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

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ABSTRACT

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

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

1. INTRODUCTION

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

Rice is one of the most frequently used cereals in gluten-free food products due to its low levels of sodium, protein, fat, fiber and high amount of easily carbohydrates (Rosell & Gómez, 2006). The enrichment of gluten-free baked products has proved to be necessary in gluten-free adherence diets, since consumers have generally a low intake of nutrients that has been associated to gluten-free diet (Lazaridou et al., 2007; Turabi et al., 2008; Marco & Rosell, 2008). Therefore, it was recommended the nutritional adequacy of various gluten-free foods and encouraged the consumption of whole-grain or enriched products (Thompson, 2009). Gluten free baked goods are mainly based on starchy compounds, and in consequence starch digestibility could be an important parameter when determining the nutritional quality of those products. In healthy individuals the polysaccharides absorption determines the appearance of nutrients in blood during the inter-digestive period (Goñi et al., 2002). Thus, it has been demonstrated that a disruption of the botanical structure in rice, legumes, or kernel-based bread products, increases the availability of starch for enzymatic digestion, and hence the glycemic response (Dartois et al., 2010, Granfeldt et al., 1994).

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

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

2. MATERIALS AND METHODS

2.1 Materials

Commercial rice flour was supplied by Harinera los Pisones (Zamora, Spain). Flours from chickpeas, peas, lentils and beans were also provided by Harinera los Pisones (Zamora, Spain). Flours had particle size lower than 210 μm. Protein and moisture content of rice, chickpea, pea, lentil and bean were 6 g/100 g, 23 g/100 g, 22 g/100 g, 22 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 g/100 g, respectively. Sugar, sunflower oil, fresh whole eggs, fresh milk and double-action baking powder were purchased from the local market.

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

2.2 Methods

2.2.1 Cake making

A single-bowl mixing procedure using a yellow layer cake recipe was used. Basic recipe contained 350g rice flour, 350g legumes flour, 420g fresh milk, 350g pasteurized whole eggs, 210g sunflower oil, 630g sucrose and 21g baking powder. A cake batter containing 700g rice flour was used as control. All ingredients were mixed during 1 min at speed 4 and 9 min at speed 6 using a Kitchen-Aid Professional mixer – KPM5 (KitchenAid, St. Joseph, MI, USA). Cake batter (180 g) was placed into rectangular (109 mm x 159 mm, capacity 430 ml) metallic, lard coated pan and baked in an electric oven for 30 min at 190 ºC. After baking, the cakes were removed from the pans, kept at room temperature for one hour to cool down, and packed in polyethylene bags to prevent drying. Seven cakes were obtained from each batter. Three different sets for each cake recipe were made in different days.

2.2.2 Batter measurements

Batter pH was measured using a pH-Tester30 (Eutech Instruments, Nijkerk, Netherland). Batter density was measured using a density cup Elcometer 1800 (Elcometer, Manchester, UK), and determined as the ratio of the weight of a standard container filled with batter to that of the same container filled with water (density, 1 g/cm^3) and calibrated at 20 ºC.

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

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

2.2.3 Cake quality assessment

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

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

2.2.4 Nutritional measurements

Nutritional parameters of gluten-free layer cake were determined following AACC methods (2000) and they include: moisture (method 44-15A), ash (method 08-01), crude fat (method 30-25) and crude protein (method 46-13) using N x 6.25. Available protein was calculated by percentage of protein digestibility of crude protein. The available carbohydrate content of the samples was calculated by difference, subtracting 100 g minus the sum of grams of moisture, protein, fat, ash and dietary fiber. The components were converted to food energy using conversion factors (4.0 kcal g^{-1} for proteins and available carbohydrates; 9.0 kcal g^{-1} for fats and 2.0 kcal g^{-1} for dietary fiber) (FAO, 2003). The *in vitro* protein digestibility of the samples was determined by the modified methods of Hsu et al. (1977) and Bilgiçli et al. (2007). Briefly, 50 ml of aqueous protein suspension having 6.25 mg protein/ml was prepared. Then, samples were placed in a 37 ºC water bath and the pH was adjusted to 8.00 using 0.1 N NaOH and/or 0.1 N HCl, while stirring. Trypsin at a concentration of 1.6 mg/ml was maintained in an ice bath and the pH was adjusted to 8.00 with 0.1 N NaOH and/or 0.1 N HCl. Five milliliters of enzyme solution were then added to the protein suspension, which was kept stirred at 37 ºC. The trypsin had an activity of 13,766 BAEE units/mg proteins. The pH drop was recorded 15 s after enzyme addition and at one minute intervals for 10 min. The enzyme solution was always freshly prepared before each series of experiments. The percent protein digestibility (Y) was calculated by using Eq. (1) (Hsu et al., 1977):

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

Where x is the change in pH after 10 min.

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

2.2.5 *In vitro* starch digestibility and expected glycaemic index

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

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

2.2.6 Statistical analysis

Experimental data were statistically analyzed by using Statgraphics V.7.1 program (Bitstream, Cambridge, MN, USA) to determine significant differences among them. When ANOVA indicated significant P-values, multiple sample comparison was also performed and Fisher's least significant difference (LSD) procedure was used to discriminate among the means, and correlation matrix was carried out by the Pearson-product moment to significant $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of legumes on batter properties

The partial (50%) replacement of rice flour by different legume flours was proposed for the nutritional enrichment of gluten free layer cakes. The level of legume flour incorporated was selected based on previous results obtained with the addition of chickpea flour to wheat flour for obtaining gluten layer and sponge cakes (Gómez et al., 2008). Batter properties of gluten-free layer cakes containing different legumes are shown in Table 1. The pH values were comprised within the range 7.1 and 7.5, and only the pea containing batter showed a significant reduction in the pH compared to the control. Legumes affected in different extent the batter viscosity and the effect was dependent on the legume origin. All the legumes induced a significant ($p<0.05$) increase of the batter viscosity at 30ºC and 50ºC, with the exception of pea flour that did not modify the viscosity at 50ºC. The incorporation of bean flour gave the highest viscosity at 30 and 50ºC. In all cases, batter viscosity decreased with the temperature increase, being at 50 ºC around half of that at 30 ºC. Likely, this decrease was related to the partial protein unfolding and aggregation (Lazaridou & Biliaderis, 2009). A significant positive correlation was observed between batter viscosity at 30ºC and 50ºC ($p=0.012$, r =0.999). The batter density decreased significantly ($p<0.05$) in the presence of lentil and bean, suggesting that more air was incorporated in the structure. That observation was corroborated with the micrographs of the batters (Figure 1A). Lentil and bean batters showed an uneven structure with larger number of gas cells. No significant differences (*p<0.05)* were detected in the case of pea and chickpea flours. Gómez et al. (2008) found that incorporation of 50% chickpea decreased batter density, but authors also pointed out the significant variation induced by the chickpea cultivar. In general, low batter density has been related to high air quantity into the batter and therefore high cake volume (Gomez et al., 2007).

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

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

The effect of legumes on the colour properties of gluten-free layer cake is shown in Table 3 and Figures 1B and 1C. Legumes affected in different extent the crust and crumb colour properties of gluten-free cakes and the effect was highly dependent on the legume nature. The *L** of crust was significantly ($p<0.05$) increased by chickpea, pea and bean. All legumes increased the reddish value (*a**) of the cake crust. Chickpea and pea showed the brightest and the most brownish crust compared to the other legumes. Protein composition of the legume flours was rather similar, thus differences in the browning colour might be ascribed to the sugar content of those flours, since crust colour depends on Maillard and caramelisation reactions that occur on the cake surface (Oliete et al., 2010). Lightness of the cake crumb was significantly decreased by lentil and bean flours. The hue red (*a**) and yellow (*b**) were significantly increased by the legumes addition, with the exception of lentil that did not affect *a** and bean flour that decreased *b**. Colour results were directly related to the original legume flours colour.

The effect of legumes on the texture properties of gluten-free layer cake is shown in Table 4. The addition of legumes induced a significant (p<0.05) increase of the hardness and chewiness of the gluten-free layer cake, except with addition of lentil that showed the same pattern of control cake. The crumb hardness was negatively correlated with specific volume (r = -0.997, p<0.05), which agree with results of Gómez et al (2010). The cohesiveness increased in all legume containing cakes likely due to the increased content in proteins, and this parameter showed positive correlation with specific volume (r = 0999, p=0.006). Springiness has been associated to fresh and aerated product, and in the case of muffins high springiness values are linked to quality (Sanz et al., 2009). A significant (p<0.05) increase in the springiness was induced in the presence of lentil and bean, but no relationship was observed between the springiness and the specific volume. Cohesiveness, related to energy required for the second compression and thus for chewing a food, increased in all the legume containing cakes compared to the control, which indicated lower crumbliness. A positive correlation was also observed between crumb cohesiveness and specific volume (r = 0.999, p = 0.006).

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

Macronutrients evaluation of cakes included the estimation of proteins (total, available and digestible), fat, dietary fiber, available carbohydrates, minerals and energy provided (Table 5). Compared to control cakes, legume containing gluten free cakes were richer in total proteins, minerals (ash) and fat, in detriment of the available carbohydrates. Utrilla-Coello et al. (2007) also reported an increase of proteins, fat and minerals when wheat bread was enriched with chickpea flour (wheat flour:chickpea flour, 60:40) due to the high protein content of legumes. No significant differences were observed in the moisture content of the cakes (24.5- 26.2 g/100 g) (results not showed). Regarding cake performance, a negative correlation between total proteins and batter density was observed (r = - 0.999, p<0.05). Schober (2009) reported a negative effect of the proteins due to their interference with the starch gel, forming points of weakness, where the crumb rupture is induced under the gas pressure and collapses. In addition, a positive correlation was also observed between the fat content and the specific volume of the cakes (r= 0.999, p<0.05). This effect might be related to the fat contribution to the interface stabilization that favors gas entrapment in the batter matrix.

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

In comparison to control sample, significant (*p*<0.05) increase of dietary fiber content was achieved with the incorporationof lentil, and the fiber was mainly insoluble. The highest content of soluble dietary fiber was found in cake enriched with chickpeas. The SDF content showed positive correlation with batter viscosity at 30 and 50 ºC, indicating the effect of soluble dietary fiber in the batter viscosity.

3.4 Effect of legumes on *in vitro* starch digestion

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

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

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

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

Regarding the hydrolysis index, all legumes significantly decreased the HI compared to control cake, with the exception of chickpea. In consequence, cakes with peas, lentil and bean will be potentially beneficial for dietary management to control diabetes, obesity, and hyperlipidemia and the glycemic index. Hoover & Zhou (2003) reported that legumes reduce glycaemic and insulinemic posprandial responses. Chung et al. (2006) reported that legume starch and rice variants produce lower GI and lower metabolic responses values than common cereal and tuber starches, likely due to starch retrogradation that increases the resistance to digestive enzymes.

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

4. Conclusion

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

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

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FIGURE CAPTIONS

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

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

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

Table 1. Effect of legumes on batter properties in gluten-free layer cakes[a].

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

[a] Mean of duplicates. Values followed by different letters within each column are significant different ($p \leq 0.05$).

Table 2. Effect of legume flour addition on quality properties of gluten-free layer cakes[a].

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

[a] Mean of four replicates. Values followed by different letters in each column are significant different ($p \leq 0.05$).

Table 3. Effect of legume enrichment of cakes on the crust and crumb colour parameters[a].

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

[a] Mean of three replicates. Values followed by different letters in each column are significantly different ($p \leq 0.05$).

Table 4. Effect of legume enrichment of gluten free cakes on instrumental hardness, springiness, cohesiveness, chewiness and resilience[a].

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

[a] Mean of three replicates. Values followed by different letters in each column are significantly different ($p \leq 0.05$).

Table 5. Nutritional composition of legume enriched gluten free cakes [a].

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

[a] Mean of three replicates. Values followed by different letters in each column are significantly different ($p \leq 0.05$).
TDF: total dietary fiber, SDF: soluble dietary fiber, IDF: insoluble dietary fiber.

Table 6. Starch fractions after enzymatic hydrolysis and kinetics of the in *vitro* starch digestibility [a].

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

[a] Mean of three replicates. Values followed by different letters in each column are significantly different ($p \leq 0.05$).
RDS: rapidly digestible starch, SDS: slowly digestible starch, eGI: estimated glycaemic index.