

# 1 Physicochemical Properties of Long Rice Grain Varieties in Relation to Gluten Free Bread

## 2 Quality

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### 10 **Abstract**

11 The aim of this study was conducted to determine the breadmaking potential of six long-grain rice  
12 varieties (INIAP 14, INIAP 15, INIAP 16, INIAP 17, F09 and F50) and to identify any flour  
13 characteristic governing their breadmaking behavior. Pasting parameters, thermal parameters  
14 assessed by differential scanning calorimetry and bread quality parameters (specific volume, color,  
15 and crumb texture profile analysis) were assessed. Results confirmed the suitability of long-grain  
16 rice varieties for breadmaking. Nevertheless, significant differences were observed in flour  
17 properties among varieties. A significant correlation was observed between specific volume of the  
18 gluten-free bread (GFB) with swelling power ( $r=0.71$ ,  $P<0.01$ ), breakdown viscosity ( $r=-0.97$ ,  
19  $P<0.01$ ) and conclusion temperature ( $T_c$ ) of gelatinization ( $r=0.81$ ,  $P<0.05$ ). Moreover, a strong  
20 correlation was found between cohesiveness and properties of rice flour such as peak temperature  
21 ( $T_p$ ) ( $r=-0.96$ ,  $P<0.001$ ),  $\Delta H$  ( $r=0.71$   $P<0.05$ ) and swelling volume ( $r=0.82$ ,  $P<0.05$ ). The quality  
22 characteristics of the gluten-free breads made of long-grain rice flour were comparable to those  
23 reported in commercial GFB. INIAP 14 and F09 were the most promising varieties for bakery

24 applications. Results suggested that the most important parameters of rice flour when defining  
25 breadmaking performance of GFB would be WBC, SP, SV, Tp, Tc and enthalpy.

26 **Key words:** rice flour; variety; bread; gluten-free; quality.

## 27 **1. Introduction**

28 Rice is mainly consumed as cooked rice, but during the last decade the consumption of rice flour  
29 has increased due to its application in breadmaking. Rice has unique sensorial and nutritional  
30 advantages for developing gluten-free foods. Specifically, rice flour has a neutral flavor, low levels  
31 of sodium, easy digestibility, hypoallergenic proteins, and does not contain gluten. These  
32 characteristics make rice flour a suitable ingredient for gluten-free bakery products (Marco &  
33 Rosell, 2008). However, features of rice based breads are greatly dependent on rice flour  
34 functionality. Physicochemical properties of rice flour, main determinants of its technological  
35 functionality, are greatly variable. In fact, these properties are significantly influenced by rice  
36 variety (Han, Cho, Kang & Koh, 2012; Sompong, Siebenhandl-Ehn, Linsberger-Martin &  
37 Berghofer, 2011; Yu, Ma, Menager & Sun, 2012), storage conditions (Park, Kim, Park & Kim,  
38 2012; Tananuwong & Malila, 2011), particle size of the flour and length of rice grain (de la Hera,  
39 Gomez & Rosell, 2013a), processing method (Guha & Ali, 2011), chemical structure and  
40 composition (Kim, Song & Shin, 2010; Zhu, Liu, Sang, Gu & Shi, 2010), among others.

41 Regarding variety, there is a general agreement that rice grain length is a factor that influences the  
42 bread quality in gluten-free breads (GFB), although discrepancies about the most convenient type of  
43 rice grain have been reported (Kadan, Robinson, Thibodeaux & Pepperman, 2001; Rosell &  
44 Gomez, 2006; de la Hera, Martinez & Gómez, 2013b). In general, the size of the grain is related to  
45 its amylose content. Long-grain rice contain higher amylose content and gelatinization temperature,  
46 as well as more tendency of retrogradation than short-grain rice. Noomhorm, Bandola & Kongseree  
47 (1994) demonstrated that rice varieties with low amylose content exhibited low gelatinization

48 temperature and soft gels, which was beneficial for baking. Kadan et al. (2001) reported that  
49 combining part of the long-grain variety with 10% of short-grain variety produces a smoother  
50 texture in bread. Furthermore, Han et al. (2012) stated that intermediate amylose content and low  
51 water absorption capacity of rice gave better rice bread physicochemical properties. Studies had  
52 shown that short and medium-grain rice varieties presented better bread textures (Rosell et al.,  
53 2006). de la Hera et al., (2013b) showed that short-grain rice produced breads with higher specific  
54 volume and lower hardness than long-grain rice. Conversely, few studies demonstrated that long-  
55 grain rice could have good breadmaking performance (Han et al., 2012; Kadan et al., 2001;  
56 Sivaramakrishnan, Senge, & Chattopadhyay, 2004). In fact, Sivaramakrishnan et al. (2004) reveal  
57 that flour of long-grain rice with 3g of hydroxypropylmethylcellulose per 100g of flour presents  
58 better texture than flour of short-grain rice. Generally, studies related to rice gluten-free bread have  
59 been carried out with commercial rice flour, without controlling the rice type or variety (de la Hera  
60 et al., 2013b; Kadan et al., 2001; Rosell et al., 2006). Since there is not knowledge about the main  
61 properties of rice flour governing breadmaking potential, it is necessary to get additional insight on  
62 the properties of rice flour from long-grain varieties.

63 Therefore, the aim of this study was to determine the breadmaking potential of six long-grain rice  
64 varieties and to assess any possible flour characteristic (physicochemical and rheological) of their  
65 breadmaking behavior.

## 66 **2. Materials and Method**

67 Six varieties of long-grain rice were selected as representative of the main production in the region.  
68 Four of them were from the National Institute of Agricultural Research from Ecuador (INIAP,  
69 Boliche, Ecuador): INIAP 14, INIAP 15, INIAP 16 and INIAP 17 and two varieties were from the  
70 company PRONACA (Guayaquil, Ecuador): F09 and F50. The average size of the rice grains was

71 7.2mm±0.1mm. All varieties were harvested between May and December of 2011. All the samples  
72 were provided by INIAP.

### 73 2.1. Flour production and chemical characterization

74 The seeds were polished and milled (Cyclotec Sample Mill, Tecator, Hoganas, Sweden) with a 500  
75 µm screen. Considering the already stated relationship between physicochemical properties of  
76 starches and their apparent amylose content (AAC), protein and lipid (Gani, Wani, Masoodi &  
77 Salim, 2013, Tester & Morrison 1990a, Kim et al. 2010), these parameters have been analyzed. The  
78 flour protein, lipid content and moisture content were analyzed following AOAC methods (AOAC  
79 18<sup>th</sup> 92087 for protein and AOAC18<sup>th</sup> 922.06 for fats). Moisture content was determined following  
80 the ISO method (ISO 712:1998). The AAC of the rice flour was measured following the iodine  
81 calorimetric method (Juliano et al., 1981). Spectrophotometer (PerkinElmer, Waltham, USA)  
82 measurements were made at 620nm after the above starch-iodine solution was incubated for 20min  
83 at ambient temperature. Standard curve was generated using starch reference with 66g of amylose  
84 per 100g of flour from the Megazyme kit K-AMYL 04/06t (Megazyme International Ltd, Wicklow,  
85 Ireland). All the analyses were made by triplicate.

### 86 2.2. Flour hydration properties

87 The water holding capacity (WHC) defined as the amount of water retained by the sample without  
88 being subjected to any stress was determined by mixing (1.000g ± 0.005g) of flour with distilled  
89 water (10ml) and kept at room temperature for 24h. The supernatant was carefully removed with a  
90 pipette. WHC was expressed as grams of water retained per gram of solid. The swelling volume  
91 (SV) was determined following the method reported by Gularte & Rosell (2011) with slight  
92 modification. The swelling volume was calculated by dividing the total volume of the swollen  
93 sample after 24h at room temperature by the powder weight of the sample. The water binding  
94 capacity (WBC) defined as the amount of water retained by the sample under low-speed

95 centrifugation was determined as described the standard method (AACC, 2010). Samples (1.000g ±  
96 0.005g) were mixed with distilled water (10ml) and centrifuged at 2,000xg for 10min. WBC was  
97 expressed as grams of water retained per gram of solid. All the analyses were made by triplicate.  
98 WHC, SV and WBC were calculated by the equations 1 to 3:

99 
$$WHC (g/g) = \frac{\text{Weight of sediment after draining supernatant} - \text{Sample dry weight}}{\text{Sample weight}} \quad \text{Eq. 1}$$

100 
$$SV (ml/g) = \frac{\text{Total volume of swollen sample}}{\text{Sample weight}} \quad \text{Eq. 2}$$

101 
$$WBC (g/g) = \frac{\text{Weight of sediment after centrifugation} - \text{Sample dry weight}}{\text{Sample weight}} \quad \text{Eq. 3}$$

### 102 2.3. Flour gelling behavior

103 Water absorption index (WAI), water solubility index (WSI) and the swelling power (SP) of  
104 different rice flour gels were determined following the method of Anderson, Conway, Pheiser &  
105 Griffin (1969), with slight modification. Briefly, flour (50.0mg ± 0.1mg) sample was dispersed in  
106 1ml of distilled water and cooked at 90°C for 15min in a water bath. The cooked paste was cooled  
107 to room temperature, and centrifuged at 3,000xg at 4°C for 10min (Thermo Scientific, Waltham,  
108 USA). The supernatant was decanted for determination of its solid content into an evaporating dish  
109 and the sediment was weighed. The weight of dry solids recovered by evaporating the supernatant  
110 overnight at 110°C was determined. Four replicates were made for each sample. WSI, WAI and SP  
111 were calculated by the equations 4 to 6:

112 
$$WAI (g/g) = \frac{\text{Weight of sediment}}{\text{Sample weight}} \quad \text{Eq. 4}$$

113 
$$WSI (g/g) = \frac{\text{Weight of dissolved solids in supernatant}}{\text{Sample weight}} \quad \text{Eq. 5}$$

114 
$$SP (g/g) = \frac{\text{Weight of sediment}}{(\text{Sample weight} - \text{Weight of dissolved solids in supernatant})} \quad \text{Eq. 6}$$

115 For the determination of oil absorption capacity (OAC), the method of Lin et al. (1974) was  
116 followed and it was expressed as grams of oil bound per gram of the sample on dry basis. Three  
117 replicates were made for each sample. OAC was calculated by the equation 7:

$$118 \quad OAC \text{ (g/g)} = \frac{\text{Weight of sediment after draining oil}}{\text{Sample weight}} \quad \text{Eq. 7}$$

#### 119 2.4. Determination of pasting properties of rice flours

120 Pasting properties of the rice flour were determined using a rapid visco analyser (RVA) (Newport  
121 Scientific model 4-SA, Warriewood, Australia) by following ICC standard method No 162 (ICC,  
122 1996). Sample (3g based on 14g of moisture per 100g of flour) was added to 25mL of water. The  
123 mixture was heated to 50°C for 1min and then heated to 95°C at a rate of 12.2°C min<sup>-1</sup>. After  
124 holding at 95°C for 2.5min, the mixture was cooled to 50°C at a rate of 11.8°C min<sup>-1</sup>. The rotational  
125 speed of the paddle was maintained at 160rpm through the run, except during the first 10s, when a  
126 960rpm speed was used. Peak viscosity, breakdown, final viscosity and setback (difference between  
127 final viscosity and peak viscosity) were evaluated. Three replicates were carried out per sample.

#### 128 2.5. Assessment of gelatinization parameters of rice flour

129 Evaluation of gelatinization was performed by using a TA instruments Q-200 differential scanning  
130 calorimeter (Newcastle, USA). Deionized water was added to rice flour (3.0mg) in aluminum pan to  
131 obtain a flour/water ratio of 1:3 (w/w, dry weight basis). The pans were hermetically sealed and the  
132 samples were allowed to stand for one hour at room temperature before analysis. The scanning  
133 temperature range was between 20-130°C to have a good assessment of thermal changes of flour. In  
134 order to increase accuracy and resolution without loss of sensitivity of results, 5°C min<sup>-1</sup> heat rate  
135 was used. The calibration was made with indium and the thermogram was recorded using an empty  
136 pan as reference. The parameters evaluated were the transition temperatures (the onset (T<sub>0</sub>), peak  
137 (T<sub>p</sub>) and conclusion (T<sub>c</sub>), gelatinization temperature range (I<sub>g</sub>) and the enthalpy of gelatinization  
138 (ΔH). In addition, the peak height index (PHI) was calculated by equation 9. PHI provides a

139 numerical value that is descriptive of the relative shape of the endotherm. A tall narrow endotherm  
140 has a high PHI than a short one does, even if the energy involved in the transition is the same  
141 (Krueger, Knutson, Inglett & Walker, 1987). High PHI value could be related to more structured  
142 starch matrix (Correia & Beirão-da-Costa, 2012). All DSC experiments were replicated at least  
143 three times.

$$144 \quad I_g = T_c - T_0 \quad \text{Eq. 8}$$

$$145 \quad PHI = \frac{\Delta H}{T_p - T_0} \quad \text{Eq. 9}$$

## 146 2.6. Breadmaking and evaluation of bread quality

147 Compressed yeast supplied by LEVAPAN (Guayaquil, Ecuador) and  
148 hydroxypropylmethylcellulose (Methocel, K4M) provided by Dow Chemical Company (Michigan,  
149 USA) were used for breadmaking. The dough was performed using the recipe reported by Marco et  
150 al. (2008) (table 1). Half of the rice flour was mixed with boiling water (half of the water) for 5min  
151 in Oster blender model 2700 (Oster, Boca Raton, USA) with dough hooks set at low speed (position  
152 1). The dough was left to rest until the temperature decreased to 30°C. Then, the rest of the flour,  
153 the other ingredients and water were added and mixed for 5min, set at low speed (position 1). Later,  
154 dough were put into pans and fermented for 40min at 35°C and 85% RH. Finally, the fermented  
155 dough was baked for 35min at 175°C. Breads were analyzed after 24h of baking.

156 The analyzed bread characteristics included specific loaf volume, crumb color and crumb texture  
157 parameters. The loaf volume was determined by rapeseed displacement, while the specific volume  
158 ( $\text{ml} \times \text{g}^{-1}$ ) was calculated as the ratio of the volume (ml) to the weight (g) of the bread. The crumb  
159 color was determined by the computer vision system (Yam & Papadakis, 2004). The computer  
160 vision system station included a light source, a camera (canon SX500 IS, 16 mega pixel, Tokio,  
161 Japan) for image acquisition and software (Adobe Photoshop CS5) for image processing and  
162 analysis. The software quantify the color of crumb in the CIE- $L^* a^* b^*$  uniform color space (CIE-

163 Lab), where  $L^*$  indicates lightness,  $a^*$  indicates hue on a green (-) to red (+) axis, and  $b^*$  indicates  
164 hue on a blue (-) to yellow (+) axis. Data from three slices per bread were averaged. Additionally  
165 the cylindrical coordinates: hue and Chroma ( $C^*_{ab}$ ) were defined by the following equations:

$$166 \quad C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \quad \text{Eq. 10}$$

$$167 \quad Hue = \arctan\left(\frac{b^*}{a^*}\right) \quad \text{Eq. 11}$$

168 Hue angle is the angle for a point calculated from  $a^*$  and  $b^*$  coordinates in the color space. Chroma  
169 is the quantitative component of the color, which reflected the purity of color in the CIELAB space.

170 Texture measurements in form of texture profile analysis (TPA) (Bourne, 1978), of the  
171 breadcrumbs was performed by a Texture Analyzer CT3 (Brookfield, Middleboro, USA). A bread  
172 slice of 1cm thickness was compressed up to 50% of its original height at a crosshead speed of  
173 1mm/s with a cylindrical acrylic probe (diameter 25.4mm). From the TPA curves, the following  
174 texture parameters were measured: hardness (N), springiness, cohesiveness, resilience and  
175 chewiness (N). Hardness was defined by peak force during first compression cycle. Cohesiveness  
176 was calculated as the ratio of the area under the second curve to the area under the first curve.  
177 Springiness was defined as a ratio of the time recorded between the start of the second area and the  
178 second probe reversal to the time recorded between the start of the first area and the first probe  
179 reversal. Chewiness was obtained by multiplying hardness, cohesiveness and springiness.  
180 Resilience was calculated as the area during withdrawal of the penetration, divided by the area of  
181 the first penetration.

## 182 2.7. Statistical analysis

183 Standardized skewness and standardized kurtosis analyses were made to verify normal distribution of  
184 the data. Multiple sample comparison was conducted to evaluate significant differences among  
185 samples by analysis of variance (ANOVA) and multiple range tests. Fisher's least significant  
186 differences (LSD) test was used to describe means with 95% confidence ( $P < 0.05$ ). Data was also



187 evaluated using Pearson correlation coefficients to establish relationship among variables. Only  
188 correlation coefficients (in absolute value) equal or greater than 0.68 were considered meaningful.  
189 All statistical analyses were performed using Statgraphics Centurion 16 (Statistical Graphics  
190 Corporation, UK).

### 191 **3. Results and Discussion**

#### 192 3.1. Protein, fat and apparent amylose content

193 In order to understand the possible role of rice grain composition on the flour functionality, protein,  
194 fat and apparent amylose content (AAC) of the rice flour varieties were determined (table 2). There  
195 was a significant difference in the chemical composition among all the varieties especially in the  
196 protein content, which ranged from 5.4 to 8.0g of protein per 100g of flour. The F09 followed by  
197 INIAP 14 and INIAP 17 showed the highest amount of protein. Apparent amylose content varied  
198 between 22.7 to 28.1g per 100g of flour. Thus, all varieties could be considered as intermediate to  
199 high amylose content, in agreement with values reported for long-grain rice (Wani et al., 2012). The  
200 highest value was found in the INIAP 17 and F09. On the other hand, the fat content ranged from  
201 0.5 to 1.0g of fat per 100g of flour. No significant difference in AAC and lipid content were found  
202 among INIAP 14, INIAP15 and INIAP 16.

#### 203 3.2. Flour and flour gel hydration properties:

204 The hydration properties of the rice flours and the rice gels are shown in table 3. No statistical  
205 difference in oil absorption capacity (OAC) and water solubility index (WSI) were observed among  
206 varieties.

207 The lowest value of water binding capacity (WBC) was displayed by INIAP 17, which is the variety  
208 with the highest amylose content. In fact, some researchers have reported that an increase in  
209 amylose level results in reduced water binding (Gani et al. 2013; Iturriaga, Lopez, & Añon, 2004).  
210 However, the INIAP 14, which has the lowest amylose content, did not follow that trend, likely

211 other intrinsic factors such as particle size, protein conformation, lipid and protein content and lipid-  
212 amylose complex are affecting this capacity (Gani et al., 2013; Tester et al., 1990a). Actually, no  
213 correlation was found between WBC with protein, fat and AAC, implying that the synergic effect of  
214 these entire factors influence the WBC.

215 The WBC of the flour has been related to stickiness of rice flour dough, high WBC reduces  
216 stickiness and produces stiff dough (Han et al., 2012). The values for WBC were higher than the  
217 ones reported by Han et al. (2012), who showed that rice lines with low WBC produce fresh bread  
218 with suitable volume and firmness. On the other hand, INIAP 17 showed the highest value of WHC,  
219 thus could retard staling in gluten-free bread (Sciarini, Ribotta, León, & Pérez, 2010). There was no  
220 significant difference on SV among INIAP 17, F09 and F50 varieties and among INIAP 14, INIAP  
221 15 and INIAP 16, which presented lower SV value.

222 INIAP 14, which had the lowest AAC, presented the highest SP value. This result agrees with  
223 previous reports describing that high amylose content inhibits swelling power (SP) of cereal starch  
224 (Tester & Morrison, 1990b; Singh, Kaur, Sandhu, Kaur & Nishinari, 2006). However, when  
225 correlation matrix was carried out, no significant correlation was found between the apparent  
226 amylose content and the SP. Therefore, result might be derived from the effect of starch content,  
227 protein content, bounding forces and molecular structure of starch, especially of the amylopectin  
228 (Kim et al., 2010).

229 The correlation analysis indicates a positive correlation parameter between SP and WAI ( $r=0.89$ ,  
230  $P<0.001$ ) and negative correlation between SP and WBC ( $r=-0.68$ ,  $P<0.01$ ) as well as WAI and  
231 WBC ( $r=-0.68$ ,  $P<0.01$ ). Previous reports indicate that WBC of flours depends on the hydrophilic  
232 parts of proteins and carbohydrates (Wani, Sogi, Wani & Gill, 2013), water is absorbed in the  
233 amorphous zone of the starch and become swollen prior to any change in the small crystallites  
234 facilitating the striping of chains and melting of crystallites (Iturraga et al., 2004). As water

235 increases in amorphous region SP increases (Kim et al., 2010). Presumably, in flours with high  
236 WBC, water become less available to hydrate the amorphous region of the starch, during the  
237 hydration and gelatinization of the flour; therefore the SP and WAI decrease. The INIAP 14 and  
238 INIAP 17 showed high values of WAI and SP. It has been reported that high absorption of water  
239 during baking can enhance initial softness and decrease firming of bread (Arendt, Moore & Dal  
240 Bello, 2008).

### 241 3.3. Pasting Properties of rice flour

242 The pasting properties and the pasting curves of the long-grain rice varieties are shown in table 4  
243 and figure 1, respectively. INIAP 14 showed the lowest peak viscosity, and INIAP 17 and F09 the  
244 highest one. Nevertheless, no significant correlation was found between pasting properties and the  
245 amylose content, which agrees with previous report (Sompong et al., 2011). Negative correlation  
246 between peak viscosity and WAI was found ( $r=-0.77$ ,  $P<0.01$ ). INIAP 17 also showed high final  
247 viscosity value, indicating high capacity to form gel, but with high retrogradation tendency due to  
248 its high setback value (Gani et al., 2013). Indeed, a significant correlation between final viscosity  
249 and AAC was found ( $r=0.70$ ,  $P<0.05$ ), although no with the setback. Result that differed from  
250 previously reported (Sompong et al., 2011; Gani et al., 2013).

251 INIAP 14 and INIAP 17 presented lower breakdown viscosity and F09 the highest. The breakdown  
252 is caused by the disintegration of gelatinized starch granule structure during continued stirring and  
253 heating. Differences in breakdown among rice starches have been related to differences in rigidity  
254 of swollen granules (Gani et al., 2013). A negative correlation was found between breakdown  
255 viscosity and SP ( $r=-0.72$ ,  $P<0.01$ ). Hence, INIAP 17 and INIAP 14 could lead to bread with high  
256 specific volume.

### 257 3.4. Gelatinization parameters of rice flour

258 The gelatinization temperatures (onset,  $T_o$ ; peak,  $T_p$ ; and conclusion,  $T_c$ ), gelatinization enthalpy  
259 ( $\Delta H$ ), gelatinization temperature range ( $I_g$ ) and peak height index (PHI) of rice flours from different  
260 varieties are shown in table 5. Significant differences were observed in the thermal properties due to  
261 varieties. Gelatinization temperatures and enthalpy were similar to those reported by other authors  
262 for rice flour and starch (Han et al., 2012; Iturriaga et al., 2004; Singh et al., 2006). Variation even  
263 of 10°C was found among the onset gelatinization temperatures ( $T_o$ ) of the INIAP 14 and INIAP 15  
264 and the other varieties.

265 Negative correlation between  $T_o$  and  $I_g$  was found ( $r=-0.97$ ,  $P<0.001$ ). INIAP 14 and INIAP 15  
266 showed high values on  $T_o$  and low on  $I_g$ . Considering that INIAP 14 and INIAP 15 showed low  
267 amylose content, this result agrees with Krueger et al. (1987), who reported that the high the  
268 amylopectin content of the starch, the low temperature range of gelatinization. In addition, negative  
269 correlation between  $T_p$  and SV was found ( $r=-0.80$ ,  $P<0.001$ ). INIAP 14, INIAP 15 and INIAP 16  
270 showed high  $T_p$  value, and likely with high resistance to swell.

271 The enthalpy of gelatinization ( $\Delta H$ ) has been used as indicator of the loss of molecular order within  
272 the granule that occurs during gelatinization (Tester et al., 1990 b). INIAP 15 showed the lowest  $\Delta H$   
273 value, indicating less stability of crystals (Chiotelli & Le Meste, 2002). Peak high index (PHI)  
274 provides a numerical value that describes the relative shape of endotherm. INIAP 16 and INIAP 17  
275 showed low peak high index (PHI) values, which can be related to lower structured starch matrix  
276 (Kruger et al., 1987). Therefore, INIAP 17 showed low  $T_o$ , PHI and broad  $I_g$  that might be due to  
277 the presence of irregularly-shaped granules (Kaur, Singh, Sandhu & Guraya 2004). The same  
278 analysis could be applied to F09 and F50 varieties. On the other hand, INIAP 14 showed the highest  
279 values of  $T_o$ ,  $T_p$ ,  $\Delta H$ , PHI and narrow  $I_g$ , which could indicate high degree of molecular order  
280 (Correia et al., 2012; Sandhu, Singh & Kaur, 2004).

281 Figure 2 shows the endothermic curves of the rice varieties. INIAP 17, INIAP 16, F09 and F50  
282 showed a shoulder peak; like it has been reported in Thai rice variety due to amylopectin structure  
283 composed by two different molecular structures (Kim et al., 2010).

284 No significant correlation was observed between gelatinization parameters and hydration properties,  
285 neither with apparent amylose content. Presumably, the presence of protein and lipid in rice flour  
286 also influence the gelatinization process, and affect the water- starch bonding (Iturriaga et al.,  
287 2004).

### 288 3.5 Evaluation of Bread

289 Figure 3 shows the cross section of breads from all rice flour varieties. INIAP 15 and INIAP 17  
290 showed smaller gas cells, which led to a more compact crumb. F09 had a more flattened surface,  
291 indicating lower driving force in the oven, possibly due to weaker mass structure.

292 The quality characteristics of the breads obtained from rice varieties are shown in table 6. Specific  
293 volume values of gluten-free breads (GFB) ranged from 1.80 to 2.41ml/g, which agrees with  
294 previously reported (de la Hera et al., 2013b; Matos & Rosell 2012; Sciarini et al., 2010). A  
295 significant correlation was observed between specific volume and SP ( $r=0.71$ ,  $P<0.01$ ) and  
296 breakdown viscosity ( $r=-0.97$ ,  $P<0.01$ ). Matos & Rosell (2013) reported also a negative correlation  
297 between specific volume and pasting properties assessed with Mixolab. In fact, INIAP 14 presented  
298 high specific volume, high SP and low breakdown viscosity. As was reported before by Han et al.  
299 (2012), rice lines with low WBC produce fresh bread with a suitable volume and firmness. Whereas  
300 INIAP 14 has low WBC and high specific volume, no correlation was found between both  
301 properties. In addition, no correlation was found between amylose content and specific volume of  
302 the bread. However, the conclusion temperature ( $T_c$ ) of gelatinization has a positive correlation  
303 ( $r=0.81$ ,  $P<0.05$ ) with the specific volume. This result could be related with the time that the bread  
304 has to increase the volume within the oven.

305 The color of the crumb has been also an important parameter for characterizing GFB.  $L^*$  value  
306 indicates the lightness of the crumb. Rice flour based breads give a very white crumb, which differs  
307 from the wheat flour based crumbs that are yellowish. The  $L^*$  range (74 to 72) agreed with the ones  
308 reported before (Matos et al., 2012). Lower crumb luminosity (65) was reported by Marco et al.  
309 (2008) when using short-grain rice flour in the same GFB recipe. The range of  $a^*$  values were  
310 indicating hue on green axis, as was obtained with short-grain rice flour (Marco et al., 2008). On the  
311 other hand, the positive range of  $b^*$  values were within the ones reported for commercial breads,  
312 showing hue on yellow axis (Matos et al., 2012). Values of  $b^*$  ranged from 18 to 21, whereas with  
313 short-grain rice flour was around 8, likely due to differences in rice polishing degree (Marco et al.,  
314 2008). All the samples showed a negative hue angle that reflected yellow-greenish hue. INIAP 15  
315 presented the major intensity of yellow component, indicated by chroma value.

316 Significant differences were observed in the crumb texture properties among rice varieties (table 7).  
317 Crumb hardness ranged from 3.5N to 10.8N, thus softer crumbs than those reported in commercial  
318 GFB (Matos et al., 2012), but harder than GFB made with short-grain rice flour ( $1.96 \pm 0.19$ N)  
319 (Marco et al., 2008). INIAP 14, F09 and F50 showed lower hardness values and INIAP 17 the  
320 highest one. The range of springiness in the studied rice varieties were in the lower limit of the  
321 ranges reported before (Marco et al., 2008; Matos et al., 2012), showing their fragility and tendency  
322 to crumble when sliced (McCarthy, Gallagher, Gormley, Schober & Arendt, 2005). High  
323 springiness values are preferred because it is related to the freshness and elasticity of the bread. Not  
324 only springiness but also resilience characterizes the loss of elasticity, because it indicates the  
325 ability of a material to return to its original shape after stressing (Onyango, Mutungi, Unbehend &  
326 Lindhauer, 2011). With the exception of INIAP 17, all the INIAP varieties showed significant lower  
327 resilience values than F09 and F50. In fact, all varieties showed lower values than the one reported  
328 for GFB from long-grain rice (Kadan et al., 2001) and close to the 0.26 resilience value reported for

329 GFB from short-grain rice (Marco et al., 2008). These results are in the upper level of the result of  
330 Matos et al. (2012) in commercial GFB. Chewiness of the rice varieties ranged between 8.97 to  
331 20.26N, those values were within the upper level of commercial GFB (Matos et al., 2012). INIAP  
332 17 showed significantly higher value.

333 F09 and F50 presented the highest cohesiveness value, which was desirable because it forms a bolus  
334 rather than disintegrates during mastication (Onyango et al., 2011). Cohesiveness characterizes the  
335 extent to which a material can be deformed before it ruptures, reflecting the internal cohesion of the  
336 material. A strong correlation was found between cohesiveness and properties of rice flour such as  
337  $T_p$  ( $r=-0.96$ ,  $P<0.001$ ),  $\Delta H$  ( $r=0.71$   $P<0.05$ ) and  $SV$  ( $r=0.82$ ,  $P<0.05$ ).

338 Therefore, results confirmed that long-grain rice varieties are suitable for obtaining gluten free  
339 breads, but rice variety had a significant impact on the GFB characteristics. Among the varieties  
340 tested, INIAP 14 and F09 gave the best GFB features, moreover considering specific volume and  
341 crumb texture properties.

#### 342 **4. Conclusion**

343 Rice flour from short-grain has been recommended for making gluten-free bread, but present results  
344 show that flour from long-grain rice is suitable for making gluten-free bread, having similar  
345 characteristics than previously reported GFB made from short-grain rice. Likely, discrepancies  
346 could be attributed to the wide use of commercial rice flours instead of using specific rice varieties.  
347 Results obtained with six different long- grain rice varieties confirmed their suitability for  
348 breadmaking performance. Significant differences were observed within varieties. Results  
349 suggested that the most important parameters of rice flour when defining breadmaking performance  
350 of GFB would be WBC, SP, SV,  $T_p$ ,  $T_c$  and enthalpy. Technological parameters (hydration and  
351 pasting) would not be able to predict specific volume or hardness. Nevertheless, regarding crumb  
352 cohesiveness and resilience, it would be advisable to select flours with high swelling value, and

353 enthalpy but low gelatinization peak temperature. In addition, no correlation has found between  
354 WBC with hardness and specific volume of the GFB. Flour properties such as SV, Tp and  $\Delta H$  were  
355 strongly related to cohesiveness value, F09 and F50 showed the highest cohesiveness value. Also,  
356 high SP and low breakdown viscosity of the rice flour, showed in INIAP 14, are related to high  
357 specific volume of the GFB. Previous reports supported that short rice grain was the only suitable  
358 for gluten-free breadmaking. Nonetheless, this study indicated that the length of the rice is not a  
359 determining factor for breadmaking, and long-grain rice could be used for bakery, indeed it seems  
360 that synergic effect of intrinsic factors such as particle size, protein conformation, lipid and protein  
361 content and lipid-amylose complex and starch structure that could affect the properties of rice flour.  
362 In addition, results suggest that by selecting specific varieties of rice it would be possible to  
363 improve the baking performance of the rice flours, and presumably breeding could also be a good  
364 tool to obtain new promising varieties for baking.

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468

469 **FIGURE CAPTIONS**

470 **Figure 1:** Rapid viscoanalyzer plots showing the pasting behavior of flours from different long-  
471 grain rice varieties. Legends: Grey line: temperature; F09 ( - - - - ); F50 (-----);  
472 INIAP 14 (.....); INIAP 15 (————); INIAP 16 (-·-·-·-); INIAP 17 (————).

473

474 **Figure 2:** DSC plots showing the endothermic curves of flours from different long-grain rice  
475 varieties. Legends: Grey line: temperature; F09 ( - - - - ); F50 (-----);  
476 INIAP 14 (.....); INIAP 15 (————); INIAP 16 (-·-·-·-); INIAP 17 (————).

477

478 **Figure 3:** Cross-section of gluten-free breads made of rice flours from different long-grain  
479 varieties. Recipe described in Table 1.

480 **Table 1.** Gluten-free bread recipe used for breadmaking.

481

<b>Ingredient</b>	<b>Percentage in flour basis</b>
Rice flour	100
Water	110
Dry yeast	3
Salt	1.8
Sugar	3
Vegetal oil	6
Baking improver (HPMC)	2

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486 **Table 2.** Proximate composition of flours from different long-grain rice varieties.  
 487

Variety	AAC (g/100g)	Protein (g/100g)	Fat (g/100g)	Moisture content (g/100g)
INIAP 14	22.67±1.88c	7.67±0.10b	0.63±0.03c	12.47±0.03d
INIAP 15	23.74±1.15bc	5.43±0.10e	0.59±0.04c	11.53±0.06c
INIAP 16	24.62±1.53bc	6.23±0.06d	0.60±0.05c	10.62±0.14b
INIAP 17	28.16±2.50a	7.67±0.16b	0.47±0.03d	11.65±0.16c
F09	25.65±1.27ab	8.01±0.02a	0.79±0.02b	10.17±0.02a
F50	23.82±0.94bc	7.04±0.04c	0.95±0.05a	10.18±0.11a

488 AAC: Apparent Amylose Content.

489 Values with different letters in the same column are significantly different ( $P<0.05$ ) (n=3).  
 490

491 **Table 3.** Hydration properties of flours and gels from different long-grain rice varieties.

492

Variety	WBC (g/g)	WHC (g/g)	SV (ml/g)	WAI (g/g)	WSI (g/g) x 100	SP (g/g)	OAC (g/g)
INIAP 14	1.28±0.04cd	1.56±0.10cd	2.99±0.00bc	13.34±0.61a	1.74±0.49a	13.58±0.61a	1.76±0.01a
INIAP 15	1.33±0.07abc	1.48±0.05d	2.79±0.19c	5.04±0.28e	2.23±0.77a	5.16±0.32d	1.73±0.05a
INIAP 16	1.38±0.05a	1.74±0.06b	2.93±0.12bc	6.26±0.81d	2.19±0.59a	6.39±0.84c	1.71±0.01a
INIAP 17	1.23±0.02d	1.95±0.15a	3.13±0.12ab	11.65±0.19b	2.73±0.75a	12.07±0.15b	1.71±0.02a
F09	1.35±0.02ab	1.51±0.08d	3.37±0.23a	5.95±0.38d	1.89±0.42a	6.07±0.38c	1.71±0.01a
F50	1.30±0.02bc	1.70±0.09bc	3.27±0.12a	7.49±0.79c	3.00±0.01a	11.73±0.61b	1.77±0.06a

493

494 WBC: Water Binding Capacity, WHC: Water Holding Capacity, SV: Swelling Volume, SP: Swelling Power, WAI: Water Absorption Index,

495 WSI: Water Solubility Index, OAC: Oil Absorption Capacity.

496 Values with different letters in the same column are significantly different ( $P<0.05$ ), (n=3).

497 **Table 4.** Pasting Properties of flours from different long-grain rice varieties determined from the  
 498 RVA plots.  
 499

<b>Variety</b>	<b>Peak viscosity (cP)</b>	<b>Breakdown (cP)</b>	<b>Final viscosity (cP)</b>	<b>Setback (cP)</b>
INIAP 14	1692±16d	287±1d	4014±18b	2609±3b
INIAP 15	2731±24c	1083±47b	3683±0d	2035±23d
INIAP 16	2883±10b	989±1b	4079±2b	2184±11c
INIAP 17	2964±29a	580±1c	5356±74a	2972±46a
F09	2971±15a	1237±40a	3803±21c	2069±19d
F50	2822±66b	1040±100b	3874±13c	2092±21d

500 Values with different letters in the same column are significantly different ( $P < 0.05$ ), (n=3).  
 501  
 502



503 **Table 5.** Gelatinization parameters of flours from different long-grain rice varieties determined  
 504 from the DSC plots.  
 505

Variety	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	Ig (°C)	PHI
INIAP 14	69.0±0.4a	74.1±0.5ab	80.6±0.4a	12.7±2.7ab	11.7±0.7b	2.44±0.31a
INIAP 15	68.5±1.0a	75.2±0.8a	79.8±2.4a	8.6±1.6b	11.4±2.7b	1.39±0.04c
INIAP 16	60.8±2.0b	74.6±0.2ab	81.6±0.8a	11.7±3.3ab	20.9±2.6a	0.84±0.12d
INIAP 17	59.0±0.8bc	73.7±0.2b	79.5±1.2a	12.9±1.8ab	20.5±0.5a	0.88±0.14d
F09	58.4±2.0c	66.8±0.8c	80.9±0.9a	13.9±3.4ab	22.5±2.9a	1.68±0.19c
F50	58.7±0.4bc	66.4±1.0c	81.3±1.2a	16.0±2.8a	22.6±1.5a	2.07±0.04b

506 To: Onset Temperature, Tp: Peak Temperature, Tc: Conclusion Temperature, ΔH: Enthalpy of gelatinization,  
 507 Ig: gelatinization temperature range, PHI: Peak Height Index.  
 508 Values with different letters in the same column are significantly different ( $P<0.05$ ), (n=3).  
 509

510 **Table 6.** Technological characteristics of GFB made of rice flour from different long-grain varieties.

511

Variety	Specific Volume (ml/g)	<i>L</i> *	<i>a</i> *	<i>b</i> *	Chroma	Hue angle (°)
INIAP 14	2.35±0.14a	74.00±0.00a	-3.77±0.58a	18.33±1.55b	18.71±1.08bc	-78.63±2.18a
INIAP 15	2.09±0.02c	72.00±1.00c	-2.67±0.58a	21.00±0.00a	23.82±4.62a	-83.51±1.44b
INIAP 16	2.28±0.03b	72.67±0.58bc	-2.67±0.58a	18.67±0.58b	18.86±0.54bc	-81.85±1.87ab
INIAP 17	1.80±0.02d	73.67±1.15ab	-3.00±0.00a	21.00±0.00a	22.53±2.29ab	-82.30±0.74ab
F09	2.41±0.19a	74.67±0.58a	-3.67±0.58a	18.00±1.00b	18.38±0.89c	-78.41±2.29a
F50	2.24±0.14bc	74.00±0.00a	-3.33±1.15a	19.33±1.53b	19.65±1.34bc	-80.05±3.92ab

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Values with different letters in the same column are significantly different ( $P < 0.05$ ), (n=3).

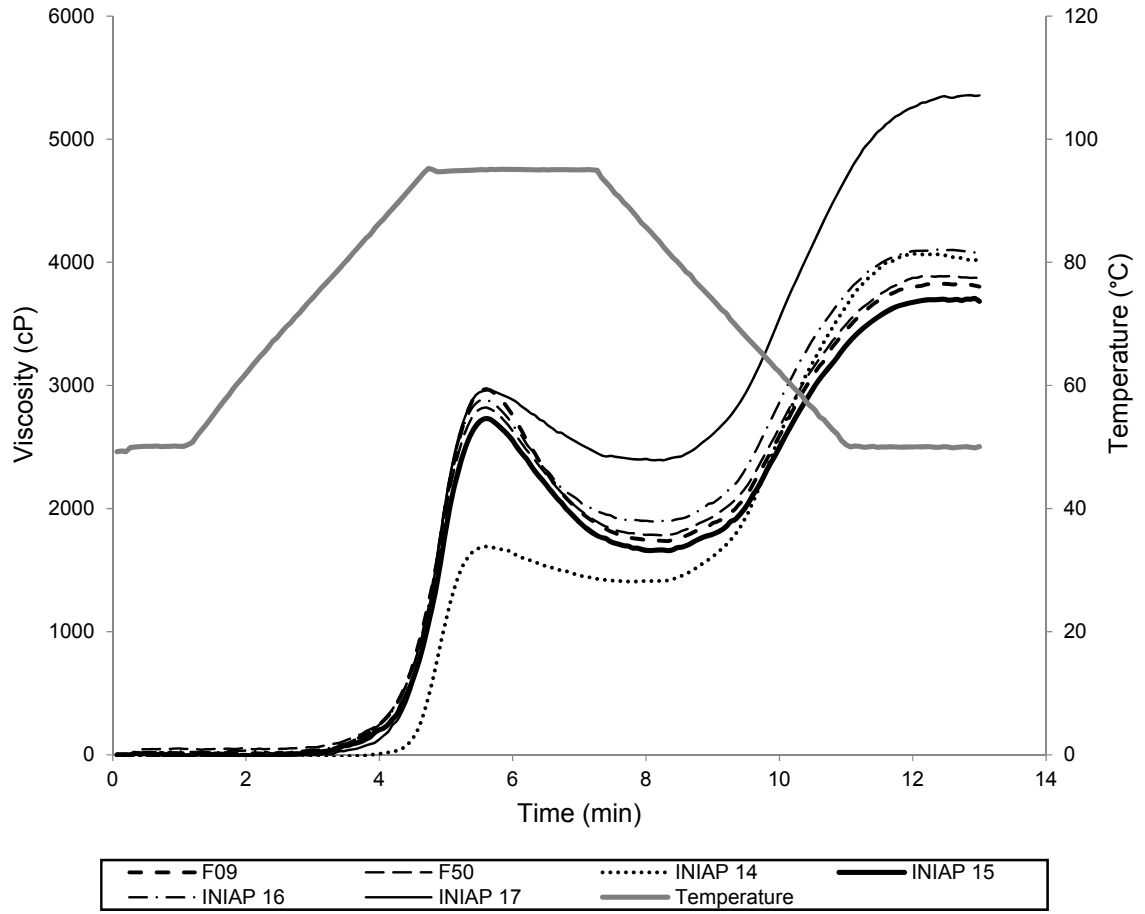
516 **Table 7.** Texture profile analysis of crumbs of GFB made of rice flours from different long-grain  
 517 varieties.  
 518

Variety	Hardness (N)	Resilience	Springiness	Chewiness (N)	Cohesiveness
INIAP 14	5.88±1.79cd	0.23±0.02c	0.73±0.06abc	8.97±2.31c	0.48±0.05bc
INIAP 15	9.67±2.06ab	0.20±0.03c	0.68±0.05c	14.95±2.97b	0.44±0.05c
INIAP 16	7.16±1.31bc	0.21±0.03c	0.70±0.00bc	11.05±2.31bc	0.45±0.04c
INIAP 17	10.84±3.21a	0.29±0.05b	0.75±0.07abc	20.26±6.11a	0.54±0.08b
F09	3.85±0.82d	0.30±0.01ab	0.80±0.00a	9.04±1.87c	0.62±0.01a
F50	3.54±1.06d	0.35±0.02a	0.75±0.06ab	9.16±2.62c	0.67±0.03a

519  
 520 Values with different letters in the same column are significantly different ( $P<0.05$ ), (n=3).  
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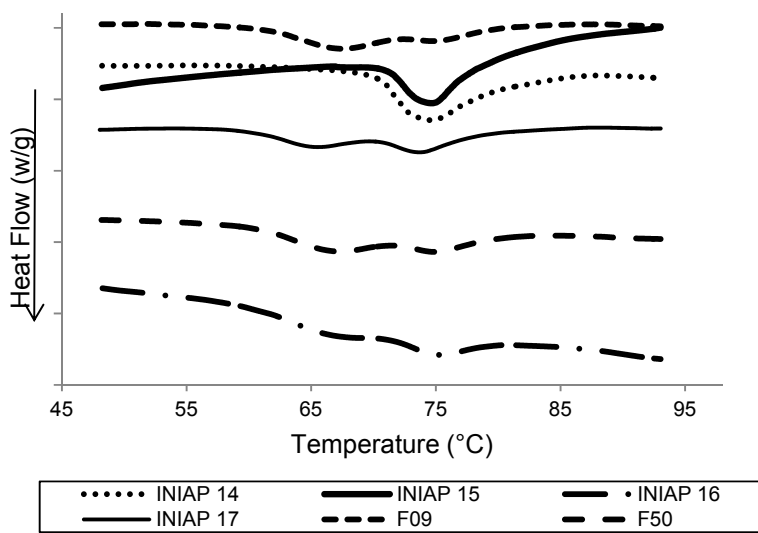
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524 **Figure 1**  
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533 Figure 2.



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537 **Figure 3.**



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