

1 **Impact of legume flours on quality and *in vitro* digestibility of starch and protein**  
2 **from gluten-free cakes**

3

4 **1) Short running head:** Enriched gluten-free cakes

5

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23

24

25 **ABSTRACT**

26 The aim of the study was to investigate the impact of incorporation of different legumes  
27 (chickpea, pea, lentil and bean) on quality, chemical composition and *in vitro* protein  
28 and starch digestibility of gluten-free layer cake (rice flour: legume flour, 50:50). The  
29 incorporation of legume flours increased the batter viscosity and, with exception of  
30 chickpea, resulted in higher specific cake volume than that in control. Chickpea and pea  
31 containing cakes had the brightest and the most yellowish crust. The legumes  
32 significantly increased the hardness and chewiness in the cakes, except with addition of  
33 lentil. Enriched cakes had higher total protein, available proteins, minerals, fat, as well  
34 as fiber content with except in the case of chickpeas. Legumes significantly affect the  
35 *in vitro* hydrolysis of starch fractions, decreasing the rapidly digestible starches yielding  
36 a reduction in the eGI, except chickpea containing samples. Overall, considering  
37 physicochemical properties and nutritional quality, lentil flour incorporation resulting  
38 in the best gluten free cakes.

39

40 **Key words:** gluten-free; cake; legume; quality; nutrition; starch hydrolysis.

41

42

## 43 1. INTRODUCTION

44 The increase in the incidence of celiac disease has prompted, a rapid growth and  
45 availability of gluten-free products on the market (Cureton & Fasano, 2009). According  
46 to these authors, an annual growth of 25% in the sale of gluten-free products is  
47 projected over the next 4 years. There are a wide variety of gluten-free cereal foods and  
48 cakes are an option within the baked goods. During the last decade numerous gluten  
49 free recipes for bakery products have been proposed, which were selected or accepted  
50 based on the instrumental and sensory qualities of the resulting products. For instance,  
51 using different starch sources (rice, corn, potato and wheat) and protein types (soy  
52 protein isolate, wheat protein) at different percentages (0%, 10%, 20%) was possible to  
53 obtain cakes with adequate volume, although the best quality was obtained with the  
54 blend starch and protein from wheat (Ronda et al., 2010), However, questions have been  
55 raised about the nutritional quality of these foods, which differ considerably with their  
56 gluten counterparts (Thompson, 2000, 2009). Those divergences have prompted the  
57 development of alternative gluten free baked goods in which the nutritional quality  
58 acquired special relevance (Marco & Rosell, 2008).

59 Rice is one of the most frequently used cereals in gluten-free food products due to its  
60 low levels of sodium, protein, fat, fiber and high amount of easily carbohydrates (Rosell  
61 & Gómez, 2006). The enrichment of gluten-free baked products has proved to be  
62 necessary in gluten-free adherence diets, since consumers have generally a low intake of  
63 nutrients that has been associated to gluten-free diet (Lazaridou et al., 2007; Turabi et  
64 al., 2008; Marco & Rosell, 2008). Therefore, it was recommended the nutritional  
65 adequacy of various gluten-free foods and encouraged the consumption of whole-grain  
66 or enriched products (Thompson, 2009). Gluten free baked goods are mainly based on  
67 starchy compounds, and in consequence starch digestibility could be an important

68 parameter when determining the nutritional quality of those products. In healthy  
69 individuals the polysaccharides absorption determines the appearance of nutrients in  
70 blood during the inter-digestive period (Goñi et al., 2002). Thus, it has been  
71 demonstrated that a disruption of the botanical structure in rice, legumes, or kernel-  
72 based bread products, increases the availability of starch for enzymatic digestion, and  
73 hence the glycemic response (Dartois et al., 2010, Granfeldt et al., 1994).

74 Balandrán-Quintana et al. (1998) reported that legumes are important constituents of the  
75 diet providing proteins and also soluble fiber. The enrichment of bakery products with  
76 legumes might be an alternative for improving the nutritional value of gluten free  
77 bakery products; besides legumes could lead to food products with modified physical  
78 characteristics, like texture and mouthfeel (Gómez et al., 2008). In fact, chickpea flour  
79 has been proposed for total or partial (50%) replacement of wheat flour in layer or  
80 sponge cakes making (Gómez et al., 2008), although volume, symmetry and color of the  
81 cakes decreased when increasing the amount of chickpea flour. However, no attempts  
82 have been carried out for obtaining enriched gluten free cakes. Cake quality depends on  
83 the batter viscosity and stability for obtaining a sponged structure that do not collapse  
84 and the batter properties are related to starch, pentosan and protein characteristics of  
85 flour (Oliete et al., 2010).

86 To the best of our knowledge, there is limited research regarding the effect of legumes  
87 addition on the nutritional and instrumental quality of gluten free cakes. The aim of this  
88 study was to investigate the possible impact of different legumes (chickpea, pea, lentil,  
89 bean) on quality properties, chemical composition, *in vitro* digestibility of protein and *in*  
90 *vitro* enzymatic hydrolysis of starch in gluten-free layer cake.

91

## 92 2. MATERIALS AND METHODS

## 93 2.1 Materials

94 Commercial rice flour was supplied by Harinera los Pisones (Zamora, Spain). Flours  
95 from chickpeas, peas, lentils and beans were also provided by Harinera los Pisones  
96 (Zamora, Spain). Flours had particle size lower than 210  $\mu\text{m}$ . Protein and moisture  
97 content of rice, chickpea, pea, lentil and bean were 6 g/100 g, 23 g/100 g, 22 g/100 g, 22  
98 g/100 g, 22 g/100 g and 11.5 g/100 g, 10.3 g/100 g, 10.9 g/100 g, 11.6 g/100 g, 11.1  
99 g/100 g, respectively. Sugar, sunflower oil, fresh whole eggs, fresh milk and double-  
100 action baking powder were purchased from the local market.

101  $\alpha$ -Amylase from porcine pancreas (Pancreatin Cat. No. P-1625, activity 3\_USP/g) and  
102 trypsin from porcine pancreas type IX (activity 13000 – 20000 BAEE units/mg protein)  
103 were purchased from Sigma Chemical Company (St. Louis, MO, USA).  
104 Amyloglucosidase (EC 3.2.1.3., 3300 U/mL) and glucose oxidase–peroxidase assay kit  
105 GOPOD (Cat. No. K-GLUC) were purchased from Megazyme (Megazyme  
106 International Ireland Ltd., Bray, Ireland).

107

## 108 2.2 Methods

### 109 2.2.1 Cake making

110 A single-bowl mixing procedure using a yellow layer cake recipe was used. Basic recipe  
111 contained 350g rice flour, 350g legumes flour, 420g fresh milk, 350g pasteurized whole  
112 eggs, 210g sunflower oil, 630g sucrose and 21g baking powder. A cake batter  
113 containing 700g rice flour was used as control. All ingredients were mixed during 1 min  
114 at speed 4 and 9 min at speed 6 using a Kitchen-Aid Professional mixer – KPM5  
115 (KitchenAid, St. Joseph, MI, USA). Cake batter (180 g) was placed into rectangular  
116 (109 mm x 159 mm, capacity 430 ml) metallic, lard coated pan and baked in an electric  
117 oven for 30 min at 190 °C. After baking, the cakes were removed from the pans, kept at

118 room temperature for one hour to cool down, and packed in polyethylene bags to  
119 prevent drying. Seven cakes were obtained from each batter. Three different sets for  
120 each cake recipe were made in different days.

121

### 122 2.2.2 Batter measurements

123 Batter pH was measured using a pH-Tester30 (Eutech Instruments, Nijkerk,  
124 Netherland). Batter density was measured using a density cup Elcometer 1800  
125 (Elcometer, Manchester, UK), and determined as the ratio of the weight of a standard  
126 container filled with batter to that of the same container filled with water (density, 1  
127 g/cm<sup>3</sup>) and calibrated at 20 °C.

128 Batter samples were imaged using a DM750 microscope (Leica Microsystems, Wetzlar,  
129 Alemania) with a magnification of 40x. An EC3 (Leica Microsystems, Wetzlar,  
130 Alemania) linked video camera provided images using LAS EZ software (Leica  
131 Microsystems, Wetzlar, Alemania).

132 Viscosity of batter was measured using a Rapid Viscoanalyser (RVA) (Newport  
133 Scientific model 4-SA, Warriewood, Australia). Batter sample (28 g) was placed in the  
134 RVA aluminum canister to register viscosity changes. The experimental settings were  
135 sample equilibration at 30 °C for one min, heating to 50 °C for 3.5 min, and holding at  
136 50 °C for 5 min. Paddle speed was 960 rpm for first 10 s and then set at 160 rpm.  
137 Parameters recorded included viscosity at 30°C and 50 °C determined at the end of the  
138 corresponding stage, expressed in cP units (1 cP=1 mPa s<sup>-1</sup>). The reported values are  
139 means of duplicate measurements.

140

### 141 2.2.3 Cake quality assessment

142 Cake characterization was carried out 24 hours after baking. The digital caliper was  
143 used to measure collapses by determining the difference in height of the cakes after  
144 being removed from the oven and one hour later. Cake volume was determined using a  
145 laser sensor with the volume analyzer BVM-L 370 (TexVol Instruments, Viken,  
146 Sweden). Cake density was calculated by the ratio between the weight of the cake and  
147 its volume, and specific volume was evaluated by the ratio between the cake volume  
148 and its weight.

149 Colour was measured using a Minolta spectrophotometer CN-508i (Minolta, Co. LTD,  
150 Osaka, Japan). Results were expressed in the CIE  $L^*a^*b^*$  colour space and were  
151 obtained using the D65 standard illuminant. Colour was measured at four different  
152 points on the flour and the crumb or crust of each cake. Crumb texture was determined  
153 by a TA-XT2 texture analyzer (Stable Microsystems, Surrey, UK) provided with the  
154 software "*Texture Expert*". An aluminum cylindrical probe (25 mm diameter) was used  
155 in a double compression test (Texture Profile Analysis, TPA) with a compression up to  
156 50% depth, at 2 mm/s speed test, and 30 s delay between first and second compression.  
157 Hardness ( $N$ ), springiness, cohesiveness, chewiness ( $N$ ) and resilience were calculated  
158 from the TPA graph (Gómez et al., 2007). Average results of four determinations (2  
159 slices from the central part) are presented. Pictures (300 dots per inch) of both sides of  
160 the cake were taken with a digital camera (Panasonic DMC-LZ7, Osaka, Japan). Images  
161 of the slices were captured using a flatbed scanner (HP scanjet 4370, Hewlett-Packard,  
162 Palo Alto, CA, USA).

163

#### 164 2.2.4 Nutritional measurements

165 Nutritional parameters of gluten-free layer cake were determined following [AACC](#)  
166 [methods \(2000\)](#) and they include: moisture (method 44-15A), ash (method 08-01),

167 crude fat (method 30-25) and crude protein (method 46-13) using  $N \times 6.25$ . Available  
168 protein was calculated by percentage of protein digestibility of crude protein. The  
169 available carbohydrate content of the samples was calculated by difference, subtracting  
170 100 g minus the sum of grams of moisture, protein, fat, ash and dietary fiber. The  
171 components were converted to food energy using conversion factors (4.0 kcal  $g^{-1}$  for  
172 proteins and available carbohydrates; 9.0 kcal  $g^{-1}$  for fats and 2.0 kcal  $g^{-1}$  for dietary  
173 fiber) (FAO, 2003). The *in vitro* protein digestibility of the samples was determined by  
174 the modified methods of Hsu et al. (1977) and Bilgiçli et al. (2007). Briefly, 50 ml of  
175 aqueous protein suspension having 6.25 mg protein/ml was prepared. Then, samples  
176 were placed in a 37 °C water bath and the pH was adjusted to 8.00 using 0.1 N NaOH  
177 and/or 0.1 N HCl, while stirring. Trypsin at a concentration of 1.6 mg/ml was  
178 maintained in an ice bath and the pH was adjusted to 8.00 with 0.1 N NaOH and/or 0.1  
179 N HCl. Five milliliters of enzyme solution were then added to the protein suspension,  
180 which was kept stirred at 37 °C. The trypsin had an activity of 13,766 BAEE units/mg  
181 proteins. The pH drop was recorded 15 s after enzyme addition and at one minute  
182 intervals for 10 min. The enzyme solution was always freshly prepared before each  
183 series of experiments. The percent protein digestibility ( $Y$ ) was calculated by using Eq.  
184 (1) (Hsu et al., 1977):

$$185 \quad Y = 210.464 - 18.1x \quad (1)$$

186 Where  $x$  is the change in pH after 10 min.

187 For the estimation of dietary fiber, the defatted residues of cake samples obtained during  
188 the course of analysis of crude fat were finally powdered to pass through a sieve of 250  
189  $\mu\text{m}$ . This fine powder of each sample was utilized for the estimation of soluble dietary  
190 fiber (SDF), insoluble dietary fiber (IDF) and total dietary fiber (TDF) contents  
191 following the method 37-02 (AACC, 2000).

192

### 193 2.2.5 *In vitro* starch digestibility and expected glycaemic index

194 Starch digestibility of cakes was determined in the freeze dried samples. Powdered  
195 sample (100 mg) was incubated with porcine pancreatic  $\alpha$ -amylase (10 mg) and  
196 amyloglucosidase (3.3 U/ml) in 4 ml of 0.1 M sodium maleate buffer (pH 6.0) in a  
197 shaking water bath at 37 °C (0.5-16 h). After incubation, ethanol (96%) was added and  
198 the sample was centrifuged and the pellet washed with 50% ethanol. The glucose  
199 content of supernatant was measured using a glucose oxidase-peroxidase (GOPOD) kit  
200 and calculated as glucose (mg) x 0.9.

201 Rapidly digestible starch (RDS) as the starch that was hydrolyzed within 30 min of  
202 incubation, slowly digestible starch (SDS) as the starch digested was expressed as the  
203 total starch hydrolyzed at different times (30, 60, 90 and 120 min) were determined as  
204 suggested by [Englyst et al. \(1996\)](#). The *in vitro* digestion kinetics was calculated in  
205 accordance with the procedure established by [Goñi et al. \(1997\)](#). A nonlinear model  
206 following the equation  $[C = C_{\infty}(1 - e^{-kt})]$  was applied to describe the kinetics of starch  
207 hydrolysis, where  $C_{\infty}$ , and  $k$  were, respectively, equilibrium concentration and kinetic  
208 constant. Using the hydrolysis curve (0–180 min), hydrolysis index (HI) was obtained  
209 by dividing the area under the hydrolysis curve of the sample by the area of standard  
210 material obtained for white bread. The expected glycemic index (eGI) was calculated  
211 using the equation described by [Grandfeldt et al. \(1992\)](#):  $eGI = 8.198 + 0.862HI$ .

212

### 213 2.2.6 Statistical analysis

214 Experimental data were statistically analyzed by using Statgraphics V.7.1 program  
215 (Bitstream, Cambridge, MN, USA) to determine significant differences among them.  
216 When ANOVA indicated significant P-values, multiple sample comparison was also

217 performed and Fisher's least significant difference (LSD) procedure was used to  
218 discriminate among the means, and correlation matrix was carried out by the Pearson-  
219 product moment to significant  $p<0.05$ .

220

### 221 **3. RESULTS AND DISCUSSION**

#### 222 3.1 Effect of legumes on batter properties

223 The partial (50%) replacement of rice flour by different legume flours was proposed for  
224 the nutritional enrichment of gluten free layer cakes. The level of legume flour  
225 incorporated was selected based on previous results obtained with the addition of  
226 chickpea flour to wheat flour for obtaining gluten layer and sponge cakes (Gómez et al.,  
227 2008). Batter properties of gluten-free layer cakes containing different legumes are  
228 shown in [Table 1](#). The pH values were comprised within the range 7.1 and 7.5, and only  
229 the pea containing batter showed a significant reduction in the pH compared to the  
230 control. Legumes affected in different extent the batter viscosity and the effect was  
231 dependent on the legume origin. All the legumes induced a significant ( $p<0.05$ ) increase  
232 of the batter viscosity at 30°C and 50°C, with the exception of pea flour that did not  
233 modify the viscosity at 50°C. The incorporation of bean flour gave the highest viscosity  
234 at 30 and 50°C. In all cases, batter viscosity decreased with the temperature increase,  
235 being at 50 °C around half of that at 30 °C. Likely, this decrease was related to the  
236 partial protein unfolding and aggregation ([Lazaridou & Biliaderis, 2009](#)). A significant  
237 positive correlation was observed between batter viscosity at 30°C and 50°C ( $p=0.012$ ,  $r$   
238  $=0.999$ ). The batter density decreased significantly ( $p<0.05$ ) in the presence of lentil and  
239 bean, suggesting that more air was incorporated in the structure. That observation was  
240 corroborated with the micrographs of the batters ([Figure 1A](#)). Lentil and bean batters  
241 showed an uneven structure with larger number of gas cells. No significant differences

242 ( $p < 0.05$ ) were detected in the case of pea and chickpea flours. Gómez et al. (2008)  
243 found that incorporation of 50% chickpea decreased batter density, but authors also  
244 pointed out the significant variation induced by the chickpea cultivar. In general, low  
245 batter density has been related to high air quantity into the batter and therefore high  
246 cake volume (Gomez et al., 2007).

247

### 248 3.2 Effect of legume flours on gluten free layer cake quality properties

249 [Table 2](#) showed that the incorporation of legume flours did not significantly affected the  
250 weight loss and collapse parameters, with exception of the increase in collapse observed  
251 when added pea flour. Specific volume showed the same trend in the presence of  
252 legume flours. Regardless chickpea cake, all legume increased specific volume, and the  
253 highest effect was observed with lentil. The volume of cake is partly consequence of the  
254 air incorporated in the batter, which agrees with described results on batter density.  
255 During heating starch gelatinization, protein denaturation and air bubbles expansion  
256 occur, and cake structure depends on the harmonization of these processes (Yang &  
257 Foegeding, 2010). The result observed with chickpeas agrees with findings of Gómez et  
258 al. (2008) when studied chickpea-wheat cake.

259 The effect of legumes on the colour properties of gluten-free layer cake is shown in  
260 [Table 3](#) and [Figures 1B and 1C](#). Legumes affected in different extent the crust and  
261 crumb colour properties of gluten-free cakes and the effect was highly dependent on the  
262 legume nature. The  $L^*$  of crust was significantly ( $p < 0.05$ ) increased by chickpea, pea  
263 and bean. All legumes increased the reddish value ( $a^*$ ) of the cake crust. Chickpea and  
264 pea showed the brightest and the most brownish crust compared to the other legumes.  
265 Protein composition of the legume flours was rather similar, thus differences in the  
266 browning colour might be ascribed to the sugar content of those flours, since crust

267 colour depends on Maillard and caramelisation reactions that occur on the cake surface  
268 (Oliete et al., 2010). Lightness of the cake crumb was significantly decreased by lentil  
269 and bean flours. The hue red ( $a^*$ ) and yellow ( $b^*$ ) were significantly increased by the  
270 legumes addition, with the exception of lentil that did not affect  $a^*$  and bean flour that  
271 decreased  $b^*$ . Colour results were directly related to the original legume flours colour.  
272 The effect of legumes on the texture properties of gluten-free layer cake is shown in  
273 Table 4. The addition of legumes induced a significant ( $p<0.05$ ) increase of the hardness  
274 and chewiness of the gluten-free layer cake, except with addition of lentil that showed  
275 the same pattern of control cake. The crumb hardness was negatively correlated with  
276 specific volume ( $r = -0.997, p<0.05$ ), which agree with results of Gómez et al (2010).  
277 The cohesiveness increased in all legume containing cakes likely due to the increased  
278 content in proteins, and this parameter showed positive correlation with specific volume  
279 ( $r = 0.999, p=0.006$ ). Springiness has been associated to fresh and aerated product, and  
280 in the case of muffins high springiness values are linked to quality (Sanz et al., 2009). A  
281 significant ( $p<0.05$ ) increase in the springiness was induced in the presence of lentil  
282 and bean, but no relationship was observed between the springiness and the specific  
283 volume. Cohesiveness, related to energy required for the second compression and thus  
284 for chewing a food, increased in all the legume containing cakes compared to the  
285 control, which indicated lower crumbliness. A positive correlation was also observed  
286 between crumb cohesiveness and specific volume ( $r = 0.999, p = 0.006$ ).

287

### 288 3.3 Effect of legumes flour on gluten free layer cake on nutritional properties

289 Macronutrients evaluation of cakes included the estimation of proteins (total, available  
290 and digestible), fat, dietary fiber, available carbohydrates, minerals and energy provided  
291 (Table 5). Compared to control cakes, legume containing gluten free cakes were richer

292 in total proteins, minerals (ash) and fat, in detriment of the available carbohydrates.  
293 [Utrilla-Coello et al. \(2007\)](#) also reported an increase of proteins, fat and minerals when  
294 wheat bread was enriched with chickpea flour (wheat flour:chickpea flour, 60:40) due to  
295 the high protein content of legumes. No significant differences were observed in the  
296 moisture content of the cakes (24.5- 26.2 g/100 g) (results not showed). Regarding cake  
297 performance, a negative correlation between total proteins and batter density was  
298 observed ( $r = - 0.999, p < 0.05$ ). [Schober \(2009\)](#) reported a negative effect of the proteins  
299 due to their interference with the starch gel, forming points of weakness, where the  
300 crumb rupture is induced under the gas pressure and collapses. In addition, a positive  
301 correlation was also observed between the fat content and the specific volume of the  
302 cakes ( $r = 0.999, p < 0.05$ ). This effect might be related to the fat contribution to the  
303 interface stabilization that favors gas entrapment in the batter matrix.

304 Incorporation of legume flours led to protein enriched cakes that contained significantly  
305 ( $p < 0.05$ ) higher amount of available protein, which showed high *in vitro* digestibility,  
306 with exception of bean containing cake ([Table 5, Figure 2](#)). A rapid decline in pH,  
307 caused by the release of the carboxyl groups during enzymatic digestion of the protein  
308 ([Hsu et al., 1977](#)), was observed in the lentil cake followed by chickpea and pea cakes,  
309 indicating an increase in the *in vitro* protein digestibility ([Figure 2](#)). The protein  
310 digestibility is indicative of its amino acids availability for evaluating the nutritive  
311 quality of a protein ([Hsu et al., 1977](#)). Variations observed in legume protein  
312 digestibility could be attributed to processing differences. Processing can improve the  
313 digestibility of proteins by destroying protease inhibitors and opening of the protein  
314 structure through denaturation ([Hsu et al., 1977](#)). In contrast, the decrease in the  
315 digestibility observed with bean maybe related to the low biological value of that type  
316 of proteins ([Baladrán-Quintana et al., 1998](#)).

317 In comparison to control sample, significant ( $p<0.05$ ) increase of dietary fiber content  
318 was achieved with the incorporation of lentil, and the fiber was mainly insoluble. The  
319 highest content of soluble dietary fiber was found in cake enriched with chickpeas. The  
320 SDF content showed positive correlation with batter viscosity at 30 and 50 °C,  
321 indicating the effect of soluble dietary fiber in the batter viscosity.

322

### 323 3.4 Effect of legumes on *in vitro* starch digestion

324 Starch hydrolysis was conducted by enzymatic assays, which allowed determining  
325 rapidly digestible starch (RDS) and slowly digestible starch (SDS) according to their  
326 rate and extent of *in vitro* digestion. The predominant fraction in gluten free cake was  
327 the RDS followed by SDS (Table 6). The swelling of the starch granules during  
328 gelatinization enhances the access of digestive enzyme inside the granules and thus  
329 increases the RDS content (Chung et al., 2008). Cakes added with pea and lentil showed  
330 lower content of RDS than the control cake, whereas chickpea increased that fraction of  
331 starch. Chickpea containing cake had the lowest content of SDS fraction. It has been  
332 reported that starch in legume flour is more sensitive to enzyme hydrolysis than the  
333 starch in boiled beans (Osorio-Díaz et al., 2008), due to the mechanical rupture of the  
334 cell walls.

335 Primary and secondary parameters derived from the *in vitro* digestion of legume  
336 containing cakes are listed in Table 6. Those parameters included equilibrium  
337 concentration of hydrolyzed starch ( $C_{\infty}$ ), kinetic constant ( $k$ ), total starch hydrolysis at  
338 90 min ( $H_{90}$ ), area under the hydrolysis curve after 180 minutes (AUC 180), hydrolysis  
339 index (HI) and estimated glycemic index (eGI). The kinetic constant ( $k$ ), which reflected  
340 the rate of starch hydrolysis, was significantly augmented in the presence of chickpea.  
341 The high hydrolysis rate agrees with the high value of RDS fraction in detriment of the

342 SDS fraction. [Goñi et al. \(2002\)](#) reported that the nature of polysaccharide determines  
343 its physico-chemical behaviour and this may affect the rate of digestion of  
344 carbohydrates and absorption of sugars in the small intestine. It has been reported that  
345 starch hydrolysis in bread and pasta enriched with chickpea flour showed lower  
346 hydrolysis rate ([Osorio-Díaz et al., 2008](#); [Utrilla-Coello et al., 2007](#)), likely the complex  
347 formulation of the gluten free cakes with egg and milk proteins, sugar and fats affected  
348 the physico-chemical behaviour of legume starches. The result of  $C_{\infty}$  parameter showed  
349 that the pea and lentil added cakes had the lowest values, indicating low equilibrium  
350 concentration of starch.

351 A comprehensive parameter for the starch digestibility is the total area under the  
352 hydrolysis curve [AUC ( $\text{mg}_{\text{glucose}}/\text{g}_{\text{sample}}$ ) x min] relating the glucose release over a  
353 hydrolysis period of 180 min ([Goñi et al., 1997](#)). The type of legume had a significant  
354 effect on the AUC 180 min values ( $p < 0.05$ ), being the value for chickpea higher than  
355 that of control, whereas the other legumes decreased it ([Figure 3](#)). The addition of pea,  
356 lentil and bean in the cakes recipe resulted in lower starch hydrolysis, and in  
357 consequence, lower glucose liberation under *in vitro* conditions took place, thus  
358 probably the intake of these cakes slows down the gastric empty and reduces the rate of  
359 intestine absorption of glucose.

360 Regarding the hydrolysis index, all legumes significantly decreased the HI compared to  
361 control cake, with the exception of chickpea. In consequence, cakes with peas, lentil and  
362 bean will be potentially beneficial for dietary management to control diabetes, obesity,  
363 and hyperlipidemia and the glycemic index. [Hoover & Zhou \(2003\)](#) reported that  
364 legumes reduce glycaemic and insulinemic postprandial responses. [Chung et al. \(2006\)](#)  
365 reported that legume starch and rice variants produce lower GI and lower metabolic

366 responses values than common cereal and tuber starches, likely due to starch  
367 retrogradation that increases the resistance to digestive enzymes.

368 Regardless chickpea, legume containing cakes showed lower estimated glycaemic index  
369 than the control cake. The incorporation of pea flour resulted in 18% reduction of eGI,  
370 followed by lentil and bean. Low-GI diets have been reported to effectively control the  
371 risk to suffer cardiovascular diseases (Chung et al., 2008, Roberts, 2000). Roberts  
372 (2000) stated that in general, high-GI foods are those with high carbohydrate content  
373 that is rapidly digested.

374

#### 375 **4. Conclusion**

376 The present study confirmed that legume flours are useful for protein and fiber  
377 enrichment of bakery foodstuff, namely gluten free cakes. Although the effect was  
378 dependent on the legume origin, in all cases nutritional improvement of those products  
379 could be obtained without a significant detriment of the quality. In fact, the addition of  
380 legume flours led to cakes with increased specific volume. Legumes significantly  
381 enhanced the protein content and its availability, moreover in the case of pea and lentil  
382 containing cakes, the *in vitro* starch hydrolysis was somewhat modified yielding a  
383 reduction of the eGI. In particular, among the legume flours tested, the incorporation of  
384 lentil flour to gluten free cakes recipe led to low density batters, besides lentil enriched  
385 cakes showed the highest specific volume, similar crumb hardness and higher  
386 springiness than the control cake. Regarding the nutritional quality, lentil flour yielded  
387 cakes with high protein content which had increased digestibility; moreover cakes  
388 showed high total dietary fiber content, low starch hydrolysis rate and lower estimated  
389 glycaemic index compared to the control cake. Overall, considering the

390 physicochemical and nutritional quality, lentil flour would be the recommended legume  
391 for enriching gluten free cakes.

392 Future studies will be undertaken for determining the sensory quality and consumer  
393 acceptance of enriched gluten free cakes by organizing a consumer test with coeliac  
394 patients.

395

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400

### 401 **References**

402 AACC International (2000). Approved Methods of the American Association of Cereal  
403 Chemists. St Paul, Mn (USA): American Association of Cereal Chemists.

404 Balandrán-Quintana R R, Barbosa-Cánovas G V, Zazueta-Morales J J, Anzaldúa-  
405 Morales A & Quintero-Ramos A (1998) Functional and nutritional properties of  
406 extruded whole pinto bean meal (*Phaseolus Vulgaris* L.). Journal of Food Science,  
407 63, 113-116.

408 Bilgiçli N, Ibanoglu S & Herken E N (2007) Effect of dietary fibre addition on the  
409 selected nutritional properties of cookies. Journal of Food Engineering, 78, 86-89.

410 Chung H-J, Lim H S & Lim S-T (2006) Effect of partial gelatinization and  
411 retrogradation on the enzymatic digestion of waxy rice starch. Journal of  
412 Cereal Science, 43, 353-359.

413 Chung H, Shin D & Lim S (2008) In vitro starch digestibility and estimated glycemic  
414 index of chemically modified corn starches. Food Research International, 41, 579-  
415 585.

416 Chung H, Liu Q, Pauls K P, Fan M Z & Yada R (2008) In vitro starch digestibility,  
417 expected glycemic index and some physicochemical properties of starch and flour  
418 from common bean (*Phaseolus vulgaris L.*) varieties grown in Canada. Food  
419 Research International, 41, 869-875.

420 Cureton P & Fasano A (2009). The increasing incidence of celiac disease and the range  
421 of gluten-free products in the marketplace. In: Gluten-free Food Science and  
422 Technology, Gallagher, E. eds. Wiley-Blackwell: USA.

423 Dartois A, Singh J, Kaur L & Singh H (2010) Influence of guar gum on the in vitro  
424 starch digestibility – reological and microstructural characteristics. Food Biophysics,  
425 5, 149-160.

426 Englyst H N, Veenstra J & Hudson GJ (1996) Measurement of rapidly available glucose  
427 (RAG) in plant foods: a potential in vitro predictor of the glycaemic response. British  
428 Journal of Nutrition, 75, 327-337.

429 FAO Food and Nutrition, Paper 77 (2003). Food energy – methods of analysis and  
430 conversion factors, Food and Agriculture Organization of the United Nation, Rome.

431 Gómez M, Oliete B, Rosell C M, Pando V & Fernández E (2008) Studies on cake  
432 quality made of wheat – chickpea flour blends. Food Science and Technology, 41,  
433 1701-1709.

434 Gómez M, Ronda F, Caballero PA, Blanco C A & Rosell C M (2007) Functionality of  
435 different hydrocolloids on the quality and shelf-life of yellow layer cakes. Food  
436 Hydrocolloids, 21, 167-173.

437 Gómez M, Ruiz-Paris E, Oliete B & Pando V (2010) Modelling of texture evolution of  
438 cakes during storage. *Journal of Texture Studies*, 41, 17-33.

439 Gómez M, Manchón L, Oliete B, Ruiz E & Caballero P A (2010) Adequacy of  
440 wholegrain non-wheat flours for layer cake elaboration. *LWT - Food Science and*  
441 *Technology*, 43, 507–513. Goñi I, Garcia-Alonso A & Saura-Calixto F (1997). A  
442 starch hydrolysis procedure to estimate glycemic index. *Nutrition Research*, 17, 427-  
443 437.

444 Goñi I, Valdivieso L & Gudiel-Urbano M (2002) Capacity of edible seaweeds to  
445 modify in vitro starch digestibility of wheat bread. *Nahrung/Food*, 46 (1), 18-20.

446 Granfeldt Y, Björck I, Drews A & Tovar J (1992) An in vitro procedure based on  
447 chewing to predict metabolic responses to starch in cereal and legume products.  
448 *European Journal of Clinical Nutrition*, 46, 649–660.

449 Granfeldt Y, Liljeberg H, Drews A, Newman R & Björck I (1994) Glucose and insulin  
450 responses to barley products: influence of food structure and amylase-amylopectin  
451 ratio. *American Journal of Clinical Nutrition*, 59, 1075-1082.

452 Hoover R & Zhou Y (2003) In vitro and in vivo hydrolysis of legume starches by  $\alpha$ -  
453 amylase and resistant starch formation in legumes-a review. *Carbohydrate Polymers*,  
454 54, 401–417.

455 Hsu H W, Vavak D L, Satterlee L D & Miller G A (1977) A multienzyme technique for  
456 estimating protein digestibility. *Journal of Food Science*, 42, 1269-1273.

457 Lazaridou A & Biliaderis C B (2009) Gluten-free doughs: rheological properties, testing  
458 procedures – methods and potential problems. In: *Gluten-free Food Science and*  
459 *Technology*, Gallagher, E. eds. Wiley-Blackwell: USA.

460 Lazaridou A, Duta D, Papageorgiou M, Belc N & Biliaderis C G (2007). Effects of  
461 hydrocolloids on dough rheology and bread quality parameters in gluten-free  
462 formulations. *Journal of Food Engineering*, 79, 1033-1047.

463 Marco C & Rosell CM (2008) Breadmaking performance of protein enriched gluten free  
464 breads. *European Food Research and Technology*, 227, 1205-1213.

465 Oliete B, Pérez, G T, Gómez M, Ribotta P D, Moiraghi M & León A E (2010). Use of  
466 wheat, triticale and rye flours in layer cake production. *International Journal of Food*  
467 *Science & Technology*, 45, 697-706.

468 Osorio-Díaz P, Agama-Acevedo E, Mendoza-Vinalay M, Tovar J & Bello-Pérez LA  
469 (2008) Pasta added with chickpea flour: chemical composition, in vitro starch  
470 digestibility and predicted glycemic index. *Ciencia y Tecnología Alimentaria*, 6, 6-  
471 12.

472 Roberts S B (2000). High-glycemic index foods, hunger, and obesity: is there a  
473 connection? *Nutrition Reviews*, 58, 163-169.

474 Ronda F, Oliete B, Gómez M, Caballero P A & Pando V (2011) Rheological study of  
475 layer cake batters made with soybean protein isolate and different starch sources.  
476 *Journal of Food Engineering*, 102, 272–277

477 Rosell CM & Gómez M Rice. In: *Bakery products: Science and Technology*. Ed Y.H.  
478 Hui. 2006. Blackwell Publishing, Ames, Iowa. USA. Pp 123-133.

479 Sanz T, Salvador A, Baixauli R, Fiszman S M (2009) Evaluation of four types of  
480 resistant starch in muffins. II. Effects in texture, colour and consumer response.  
481 *European Food Research and Technology*, 229, 197-204.

482 Schober T (2009). Manufacture of gluten-free specialty breads and confectionery  
483 products. In: *Gluten-free Food Science and Technology*, Gallagher, E. eds. Wiley-  
484 Blackwell: USA.

485 Turabi E, Sumnu G & Sahin S (2008) Rheological properties and quality of rice cakes  
486 formulated with different gums and an emulsifier blend. *Food Hydrocolloids*, 22,  
487 305-312.

488 Thompson T (2000) Folate, iron and dietary fibre contents of the gluten-free diet.  
489 *Journal of the American Dietetic Association*, 1000, 1389-1396.

490 Thompson T (2009). The nutritional quality of gluten-free foods. In: *Gluten-free Food*  
491 *Science and Technology*, Gallagher, E. eds. Wiley-Blackwell: USA.

492 Utrilla-Coello RG, Osorio-Díaz P & Bello-Pérez LA (2007) Alternative use of chickpea  
493 flour in breadmaking: chemical composition and starch digestibility of bread. *Food*  
494 *Science Technology International*, 13, 323-327.

495 Yang X & Foegeding E A (2010). Effects of sucrose on egg white protein and whey  
496 protein isolate foams: Factors determining properties of wet and dry foams (cakes).  
497 *Food Hydrocolloids*, 24, 227-238.

498

499 **FIGURE CAPTIONS**

500 **Figure 1.** Effect of different legumes in gluten-free layer cakes. A: image microscopy  
501 of cake batters, B: cross section of gluten free layer cakes, and C: front picture of  
502 cakes.

503 **Figure 2.** *In vitro* digestibility of proteins in legume enriched gluten free cakes. Legend:  
504 control (●), chickpea (◆), pea (▲), lentil (■), bean (x).

505 **Figure 3.** *In vitro* digestibility of starch in legume enriched gluten free cakes Legend:  
506 control (●), chickpea (◆), pea (▲), lentil (■), bean (x).

507

508 **Table 1.** Effect of legumes on batter properties in gluten-free layer cakes<sup>a</sup>.

<b>Legumes</b>	<b>pH</b>	<b>Batter density (g/cm<sup>-3</sup>)</b>	<b>Viscosity at 30°C (cP)</b>	<b>Viscosity at 50 °C (cP)</b>
<b>Control</b>	7.5± 0.0 <sup>a</sup>	1.0± 0.0 <sup>a</sup>	2131± 17 <sup>e</sup>	1268± 11 <sup>d</sup>
<b>Chickpea</b>	7.3± 0.2 <sup>ab</sup>	1.0± 0.0 <sup>a</sup>	3340± 80 <sup>b</sup>	1735± 60 <sup>b</sup>
<b>Pea</b>	7.1± 0.1 <sup>b</sup>	1.0± 0.0 <sup>a</sup>	2676± 85 <sup>d</sup>	1332± 64 <sup>d</sup>
<b>Lentil</b>	7.5± 0.1 <sup>a</sup>	0.9± 0.0 <sup>b</sup>	2996± 54 <sup>c</sup>	1519± 40 <sup>c</sup>
<b>Bean</b>	7.2± 0.0 <sup>ab</sup>	0.9± 0.0 <sup>b</sup>	6349± 74 <sup>a</sup>	3059± 55 <sup>a</sup>

<sup>a</sup> Mean of duplicates. Values followed by different letters within each column are significant different ( $p \leq 0.05$ ).

509

510

511 **Table 2.** Effect of legume flour addition on quality properties of gluten-free layer  
 512 cakes<sup>a</sup>.

<b>Legumes</b>	<b>Collapse (cm)</b>	<b>Weight loss (g/100 g)</b>	<b>Specific volume (cm<sup>3</sup>/g)</b>
<b>Control</b>	1.5± 0.2 <sup>b</sup>	10.6± 0.8 <sup>a</sup>	2.7± 0.1 <sup>c</sup>
<b>Chickpea</b>	1.8± 0.5 <sup>b</sup>	10.6± 0.9 <sup>a</sup>	2.7± 0.1 <sup>c</sup>
<b>Pea</b>	4.1± 1.1 <sup>a</sup>	11.0± 1.1 <sup>a</sup>	2.9± 0.1 <sup>b</sup>
<b>Lentil</b>	2.1± 1.0 <sup>b</sup>	10.4± 0.9 <sup>a</sup>	3.2± 0.0 <sup>a</sup>
<b>Bean</b>	2.0± 1.3 <sup>b</sup>	11.1± 1.4 <sup>a</sup>	2.9± 0.1 <sup>b</sup>

<sup>a</sup> Mean of four replicates. Values followed by different letters in each column are significant different ( $p \leq 0.05$ ).

513

514 **Table 3.** Effect of legume enrichment of cakes on the crust and crumb colour parameters<sup>a</sup>.

Legumes	Crust			Crumb		
	<i>L</i> *	<i>a</i> *	<i>b</i> *	<i>L</i> *	<i>a</i> *	<i>b</i> *
<b>Control</b>	46.2± 2.3 <sup>c</sup>	11.9± 1.1 <sup>c</sup>	21.0± 2.1 <sup>bc</sup>	78.8± 0.5 <sup>a</sup>	0.7± 0.2 <sup>c</sup>	16.1± 1.3 <sup>b</sup>
<b>Chickpea</b>	59.2± 0.7 <sup>a</sup>	16.6± 0.8 <sup>a</sup>	26.1± 0.3 <sup>a</sup>	75.6± 0.6 <sup>ab</sup>	1.9± 0.1 <sup>b</sup>	21.5± 0.4 <sup>a</sup>
<b>Pea</b>	58.9± 0.8 <sup>a</sup>	15.5± 0.5 <sup>ab</sup>	24.3± 1.4 <sup>ab</sup>	76.4± 0.4 <sup>a</sup>	2.1± 0.2 <sup>b</sup>	21.3± 0.7 <sup>a</sup>
<b>Lentil</b>	47.2± 1.9 <sup>c</sup>	15.0± 1.2 <sup>b</sup>	16.6± 2.3 <sup>d</sup>	70.0± 2.6 <sup>c</sup>	1.0± 0.7 <sup>c</sup>	20.7± 1.8 <sup>a</sup>
<b>Bean</b>	55.5± 0.8 <sup>b</sup>	15.6± 0.5 <sup>ab</sup>	20.7± 2.4 <sup>c</sup>	71.4± 0.6 <sup>bc</sup>	3.1± 0.1 <sup>a</sup>	13.0± 0.5 <sup>c</sup>

515

516 <sup>a</sup> Mean of three replicates. Values followed by different letters in each column are significantly different ( $p \leq 0.05$ ).

517

518

519 **Table 4.** Effect of legume enrichment of gluten free cakes on instrumental hardness, springiness, cohesiveness, chewiness and resilience<sup>a</sup>.

Legumes	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience
<b>Control</b>	4.5± 0.4 <sup>b</sup>	0.77± 0.01 <sup>bc</sup>	0.31± 0.10 <sup>b</sup>	1.5± 0.2 <sup>b</sup>	0.17± 0.02 <sup>a</sup>
<b>Chickpea</b>	6.3± 0.4 <sup>a</sup>	0.80± 0.02 <sup>ab</sup>	0.43± 0.03 <sup>a</sup>	2.1± 0.1 <sup>a</sup>	0.15± 0.01 <sup>b</sup>
<b>Pea</b>	6.6± 0.4 <sup>a</sup>	0.76± 0.10 <sup>c</sup>	0.45± 0.01 <sup>a</sup>	2.2± 0.2 <sup>a</sup>	0.16± 0.03 <sup>ab</sup>
<b>Lentil</b>	4.3± 0.5 <sup>b</sup>	0.81± 0.03 <sup>a</sup>	0.45± 0.02 <sup>a</sup>	1.6± 0.2 <sup>b</sup>	0.17± 0.01 <sup>a</sup>
<b>Bean</b>	6.4± 0.3 <sup>a</sup>	0.81± 0.01 <sup>a</sup>	0.43± 0.03 <sup>a</sup>	2.2± 0.1 <sup>a</sup>	0.15± 0.01 <sup>b</sup>

520  
 521 <sup>a</sup> Mean of three replicates. Values followed by different letters in each column are significantly different ( $p \leq 0.05$ ).

522 **Table 5.** Nutritional composition of legume enriched gluten free cakes <sup>a</sup>.

Legumes	Available		Digestibility of protein (g/100 g)	Ash (g/100 g)	Fat (g/100 g)	Available		Energy (Kcal)	TDF (g/100 g)	IDF (g/100 g)	SDF (g/100 g)
	Total protein (g/100 g)	protein (g/100 g)				carbohydrate (g/100 g)					
<b>Control</b>	6.2± 0.0 <sup>d</sup>	4.8± 0.0 <sup>d</sup>	78± 1 <sup>b</sup>	1.7± 0.0 <sup>c</sup>	12.9± 0.1 <sup>c</sup>	52.2± 0.0 <sup>a</sup>	354± 0 <sup>a</sup>	2.2± 0.0 <sup>d</sup>	1.3± 0.0 <sup>d</sup>	1.0± 0.0 <sup>b</sup>	
<b>Chickpea</b>	9.3± 0.0 <sup>a</sup>	7.3± 0.0 <sup>a</sup>	79± 1 <sup>a</sup>	2.2± 0.0 <sup>a</sup>	14.3± 0.3 <sup>a</sup>	45.6± 0.0 <sup>b</sup>	351± 0 <sup>b</sup>	1.4± 0.0 <sup>e</sup>	0.1± 0.0 <sup>e</sup>	1.4± 0.1 <sup>a</sup>	
<b>Pea</b>	8.7± 0.0 <sup>c</sup>	6.8± 0.0 <sup>c</sup>	79± 1 <sup>a</sup>	2.0± 0.0 <sup>b</sup>	13.7± 0.1 <sup>b</sup>	46.2± 0.0 <sup>c</sup>	349± 0 <sup>c</sup>	2.3± 0.0 <sup>c</sup>	1.6± 0.1 <sup>c</sup>	0.7± 0.0 <sup>d</sup>	
<b>Lentil</b>	9.1± 0.0 <sup>b</sup>	7.2± 0.0 <sup>ab</sup>	79± 1 <sup>a</sup>	2.0± 0.0 <sup>b</sup>	13.8± 0.2 <sup>b</sup>	46.0± 0.0 <sup>c</sup>	349± 0 <sup>c</sup>	2.8± 0.0 <sup>a</sup>	2.1± 0.0 <sup>a</sup>	0.7± 0.0 <sup>d</sup>	
<b>Bean</b>	9.4± 0.0 <sup>a</sup>	7.1± 0.0 <sup>b</sup>	76± 0 <sup>c</sup>	2.2± 0.0 <sup>a</sup>	13.5± 0.2 <sup>b</sup>	45.5± 0.0 <sup>b</sup>	347± 0 <sup>d</sup>	2.5± 0.0 <sup>b</sup>	1.7± 0.0 <sup>b</sup>	0.8± 0.0 <sup>c</sup>	

523

524

525 <sup>a</sup> Mean of three replicates. Values followed by different letters in each column are significantly different ( $p \leq 0.05$ ).

526 TDF: total dietary fiber, SDF: soluble dietary fiber, IDF: insoluble dietary fiber.

527 **Table 6.** Starch fractions after enzymatic hydrolysis and kinetics of the *in vitro* starch digestibility <sup>a</sup>.

<b>Legumes</b>	<b>RDS (g/100 g)</b>	<b>SDS (g/100 g)</b>	<b>C<sub>∞</sub> (g/100g)</b>	<b>K (min<sup>-1</sup>)</b>	<b>AUC180</b>	<b>eGI</b>	<b>HI</b>
<b>Control</b>	75.8± 2.6 <sup>b</sup>	14.8± 3.4 <sup>a</sup>	90.7± 1.6 <sup>b</sup>	0.0611± 0.0012 <sup>b</sup>	3626± 52 <sup>b</sup>	85± 1 <sup>b</sup>	89± 1 <sup>b</sup>
<b>Chickpea</b>	86.6± 3.8 <sup>a</sup>	7.3± 5.4 <sup>b</sup>	92.2± 1.0 <sup>a</sup>	0.1192± 0.0101 <sup>a</sup>	3777± 30 <sup>a</sup>	88± 0 <sup>a</sup>	93± 1 <sup>a</sup>
<b>Pea</b>	61.3± 4.8 <sup>c</sup>	11.1± 5.0 <sup>a</sup>	72.4± 1.2 <sup>d</sup>	0.0667± 0.0021 <sup>b</sup>	2892± 58 <sup>e</sup>	69± 1 <sup>e</sup>	71± 1 <sup>e</sup>
<b>Lentil</b>	63.7± 2.2 <sup>c</sup>	14.6± 2.0 <sup>a</sup>	78.3± 0.8 <sup>c</sup>	0.0561± 0.0030 <sup>b</sup>	3102± 42 <sup>d</sup>	74± 1 <sup>d</sup>	76± 1 <sup>d</sup>
<b>Bean</b>	71.5± 3.6 <sup>b</sup>	12.6± 6.7 <sup>a</sup>	84.3± 3.8 <sup>b</sup>	0.0693± 0.0029 <sup>b</sup>	3370± 50 <sup>c</sup>	79± 2 <sup>c</sup>	83± 2 <sup>c</sup>

528

529 <sup>a</sup> Mean of three replicates. Values followed by different letters in each column are significantly different ( $p \leq 0.05$ ).

530 RDS: rapidly digestible starch, SDS: slowly digestible starch, eGI: estimated glycaemic index.

