

1 **MAIZE BASED GLUTEN FREE BREAD: INFLUENCE OF PROCESSING**  
2 **PARAMETERS ON SENSORY AND INSTRUMENTAL QUALITY**

3

4

5 **Carla Brites<sup>1\*</sup>, Maria João Trigo<sup>1</sup>, Carla Santos<sup>1</sup>, Concha Collar<sup>2</sup>, Cristina M.**  
6 **Rosell<sup>2</sup>**

7

8 <sup>1</sup>**Instituto Nacional dos Recursos Biológicos, I.P., L-INIA, Unidade Tecnologia**  
9 **Alimentar, Quinta do Marquês, 2784-505 Oeiras, Portugal.**

10 <sup>2</sup>**Institute of Agrochemistry and Food Technology (IATA-CSIC). PO Box 73, 46100-**  
11 **Burjasot (Valencia), Spain.**

12

13 **Running title: Maize based gluten free breads**

14

15 **\* Corresponding author. Tel: +351214403684; fax:+351214413208**

16 **E-mail address: [carlabrites@mail.telepac.pt](mailto:carlabrites@mail.telepac.pt)**

17 **Abstract**

18 The performance of maize bread with spongy texture is still a technological challenge due  
19 to the absence of a natural network required for holding the carbon dioxide released  
20 during the fermentation process. The objective of this research was to investigate the  
21 influence of different maize varieties (regional and hybrid), milling process (electric and  
22 water mill), formulation and processing variables on the sensory and instrumental  
23 (specific volume, texture and colour) quality attributes of corn bread. For that purpose, the  
24 traditional breadmaking process applied to the development of the ethnic Portuguese bread  
25 (*broa*) obtained from composite maize-rye-wheat flour was modified to produce gluten-  
26 free *broa*. Significant differences ( $p < 0.05$ ) between regional and hybrid maize were  
27 detected in terms of protein, amylose, and maximum, minimum and final viscosities as  
28 evaluated by Rapid Visco Analyser. Concerning the effect of milling process, the grinding  
29 in a water mill occurs at slower rate than it does in the electrical mill, in consequence the  
30 flour from water milling had lower ash content and higher maximum, minimum and final  
31 viscosities than the one obtained from electrical milling. An important point in the  
32 breadmaking process was the flour blanching that resulted in doughs with higher  
33 consistency, adhesiveness, springiness and stickiness as measured by texture analyser, due  
34 to the partial gelatinization of the corn starch. Baking assays demonstrated that *broa*  
35 breadmaking technology could be satisfactorily applied to produce gluten-free *broa* with  
36 acceptable quality characteristics better than bread made from regional maize varieties.

37

38 **Keywords:** maize flours, blanching, rheology, *broa*, maize bread, gluten free bread

39 **1. Introduction**

40 Wheat proteins have the unique properties of developing a viscoelastic matrix when wheat  
41 flour is mechanically mixed with water. This viscoelastic network enables the dough to  
42 hold the gas produced during the fermentation process, leading to an aerated crumb bread  
43 structure. Unfortunately, gluten must be kept apart from the diet of celiac patients, who  
44 suffer very important intestinal damage when they ingest gluten containing products. This  
45 technological obstacle has been overcome by using complex bread recipes with different  
46 starches and cereal flours like corn starch, brown rice, soy and buckwheat flour (Gallagher  
47 et al., 2004; Moore et al., 2006), or a composite blend of rice flour with corn and cassava  
48 starches obtaining gluten-free bread with a well structured crumb and pleasant flavour and  
49 appearance (Sanchez et al., 2002; Lopez et al., 2004). Generally, in the performance of  
50 gluten free bread, a variety of hydrocolloids or gums have been used for creating a  
51 polymer network with similar functionality than the wheat gluten proteins. In fact, gluten  
52 free breads have been successfully developed using several combinations of cellulose  
53 derivatives (Gujral & Rosell, 2004a, Schober et al., 2007). With the same purpose,  
54 crosslinking enzymes (glucose oxidase and transglutaminase) have been used as  
55 processing aids for improving rice based gluten free bread quality (Gujral and Rosell,  
56 2004a,b; Moore et al., 2006). Lately, different proteins have been proposed as alternative  
57 for both playing the polymer role and increasing the nutritional value of gluten free  
58 products (Marco & Rosell, 2008a, b, c).

59 It is clear that the common player when gluten free breads are developed is the presence of  
60 a polymer with certain viscoelasticity and ability to entrap the other components of the  
61 system; and usually they are incorporated as ingredients of the recipe. Nevertheless, an  
62 attractive alternative would be to perform gluten free breads by using only gluten free

63 cereals and to generate ‘in situ’ during the breadmaking process the required holding  
64 biopolymer.

65

66 *Broa* is Portuguese ethnic bread made with more than fifty per cent of maize mixed with  
67 wheat or rye flours, highly consumed in the north and central zones of Portugal (Brites et  
68 al., 2007a). Bread making process is mainly empirical and several types of *broa* are  
69 produced depending on maize types and blending flours, although local maize landraces  
70 are usually preferred (Vaz Patto et al., 2007). Maize flour for breadmaking was  
71 traditionally obtained in stone wheel mills, moved by water or wind, and nowadays  
72 frequently by electricity. There are many recipes to prepare *broa*, but the traditional  
73 process (Lino et al., 2007) involves adding maize flour (sieved whole meal flour), hot  
74 water, wheat flour, yeast and leavened dough from the late *broa* (acting as sourdough).  
75 After mixing, resting and proofing, the dough is baked in a wood-fired oven. This  
76 empirical process leads to an ethnic product highly accepted for its distinctive sensory  
77 characteristics. Nevertheless scarce scientific studies on *broa* breadmaking have been  
78 reported, and research have been focused on the partial replacement of wheat flour by  
79 maize flour (Martínez & el-Dahs, 1993) or maize starch (Miyazaki & Morita, 2005) or  
80 developing formulations based on maize starch (Sanni et al., 1998; Özboy, 2002).

81

82 Maize is a gluten free cereal, thus suitable to produce foods addressed to celiac patients.  
83 The acquired knowledge on *broa* (made from composite maize-rye-wheat flour) is  
84 important for facing the challenges in producing gluten-free bread that usually exhibits  
85 compact crumb texture and low specific volume (Rosell & Collar, 2007; Rosell & Marco,  
86 2008). Therefore, a better understanding of this breadmaking process would provide the  
87 basis for developing gluten free bread based on maize flour.

88 The objective of this study was to assess the impact of different factors as maize variety,  
89 type of milling, water mixing temperature on maize dough rheology and to define and  
90 optimize the maize breadmaking and to identify their effect on specific volume, texture  
91 and sensory quality of the maize bread performed by applying the technology of *broa*.

92

## 93 **2. Material and Methods**

### 94 **2.1. Maize flours characteristics**

95 Four maize varieties selected based on genetic background (Moreira, 2006) were used in  
96 this study (Table 1). Whole meal maize flour was obtained after milling the selected  
97 varieties in artisan water-mill and electric-mill (model M-50, Agrovil, Portugal), both  
98 having millstones. Whole meal flour was sieved through 0.5mm screen, and larger  
99 particles were discarded to obtain maize flour.

100 Flour protein and ash content were determined in triplicate following ICC standard  
101 105/2:1994 and 104/1:1990 methods. Apparent amylose content was determined  
102 following the ISO 6647-2:2007 using 720nm as wavelength, and a calibration curve  
103 previously performed with maize flour samples according to ISO 6647-1:2007.

104 Viscosity profiles were obtained with a Rapid Viscosity Analyser (RVA, Newport  
105 Scientific, Australia), according to Almeida-Dominguez et al. (1997) at 15% solids, using  
106 the following time (min): temperature (°C) settings 0:50, 2:50, 6.5:95, 11:95, 15:50, 25:50,  
107 the time (min):speed (rpm) programme were 0:960, 10:160. Maximum, minimum (or  
108 trough) and final viscosities (cP units) were recorded and the breakdown calculated as  
109 maximum viscosity-minimum viscosity.

110 Maize flour colour was determined on 10-12g of sample in an opaque recipient by using a  
111 Minolta Chromameter CR-2b. Maize flour tristimulus colour parameters included: L\* -  
112 lightness, a\* - red/green index, b\* - yellow/blue index and  $\Delta E$  – colour total variation

113 relative to a white surface reference ( $L^*=97.5$   $a^*=-0.13$   $b^*=1.63$ ). Values are the mean of  
114 10 replicates.

115

## 116 **2.2. Dough rheological properties**

117 Dough rheological properties were evaluated in a conventional Brabender farinograph®  
118 (Duisburg, Germany) following the method ISO 5530-1:1997 with some modifications.

119 Maize flour (40g) were mixed in the Farinograph 50g bowl with 44mL of distilled water  
120 (110% flour basis) during 20min. Assays were carried out under two different conditions,  
121 at 25°C and by adding boiling water (100°C). The parameters obtained from the  
122 farinogram included Td (development time in minutes, time to reach the maximum  
123 consistency), the dough consistency at the Td ( $C_{Td}$ ) and the consistency after 20min  
124 mixing ( $C_{20}$ ), both in BU (Brabender Units).

125 Mechanical and surface related properties were determined in the resulting dough, either  
126 from 25°C and 100°C. Dough machinability was determined by assessing the texture  
127 profile analysis (TPA) and dough stickiness in a TA-XT2i texturometer (Stable Micro  
128 Systems, Godalming, UK) as described by Armero & Collar (1997) using the Chen &  
129 Hosney cell. Primary textural properties were measured in absence of dough  
130 adhesiveness by using a plastic film on the dough surface to avoid the distortion induced  
131 by the negative peak of adhesiveness (Collar & Bollaín, 2005). The adhesiveness was  
132 measured without the plastic film. Three and ten repetitions for the TPA parameters and  
133 stickiness were done, respectively. Compression test was performed with a 50mm of  
134 diameter cylindrical aluminium probe, a 60% compression rate followed of 75s interval.  
135 TPA profile recorded the following parameters: hardness (g/force), adhesiveness (g/s),  
136 cohesiveness and springiness. For dough stickiness (g/force) determination was used the

137 Chen & Hosney cell with a cylindrical probe of 25mm diameter (Armero & Collar,  
138 1997).

139

### 140 **2.3. Breadmaking process**

141 The traditional *broa* formulation included 70 % of maize flour, 20 % of commercial rye  
142 flour (Concordia type 70, Portugal), 10 % of commercial wheat flour (National type 65,  
143 Portugal), 95 % (v/w, flour basis) of water, 3.6 % (w/w, flour basis) sugar, 2.2 % (w/w,  
144 flour basis) salt, 0.5 % (w/w, flour basis) of improver (S500 Acti-plus, Puratos) and 0.8 %  
145 (w/w, flour basis) dry yeast (Fermipan, DSM, Holland). Sourdough was prepared using  
146 the same recipe of *broa* and adding enough bacteria suspension (*Lactobacillus brevis* and  
147 *plantarum* previously isolated) to yield  $10^7$  CFU (colony formed units)/g mass  
148 concentration. Sourdough was kept at 25°C during 12h before its use. Traditional *broa*  
149 baking trials were performed with the four maize varieties milled with two different mills,  
150 which gave a total of eight different maize flours (n=8).

151 Breadmaking process consisted in mixing the maize flour with 77% (v/w, flour basis)  
152 boiling water containing 2.2% salt, for 5 minutes in the bowl of Kenwood kitchen  
153 Machine. Dough was left idle till cooling to 27°C, then the remaining ingredients  
154 (including 18 % water containing 2.2 % salt and 10 % w/w flour basis of sourdough) were  
155 added and dough was kneaded again for 8 min and left resting for bulk fermentation at  
156 25°C for 90min. After fermentation, the dough was manually moulded in balls of 400g and  
157 baked in the oven (Matador, Werner & Pfleiderer Lebensmitteltechnik GmbH) at 270°C  
158 for 40min. For each trial, three samples were produced and analysed separately.

159 An adapted breadmaking process was carried out for obtaining gluten free maize bread, in  
160 which rye and wheat flours were replaced by maize flour and recipe contained 110 %  
161 (v/w, flour basis) of water, identical proportion of the other traditional *broa* ingredients

162 (sugar, salt, improver, dry yeast). Gluten free baking trials were performed with *Pigarro*  
163 and *Fandango* maize varieties milled in artisan water mills (n=2).

164

#### 165 **2.4. Bread analyses**

166 Quality technological parameters of breads were determined the following day to its  
167 production. Quality parameters included: weight (g), volume (cm<sup>3</sup>) using polyethylene  
168 spheres displacement method (Esteller & Lannes, 2005). Specific volume was then  
169 calculated in cm<sup>3</sup>/g.

170 The tristimulus colour parameters (L\*, a\*, and b\*) of crumbs were determined using  
171 Minolta Chromameter Model CR-2b colorimeter.

172 Bread slices (25mm thickness) were used for crumb firmness determination through  
173 compression test in a texture analyser (TA-Hdi, Stable Micro Systems, Godalming, UK)  
174 using adapted American Institute of Baking- AIB Standard Procedure (2007): 12.5mm  
175 cylindrical probe, 2.0mm/s test speed, 10g trigger force, 6.2cm compression distance after  
176 detecting resistance (crumb surface) and final speed test of 10mm/s. Firmness in g-force  
177 was automatically recorded by the data processing software.

178 Sensory analysis (ISO 8587, 1988) was conducted with a panel of twelve trained judges  
179 that quantify the influence of different maize varieties on overall differentiation of *broa*  
180 and maize bread. Triangular assays (AACC 33-50A, 1999) were carried out for each  
181 maize variety subjected to the two types of milling. Paired comparison tests (ISO 5495,  
182 1983) were conducted to compare different varieties (within each colour group- white or  
183 yellow), panelists were asked to ranking the samples based on overall texture, taste and  
184 aroma. Traditional *broa* was compared with gluten free maize bread.

185

#### 186 **2.5 Statistical analysis**



187 The effect of different flour (maize variety, type of milling) and dough (water mixing  
188 temperature) variables on respectively flour chemical composition, colour, viscosity  
189 profile, dough rheological and bread technological quality parameters were analysed by  
190 analysis of variance (ANOVA). Means comparisons were performed by Duncan's test  
191 also used for compared traditional *broa* with gluten free maize bread. Significant  
192 correlations between flour composition, viscosities and dough rheological parameters  
193 were determined with Pearson correlations analysis. All statistical analyses were  
194 conducted at a significant level of  $P \leq 0.05$  with Statistical Analysis System (SAS Institute,  
195 Cary, NC, 1999).

196

### 197 **3. Results and discussion**

#### 198 **3.1. Effects of maize varieties and milling types on flour composition, colour and** 199 **viscosities**

200 The viscosity profile of a wide germplasm collection of pure lines, hybrids and local  
201 maize populations was previously characterized by Santos (2006) and four varieties  
202 (*Pigarro*, *Fandango*, Yellow Hibrid and White Hibrid) (Table 1) were selected for bread  
203 production with and without composite rye-wheat flours. The effect of milling type on the  
204 flour and dough characteristics was also studied to assess the possible influence of the new  
205 practices (electrical mill) compared to the traditional ones (stone mill).

206

207 Significant differences between maize varieties were detected for protein and amylose  
208 contents ( $P < 0.05$ ) (Table 2). Regional varieties (*Fandango*, *Pigarro*) exhibited significant  
209 higher protein content and lower amylose content than hybrids. *Pigarro* flour had the  
210 highest ash content probably due to its endosperm of flint type. Type of milling influenced  
211 significantly ( $P < 0.05$ ) the ash content that affects pH profile during fermentation and, in

212 turn, will influence bread quality. The type of grinding did not have any influence on  
213 protein and amylose content.

214 As expected, type of variety showed greater significance ( $P < 0.05$ ) on flour colour  
215 parameters than milling type (Table 3). Despite of testing two yellow maize (*Fandango*,  
216 Yellow Hibrid) and two white maize (*Pigarro*, White Hibrid), there were significant  
217 differences ( $P < 0.05$ ) concerning  $a^*$  and  $b^*$  parameters between the yellow varieties and  
218 only significant differences in  $a^*$  values between the white varieties. The type of milling  
219 affected significantly ( $P < 0.05$ ) the lightness (L) of the maize flours (data not shown).

220 Viscosity profile of four maize flours during a heating-cooling cycle was recorded by  
221 using the rapid viscoanalyzer (RVA) (Figure 1). Compared with commercial wheat flour  
222 (results not shown) maize flour exhibited lower pasting temperature, lower maximum  
223 viscosity and higher final viscosity, therefore higher setback was obtained. Similar results  
224 were obtained by Martínez & el-Dahs (1993), who detected a reduction of the maximum  
225 viscosity and an increase in the final viscosities of the wheat flour when adding instant  
226 maize flour (up to 25%). When compared the viscosity profile of the different maize  
227 varieties, maximum and final viscosities values from hybrid varieties were significantly  
228 ( $P < 0.05$ ) higher than those of the regional ones (*Fandango*, *Pigarro*) (Table 4). Flint  
229 maize varieties have harder endosperm than the dent varieties and their flours have distinct  
230 viscosity profile (Brites et al., 2007a,b). *Fandango* maize flour variety (regional dent type)  
231 presented superior values than *Pigarro* (regional flint type), agreeing to previously data  
232 (Santos, 2006; Brites, 2006). Previous findings reported that the flint maize shows lower  
233 maximum viscosity and lower setback than dent varieties (Almeida-Domingués et al.,  
234 1997; Sandhu et al., 2007; Brites et al., 2007b).

235 The milling type variation influenced maximum viscosity (Table 4) and also breakdown,  
236 being the average values of the flour from water mill higher and significantly different

237 than the results of flour from the electric mill. The electric milling process yielded flour  
238 with lower viscosity profile likely associated to the negative impact of damage starch on  
239 the ability to absorb water.

240

### 241 **3.2. Effects of maize varieties, milling type and water temperature on dough** 242 **farinograph and texture parameters**

243 The behaviour of the dough during mixing and handling was analysed by using the  
244 Farinograph and texturometer respectively, considering the effect of the variety and  
245 milling type as well as the mixing water temperature. No influence of variety and milling  
246 type was detected (Table 5) on those parameters, with exception of the significant  
247 ( $P<0.05$ ) effects of the milling process on dough hardness.

248 The temperature of the added water for making the dough was the major factor of  
249 variability (Table 6). Water temperature significantly ( $P<0.05$ ) affected development time  
250 ( $T_d$ ), the consistency of the dough ( $C_{Td}$ ) and the stability of the dough (related to the  
251 consistency after 20 min mixing). Concerning dough machinability, the temperature of the  
252 dough did not significantly affect hardness, but resulted in a significant ( $P<0.05$ ) effect on  
253 adhesiveness, gumminess and stickiness of the dough. When boiling water was used for  
254 dough mixing, maize dough showed significantly ( $P<0.05$ ) higher consistencies with  
255 minor development times compared to doughs mixed at 25°C dough. Associated with the  
256 increase of the water temperature was the increase of mechanical and surface related  
257 parameters adhesiveness, elasticity, and stickiness and subsequent reduction of  
258 cohesiveness. These results were not unexpected since previous studies reported that  
259 dough rheological parameters were particularly affected by starch gelatinisation (Miyazaki  
260 & Morita, 2005). The addition of boiling water to the maize flour promoted the partial  
261 gelatinization of the starch, increasing the viscosity of the dough, consequently, leading to

262 higher dough consistency. The gelatinization occurs as the temperature rises, which  
263 increases mechanical strength of dough. This is an important factor to consider when  
264 maize flours are destined to gluten free breadmaking obtaining a viscous system that holds  
265 the components of the system. In fact, Rosell & Marco (2007) observed a decrease in the  
266 peak of maximum viscosity after heating rice flour dough prepared by using heated water  
267 during mixing, due to the previous partial gelatinization when warm water was added.  
268 Similar effects have been observed when the pasting characteristics of native and heat-  
269 moisture treated maize starches were compared (Hoover & Manuel, 1996). As a  
270 consequence of the initial starch gelatinization, dough consistency increases, improving  
271 the mechanical and handling properties of the rice flour dough compared to those of the  
272 dough mixed with water at 25°C (Marco & Rosell, 2008a)

273 Therefore, an alternative for improving gluten-free dough consistency is to promote the  
274 partial starch gelatinisation through the addition of boiling water when mixing.  
275 Relationships between flour composition and viscosity and dough rheological parameters  
276 were particularly significant for dough textural parameters vs flour parameters. Significant  
277 correlations ( $P < 0.05$ ) between amylose and cohesiveness were detected ( $r = 0.72$ ), whereas  
278 springiness and stickiness parameters were associated to gelatinization and retrogradation  
279 phenomena ( $r > 0.71$ ), as were previously found for wheat doughs (Collar & Bollaín 2005;  
280 Collar et al., 2007).

281

### 282 **3.3. Effect of maize varieties and milling types on bread specific volume, colour,** 283 **firmness and sensory assessment**

284 A preliminary breadmaking study was performed varying the temperature (25°C or  
285 100°C) of the water added to the maize flour during mixing, *broa* obtained by adding  
286 water at 100°C showed superior crumb texture quality than the ones obtained at 25°C

287 water temperature (results not showed). Further breadmaking trials were made following  
288 the traditional *broa* making procedure, using boiled water for mixing maize flour.

289

290 Traditional ethnic bread, *Broa*, was made for defining breadmaking conditions prior to the  
291 performance of gluten free maize bread because although does The specific volume of the  
292 *broa* ranged from 1.40 to 1.57cm<sup>3</sup>/g (Table 7), which could be considered low values if  
293 compared with wheat bread loaves. Traditionally, *Broa* is a type of bread with high  
294 density and closed crumb cells, thus high specific volume is not desirable. Besides breads  
295 made or containing high amounts of gluten free cereals show low specific volume  
296 compared to the ones obtained with wheat flour (Marco & Rosell, 2008a).

297 Regarding the effect of maize varieties and milling type on the specific volume of *broa*,  
298 no significant differences were detected (Table 7). Significant differences (P<0.05) were  
299 induced by maize varieties in the colour parameters and firmness, by contrast no  
300 significant differences (exception to blue/yellow parameter -b\*) were obtained between  
301 flours obtained from water and electric mills. Maize varieties had a significant effect on  
302 the firmness of the bread crumb, being the crumbs from *Pigarro* maize variety  
303 significantly harder than the ones from *Fandango*.

304 Sensory triangular assays of *broa* showed no significant differences ascribed to the type of  
305 mill (data not shown). Sensory rank sums and paired comparison test of regional and  
306 hybrid maize varieties within each colour (white or yellow type) showed the preference of  
307 regional maize varieties in detriment of hybrids (22.0 vs 14.0 in the case of yellow types  
308 and 20.0 vs 16.0 in the case of white types). The judges defined *Fandango* variety *broa*  
309 with better characteristics of mouth feel flavour and texture, even though *broas* produced  
310 with the hybrid varieties had higher specific volume.

311

312 From the above results, *Fandango* and *Pigarro* varieties were selected for performing  
313 gluten free maize bread, since they were the preferred varieties by the judges. The study  
314 was restricted to the maize flours from water mill, because milling type did not induce  
315 significant differences on the *broa* quality. The specific volume of gluten free maize bread  
316 ranged from 1.02 to 1.12 cm<sup>3</sup>/g. As expected the gluten free breads presented from 20 to  
317 30% less specific volume than their counterparts produced with the traditional recipe  
318 (obtained from composite maize-rye-wheat flour). Sanni *et al* (1998) obtained maize bread  
319 containing egg proteins with 0.95 cm<sup>3</sup>/g specific volume. Similar bread specific volume  
320 had been reported in rice based breads, which were improved by using crosslinking  
321 enzymes (Gujral & Rosell, 2004 a,b), hydrocolloids (Marco *et al*, 2007) or proteins  
322 (Marco & Rosell, 2008 a).

323 Gluten free maize bread displayed smaller volume with slightly more compact structure  
324 than the traditional *broa*, which showed defined gas cells in the crumb (Figure 2). Gluten  
325 free breads due to the absence of a protein network cannot retain the carbon dioxide  
326 produced during the fermentation, leading to a product with low specific volume and  
327 compact crumb (Rosell & Marco, 2008), which has a close appearance resemblance to the  
328 Portuguese ethnic bread.

329 A comparison was made between the quality parameters of the *broa* and the gluten free  
330 bread. Significant differences were detected in the colour and texture parameters between  
331 gluten free and traditional *broa*. As was expected, crumb firmness of gluten free bread  
332 was significantly higher (+ 50%) than the one obtained in the traditional *broa*, which agree  
333 with the specific volume results obtained. Reduced loaf volume and firmer crumb texture  
334 of gluten free bread when compared with traditional *broa* was attributed to maize gluten  
335 absence, as has been previously observed in other gluten free bread recipes (Rosell &  
336 Marco, 2008).

337 Sensory ordinance test showed contradictory results depending on the maize variety used  
338 for breadmaking. Significant differences ( $P<0.05$ ) between gluten free and traditional *broa*  
339 obtained from *Pigarro* maize flour were obtained in the sensory paired preference test, the  
340 sensory panel preferred traditional *broa* (22.0 vs 14.0). Conversely, in the case of  
341 *Fandango* (yellow variety), no significant differences ( $P<0.05$ ) were observed between the  
342 scores that received the traditional *broa* and the gluten free maize bread. *Fandango* variety  
343 was sweeter than *Pigarro* and it seems to perform better in breadmaking process that  
344 includes sourdoughs.

345

#### 346 **4. Conclusions**

347 Breads were obtained from maize and composite maize-rye-wheat flour, studying the  
348 effect of maize varieties, milling process, and processing variables on the dough  
349 characteristics and bread quality. Significant differences between regional and hybrid  
350 maize were detected regarding protein, amylose and RVA viscosity profiles. Concerning  
351 the effect of milling process, the grinding in a water mill occurs at slower rate than in the  
352 electrical, obtaining flour with lower ash content and higher viscosities. Nevertheless the  
353 influence of milling type on flour parameters, no significant differences were detected in  
354 *broa* sensory triangular tests and ordinance tests had neglected hybrid maize in relation to  
355 the regional ones.

356 Baking assays demonstrated that *broa* breadmaking technology could be satisfactorily  
357 applied to produce gluten free *broa*. An important point in the breadmaking process was  
358 the blanching that resulted in doughs with higher consistency, because in the absence or  
359 reduced amount of gluten the dough rheological properties are provided by the starch  
360 gelatinisation. Maize based gluten free bread were obtained following *broa* breadmaking

361 process, obtaining bread with satisfactory sensory characteristics and similar appearance  
362 than the traditional *broa*.

363

## 364 **5. Acknowledgements**

365 Authors thank the Cerealis Group enterprises (Nacional, Lisboa, Portugal) for supplying  
366 ingredients and baking facilities, Armando Ferreira for support on *broa* crumb texture  
367 assessment and colleagues of department for sensory analysis. Authors thank the financial  
368 support of the joint research program between the Spanish/Portugal Scientific Research  
369 Council (CSIC, Spain; Grices, Portugal); Comissão Interministerial de Ciência y  
370 Tecnología Project (MCYT, AGL2005-05192-C04-01) and Programa Operacional  
371 Ciência e Inovação (PPCDT/AGR/57994/2004).

372

## 373 **References**

- 374 Almeida-Dominguez HD, Suhendro EL & Rooney LW (1997) Factors affecting rapid  
375 visco analyser curves for the determination of maize kernel hardness. *Journal of*  
376 *Cereal Science*, 25,93-102.
- 377 American Association of Cereal Chemists, Approved methods AACC 10<sup>th</sup> Ed. (2000),  
378 method 33–50A (1999) Sensory Evaluation – Triangle difference test, 9pp.
- 379 American Institute of Baking Standard Procedure (2007). White pan bread firmness  
380 measurement, Manhattan, Kansas, USA. Available at [www.aibonline.org/](http://www.aibonline.org/researchandtechnical/services/prodqualityeval/AIBTextureAnalysis%20Procedures.pdf)  
381 [researchandtechnical/services/prodqualityeval/AIBTextureAnalysis%20Procedures](http://www.aibonline.org/researchandtechnical/services/prodqualityeval/AIBTextureAnalysis%20Procedures.pdf)  
382 [.pdf](http://www.aibonline.org/researchandtechnical/services/prodqualityeval/AIBTextureAnalysis%20Procedures.pdf). Accessed 7 April 2007.
- 383 Armero E & Collar C (1997) Texture properties of formulated wheat doughs.  
384 Relationships with dough and bread technological quality. *European Food*  
385 *Research Technology*, 204,136–145.



386 Brites C (2006) Melhoria da qualidade do pão de milho no contexto de alimento funcional.  
387 In R. Rezzónico, *Proceedings Congreso Internacional de Ciencia y Tecnología de*  
388 *los Alimentos*, (pp33) Córdoba, Argentina: Agencia Córdoba Ciencia.

389 Brites C, Haros M, Trigo MJ & Islas RP (2007a) Maíz. In AE León & CM Rosell (eds) *De*  
390 *tales harinas, tales panes. Granos, harinas y productos de panificación en*  
391 *Iberoamérica*, pp 74-121. Córdoba, Argentina: Hugo Báez.

392 Brites C, Trigo MJ, Santos C, Collar C & Rosell CM (2007b) Factores que influenciam as  
393 propriedades reológicas de farinhas de milho. In S.F. Palma, J. Canada, J. Dias,  
394 S.P. Ferro, & N.B. Alvarenga, *Proceedings 8º Encontro de Química dos Alimentos*,  
395 (pp 609-612). Beja, Portugal: GTO 2000, Soc. Artes Gráficas, Lda.

396 Collar C & Bollaín C (2005) Relationships between dough functional indicators along  
397 breadmaking steps in formulated samples. *European Food Research and*  
398 *Technology*, 220, 372–379.

399 Collar C, Bollaín C & Rosell CM (2007) Rheological behaviour of formulated bread  
400 doughs during mixing and heating. *Food Science and Technology International*,  
401 13(2), 99-107.

402 Esteller MS & Lannes SCS (2005) Parâmetros complementares para fixação de identidade  
403 e qualidade de produtos panificados. *Ciência e Tecnologia de Alimentos*, 25(4),  
404 802-806.

405 Gallagher E, Gormley TR & Arendt EK (2004) Recent Advances in the Formulation of  
406 Gluten-free Cereal-based Products. *Trends in Food Science and Technology*, 15,  
407 143-152.

408 Gujral HS & Rosell CM (2004a) Improvement of the breadmaking quality of rice flour by  
409 glucose oxidase. *Food Research International*, 37 (1), 75-81.

410 Gujral HS & Rosell CM (2004b) Functionality of rice flour modified with a microbial  
411 transglutaminase. *Journal of Cereal Science*, 39, 225-230.

412 Hoover R & Manuel H (1996) The effect of heat-moisture treatment on the structure and  
413 physicochemical properties of normal maize, waxy maize, dull waxy maize and  
414 amylo maize V starches. *Journal of Cereal Science*, 23, 153-162.

415 International Association for Cereal Science and Technology, ICC Standard Methods.  
416 Standard 104/1:1990, Determination of Ash in Cereals and Cereal Products;  
417 Standard 105/2: 1994, Determination of Crude Protein in Cereals and Cereal  
418 Products for Food and Feed.

419 International Organization for Standardization, ISO. Approved Methods of ISO. Method  
420 5530-1:1997 Wheat flour, Physical characteristics of doughs, Part 1: Determination  
421 of water absorption and rheological properties using a farinograph. ISO/ 6647-  
422 1:2007. Rice, Determination of amylose content, Part 1: Reference method ISO/  
423 6647-2:2007. Rice, Determination of amylose content, Part 2: Routine methods.  
424 ISO 8587:1988 Sensory analysis, Methodology, Ranking; ISO 5495:1983 Sensory  
425 analysis, Methodology, Paired comparison test. The Association: Geneva,  
426 Switzerland.

427 Lino CM, Silva LJG, Pena A, Fernández M & Mañes J (2007) Occurrence of fumonisins  
428 B1 and B2 in *broa*, typical Portuguese maize bread. *International Journal of Food*  
429 *Microbiology*, 118,79-82.

430 Lopez ACB, Pereira AJG & Junqueira RG (2004) Flour mixture of rice flour, corn and  
431 cassava starch in the production of gluten-free white bread. *Brazilian Archives of*  
432 *Biology and Technology*, 47, 63-70.

433 Marco C, Brites C, Collar C & Rosell CM (2007) Influence of hydrocolloids on  
434 rheological parameters of gluten free corn bread. In ICC (ed), Proceedings of 1<sup>st</sup>  
435 Latin American Conference ICC, pp217. ICC, Rosario, Argentina.

436 Marco C & Rosell CM (2008a) Breadmaking performance of protein enriched gluten free  
437 breads. European Food Research International. DOI: 10.1007/s00217-008-0838-6

438 Marco C & Rosell CM (2008b) Modification of rice proteins functionality by crosslinking  
439 with different protein isolates. Journal of Food Engineering, 84, 132-139.

440 Marco C & Rosell CM (2008c) Functional and rheological properties of protein enriched  
441 gluten free composite flours. Journal of Food Engineering, 88, 94-103.

442 Martínez BF & el-Dahs AA (1993) Elaboration of instant corn flour by hydrothermal  
443 process. Archivos Latinoamericanos de Nutricion, 43(4), 321-326.

444 Miyazaki M & Morita N (2005) Effect of heat-moisture treated maize starch on the  
445 properties of dough and bread. Food Research International, 38, 369-376.

446 Moore MM, Heinbockel M, Dockery P, Ulmer HM & Arendt EK (2006) Network  
447 formation in gluten-free bread with application of transglutaminase. Cereal  
448 Chemistry, 83(1), 28-36.

449 Moreira PM (2006) Participatory maize breeding in Portugal. A Case Study. Acta  
450 Agronomica Hungarica, 54(4), 431-439.

451 Özboy Ö (2002) Development of corn starch-gum bread for phenylketonuria patients. Die  
452 Nahrung, 46(2), 87-91.

453 Rosell CM & Collar C (2007) Rice based products. In: YH Hui (ed) Handbook of food  
454 products manufacturing, pp 523-538. Willey-Intersciences, Hoboken, New Jersey.  
455 USA.

456 Rosell CM & Marco C (2007) Different strategies for optimizing rice based bread:  
457 ingredients, structuring agents and breadmaking process. In JF Panozzo & CK

458 Black (eds), Proceedings of 57th Australian Cereal Chemistry Conference (pp.  
459 155-158). Impact Printing, Victoria, Australia.

460 Rosell CM & Marco C (2008) Rice. In: EA Arendt & F Dal Bello (eds), Gluten free cereal  
461 products and beverages, pp 81-100. Elsevier, Oxford, UK.

462 Sánchez HD, Osella CA & de la Torre MA (2002) Optimization of gluten-free bread  
463 prepared from cornstarch, rice flour, and cassava starch. *Journal of Food Science*,  
464 67(1), 416-419.

465 Sandhu KS, Singh N & Malhi NS (2007) Some properties of corn grains and their flours I:  
466 Physicochemical, functional and chapatti-making properties of flours. *Food*  
467 *Chemistry*, 101, 938-946.

468 Sanni AI, Onilude AA & Fatungase MO (1998) Production of sour maize bread using  
469 starter-cultures. *World Journal of Microbiology & Biotechnology*, 14, 101-106.

470 Santos CAL (2006) Relatório de actividades de bolsa de investigação. Estação  
471 Agronómica Nacional. Oeiras, Portugal.

472 SAS (1999) SAS/STAT<sup>®</sup> User's guide-release 8 edition. SAS Institute Inc. Cary, North  
473 Carolina, USA.

474 Schober TJ, Bean SR, & Boyle DL (2007) Gluten-free sorghum bread improved by  
475 sourdough fermentation: Biochemical, rheological, and microstructural  
476 background. *Journal of Agricultural and Food Chemistry*, 55 (13), 5137-5146.

477 Vaz Patto MC, Moreira PM, Carvalho V & Pego S (2007) Collecting maize (*Zea mays* L.  
478 *convar. mays*) with potential technological ability for bread making in Portugal.  
479 *Genetic Resources and Crop Evolution*, 54(7), 1555-1563.

480

**Table 1** – Characteristics of maize varieties used in this study.

481

<b>Variety</b>	<b>Type</b>	<b>Endosperm</b>	<b>Color</b>
<i>Pigarro</i>	Regional, local germplasm, open pollinated	Flint	White
<i>Fandango</i>	Regional, exotic germplasm, open pollinated	Dent	Yellow
Yellow Hybrid	Hybrid	Dent	Yellow
White Hybrid	Hybrid	Dent	White

482

483 **Table 2** – Effects of maize variety and milling type on protein, ash and amylose contents  
 484 of maize flours

485

Factor	Level	Protein (% db)	Ash (% db)	Amylose (% db)
Variety	<i>Fandango</i>	9.5 <sup>b</sup>	1.50 <sup>b</sup>	28.6 <sup>b</sup>
	<i>Pigarro</i>	10.5 <sup>a</sup>	1.94 <sup>a</sup>	29.2 <sup>b</sup>
	Yellow Hybrid	8.3 <sup>d</sup>	1.49 <sup>b</sup>	32.7 <sup>a</sup>
	White Hybrid	8.8 <sup>c</sup>	1.39 <sup>b</sup>	32.3 <sup>a</sup>
Milling type	Water mill	9.31 <sup>a</sup>	1.48 <sup>b</sup>	31.10 <sup>a</sup>
	Electric mill	9.27 <sup>a</sup>	1.68 <sup>a</sup>	30.29 <sup>a</sup>

486

487 For each parameter and single factor, values followed by the same letter are not significantly different at  
 488  $p < 0.05$ .

489

490

**Table 3** – Effect of maize variety on colour parameters.

491

492

Variety	L*	a*	b*
<i>Fandango</i>	87 <sup>b</sup>	-1.49 <sup>c</sup>	38.7 <sup>a</sup>
<i>Pigarro</i>	89 <sup>b</sup>	-0.04 <sup>a</sup>	12.6 <sup>c</sup>
Yellow hibrid	88 <sup>b</sup>	-1.86 <sup>d</sup>	33.7 <sup>b</sup>
White hibrid	92 <sup>a</sup>	-0.07 <sup>b</sup>	10.5 <sup>c</sup>

493

494 For each parameter, values followed by the same letter are not significantly different ( $p < 0.05$ ).

495

496 **Table 4** – Effects of maize variety and milling process on RVA parameters (maximum,  
 497 minimum and final viscosities and breakdown) of flour.

498

Factor	Level	Maximum Viscosity (cP)	Minimum Viscosity (cP)	Final Viscosity (cP)	Breakdown (cP)
Varieties	<i>Fandango</i>	2999 <sup>b</sup>	1391 <sup>c</sup>	4675 <sup>b</sup>	1609 <sup>b</sup>
	<i>Pigarro</i>	1580 <sup>c</sup>	1088 <sup>d</sup>	3168 <sup>c</sup>	492 <sup>c</sup>
	Yellow Hybrid	5342 <sup>a</sup>	2004 <sup>b</sup>	6344 <sup>a</sup>	3338 <sup>a</sup>
	White Hybrid	5484 <sup>a</sup>	2340 <sup>a</sup>	6745 <sup>a</sup>	3144 <sup>a</sup>
Milling Type	Water mill	4140 <sup>a</sup>	1764 <sup>a</sup>	5387 <sup>a</sup>	2376 <sup>a</sup>
	Electric mill	3562 <sup>b</sup>	1647 <sup>a</sup>	5078 <sup>a</sup>	1915 <sup>b</sup>

499

500 For each parameter and single factor, values followed by the same letter are not significantly different at  
 501  $p < 0.05$ .

502



503 **Table 5-** Farinograph and texturometer dough mean parameters from four maize varieties, two types of milling and two mixing temperatures.

504

<b>Variety</b>	<b>Milling type</b>	<b>Water temperature (°C)</b>	<b>Td (min.)</b>	<b>C<sub>Td</sub> (BU)</b>	<b>C<sub>20</sub> (BU)</b>	<b>Hardness (g/force)</b>	<b>Adhesiveness (g/s)</b>	<b>Gumminess</b>	<b>Stickiness (g/force)</b>
<i>Fandango</i>	Water	25	13.0	55	60	2502	1016	300	16.1
		100	6.5	95	80	2058	2211	121	21.4
	Electric	25	6.5	80	90	2661	2831	286	20.9
		100	4.0	210	145	2824	12758	223	25.2
<i>Pigarro</i>	Water	25	7.5	60	65	4179	1341	260	16.1
		100	8.8	260	260	6500	5867	735	20.2
	Electric	25	10.0	75	75	2131	1098	201	19.1
		100	3.5	120	195	2258	4137	137	25.3
Yellow hibrid	Water	25	10.0	75	80	2948	2244	353	21.0
		100	7.5	185	165	2876	11458	241	26.2
	Electric	25	6.5	80	80	2012	3529	178	20.5
		100	6.8	150	140	2541	5348	203	29.0
White hibrid	Water	25	14.5	90	100	3825	4297	464	17.0
		100	5.0	150	160	3008	7514	288	30.3
	Electric	25	7.5	50	55	2078	1785	270	16.9
		100	7.8	100	90	2256	4980	176	24.7

505

Td – development time (min), C<sub>Td</sub> – consistency at development time, C<sub>20</sub> – consistency at 20min

506

507 **Table 6** – Effect of water temperature on dough Farinograph and texturometer parameters.

508

	Water temperature	
	25°C	100°C
Td (min)	9.4 <sup>a</sup>	6.2 <sup>b</sup>
C <sub>Td</sub> (UB)	71 <sup>b</sup>	159 <sup>a</sup>
C <sub>20</sub> (UB)	76 <sup>b</sup>	154 <sup>a</sup>
Adhesiveness (g/s)	2267 <sup>b</sup>	6784 <sup>a</sup>
Cohesiveness	0.11 <sup>a</sup>	0.08 <sup>b</sup>
Springiness	0.25 <sup>b</sup>	3.2 <sup>a</sup>
Stickiness (g/force)	18.5 <sup>b</sup>	25.3 <sup>a</sup>

509

510 Td – development time, C<sub>Td</sub> – consistency at development time, C<sub>20</sub> – consistency at 20

511 min. For each parameter, values followed by the same letter are not significantly different

512 at ( $p < 0.05$ ).

513 **Table 7** – Effect of maize variety and milling type on specific volume, colour parameters

514 and crumb firmness of *broa*

515

Variety	Specific			b*	Firmness (g force)
	Volume (cm <sup>3</sup> /g)	L*	a*		
<i>Fandango</i>	1.44 <sup>a</sup>	66.7 <sup>c</sup>	-1.05 <sup>c</sup>	30.9 <sup>a</sup>	1503 <sup>b</sup>
<i>Pigarro</i>	1.46 <sup>a</sup>	71.1 <sup>a</sup>	-0.34 <sup>a</sup>	16.2 <sup>c</sup>	1800 <sup>a</sup>
Yellow hibrid	1.40 <sup>a</sup>	65.6 <sup>d</sup>	-1.25 <sup>d</sup>	27.1 <sup>b</sup>	1778 <sup>ab</sup>
White hibrid	1.57 <sup>a</sup>	68.9 <sup>b</sup>	-0.74 <sup>b</sup>	15.6 <sup>d</sup>	1611 <sup>ab</sup>

516 For each parameter, values followed by the same letter are not significantly different at  $p < 0.05$ .

517

518 **Figure captions**

519

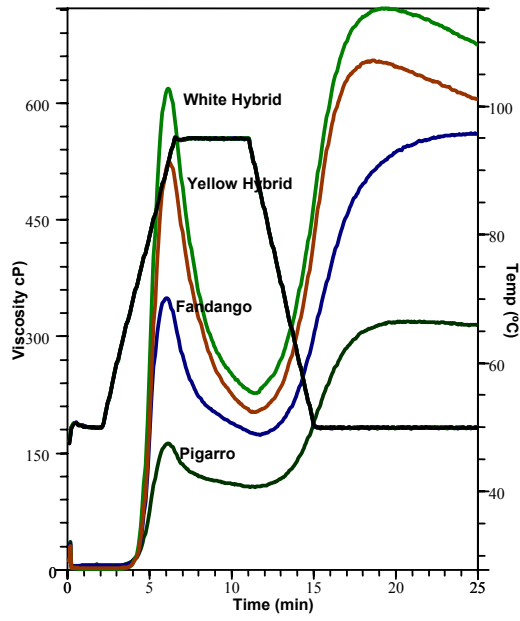
520 **Figure 1.** Viscosities profiles of maize flours obtained from electric mill determined by  
521 RVA (Rapid Visco Analyser).

522 **Figure 2–** Crumbs of *broa* produced with traditional and gluten free formulation. Upper  
523 pictures: *Fandango* maize variety, and lower pictures: *Pigarro* maize variety.

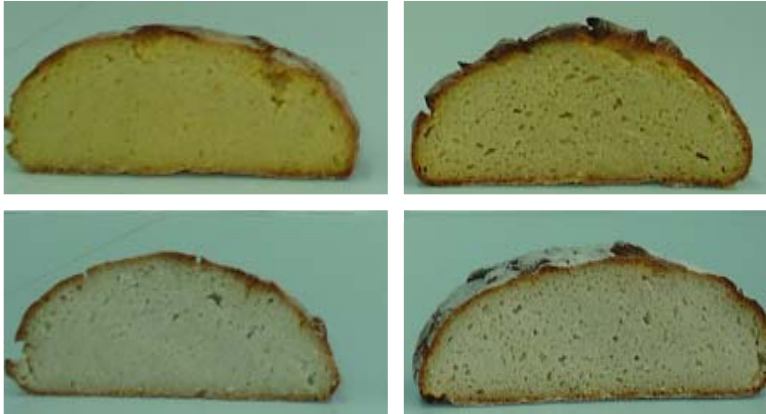
524

525 Figure 1.

526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546



547 Figure 2.  
548  
549



**Gluten free**

**Traditional**

550  
551