

1 **Relationship between instrumental parameters and sensory characteristics in**  
2 **gluten-free breads**

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9 **Running head:** Gluten free bread characteristics

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17 **Abstract**

18 Numerous bread-like gluten free products have been lately developed due to the rising  
19 demand on wheat free foods. A range of parameters has been used to describe these  
20 products, but there is no general agreement about the most suitable assessment to  
21 characterize them. The objective of this research was to characterize diverse gluten free  
22 like breads (GFB) in order to discriminate them and to establish possible correlations  
23 among descriptive parameters of GFB features determined by instrumental methods and  
24 sensory analysis. Statistical analysis showed that all physical, physicochemical  
25 characteristics (specific volume, moisture content, water activity,  $L^*$ ,  $a^*$ ,  $b^*$ , hue and  
26 chroma), hydration properties (swelling, water holding capacity and water binding  
27 capacity), texture profile analysis (TPA) parameters (hardness, springiness, chewiness,  
28 cohesiveness and resilience) and structural analysis of the crumbs (number of cells and  
29 total area) significantly ( $p < 0.05$ ) discriminated between the GFB types tested. Sensory  
30 analysis revealed great divergences in crumb appearance, odour, springiness,  
31 crumbliness and colour of samples, but not significant differences ( $p < 0.05$ ) in flavour,  
32 aftertaste and hardness of them. Certain significant correlations were established within  
33 the parameters determined by instrumental methods. Hydration properties of the crumb  
34 showed to be positively correlated with cohesiveness and resilience. Significant  
35 correlations, but scientifically meaningless, were observed among the instrumental and  
36 sensory parameters, because correlation coefficients were rather low, which represent  
37 very weak or low linear correlations ( $r \leq 0.35$ ). The principal component analysis showed  
38 that sensory parameters described in this study and also hydration properties besides  
39 texture parameters would be suitable for characterizing bread like gluten free products.

40 **Highlights:**

- 41
- Gluten-free breads are evaluated by instrumental and sensory parameters

- 42      • Physicochemical characteristics discriminate gluten-free breads
- 43      • Correlations among sensory and instrumental characteristics are established
- 44    **Key words:** gluten-free, bread, quality, crumb, sensory characteristics.
- 45

46 **Introduction**

47

48 Celiac disease (CD), also known as gluten-sensitive enteropathy, is a chronic disorder  
49 of the small intestine caused by exposure to gluten in the genetically predisposed  
50 individuals [1,2]. It is characterized by a strong immune response to certain amino acid  
51 sequences found in the prolamin fractions of wheat, barley, rye, and certain varieties of  
52 oats, resulting in inflammation and damage of the small-intestine mucosa and leading to  
53 malabsorption of nutrients [1,3]. Nowadays, the general prevalence of CD was  
54 estimated to be 1 in 300, although population-based screening studies carried out in  
55 2008 suggest that the prevalence may be 1 in 100 [4]. Persons with CD are unable to  
56 consume some of the most common products in the market, including breads, baked  
57 goods, and other food products made with wheat flour. Until now, the only effective  
58 treatment for CD is strict adherence to gluten-free (GF) diet throughout the patient's  
59 lifetime [4].

60 The apparent or real increase in celiac disease or other allergic reactions and  
61 intolerances to gluten consumption has prompted the rising demand for gluten-free  
62 products. A range of bread-like gluten-free products has been designed trying to  
63 resemble wheat bread. The gluten-free bread recipes contain mainly rice or maize flours  
64 combined with potato, maize or wheat starches [5-7]. In recent years there has been  
65 extensive research for the development of gluten-free bread, involving diverse  
66 approaches, like the use of different starches (maize, potato, cassava or rice), dairy  
67 products, gums and hydrocolloids, emulsifiers, other non-gluten proteins, prebiotics or  
68 combinations thereof, as alternatives to gluten, to improve the structure, mouthfeel,  
69 acceptability and shelf-life of gluten-free bakery products [5-6, 8-16]. The development  
70 of such bread is frequently difficult having in mind that gluten is the main structure-

71 forming protein in wheat flour, responsible for the elastic and extensible properties to  
72 produce good quality bread [17].

73 In those researches, different features of the gluten free breads have been evaluated to  
74 assess their quality. Despite the different characteristics of the gluten free bread  
75 compared to its wheat counterparts, the same evaluation methods have been usually  
76 applied. Instrumentals analysis, including loaf weight and volume, specific volume,  
77 colour parameters, and textural parameters have been frequently used to characterize  
78 gluten-free breads [12, 14, 16, 18-22]. Sensory analysis has been also considered in  
79 some of the studies when developing gluten-free breads [7, 10, 13-15, 20, 23, 24]. Other  
80 researches have also characterized the crumb microstructure by using image analysis  
81 [19, 23] or scanning electron microscopy [12].

82 Therefore, instrumental measurements and sensory analysis have been applied to  
83 characterize gluten free breads. However, no correlation between instrumental  
84 parameters and sensory analysis has been previously established in this type of  
85 products, which would be very helpful for defining the best quality attributes of gluten-  
86 free breads. Additionally, principal components analysis (PCA) could be used to  
87 identify the best parameters or descriptors of the quality of gluten-free breads that allow  
88 the discrimination among bread features.

89 The aim of this research was to characterize a range of gluten free breads in order to  
90 establish possible correlations among descriptive parameters of gluten free bread like  
91 features determined by instrumental methods and sensory analysis. For that purpose,  
92 eleven gluten-free breads like products, which represent a large range of commercial  
93 gluten-free breads, were evaluated regarding physicochemical analysis, hydration  
94 properties, crumb microstructure, crumb texture and sensory analysis.

95 **Materials and methods**

96

97 **Materials**

98

99 Eleven specialties of gluten-free breads (GFB) with either loaf or sliced presentations  
100 were selected and purchased in general and specialized supermarkets. Gluten-free  
101 breads are marketed in polyethylene pouches and packaged under modified atmosphere  
102 for keeping their characteristics during at least four months. All breads were purchased  
103 within the first month after its production. Breads were kept at 20°C till analysis.  
104 Information on the ingredients of each bread type, according to the labeling is given in  
105 Table 1. Due to commercial sensitivity the branded bread (n=11) varieties were labeled  
106 as GFB. Abbreviations of the samples are listed in Table 1. Samples from two different  
107 batches were used for the characterization.

108

109 **Physicochemical analysis**

110

111 Bread moisture content was determined following the ICC Standard Methods (110/1)  
112 [25]. Volume was determined by rapeseed displacement method and specific volume  
113 ( $\text{cm}^3/\text{g}$ ) of the individual loaf was calculated by dividing volume by weight. Water  
114 activity ( $a_w$ ) of bread samples was measured using an Aqua Lab Series 3 (Decagon  
115 devices Pullman, USA) at 22°C. The colour of the bread crumbs was measured at three  
116 different locations by using a Minolta colorimeter (Chromameter CR-400/410. Konica  
117 Minolta. Japan) after standardization with a white calibration plate ( $L^*= 96.9$ ,  $a^*= -$   
118  $0.04$ ,  $b^*=1.84$ ). The colour was recorded using CIE- $L^*a^*b^*$  uniform colour space (CIE-  
119 Lab) where  $L^*$  indicates lightness,  $a^*$  indicates hue on a green (-) to red (+) axis, and  $b^*$

120 indicates hue on a blue (-) to yellow (+) axis. Data from three slices per bread were  
121 averaged. Additionally the cylindrical coordinates: hue or hue angle ( $h_{ab}$ ) and Chroma  
122 ( $C^*_{ab}$ ) were defined by the following equations:

$$123 \quad C^*_{ab} = \sqrt{(a^*)^2 + (b^*)^2}$$

$$124 \quad h_{ab} = \arctan(b^*/a^*)$$

125 Hue angle is the angle for a point calculated from  $a^*$  and  $b^*$  coordinates in the colour  
126 space. Chroma is the quantitative component of the colour [26], which reflected the  
127 purity of colour in the CIELAB space.

128

129 Hydration properties

130

131 Swelling or the volume occupied by a known weight of sample was evaluated by  
132 mixing 5g ( $\pm 0.1$  mg) of dried gluten-free bread with 100 mL distilled water and  
133 allowing it to hydrate during 16h. Water holding capacity (WHC) defined as the amount  
134 of water retained by the sample without being subjected to any stress was determined by  
135 suspending 5g ( $\pm 0.1$  mg) of commercial gluten-free bread sample with 100mL distilled  
136 water and allowing them to hydrate overnight. After removing the excess of water, the  
137 hydrated solid was weighed and expressed per one gram of solid. Water binding  
138 capacity (WBC) or the amount of water retained by the bread after being subjected to  
139 centrifugation was measured as described the [AACC International method \(56-30.01\)](#)  
140 [27].

141

142 Crumb cell analysis

143

144 Images of the gluten-free bread slice (10-mm thick) were captured using a flatbed  
145 scanner equipped with the software HP PrecisoScan Pro version 3.1 (HP scanjet 4400C,  
146 Hewlett–Packard, USA). The default settings for brightness (midtones 2.2) and contrast  
147 (highlights 240, midtones 2.2, and shadows 5) of the scanner software were used for  
148 acquiring the images. The images were scanned full scale at 1200 pixels per inch and  
149 analysed in levels of grey (8 bits, readout 0–255) and captured in jpeg format for each  
150 measurement. A 30x30-mm square field of view (FOV) was evaluated for each image.  
151 This FOV captured the majority of the crumb area of each slice. Images were analysed  
152 by Image J software (National Institutes of Health, Bethesda, MD, USA) using the  
153 Otsu’s algorithm for assessing the threshold according to [Gonzales-Barron and Butler](#)  
154 [\[28\]](#). Data derived from the crumb structure analysis included: number of cells or  
155 alveoli, average cells area and cell circularity, and were used for comparing purposes  
156 among different samples. Circularity was calculated using the following equation:

157 
$$\text{Circularity} = 4 \times \pi \times \text{area} / (\text{perimeter})^2$$

158 A value of 1.0 indicates a perfect circle.

159

160 Crumb texture analysis

161

162 Crumb texture analysis was measured on uniform slices of 10mm thickness. Three  
163 slices from the center of each loaf were taken for evaluation [\[29\]](#). Texture profile  
164 analysis (TPA) was performed using a universal testing machine TA-XT2i (Stable  
165 Micro Systems, Surrey, UK) equipped with a 30 Kg load cell and 25 mm aluminium  
166 cylindrical probe. The settings used were test speed of 2.0 mm/s with a trigger force of  
167 5 g to compress the middle of the bread crumb to 50% of its original height at a  
168 crosshead speed of 1mm/s. Values were the mean of three replicates.



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170 Sensory evaluation

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172 A descriptive sensory analysis was performed for evaluating the sensory characteristics  
173 of commercial gluten-free breads. Bread slices, including crust and crumb, were  
174 presented (1cm thick) on plastic dishes coded and served in randomised order. A  
175 quantitative descriptive sensory analysis was carried out with twelve trained panellists  
176 under normal lightening conditions and at room temperature. The range of time that test  
177 panellist had participated in descriptive analysis and scale rating of a wide range of  
178 bread products varied from 3 to 20 years. Preliminary training test was performed, in  
179 which they were sat in a round table and after evaluating the sample, an open discussion  
180 was initiated for defining and describe the best descriptors for characterizing the  
181 product. Evaluation included perception at first glance of the bread slice (crust and  
182 crumb included) and mastication with the molar teeth up to swallowing. The attributes  
183 assessors finally agree were appearance (by observing the product slice), flavour,  
184 colour, taste, aftertaste (taste remaining in the mouth after swallowing), texture  
185 attributes during chewing and springiness (ability to regain original shape after pressing  
186 down the crumb with the middle finger). The descriptors for each attributes were  
187 appearance (visually liking or disliking), flavour (scale goes from high when typical of  
188 bread or bakery products to low, uncharacteristic of bakery products), colour (scales  
189 goes from high yellow/beige to low when brown or grey), taste (scale goes from high  
190 when typical taste of bread or bakery products to low, uncharacteristic of bakery  
191 products), aftertaste (scale goes from high when agreeable taste to low when distaste  
192 after swallowing), texture attributes during chewing (scales goes from hard-soft,  
193 crumbly-cohesive). Attribute intensity was scored on a scale varying from 1 (disliked

194 extremely) to 5 (like extremely). Two samples were evaluated during one session.  
195 Breads were considered acceptable if their means score for overall acceptance were  
196 above 2.5.

197

198 Statistical analysis

199

200 The results were expressed as mean values. For each quality parameter, a one way  
201 analysis of variance (ANOVA) was applied using Statgraphics Plus V 7.1 (Statistical  
202 Graphics Corporation, UK). Fisher's least (LSD) test was used to assess significant  
203 differences ( $p < 0.05$ ) among samples that might allow discrimination among them.  
204 Simple correlations were performed using Statgraphics V.7.1 software. Principal  
205 component analysis (PCA) was also performed to determine the number of principal  
206 components that significantly ( $p < 0.05$ ) discriminated samples.

207

## 208 **Results and discussion**

209

210 Technological and sensory characteristics of gluten free bread

211

212 The characterization of diverse gluten-free breads was carried out to identify the most  
213 discriminating parameters. With that purpose, an in-depth analysis of the gluten free  
214 breads was carried out (Table 2, 3). The analysis included physical, physicochemical  
215 properties, crumb structure analysis, also hydration properties of the crumb and sensory  
216 analysis. Mean values from two different batches for each sample are showed in table 2.  
217 Analysis of data collated using ANOVA showed that all physicochemical  
218 characteristics significantly ( $p < 0.05$ ) discriminated between the breads tested. GFB

219 samples presented specific volume values that ranged from 1.54 to 4.79 mL/g. Those  
220 agree with the ones reported by Sabanis, Lebesi and Tzia [13] when they evaluated  
221 enrichment of gluten-free baked products with different cereal fibres (2.7 to 3.9 mL/g),  
222 or with Marco and Rosell [12] findings (1.57 to 2.71 mL/g). Moisture content values  
223 ranged from 21.10 g/100g (GFB8) to 42.03 g/100g (GFB11). The present study  
224 included a range of marketed GFB specialties, thus probably differences might be  
225 attributed to the different bread formulations. In general, the moisture content values  
226 reported for gluten-free breads obtained from different formulations are rather high, for  
227 instance rice based bread enriched with proteins showed values of 41.66- 46.13 g/100g  
228 [12] and the enrichment of gluten-free breads with fibres even enhances those values  
229 (49-53 g/100g) [13]. Water activity values of crumb were also high (Table 2). Those  
230 values agree with the findings of Lazaridou, Duta, Papageorgiou, Belc and Biliaderis  
231 [10], that reported water activity values of GFB crumb in the range of 0.97-0.99. Likely,  
232 the high water activity as well as the moisture retention might be ascribed to the high  
233 water holding capacity of the incorporated hydrocolloids [30] that are usually added to  
234 GFB formulations as thickeners for improving volume (see Table 1). It has been  
235 reported 0.95 as typical aw value for breads [31]. Therefore, GFB samples tested,  
236 according to the above results, covered a good range of characteristics previously  
237 reported for this type of breads.

238 The colour of the crumb has been also an important parameter for characterising GFB.  
239 Lower  $L^*$  value indicates darker crumb,  $a^*$  positive value is associated with crumb  
240 redness, whereas  $b^*$  positive value indicates yellow colour. To obtain a good  
241 characterisation of the colour, it is necessary to bear in mind the psychophysical  
242 parameters, which correspond with the cylindrical coordinates: hue ( $h_{ab}$ ) and chroma  
243 ( $C^*_{ab}$ ). Great variability was observed in lightness. GFB8 and GFB9 showed the highest

244 values (83.83 and 80.20, respectively), indicating more reflectance of light when  
245 compared with the rest of the breads. Additionally, darker crumb was observed for  
246 GFB1, GFB4, GFB5 and GFB7. The darkening of the crumb colour is desirable as  
247 gluten-free breads usually tend to have lighter colour than wheat breads [23], and darker  
248 bread are usually associated with whole grains and wholesomeness [15]. Regarding  $a^*$ ,  
249 only GFB2 and GFB3 showed low positive value indicating hue on red axis, whereas  
250 the other breads presented negative  $a^*$  value (hue on green axis). In addition, all  
251 samples presented positive  $b^*$  value (indicating hue on yellow axis), showing  
252 significant differences among them ( $p < 0.05$ ). In relation to hue ( $h_{ab}$ ) and chroma ( $C^*_{ab}$ )  
253 colour attributes, great variation was observed (Table 3). The majority of the GFB  
254 samples presented negative hue values that reflected yellow-greenish hue, with the  
255 exception of GFB2 and GFB3 samples that presented hue positive values, which  
256 reflected yellow-orange hue. Chroma is the quantitative component of the colour  
257 associated to the colour purity in the CIELAB space. Both GFB2 and GFB3 showed  
258 chroma values higher than the other samples, which revealed its higher purity of colour  
259 related to major intensity of the yellow component (Figure 1).

260 Gluten-free breads have low ability to retain moisture during storage [11], thus  
261 hydration properties of the bread crumbs might be interesting properties to characterize  
262 this type of products. Hydration parameters are generally used for assessing the water  
263 uptake ability of different ingredients like hydrocolloids or fibers. GFB9 exhibited the  
264 highest values for swelling, WHC and WBC indicating that it can retain significantly  
265 more water than the other breads (Table 2). In addition, GFB4 showed the lowest value  
266 for swelling while GFB3 presented lowest values to WHC and WBC. In GFB, dietary  
267 fibre (mainly hydrocolloids incorporated as ingredient into gluten-free bread  
268 formulations) might be a major determinant of the water retention capacity of these

269 products. Significant differences were found among the samples, which could be useful  
270 for discriminating GFB and maybe those properties could be related to sensory  
271 attributes. Presumably, water retention capacity of the crumb could affect the perception  
272 of textural properties when these samples are eaten.

273 Parameters from the image analysis of the gluten-free bread crumbs (Figure 1) showed a  
274 large variability among crumb bread structures (Table 3). GFB6 exhibited significantly  
275 high cells or alveoli number value and total area value, whereas lower values were seen  
276 for GFB5 and GFB7. The unique reported values of this parameter in gluten-free breads  
277 ranged from 15 to 20 cells/cm<sup>2</sup> [32]. No significant differences were observed for  
278 average cell area (mm<sup>2</sup>). Nevertheless, significant differences were found for circularity  
279 values ( $p < 0.05$ ). It has been described that up to certain limit, the number of cells/cm<sup>2</sup>  
280 increases as HPMC and water increase [24]. Nonetheless, the combination of high  
281 levels of both decreases the cell/cm<sup>2</sup>, likely due to the coalescence of many gas cells  
282 into one large cell. Carboxymethyl cellulose and xanthan gum has been associated with  
283 higher cell average size, while breads with carrageenan and alginate had smaller cell  
284 sizes [22]. Gluten free crumbs had circularity values ranging from 0.60 to 0.81,  
285 indicating less uniform shape (Figure 1). Beside, cell (air) total area of bread crumbs  
286 showed significant differences among gluten-free breads.

287 In addition, significant differences were observed in the crumb texture properties of the  
288 different gluten free breads (Table 3). Gluten free bread like products due to their  
289 complex formulation, mainly based in carbohydrates [33], present high crumb hardness,  
290 which agree with the results of crumb image analysis. The majority of GFBs presented  
291 hardness values ranging from 10.33N to 14.60N; however GFB2 and GFB11 had the  
292 highest and lowest values, respectively. With respect to springiness, GFB8 showed the  
293 highest value, while GFB5 presented the lowest. Springiness is associated to a fresh and

294 elastic product; therefore high quality bread will be related to high springiness values.  
295 Marco and Rosell [12] found springiness values that ranged from 0.77 to 0.94 when  
296 study the protein enrichment of rice based gluten-free breads. Low springiness value is  
297 indicative of brittleness and this reflects the tendency of the bread to crumble when is  
298 sliced [24]. Cohesiveness characterises the extent to which a material can be deformed  
299 before it ruptures, reflecting the internal cohesion of the material. Bread with high  
300 cohesiveness is desirable because it forms a bolus rather than disintegrates during  
301 mastication, whereas low cohesiveness indicates increased susceptibility of the bread to  
302 fracture or crumble [16]. With the exception of the GFB8 and GFB9, low cohesiveness  
303 values (0.20-0.44) were observed, which implies that lower compression energy was  
304 required and consequently those breads more easily crumbled. Chewiness varied from  
305 1.69 to 32.90 N, but the majority of breads presented values comprised between 2.33 to  
306 5.77N and only GFB2 showed higher value. Therefore, the time required masticating a  
307 bread piece prior to swallow showed great variation. Low chewing value means easy  
308 break of the bread in the mouth like a biscuit. It was also observed that hardness and  
309 chewiness showed similar traits for all breads. Resilience values showed that GFB7 had  
310 the lowest elasticity, whereas GFB8 and GFB9 presented the highest values. It has been  
311 reported that the reduction in resilience or springiness characterizes loss of elasticity  
312 [16].

313 A quantitative descriptive analysis was performed for the sensory evaluation of the  
314 breads. Although 50 panellist are recommended for this analysis, in this study 12 long  
315 trained judges participate in the sensory evaluation, which agree with method of Heenan  
316 et al [34]. According to ANOVA results, the gluten-free breads differed significantly  
317 ( $p<0.05$ ) in crumb appearance, odour, springiness and crumbliness, also significant  
318 differences ( $p<0.1$ ) were found in colour (Table 4). Conversely, no significant

319 differences were observed in taste, aftertaste and hardness. GFB6 showed the highest  
320 appearance score. The less intense odour was perceived in GFB9. GFB4 received the  
321 highest score for springiness. In general, GFB6 was scored higher for majority of the  
322 sensorial attributes evaluated. Conversely, GFB9 and GFB10 were scored lower for  
323 most of the sensory attributes. These results clearly revealed great variability on sensory  
324 quality.

325

326 Relationship among technological and sensory parameters of gluten free bread like  
327 products

328

329 The assessment of technological or instrumental quality is the most preferred analysis  
330 for characterizing gluten-free breads because they are not subjected to consumer  
331 perceptions, which are greatly dependent on individual backgrounds, locations and so  
332 on. Therefore, the establishment of possible relationship between sensory and quality  
333 parameters or within the technological parameters would be very useful. With that  
334 purpose multivariate data handling was applied by using Pearson correlation analysis.

335 Significant correlations were observed within the parameters used for characterizing  
336 gluten free bread like products, but they were mainly obtained within the instrumental  
337 parameters (Table 5). Strong linear relationships were observed within the colour  
338 parameters, but also a strong positive linear relationship was obtained between  $L^*$  and  
339 cohesiveness ( $p < 0.001$ ) and resilience ( $p < 0.001$ ). Presumably, crumb structure has great  
340 influence on the texture properties and the luminosity of the crumb. The initial  
341 observation about the hardness and chewiness trend was confirmed with the high  
342 relationship ( $r < 0.9043$ ) detected between those parameters. Additionally, cohesiveness  
343 was strongly linear related to resilience ( $r < 0.9895$ ), showing the importance of the

344 internal cohesion of the crumb on the ability to recover after compressing. In this type  
345 of products, water activity showed a significant positive relationship with the moisture  
346 content. It must be highlighted the relationships observed among the crumb hydration  
347 properties and some other parameters, since those properties have not been previously  
348 determined in bread crumbs. Water hydration properties (swelling, WHC and WBC)  
349 were significant positively related within them. Moreover, strong positive relationships  
350 were observed between the WHC with resilience ( $r < 0.7020$ ) and between WBC with  
351 cohesiveness ( $r < 0.7633$ ) and resilience ( $r < 0.7901$ ).

352 Some relationships between sensorial parameters and instrumental parameters were  
353 statistically significant, although the correlation coefficients were rather low, which  
354 represent very weak or low linear correlations ( $r \leq 0.35$ ). With these type of products no  
355 linear relationships were detected between the instrumental and sensory parameters  
356 likely due to their complex formulations.

357 In order to propose a small number of parameters that allow gluten free bread  
358 characterization, a principal component analysis (PCA) with the significant quality  
359 parameters was carried out. Significant quality parameters analysed by PCA indicated  
360 that six principal components significantly ( $p < 0.05$ ) discriminated between breads,  
361 which accounted for 91% of the variability in the original data (data not showed). This  
362 analysis described 35% and 18% of variation on principal components 1 (PC1) and 2  
363 (PC2), respectively (Figures 2 and 3). Component 1 was defined by hydration  
364 properties, instrumental cohesiveness, resilience and springiness, and luminosity ( $L^*$ )  
365 along the positive axis, which were present in GFB8 and GFB10. Along the negative  
366 axis, PC1 was described by sensory parameters, moisture content and area and number  
367 of alveoli that were present in the majority of the gluten free breads tested. Conversely,  
368 the component 2 was mainly defined by specific volume, colour parameters ( $a^*$ ,  $b^*$ ,



369 chroma and hue) and hardness, along the positive and negative axis, respectively. GFB8  
370 and GF10 were positively located along PC1 and PC2 (Figure 3). On the other hand, the  
371 breads located along the negative axis of PC1 and PC2 were GFB2 and GFB3.  
372 Therefore, PCA allowed discriminating among gluten free breads and it showed that  
373 crumb hydration properties, besides texture parameters like cohesiveness, resilience and  
374 springiness could be of great importance for characterizing gluten free breads. In  
375 addition, most of the gluten free breads tested (GFB1, GFB4, GFB5, GFB6, GFB7,  
376 GFB11) were mainly grouped by the sensory parameters. Descriptive sensory attributes  
377 have been reported for discriminating among different wheat bread types [34]. In that  
378 study, porous appearance and odour attributes were the most important descriptors.  
379 Simultaneously, quality parameters obtained from instrumental analysis have been  
380 selected for defining the consumers' acceptability of wheat breads, which have been  
381 useful for identifying the main discrepancies of wheat breads produced by different  
382 breadmaking processes [35].

383

## 384 **Conclusions**

385

386 The assessment of the physicochemical, hydration properties, crumb texture and  
387 microstructure of a range of gluten free breads showed great divergence among their  
388 properties and the same observation was perceived in the sensory analysis. Sensory  
389 analysis revealed also great divergences in crumb appearance, odour, springiness,  
390 crumbliness and colour. Among all the assessed parameters, from the correlation matrix  
391 it was observed that colour, texture and hydration parameters were highly correlated  
392 within them. In addition, hydration properties were significantly positive correlated with  
393 cohesiveness and resilience. Significant but scientifically meaningless correlations were

394 found between sensory and instrumental parameters. According to the principal  
395 component analysis, gluten free breads could be classified along the first component on  
396 the basis of sensory properties (negative side) and hydration properties, instrumental  
397 cohesiveness, resilience and springiness (positive side). Therefore, sensory parameters  
398 described in this study and also hydration properties besides texture parameters would  
399 be suitable for characterizing bread-like gluten free products.

400

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407

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508 **FIGURE CAPTIONS**

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510 **Figure 1.** Digital images of commercial gluten-free bread crumb samples (30x30 mm  
511 field of view of GFB).

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513 **Figure 2.** Correlation loadings plot from principal component analysis showing the  
514 quality parameters of the eleven gluten free breads evaluated.

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516 **Figure 3.** Scores plot from principal component analysis of the eleven gluten free  
517 breads evaluated.

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**Table 1.** Ingredients in gluten-free breads (GFBs) according to supplier information

Product code	Ingredients
GFB1	Corn starch, water, sugar, egg, vegetal margarine, acidifier, preservative, aromas and colorant, yeast, thickener, emulsifier, salt, preservative, raising agents, antioxidants. May contain traces of soy.
GFB2	Corn starch, water, vegetal margarine, emulsifiers, salt, acidifier, preservative, antioxidants, aromas and colouring (betacarotene), egg, sugar, yeast, dextrose, humidifier, stabilizers, salt.
GFB3	Corn starch, water, vegetal margarine, emulsifiers, salt, acidifier, preservative, antioxidants, aromas and colorant, egg, sugar, yeast, dextrose, humidifier, stabilizers, salt.
GFB4	Potato starch, water, corn starch, caseinate (milk protein), sugar, vegetal oil, corn flour, yeast, soy protein, stabilizers, salt, preservative.
GFB5	Corn starch, water, sugar, egg, vegetal margarine, acidifier, preservative, aromas and colorant, stabilizers, yeast, emulsifiers, salt, raising agents, anise, cinnamon, and antioxidant.
GFB6	Corn starch, water, rice flour, vegetal oil, sugar, stabilizer, lupine protein, yeast, salt, vegetal fibre, aroma, emulsifiers.
GFB7	Corn starch, water, sugar, egg, vegetal margarine, acidifier, preservative, aromas and colorant, yeast, thickener, emulsifier, salt, raising agents, antioxidants. May contain traces of soy.
GFB8	Corn starch, water, sugar, yeast, thickeners, salt, raising agent, preservative.
GFB9	Corn starch, water, sugar, thickeners, emulsifier, salt, yeast, preservative, raising agents, antioxidants. May contain traces of egg.
GFB10	Corn starch, vegetal margarine, salt, sugar, emulsifier, raising agents, antioxidant, thickener, preservative, and yeast.
GFB11	Corn starch, vegetal margarine, salt, sugar, emulsifier, raising agents, antioxidant, thickener, preservative, and yeast.

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**Table 2.** Different quality characteristics of different gluten-free breads.

Sample	Specific volume	Moisture	$a_w$	Swelling	WHC	WBC	$L^*$	$a^*$	$b^*$	Chroma	Hue angle
codes	ml/g	g/100g		ml/g	g water/g solid	g water/g solid					°
GFB1	3.37 <sup>cd</sup>	29.63 <sup>d</sup>	0.91 <sup>b</sup>	1.49 <sup>a</sup>	2.55 <sup>ab</sup>	2.31 <sup>a</sup>	64.71 <sup>a</sup>	-2.01 <sup>cd</sup>	11.85 <sup>a</sup>	12.02 <sup>a</sup>	-80.36 <sup>def</sup>
GFB2	3.47 <sup>de</sup>	31.63 <sup>f</sup>	0.95 <sup>e</sup>	1.58 <sup>bc</sup>	2.63 <sup>ab</sup>	2.47 <sup>ab</sup>	72.93 <sup>f</sup>	0.50 <sup>d</sup>	21.78 <sup>g</sup>	21.78 <sup>f</sup>	88.67 <sup>h</sup>
GFB3	1.54 <sup>a</sup>	29.50 <sup>d</sup>	0.94 <sup>d</sup>	1.49 <sup>a</sup>	2.41 <sup>a</sup>	2.39 <sup>a</sup>	71.86 <sup>ef</sup>	0.97 <sup>d</sup>	19.86 <sup>f</sup>	19.88 <sup>e</sup>	87.20 <sup>g</sup>
GFB4	4.79 <sup>f</sup>	27.17 <sup>c</sup>	0.94 <sup>d</sup>	1.38 <sup>a</sup>	2.50 <sup>ab</sup>	2.60 <sup>bc</sup>	65.77 <sup>a</sup>	-1.63 <sup>abc</sup>	10.72 <sup>a</sup>	10.84 <sup>a</sup>	-81.37 <sup>cd</sup>
GFB5	3.88 <sup>e</sup>	26.27 <sup>b</sup>	0.89 <sup>a</sup>	1.99 <sup>de</sup>	3.23 <sup>c</sup>	2.90 <sup>d</sup>	67.95 <sup>b</sup>	-0.25 <sup>bcd</sup>	15.97 <sup>de</sup>	15.97 <sup>c</sup>	-89.10 <sup>a</sup>
GFB6	2.89 <sup>c</sup>	41.66 <sup>i</sup>	0.97 <sup>g</sup>	1.59 <sup>ab</sup>	2.84 <sup>b</sup>	2.70 <sup>c</sup>	72.77 <sup>f</sup>	-2.74 <sup>a</sup>	17.17 <sup>e</sup>	17.39 <sup>d</sup>	-80.93 <sup>cde</sup>
GFB7	3.14 <sup>cd</sup>	33.60 <sup>g</sup>	0.94 <sup>d</sup>	1.79 <sup>bc</sup>	2.72 <sup>ab</sup>	2.41 <sup>ab</sup>	69.21 <sup>bc</sup>	-2.44 <sup>a</sup>	13.97 <sup>b</sup>	14.18 <sup>b</sup>	-80.09 <sup>ef</sup>
GFB8	4.77 <sup>f</sup>	21.10 <sup>a</sup>	0.92 <sup>c</sup>	2.58 <sup>e</sup>	3.49 <sup>c</sup>	3.19 <sup>e</sup>	83.83 <sup>h</sup>	-2.21 <sup>a</sup>	11.92 <sup>a</sup>	12.13 <sup>a</sup>	-79.44 <sup>f</sup>
GFB9	2.31 <sup>b</sup>	31.33 <sup>e</sup>	0.96 <sup>f</sup>	3.48 <sup>f</sup>	3.86 <sup>d</sup>	3.35 <sup>e</sup>	80.20 <sup>g</sup>	-2.28 <sup>a</sup>	15.86 <sup>de</sup>	16.02 <sup>cd</sup>	-81.82 <sup>c</sup>
GFB10	3.70 <sup>e</sup>	36.13 <sup>h</sup>	0.97 <sup>g</sup>	2.09 <sup>d</sup>	3.25 <sup>c</sup>	2.78 <sup>cd</sup>	71.13 <sup>de</sup>	-1.99 <sup>a</sup>	14.09 <sup>bc</sup>	14.23 <sup>b</sup>	-81.99 <sup>bc</sup>
GFB11	3.47 <sup>de</sup>	42.03 <sup>j</sup>	0.97 <sup>g</sup>	1.90 <sup>cd</sup>	3.24 <sup>c</sup>	2.72 <sup>cd</sup>	70.37 <sup>cd</sup>	-1.90 <sup>ab</sup>	15.44 <sup>cd</sup>	15.55 <sup>bc</sup>	-83.00 <sup>b</sup>
<i>p</i> -value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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For each parameter values followed by the same letter are not significantly different at  $p \leq 0.05$ .

WHC: water holding capacity (ml/g); WBC: water binding capacity (g water/g solid).

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**Table 3.** Analysis of crumb microstructure and texture.

Sample	Number of alveoli/cm <sup>2</sup>	Total area alveoli	Hardness	Springiness	Chewiness	Cohesiveness	Resilience
codes		mm <sup>2</sup> /cm <sup>2</sup>	N		N		
GFB1	4 ab	9.07 a	20.50 e	0.95 de	5.77 d	0.29 b	0.11 abc
GFB2	6 ab	7.53 a	80.20 g	0.95 de	32.90 g	0.43 c	0.17 d
GFB3	6 ab	36.70 b	14.53 c	0.85 bc	3.53 abc	0.29 b	0.09 ab
GFB4	6 ab	24.26 ab	14.60 cd	0.90 cd	4.83 cd	0.37 c	0.13 bcd
GFB5	2 a	2.50 a	11.27 abc	0.76 a	2.33 ab	0.24 ab	0.84 ab
GFB6	16 c	130.03 c	11.47 abc	0.88 c	4.04 bcd	0.37 c	0.15 cd
GFB7	2 a	8.80 a	10.83 ab	0.79 ab	1.69 a	0.20 a	0.06 a
GFB8	5 ab	18.70 ab	18.23 de	1.00 f	14.94 e	0.82 d	0.39 e
GFB9	4 <sup>ab</sup>	23.50 ab	32.77 f	0.96 de	24.07 f	0.77 d	0.40 e
GFB10	7 b	21.33 ab	12.57 bc	0.95 de	3.74 abcd	0.38 c	0.15 cd
GFB11	6 ab	3.17 a	8.47 a	0.87 c	3.60 abc	0.44 c	0.18 d
<i>p</i> -value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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For each parameter values followed by the same are not significantly different at  $p \leq 0.05$ .

532 **Table 4.** Sensory analysis of different gluten-free bread like products.  
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Sample codes	Crumb appearance		Taste	Odour		Color	Aftertaste	Springiness	Hardness	Crumblines		
GFB1	2.57	bc	2.71	2.28	bc	3.43	3.14	1.86	a	3.14	2.29	ab
GFB2	2.83	abc	2.33	2.67	c	3.00	2.67	3.33	bc	2.67	3.00	bc
GFB3	2.33	ab	2.66	3.00	c	3.00	3.33	2.50	ab	3.33	2.50	ab
GFB4	2.83	bcd	1.33	2.00	abc	3.50	2.50	4.83	d	4.17	1.33	a
GFB5	3.33	bcd	3.00	2.16	abc	3.83	2.67	2.67	ab	3.67	3.83	c
GFB6	4.00	d	2.66	2.83	c	3.83	3.83	4.17	cd	3.67	2.50	ab
GFB7	3.50	bcd	2.83	3.00	c	3.50	3.33	3.33	bc	3.16	2.33	ab
GFB8	3.16	bcd	2.66	2.66	c	3.17	3.50	4.33	cd	3.33	2.00	ab
GFB9	1.16	a	2.16	1.16	a	1.83	2.67	2.17	ab	2.16	2.83	bc
GFB10	3.50	bcd	1.83	1.50	ab	2.83	1.83	1.33	a	2.17	2.33	ab
GFB11	3.67	cd	2.50	2.66	c	3.67	3.83	4.17	cd	3.83	2.33	ab
p-value	0.01		0.24	0.030		0.078	0.101	0.000		0.130	0.033	

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 535 For each parameter values followed by the same are not significantly different at  $p \leq 0.05$ .

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**Table 5.** Correlation matrix (correlation coefficients and *p*-value) between characterizing parameters of gluten-free bread like products.

	Specific volume	<i>L</i> *	<i>a</i> *	<i>b</i> *	Chroma	Tono °	Hardness	Springiness	Cohesiveness	Chewiness	Resilience	Moisture content	Swelling	WHC	WBC
<i>Instrumental parameters</i>															
<i>b</i> *	-0.6049***		0.6375***												
Chroma	-0.6049***		0.6232***	0.9998***											
Tono °	-0.6049***		0.8082***	0.7737***	0.7688***										
Hardness			0.4333***	0.5434***	0.5413***	0.6235***									
Springiness		0.4659***	-0.2515*				0.3569**								
Cohesiveness		0.8650***	-0.2829*					0.6643***							
Chewiness				0.4103***	0.4111***	0.4364***	0.9043***	0.5273***	0.6002***						
Resilience		0.858***	-0.3076*					0.6197***	0.9895***	0.6034***					
Moisture content	-0.3628**		-0.296*	0.2846*	0.2934*				-0.2707*		-0.2579*				
Aw	-0.2781*		-0.2823*	0.2417*	0.2511*			0.2859*				0.7431***			
Total area			-0.3173**									0.4118***			
Swelling		0.5210***	-0.4993***	-0.3849**	-0.3801**	-0.5864***	-0.4517***		0.5613***		0.6195***				
WHC		0.6186***	-0.3422**			-0.4446***			0.6604***	0.2442*	0.7020***		0.8146***		
WBC		0.7083***	-0.2905*			-0.3943***			0.7633***	0.3017*	0.7901***		0.8014***	0.9323***	
<i>Sensory parameters:</i>															
Appearance										-0.3184**					
Odour													-0.3086*	-0.3321**	-0.3098*
Colour		-0.2662*							-0.2860*	-0.2909*				-0.2493*	
Springiness	0.2829*	0.4659***													
Crumblieness				0.3047*	0.3034*										

p≤0.05 \*; p≤0.01\*\*; p≤0.001\*\*\*

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