.67 (0.33)	2.67 (0.33)	1618 ± 81^{c}	18.4 ± 0.4^{a}	$2.56 \pm 0.06^{\circ}$	6.8 ± 0.6^{a}
6	0 (0)	4 (0.5)	4 (0.5)	1315 ± 66^{ab}	18.9 ± 0.3^{a}	1.53 ± 0.19^{ab}	11.9 ± 0.8^{bc}
7	4 (0.5)	4 (0.5)	0 (0)	$1615 \pm 81^{\circ}$	20.1 ± 1.1^{a}	1.55 ± 0.12^{ab}	12.7 ± 0.8^{bc}

2 Experimental design and bread quality as function of formulation.

³ ¹ A linear relationship among experimental and coded factors was used.

4 *Central point of experimental design (triplicate).

5 XG: Xanthan gum; SA: Sodium alginate; GG: Guar gum.

res	J ₀	$\mathbf{J}_{\mathbf{m}}$	μ_0	λ	D ²
Mixtu	(10 ⁻⁴ Pa ⁻¹)	$(10^{-4} \text{ Pa}^{-1})$	(Pa·s)	(s)	K
Creep	phase				
1	$2.02\pm0.05^{\rm bc}$	4.92 ± 0.12^{ab}	$99486 \pm 1890^{\rm e}$	6.46 ± 0.12^{a}	0.9992
2	1.42 ± 0.10^{a}	6.67 ± 0.83^{a}	$65896 \pm 977^{\circ}$	7.81 ± 0.09^{d}	0.9994
3	$8.96 \pm 0.37^{\rm e}$	28.48 ± 0.83^{d}	$23601 \pm 288^{\mathrm{a}}$	6.53 ± 0.03^{a}	0.9989
4	$1.67\pm0.00~^{ab}$	$4.96\pm0.07^{\rm a}$	78495 ± 481^d	$6.98 \pm 0.00^{\rm bc}$	0.9993
5*	$2.42 \pm 0.04^{\circ}$	7.82 ± 0.23^{b}	54129 ± 1165^{b}	$7.09 \pm 0.06^{\circ}$	0.9992
6	$3.12\pm0.13^{\rm d}$	$16.18 \pm 0.93^{\circ}$	26482 ± 1060^{a}	7.90 ± 0.16^{d}	0.9994
7	$3.37\pm0.08^{\rm d}$	$8.53\pm0.1^{\rm b}$	$62663 \pm 1358^{\circ}$	6.70 ± 0.05^{ab}	0.9991
res	J ₀	J _m	J _{max}	λ	
Mixtures	J_0 (10 ⁻⁴ Pa ⁻¹)	J _m (10 ⁻⁴ Pa ⁻¹)	J_{max} (10 ⁻³ Pa ⁻¹)	λ (s)	R ²
Wixtures <i>Recove</i>	J ₀ (10 ⁻⁴ Pa ⁻¹) ery phase	J _m (10 ⁻⁴ Pa ⁻¹)	J _{max} (10 ⁻³ Pa ⁻¹)	λ (s)	R ²
Secove 1	J_0 (10 ⁻⁴ Pa ⁻¹) ery phase 2.57 ± 0.06 ^a	J_{m} (10 ⁻⁴ Pa ⁻¹) 2.95 ± 0.07 ^a	$ \begin{array}{c} J_{max} \\ (10^{-3} \text{ Pa}^{-1}) \\ 1.27 \pm 0.03^{a} \end{array} $	λ (s) 26.49 ± 0.66 ^a	R ² 0.9929
Secove 1 2	$ \begin{array}{r} J_0 \\ (10^{-4} \text{ Pa}^{-1}) \\ ery \ phase \\ 2.57 \pm 0.06^a \\ 2.21 \pm 0.28^a \end{array} $	$ J_m (10^{-4} Pa^{-1}) 2.95 \pm 0.07^a 3.39 \pm 0.44^a $	J_{max} (10 ⁻³ Pa ⁻¹) 1.27 ± 0.03 ^a 1.69 ± 0.28 ^{ab}	λ (s) 26.49 ± 0.66 ^a 26.12 ± 0.02 ^a	R ² 0.9929 0.9931
Recove 1 2 3	$ \begin{array}{r} J_0 \\ (10^{-4} \text{ Pa}^{-1}) \\ ery phase \\ 2.57 \pm 0.06^a \\ 2.21 \pm 0.28^a \\ 10.78 \pm 0.25^e \\ \end{array} $	J_{m} (10 ⁻⁴ Pa ⁻¹) 2.95 ± 0.07 ^a 3.39 ± 0.44 ^a 10.40 ± 0.24 ^d	J_{max} (10 ⁻³ Pa ⁻¹) 1.27 ± 0.03 ^a 1.69 ± 0.28 ^{ab} 6.17 ± 0.15 ^d	λ (s) 26.49 ± 0.66 ^a 26.12 ± 0.02 ^a 25.79 ± 0.13 ^a	R ² 0.9929 0.9931 0.9907
Recove 1 2 3 4	$ \begin{array}{r} J_0 \\ (10^{-4} \text{ Pa}^{-1}) \\ ery phase \\ 2.57 \pm 0.06^a \\ 2.21 \pm 0.28^a \\ 10.78 \pm 0.25^e \\ 2.57 \pm 0.01^a \end{array} $	J_{m} (10 ⁻⁴ Pa ⁻¹) 2.95 ± 0.07 ^a 3.39 ± 0.44 ^a 10.40 ± 0.24 ^d 3.23 ± 0.02 ^a	J_{max} (10 ⁻³ Pa ⁻¹) 1.27 ± 0.03 ^a 1.69 ± 0.28 ^{ab} 6.17 ± 0.15 ^d 1.40 ± 0.01 ^a	λ (s) 26.49 ± 0.66 ^a 26.12 ± 0.02 ^a 25.79 ± 0.13 ^a 25.72 ± 0.60 ^a	R ² 0.9929 0.9931 0.9907 0.9923
Recove 1 2 3 4 5*	$ \begin{array}{r} J_0 \\ (10^{-4} \text{ Pa}^{-1}) \\ ery phase \\ 2.57 \pm 0.06^a \\ 2.21 \pm 0.28^a \\ 10.78 \pm 0.25^e \\ 2.57 \pm 0.01^a \\ 3.63 \pm 0.04^b \\ \end{array} $	J_{m} (10 ⁻⁴ Pa ⁻¹) 2.95 ± 0.07 ^a 3.39 ± 0.44 ^a 10.40 ± 0.24 ^d 3.23 ± 0.02 ^a 4.74 ± 0.23 ^b	J_{max} (10 ⁻³ Pa ⁻¹) 1.27 ± 0.03 ^a 1.69 ± 0.28 ^{ab} 6.17 ± 0.15 ^d 1.40 ± 0.01 ^a 2.09 ± 0.05 ^b	λ (s) 26.49 ± 0.66 ^a 26.12 ± 0.02 ^a 25.79 ± 0.13 ^a 25.72 ± 0.60 ^a 26.15 ± 0.32 ^a	R ² 0.9929 0.9931 0.9907 0.9923 0.9931
secove 1 2 3 4 5* 6	$\begin{array}{r} \textbf{J_0} \\ (10^{-4} \text{ Pa}^{-1}) \\ \hline ery \ phase \\ 2.57 \pm 0.06^a \\ 2.21 \pm 0.28^a \\ 10.78 \pm 0.25^e \\ 2.57 \pm 0.01^a \\ 3.63 \pm 0.04^b \\ 5.00 \pm 0.17^d \end{array}$	$\begin{tabular}{ c c c c }\hline & J_m \\ (10^{-4} \mbox{ Pa}^{-1}) \\ \hline & 2.95 \pm 0.07^a \\ \hline & 3.39 \pm 0.44^a \\ 10.40 \pm 0.24^d \\ \hline & 3.23 \pm 0.02^a \\ \hline & 4.74 \pm 0.23^b \\ \hline & 7.38 \pm 0.49^c \end{tabular}$	J_{max} (10 ⁻³ Pa ⁻¹) 1.27 ± 0.03 ^a 1.69 ± 0.28 ^{ab} 6.17 ± 0.15 ^d 1.40 ± 0.01 ^a 2.09 ± 0.05 ^b 4.11 ± 0.2 ^c	λ (s) 26.49 ± 0.66 ^a 26.12 ± 0.02 ^a 25.79 ± 0.13 ^a 25.72 ± 0.60 ^a 26.15 ± 0.32 ^a 27.28 ± 0.23 ^a	R ² 0.9929 0.9931 0.9907 0.9923 0.9931 0.9924

2 Burger's model parameters (equations 2-3) of doughs as function of formulation.

3 J₀: Instantaneous compliance, J_m: Viscoelastic compliance, J_{max}: Maximum creep

4 compliance, λ : Retardation time, μ_0 : Steady state viscosity.

5 *Central point of experimental design (triplicate).







80 Time (s)





XG-GG-SA



SA-GG













Highlights

- Several rice flours and gluten substitutes were evaluated to produce rice bread.
- Bread quality was estimated from bread volume and gas cell parameters.
- Mixture design was adopted to perform the optimization of bread formulation.
- The effect of formulation on dough rheology was also studied.
- Correlations among bread quality attributes and dough viscosity were obtained.