Improving carob flour performance for making gluten free breads by particle size fractionation and jet milling

Kleopatra Tsatsaragkou[1], Theodora Kara[1,3], Christos Ritzoulis[2], Ioanna Mandala[1], Cristina M. Rosell[3*]

[1] Laboratory of Food Engineering, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

[2] Department of Food Technology, ATEI of Thessaloniki, 57400 Thessaloniki, Greece

[3] Institute of Agrochemistry and Food Technology (IATA/CSIC), Valencia, Spain

Institute of Agrochemistry and Food Technology (IATA-CSIC),

C/ Agustin Escardino 7, Paterna 46980, Valencia, Spain.

[*]Corresponding author: e-mail: crosell@iata.csic.es, Tel +34963900022, Fax +34 963636301.

Abstract

Many different raw materials have been proposed for producing nutritious gluten free breads, but rarely there is a parallel analysis of the effect of physical treatment on those ingredients. The aim of this study was to incorporate carob flour fractions of varying particle size on rice gluten-free breads prepared with carob: rice (15:85) flour blends. Carob flour particle size was controlled by fractionation or jet milling application. Quality features of gluten- free breads containing carob flour and commercially available gluten free breads were compared. Carob flour addition led to breads with improved colour parameters, crumb structure, retarded firming and lower moisture loss compared to rice bread. Further improvement in specific volume, crumb hardness, protein and ash content and estimated glycaemic index (eGI) could be obtained by a careful selection of the particle size distribution of the carob flour. Carob breads prepared either with the coarsest or the finest fraction prepared using jet milling led to end products with the highest specific volume (≈ 2.2 g/cm^3) and the lowest crumb hardness (≈ 5.5 N), although they had lower specific volume and harder crumbs than breads from commercial blends (≈3 – 4 g/cm^3, 0.6-3.8 N). Nevertheless, rice based bread made with the finest carob flour was superior considering its slower firming, protein content and lower eGI. The incorporation of carob flour obtained by jet milling in rice-based gluten free breads led to end products with quality characteristics and sensory acceptance resembling commercial breads and high nutritional value.

Keywords: carob flour; particle size; jet milling; bread; gluten free; digestibility

INTRODUCTION

Coeliac disease is a systemic immune-mediated disorder caused by the ingestion of gluten containing grains in genetically susceptible persons. In the past, it was considered a rare disorder, mostly affecting individuals of European origin. Nowadays, coeliac disease is also a common lifelong disorder in North Africa, Middle East and India, affecting approximately 1% of the general population (Lionetti et al. 2015). As a consequence, persons with celiac disease must adhere to what is commonly referred to as a gluten-free diet. This diet involves the strict avoidance of wheat, barley and rye-based foods and consume instead foods made from gluten-free grains, including rice, corn, sorghum, millet, teff, amaranth, buckwheat, quinoa, wild rice and oats (in countries that allow their use) (Thompson, 2009). Gluten free breads are mostly characterized by low specific volume, a crumbling texture, the detection of particles in the mouth during consumption, a dry mouth feeling, short shelf life and not really satisfying taste (Houben et al. 2012, Matos and Rosell 2015). Additionally, many gluten free breads available in the market present low nutritional value particularly when compared to their wheat counterparts (Matos and Rosell 2011; Miranda et al. 2014), being too rich in digestible carbohydrates and poor in proteins, amino acids and fibres (Thompson, 2009).

Therefore, the gluten free bread market is facing three main challenges comprising the improvement of fresh bread technological quality, the extension of their shelf-life and the increase of their nutritional value and healthy pattern. Numerous studies have been carried out for improving the volume and texture using different recipes (only a tiny selection is cited) supplemented with hydrocolloids (Lazaridou et al. 2007), other carbohydrates or fibres (Phimolsiripol et al. 2012, Ziobro et al. 2012), proteins (Storck et al. 2013), enzymes (reviewed by Renzetti and Rosell, 2016), sourdoughs (Moroni et al. 2009, Novotni et al. 2012), additives (Sciarini et al. 2012) and so on. Other interesting approach has been the search for alternative flours with better technological and nutritional performance that comprised other cereals, pseudocereals, roots and tubers, and leguminous flours (Dini et al. 2012) or even nuts (Moreira et al. 2013). In this scenario, carob flour is an alternative pseudocereal containing high amounts of dietary fibre, micronutrients, and caroubin, the protein found in carob germ, that could be used as a protein source, enhancing the overall nutritional value of the gluten-free products and strengthening at the same time the produced bread dough structure (Tsatsaragkou et al., 2014a). Simultaneously, physical treatments like high hydrostatic pressure (Barcenas et al. 2010, Vallons et al. 2011), germination (Omary et al. 2012, Cornejo et al. 2015) or particle size reduction of flours (de la Hera et al. 2014, Protonotariou et al. 2015) have been proposed for improving flours breadmaking performance or bread nutritional

characteristics. Flour particle size influence particle hydration and in consequence dough rheology that play a significant role for the production of high-quality gluten free products (Tsatsaragkou et al. 2014b). Moreover, particle size might impact physiological response, particularly in the glycaemic-insulin response and the satiety rating (Holt and Miller 1994), which has been also observed in *in vitro* test increasing enzymatic digestibility of rice with the reduction of the particle size (de la Hera et al. 2014). Drastic effect can be obtained by jet milling that allows the micronisation of the flour to the level of starch granules size, which had a noticeable effect on the characteristics of the wheat flour (Protonotariou et al. 2014). Presumably, a holistic approach combining nutritive flours and physical treatments might enhance the benefits from the technological and nutritional point of view.

Gluten free bread of enhanced nutritional value and improved quality can be obtained with the use of nutrient-dense ingredients and by controlling process conditions. This demand could be satisfied by the use of carob flour, highly nutritious flour for making gluten free foodstuff. Carob flour has been successfully incorporated in rice based gluten-free breads (Tsatsaragkou et al. 2012, 2014a) that had higher content of proteins, fibres and minerals compared to commercial gluten free breads, besides softer crumbs and large loaf volumes. Nevertheless, taking into account the positive effects of controlling particle size distribution of flours on gluten free breads, it was worthy to explore the possible further improvement joining a nutritious flour and physical treatment. To our knowledge there is scarce information concerning the effect of jet milling application on the nutritional characteristics of gluten free flours and specifically on carob flour. It would be an interesting approach to study the use of jet milled carob flour in gluten free breadmaking for producing end-products of enhanced nutritional and physical characteristics. Furthermore, the effect of flour milling procedure on gluten free bread properties would be analysed for the first time through a comparison between conventional and air compressed milling of carob flour. The main goal of this study was to determine the influence of the particle size of carob seed flour on the quality and digestibility of fresh and stored gluten free rice based breads. Gluten free carob breads were also compared in terms of physical and nutritional quality to commercial gluten free breads aiming at the development of gluten free breads that meet the nutritional and quality requirements of consumers.

MATERIALS AND METHODS

Materials

Rice flour (moisture content 13.10%, protein 7.39%, dietary fiber 0.5%, lipid 0.39%, ash 0.8%) was obtained from (Kaplanidis mill group S.A., Serres, Greece). Carob seeds were obtained from Cypriots local producers. Compressed yeast (L'Hirondelle, S.L. Lesaffre, France), sugar, salt (iodised sea salt, Kallas, Greece), shortening (Vitam, Unilever S.A, Greece), egg white powder (Laffort S.A., Bordeaux, France), whey protein concentrate (Nutrilac®DR-7015, Arla Foods Ingredients Amba-Denmark), emulsifier (DATEM:Diacetyl-tartaric esters of mono- and diglycerides, Danisco, Copenhagen), locust bean gum (LBG) (Sigma-Aldrich Chemie GmbH, Germany), and processing aid containing alpha-amylase, transglutaminase and hemicellulase activity (VERON CLX AB Enzymes, Darmstadt, Germany) were used for breadmaking.

Three commercially available gluten free bread mixtures were obtained from the local market. All-purpose gluten free flour (C1): mixture of rice flour, maize flour and potato starch (Loulis Mills S.A, Greece). Gluten free flour with fibre mix (C2): wheat starch, sugar beet fibre, HPMC, guar gum (Glutafin, Dr. Schär UK). Gluten free flour for making rustic bread (C3): corn starch, rice flour, buckwheat flour, dextrose, thickeners (carob seeds, carrageenan, HPMC), (Valpiform S.A, France).

Carob flour production

Carob seeds were grounded in a laboratory attrition mill. The resulting flour was sieved and fractions above 500 μm were discarded. Three fractions were separated using sieves (A: 315-500 μm, B: 250-315 μm and C: 125-250 μm). Ultrafine flour powder (D) was obtained passing carob flour below 500 μm through a Jet-O-Mizer Milling (Model 0101S, Fluid Energy Processing and Equipment Company, Telford, Pennsylvania, USA) with air pressure at 800 kPa.

Flour characterisation

Moisture content was determined by method 935.36 (AOAC 1990). Protein content of carob flour fractions was determined by the Kjeldahl method for Nitrogen determination (ISO 937-1978) and dietary fibre content of carob flour fractions was determined using AOAC (1990) method 985.29. Flour particle size distribution was determined according to the method of Protonotariou et al. (2015) using a Malvern Mastersizer 2000 (Malvern Instruments, Worcestershire, UK), equipped with a Scirocco dry powder accessory (Malvern Instruments, Worcestershire, UK). This set-up allows for the direct exposure of the dry particles to the laser beamline. The results have been calculated using a refractive index of 1.53 and an absorption parameter of 0.7 were used for the dispersed phase, setting cut off between 0.02 and 2000 μm. Chemical composition of the commercial gluten free blends was provided by the supplier. Water

holding capacity (WHC) of flour blends was determined according to the method of Niba et al. (2001). Flour samples (0.5 g) were suspended in distilled water (5 mL), mixed in a vortex for 1 min and then centrifuged at 1000 *g* for 30 min. The supernatant was decanted and the absorbed water was calculated by difference (sediment weight minus sample weight x 100). Determinations were carried out in duplicate.

Breadmaking

The breadmaking procedure of gluten free breads containing carob flour was described in our previous studies (Tsatsaragkou et al. 2012, 2014a). The basic ingredient for the preparation of gluten free breads was rice flour. Gluten-free bread containing only rice flour was used as a control sample. Carob flour substituted rice flour at 15% (based on the weight of the flour). The basic recipe for the dough (based on the weight of the flour) was 6% fresh yeast, 4% egg white powder, 4% whey protein, 3.5% shortening, 3% sugar, 2% salt, 0.5% Datem, 0.5% LBG and 90 mg enzyme. Preliminary experiments were carried out to determine the optimum amount of water for each blend (Table 1) (unpublished data). Bread preparation included mixing of dry ingredients in a Hobart mixer (Hobart N50, Hobart Co., Troy, OH, USA), followed by addition of melted shortening. Yeast was progressively mixed with water and added to the final blend. Dough was mixed for 3 min at a speed of 475 rpm. After complete mixing, 650 g of dough was divided at 8 portions of 80 g and placed into fat coated aluminium pans (80 mm x 45 mm x 37 mm, LxWxH) and fermented at 35 ^{o}C, 85% RH for 50 min. Then, samples were baked at 170 ^{o}C for 20 min in a convection oven. After baking, loaves were removed from the pans, cooled at room temperature for 1 h and subjected to physical and sensory analysis (day 0). Commercial gluten free breads were prepared according to supplier's instructions. Codes of breads (B) were based on the notation of the flour fractions (Table 1). For each bread recipe 8 loaves were prepared.

Bread quality evaluation

Bread specific volume (cm^3/g) was determined as a mean of four loaves for each bread recipe, by a volumetric displacement method using solid-glass beads with 2 mm diameter as suggested by Hwang and Hayakawa (1980). Crumb grain measurements were conducted using 4 slices of 1 cm thickness, cut from the centre of the loaf. Images of the slices were captured using a flatbed scanner (HP Scanjet 4370, Hewlett–Packard, U.S.A.). Image analysis of bread slices was carried out using Image analysis software (ImageProPlus 7, Media Cybernetics, U.S.A.). Crumb colour, average cell size (mm^2) and cell density ($cells/cm^2$) were determined, as described in Tsatsaragkou et al. (2012, 2014a). The chemical composition of breads was determined. Ash, protein, insoluble dietary fibre and total fibre contents were measured by

AACC International methods (AACC International 2012). Chemical composition determinations were carried out in duplicate.

Bread storage

Storage stability was studied in the gluten free breads. Samples were placed in polyethylene bags 1 h after baking to prevent moisture loss and stored at 25 °C and 60% RH for 3 days. The staling rate was estimated by determining crumb firmness on 0, 1, 2 and 3 days of storage at 25 °C. The firmness of crumb was estimated with the 74-09 method of the American Association of Cereal Chemists (2000) (with modifications) with a Universal Testing Machine (Instron, Model 1100, Massachusetts, USA) equipped with a 50 N load cell. Crumb cubes of 2 x 2x 2 cm (length x width x height) were compressed to 50% of initial height with a 4 cm diameter probe and at speed of 101 mm/min, recording the maximum force (firmness) in newtons. Each storage day, two bread loaves from each recipe were used for testing. Crumb firmness measurements were done in quadruplicate, using two crumb cubes from each loaf. The rate of moisture loss during the storage time was also determined in quadruplicate using two central slices from each loaf. The evaluation of moisture was performed using AOAC method 935.36.

***In vitro* starch digestibility and estimated glycaemic index**

Three slices from three different breads were freeze dried for determining the *in vitro* digestibility. Enzymatic hydrolysis of gluten-free breads was determined following the method reported by Gularte and Rosell (2011) using 100 mg of powdered (<0.5 μm sieve opening) freeze dried breads with minor modifications. Duplicates were carried out for each bread sample. Briefly, for free sugars removal, samples (0.100 ± 0.005 g) were weight in 30 ml Pyrex tubes and suspended in 2 mL of 80 % ethanol. Tubes were tightly capped and then, they were kept in a shaking water bath at 85 °C for 5 min, and centrifuged for 10 min at 1000×*g* and 4 ºC. Supernatant was separated to measured free glucose (FS). Tubes containing the remaining pellet were incubated with porcine pancreatic –α-amylase (0.24 U/mg sample) (Type VI-B, ≥10 units/mg solid, Sigma Chemical, St. Louis, USA) in 4 mL of 0.1 M sodium maleate buffer (pH 6.9) in a shaking water (140 strokes/min) bath at 37 °C. Aliquots of 200 μL were withdrawn during the incubation period (0.25–16 h) and mixed with 200 μL of ethanol (96 %, w/w) to stop the enzymatic reaction and the sample was centrifuged at 10,000 ×*g* for 5 min at 4 °C. Supernatant was recovered and the precipitate was washed twice with 50 % ethanol (200 μL) and centrifuged as described earlier. The supernatants were pooled together and kept at 4 °C for further glucose enzymatic determination. Digestible starch (DS) was determined in the supernatant after 16 h of incubation.

Resistant starch (RS) was the remnant after 16 h hydrolysis. For quantifying RS, the sediment was solubilized with 2 mL of 2 M KOH using a Polytron ultraturrax homogenizer IKA-T18 (IKA works, Wilmington, NC, USA) during 1 min at speed 3. The homogenate was diluted with 8 mL 1.2 M sodium acetate (pH 3.8) and incubated with 100 μL amyloglucosidase (33 U/mL) at 50 °C for 30 min in a shaking water bath. After centrifuging at 2,000 ×*g* for 10 min, supernatant was kept for glucose determination. The glucose content was measured using a glucose oxidase–peroxidase (GOPOD) kit (Megazyme, Dublin, Ireland) following supplier method. The absorbance was measured using an Epoch microplate reader (Biotek Instruments, Winooski, USA) at 510 nm. Starch was calculated as glucose (mg)×0.9. Replicates (n= 4) were carried out for each determination.

The *in vitro* digestion kinetics were calculated in accordance with the procedure established by Goñi et al. (1997). A non-linear model following the equation [$C_t = C_\infty\ 1-e^{-kt}$] was applied to describe the kinetics of starch hydrolysis, where C_t is the concentration of product at time t, C_∞ is the concentration at the end point, and k is the pseudo-first order rate constant. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve (0–180 min) of the sample by the area of a standard material (white wheat bread) over the same period of time. Three commercial white breads were used to have more reliable standard material, which were treated as the samples. The estimated glycaemic index (*eGI*) was calculated using the equation described by Granfeldt et al. (1992): eGI = 8.198 + 0.862HI.

Sensory evaluation

For the sensorial evaluation, carob breads BA and BD were selected among all carob breads produced, since those exhibited the best quality parameters in terms of specific volume, crumb hardness and crumb grain measurements. Commercial samples were also subjected to sensorial evaluation. Sensory evaluation of gluten free fresh breads (0 day) was performed using a 9-point hedonic scale (consumer testing). Fifty untrained panellists students and staff members of the Agricultural University of Athens, Greece participated. One slice of bread, identified by code numbers, was served to each panellist under normal (daylight) illumination. They evaluated each product for quality attributes: appearance (volume-grain), colour, flavour (aroma- taste), texture and overall acceptability. Acceptability of each quality attribute was rated with a score 1 (lowest) to 9 (highest). Products were considered acceptable if their mean scores for overall acceptability were above 5 (neither like nor dislike). Similar consumer sensorial testing in gluten free breads was described by Lazaridou et al. (2007).

Statistical analysis

Statistical analysis was performed using the Statgraphics Statistical Graphics System, Centurion XV.II (Statgraphics, Rockville, Md., USA). A multiple sample comparison was carried out for each experimental variable. When the F-test in the ANOVA table shows any significant differences amongst the means, the Multiple Range Tests was carried out to determine which means were significantly different from which others. Samples means differ significantly for a *P*-value of less than 0.05 according to Fisher's LSD analysis.

RESULTS AND DISCUSSION

Flour characterisation

Table 2 shows the physical and chemical properties of the carob flour fractions and the commercial gluten free blends. Concerning the carob flour fractions, the mean particle size (D_{50}) decreased as did the sieve size. Carob fraction FD exhibited the lowest particle size, as a result of the jet milling process used for reducing its particle size. Wheat bran and insoluble fibres showed average particle size close to 20μm after subjecting their raw material (particle size between 60 μm and 150 μm) to jet milling (Chau et al. 2006; Kim et al. 2013; Protonotariou et al. 2014). Herein, jet milling application had successfully reduced carob flour particle size to 80.36 μm, likely because carob seed coat is very tough and hard and difficult to be milled in fine granulometry (Karababa and Coşkuner 2013; El Batal and Hasib 2013).

Significant differences in chemical properties among carob fractions were observed (Table 2). Sieving influenced markedly the protein and dietary content of carob flour. Comparing carob fractions FA, FB and FC, protein content was higher in finer fractions, in opposition to dietary fibre trend. This effect can be ascribed to the morphological characteristics of the carob seed. The seed coat and the endosperm are harder than the embryo and therefore a finer particle size is difficult to be achieved in contrast to the germ, which is more friable (El Batal and Hasib 2013). In addition, the germ is rich in proteins, while the seed coat consists of cellulose, lignin and tannin and the endosperm of galactomannan-type polysaccharides (Battle and Tous 1997). Through sieving, the coarser fractions consisted mainly by the seed coat and the endosperm, which contained mainly dietary fibres.

Carob flour fractions were used for making blends with rice flour in order to improve nutritionally the gluten free cereal flour. Rice/carob flour blends contained higher amount of proteins and dietary fibre than the commercial gluten free blends analysed (Table 2). In gluten free breadmaking, hydration properties play a crucial role for obtaining aerated breads (Marco and Rosell 2008). Rice/carob flour

mixtures exhibited significantly increased water holding capacity in relation to the commercial mixtures. Among carob flour fractions, a decreasing trend of the WHC was observed when decreasing carob flour particle size, which could be related with their lower content in dietary fibre knowing its ability to retain more water compared to carob protein. Caroubin, the protein isolated from the carob bean embryo, is considered to absorb ≈3g water/g protein (at 25 ^{o}C) (Wang et al., 2001) and locust bean gum (from the endosperm) is considered to bind 10g water/g locust bean gum, (measured by the authors using locust bean gum sample from Sigma-Aldrich Chemie GmbH, Germany, the one also used for preparing gluten free breads).

Bread quality evaluation

All gluten free breads presented significant differences regarding volume, crumb colour and appearance (Figure 1, Table 3). A large variation in specific volume between the commercial samples and breads made with carob flour can be observed, due to all commercial mixtures contained more than one hydrocolloid, which may act synergistically increasing loaf volume. Values for specific volume of breads with carob flour and commercial samples agree with values of studies for gluten free breads (Marco and Rosell 2008; Matos and Rosell 2013). Among the carob breads, the incorporation of coarser (FA) and finer (FD) fraction led to the production of breads with increased specific volume. Considering their chemical composition and particle size, the only coincidence was their lower amount of protein compared to the other fractions. Nevertheless, also the higher amount of dietary fibres (polysaccharides from the endosperm of the carob seed), might contribute to improve the specific volume as was the case of hydrocolloids (Lazaridou et al. 2007).

The L^*, a^* and b^* values for crumb colour showed significant differences (P<0.05) among gluten-free bread products, ascribed to the differences in the ingredients of the recipes. In general, the addition of carob flour for the production of gluten-free rice breads (BA, BB, BC, BD) significantly modified the crumb colour, leading to darker colour as reflected the lower L^* values compared to rice bread (control). The darkening effect was desirable for gluten free breads, particularly for those based on rice flour, which tend to have lighter colour than wheat breads (Gallagher et al. 2003). The lowest value of L^* (lightness) was obtained for BD. L^* value for GF C3 was similar to the one of BA and BB likely due to the presence of locust bean gum. Similar L^* and b^* values to the ones obtained for GF C3 were reported for gluten free breads from commercial mixtures containing buckwheat flour (Mariotti et al. 2013). Particle size decrease of carob flour resulted in breads with increasing a^* and b^*, due to the native dark colour of

carob flour. The effect of the increased protein content, with carob decreasing particle size was depicted at the increment of the *b** parameter of the breads. The bread prepared with carob fraction FD exhibited the darkest crumb colour, with increased *a** and *b** values, which can be ascribed to the jet milling application for the reduction of particle size. According to Protonotariou et al. (2015), whole wheat breads made from jet milled flour exhibited a decrease in bread crumb lightness with a simultaneous increase in redness and yellowness compared to control bread (no jet mill application). The highest yellowness (high *b** values) was found for bread sample GF C1, due to the presence of maize flour in its blend.

The crumb grain characteristics and more specifically average cell size and cell density confirmed their different structure (Table 3). Rice bread crumb consisted of a very high number of tiny pores leading to a very dense structure (Figure 1). The addition of carob flour increased the cell size, with a parallel reduction of the cell density. The BC bread presented the lowest cell size and highest cell density, although not statistically significant differences were observed. According to the literature, cell size can be dimensionally distributed in the following classes; small: cell size < 0.8 mm^2; medium : 0.8 < cell size < 4.0 mm^2; large: cell size > 4.0 mm^2) (Mariotti et al., 2013). The presence of a higher number of large cells has been correlated to low bread volume while high amount of medium cells (and of smaller cells) have been found to produce loaves of high specific volume (Gallagher et al. 2003; Mariotti et al. 2013). In this context, cell density (ratio of cells per slice area) measurement attempts to provide standardization for variations in specific volume per loaf. Nevertheless, this standardization tends to diminish visible quality differences in final products, which should not be neglected (Trappey et al. 2015). More specifically, for BC bread, high specific volume would be expected considering the presence of small sized and uniformly distributed cells (Table 3, Figure 1), in opposition, BA and BD presented an open and heterogeneous crumb structure with increased number of medium-sized gas cells and higher specific volumes (Table 3, Figure 1). The crumb grain characteristics of carob breads BA and BD exhibited no statistically significant differences in average cell size and cell density compared to commercial bread samples, with the exception of GF C1 that exhibited the smallest cell size. The use of coarse particle size carob flour and the application of jet milling for reducing carob flour particle size could lead to the production of gluten free breads that resemble the physical attributes of commercial gluten free breads.

Table 4 showed the chemical composition of the gluten free breads. Rice based breads had significantly higher moisture content than the breads obtained from commercial blends, which was expected

300 considering the amount of water used for bread making, in fact a significant relationship was found
301 between them (r=-0.9957, P=0.0003). The amount of protein was significantly higher in the experimental
302 breads than in the commercial ones and it was highly correlated with the specific volume of the breads
303 (r=-0.9201, P=0.0268). Among carob fractions, only breads BB and BC led to a significant increase in
304 the protein content, corresponding with the initial higher protein content of those carob fractions. The
305 amount of total dietary fibre did not differ significantly and all gluten free breads contained good amount
306 of dietary fibre (>3 g/100 g). Literature data concerning the total dietary fibre content of gluten free
307 breads were in the range of 3.60 to 7.20 g/100 g for commercial samples and 3.61 to 6.30 g/100 g for
308 resistant starch enriched gluten free breads (Korus et al. 2009; Segura and Rosell, 2011). The same trend
309 was observed for the insoluble dietary fibre, although lower absolute values were obtained for the
310 commercial gluten free breads GF C1 and GF C2. Concerning the use of jet mill (BD), a decrease,
311 although no significant, was observed in the insoluble dietary fibre content, ascribed possibly to the
312 milling technique, which was reported to cause a redistribution of fibre components from insoluble to
313 soluble fractions (Chau et al. 2007; Zhu et al. 2010). Ash content was significantly different among the
314 produced breads due to the ingredient variability of each bread recipe. With the exception of BA, the
315 inclusion of carob flour in breads increased the amount of minerals, and they were within the range of
316 minerals obtained for the commercial breads. Experimental gluten free breads were more equilibrated in
317 nutrients than those from commercial blends, with better balance of proteins, total and insoluble dietary
318 fibre and minerals.

319 **Bread storage**

320 A major problem in gluten free breads is their fast staling, thus anti-staling alternatives are pursued.
321 During storage the most evident changes are related to moisture loss and crumb hardening mainly
322 ascribed to starch retrogradation and drying. Changes in hardness and moisture content are recorded in
323 Table 5. Significant differences (P<0.05) in the initial crumb moisture were found among the different
324 gluten-free breads (Table 4) but also in the rate of drying during storage (Table 5), and a negative
325 correlation between them (r=-0.9040, P=0.0352). Moisture losses along storage were lower for carob
326 containing breads than either control or commercial gluten free breads. Similar to GF C3 moisture losses
327 were reported for breads containing buckwheat (≈5.4%) (Mariotti et al. 2013). Among breads containing
328 carob flour, no trend was observed with the progressive reduction of particle size. Therefore, results could
329 not be directly explained based on the initial moisture content, chemical composition or the particle size,

330 solely in the case of the bread containing jet milled flour the reduced drying could be attributed to the
331 lowest particle size.
332 Initial crumb hardness showed significant difference within samples (Table 5), being the control and BC
333 sample the hardest breads. Initial crumb hardness was negatively correlated with the specific volume (r=-
334 0.8996, P=0.0376). Control bread exhibited the highest firming rate indicating the tendency of the rice
335 containing breads to stale faster. Breads containing carob flour presented lower firming rate, thus the
336 presence of carob flour had an antistaling effect that was independent on the particle size distribution.
337 Among carob breads, the softest bread crumb was accounted for BA and BD breads. A significant
338 negative relationship was obtained between the initial crumb hardness (r=-0.8996, P=0.0376) and the
339 firming rate. Sciarini et al. (2010) described a negative correlation between crumb firmness and specific
340 volume of breads, but it was not observed in this study. Concerning the gluten free breads made from
341 commercial blends, despite their low initial crumb firmness values after 3 days of storage their crumb
342 hardness equalizes with that of BA and BD breads. Firming has been also related to the decrease in
343 moisture content because it favours the rapid formation of cross links between starch molecules and
344 proteins (He and Hoseney 1990). Nevertheless, no relationship was encountered between the firming rate
345 and the water loss, but a significant relationship was observed with the protein content of the breads (r=-
346 0.9420, P=0.0166). Therefore, addition of carob flour of coarse particle size and the use of the jet milling
347 technique for the reduction of the carob flour particle size, instead of sieving, could lead to end-products
348 with reduced firming rate compared to commercially available samples.
349 ***In vitro* starch digestibility and estimated glycaemic index**
350 The parameters derived from the *in vitro* digestion of the produced breads are presented in Table 6.
351 Gluten free breads are considered starchy foods, resulting in rapid degradation in the small intestine since
352 the starch is largely gelatinized (Parada and Aguilera 2011). The maximum hydrolysis, C_∞, or hydrolysis
353 degree when the enzymatic reaction reaches a plateau of gluten-free breads, was associated with the
354 levels of rapidly hydrolyzed starch (Segura and Rosell 2011). Breads displayed low values of C_∞
355 compared to the ones described for rice based breads (>95 g/100g) (de la Hera et al. 2014), and they were
356 significantly lower for the bread containing jet milled carob flour or the commercial blends. The kinetic
357 constant (k), indicative of the hydrolysis rate in the early stage, showed no significant differences among
358 the produced breads. De la Hera et al. (2013) reported slower enzymatic hydrolysis when increasing flour
359 particle size, since the larger the granules, the smaller is the surface area to volume ratio and hence the

potential surface to be attacked and hydrolysed by enzymes. Herein, among carob breads a decreasing trend of *k* could be seen with increasing carob flour particle size, although no statistically significant. Values agree with those found for gluten free breads (Segura and Rosell 2011; de la Hera et al. 2014). Digestible starch varied between 20.61 to 36.14 g/100g and resistant starch varied from 4.55 to 8.97 g/100 g. However, previous studies reported values of digestible starch ranging from 75.6 to 92.5 g/100 g and very low values of resistant starch 1.0−2.9 g/100 g (Segura and Rosell 2011), and glucose content was only significantly higher in GF C3. The number and variety of ingredients of gluten-free bread recipes could explain the large variation found for gluten free bread digestibility. The *eGI* of gluten free breads was reported to be significantly higher than that of wheat bread due to the higher starch digestibility of Control bread (rice based bread) exhibited similar *eGI* to those reported for rice and corn based breads (103.98 for rice and 107.05 for corn) compared to white bread (100) (Gelencsér et al. 2008; Segura and Rosell 2011). The presence of carob flour decreased the *eGI*, but solely in the case of flours of fine particle size. The lowest *eGI* (≈47) was accounted for bread sample BD, which contained jet milled carob flour. Protonotariou et al. (2015) reported a decreasing tendency of *eGI* for whole wheat breads when decreasing flour particle size. The *eGI* of the breads obtained from commercial blends (56 -82) was lower than the values reported in the literature for other commercial gluten free breads (≈90 and above) (Segura and Rosell 2011). Consequently carob bread BD could be considered moderate GI foods with a health promoting effect.

Sensory evaluation

Taking into account the quality parameters (specific volume and crumb hardness) obtained for carob flour containing breads, BA and BD were selected as the most promising gluten free breads. Therefore, sensorial evaluation was carried out of those breads compared to those obtained from commercial blends. The scores for the sensory attributes (appearance, colour, flavour, texture and overall acceptance) of the gluten free breads are shown in Table 7. Carob breads were scored similarly to gluten free breads made from commercial breads, with the exception of GF C3, which scored higher values in all the parameters tested due to its spongy-cake structure. Although carob breads exhibited lower specific volumes, they were highly accepted. Although carob flour conferred a distinctive taste, panellists equally preferred carob breads and commercial samples in terms of flavour and texture, maybe due to the protein and amino acids contribution of the carob seed. All formulations were considered acceptable from panellists, since they scored over 5 in overall acceptability. Even though panellists were not coeliacs, according to

Laureati et al. (2012) coeliac and non-coeliac individuals use the same sensory descriptors to express their preference for gluten-free products. Therefore, the incorporation of carob flour in gluten free breads led to end-products that can fulfil consumer needs.

CONCLUSIONS

The incorporation of carob flour in gluten free rice- based bread, leaded to the production of gluten free breads with improved physical (specific volume, color parameters, hardness, retarded firming) and nutritional characteristics and similar sensorial acceptance than the ones obtained from commercial blends. Furthermore, particle size distribution of carob flour significantly affected the physico-chemical features of resulting breads. The incorporation of the coarser carob flour fraction (FA) obtained from sieving or the jet milled carob flour fine powder (FD) yielded the best breads, but when considering also their digestibility carob breads prepared with the finest flour particle size (BD) exhibited moderate glycaemic index. Carob flour could be a promising ingredient for gluten free bread production, since their quality matched that of commercial gluten free breads.

ACKNOWLEDGMENTS

Financial support of Spanish Ministry of Economy and Competitiveness (Project AGL2014-52928-C2-1-R) and the European Regional Development Fund (FEDER) is acknowledged.

REFERENCES

AACC International. (2012). *Approved Methods of Analysis. 11th Ed. Methods: 08-01.01; 46-13.01; 32-07.01; 74-09.01.* AACC International. St. Paul. MN. U.S.A.

AOAC. (1990). *Method 935.36 Moisture content determination and method 985.29 Total dietary fiber in foods (enzymatic–gravimetric method), vol. II* (15th ed.). Arlington, VA: Association of Official Analytical Chemists.

Barcenas, M., Altamirano-Fortoul, R., & Rosell, C. (2010). Effect of high pressure processing on wheat dough and bread characteristics. *LWT- Food Science and Technology, 43*(1), 12-19.

Batlle, I., & J. Tous. (1997). *Carob tree. Ceratonia siliqua L. Promoting the conservation and use of underutilized and neglected crops. 17.* Institute of Plant Genetics and Crop Plant Research, (pp. 78). Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.

Chau, C.F., Wang, Y.T., & Wen, Y.L. (2007). Different micronization methods significantly improve the functionality of carrot insoluble fibre. *Food Chemistry, 100*, 1402–1408.

Chau, C.F., Wen, Y.L., &Wang, Y.T. (2006). Effects of micronisation on the characteristics and physicochemical properties of insoluble fibres. *Journal of the Science of Food and Agriculture, 86*(14), 1380–1386.

Cornejo, F., Caceres, P. J., Martinez-Villaluenga, C., Rosell, C. M., & Frias, J. (2015). Effects of germination on the nutritive value and bioactive compounds of brown rice breads. *Food Chemistry, 173*, 298-304.

de la Hera, E., Rosell, C.M., & Gomez, M. (2014). Effect of water content and flour particle size on gluten-free bread quality and digestibility. *Food Chemistry, 151*, 526–531.

de la Hera, E., Talegon, M., Caballero, P., & Gomez, M. (2013). Influence of maize flour particle size on gluten-free breadmaking. *Journal of the Science of Food and Agriculture, 93*(4), 924–32.

Dini, C., Garcia, M. A. & Vina, S. Z. (2012). Non-traditional flours: frontiers between ancestral heritage and innovation. *Food & Function, 3*(6), 606-620.

El Batal, H., & Hasib, A. (2013). Optimization of Extraction Process of Carob Bean Gum Purified from Carob Seeds by Response Surface Methodology. *Chemical and Process Engineering Research, 12*, 1-8.

Gallagher, E, Gormley, T.R., & Arendt, E.K. (2003). Crust and crumb characteristics of gluten free breads. *Journal of Food Engineering, 56*(2–3), 153–161.

Gelencsér, T., Gál, V., Hódsági, M., & Salgó, A. (2008) Evaluation of quality and digestibillity characteristics of resistant starch-enriched pasta. *Food and Bioprocess Technology, 1*, 171–179.

Goñi, I., Alonso, A.G. & Calixto, F.S. (1997). A Starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*(3), 427-437.

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

Gularte, M. A., & Rosell, C. M. (2011). Physicochemical properties and enzymatic hydrolysis of different starches in the presence of hydrocolloids. *Carbohydrate Polymers, 85*, 237–244.

He, H., & Hoseney, R.C. (1990). Changes in bread firmness and moisture during long-term storage. *Cereal Chemistry, 67*, 603–605.

Houben, A., Hochstotter, A., & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: an overview. *European Food Research and Technology, 235*(2), 195-208.

448 Hwang, M.P., & Hayakawa, K.I. (1980). Bulk density of cookies undergoing commercial baking
449 processes. *Journal of Food Science, 45*(5), 1400–1402.
450 ISO 937-1978. Determination of Nitrogen (Reference Method).
451 Karababa, E., & Coşkuner Y. (2013). Physical properties of carob bean (Ceratonia siliqua L.): An
452 industrial gum yielding crop. *Industrial Crops and Products, 42*, 440–446.
453 Kim, B.K., Cho, A.R., Chun, Y.G., & Park, D.J. (2013). Effect of microparticulated wheat bran on the
454 physical properties of bread. *International Journal of Food Sciences and Nutrition, 64*(1), 122–129.
455 Korus, J., Witczak, M., Ziobro, R., & Juszczak, L. (2009). The impact of resistant starch on
456 characteristics of gluten-free dough and bread. *Food Hydrocolloids, 23*(3), 988–995.
457 Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. (2007). Effects of hydrocolloids on
458 dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering,*
459 *79*(3), 1033–1047.
460 Lionetti, E., Gatti, S., Pulvirentic, A., & Catassi, C. (2015). Celiac Disease From A Global Perspective.
461 *Best Practice & Research Clinical Gastroenterology, 29*, 365-379.
462 Laureati, M., Giussani, B., & Pagliarini E. (2012). Sensory and hedonic perception of gluten-free bread:
463 Comparison between celiac and non-celiac subjects. *Food Research International, 46*(1), 326–333.
464 Mariotti, M., Pagani, M. A., & Lucisano, M. (2013). The role of buckwheat and HPMC on the
465 breadmaking properties of some commercial gluten-free bread mixtures. *Food Hydrocolloids, 30*(1), 393–
466 400.
467 Marco, C., & Rosell, C. M. (2008). Breadmaking performance of protein enriched, gluten-free breads.
468 *European Food Research and Technology, 227*, 1205–1213.
469 Matos, M.E., & Rosell, C.M. (2011). Chemical composition and starch digestibility of different gluten
470 free breads. *Plant Foods for Human Nutrition, 66*, 224–230.
471 Matos, M.E., & Rosell, C.M. (2013). Quality Indicators of Rice-Based Gluten-Free Bread-Like Products:
472 Relationships Between Dough Rheology and Quality Characteristics. *Food and Bioprocess Technology,*
473 *6*, 2331–2341.
474 Matos, M.E., & Rosell, C.M. (2015). Understanding gluten-free dough for reaching breads with physical
475 quality and nutritional balance. *Journal of the Science of Food and Agriculture, 95*(4), 653-661.

476 Miranda, J., Lasa, A., Bustamante, M.A, Churruca, I., & Simon, E. (2014). Nutritional differences
477 between a gluten-free diet and a diet containing equivalent products with gluten. *Plant Foods for Human*
478 *Nutrition, 69*(2), 182-187.
479 Moreira, R., Chenlo, F., & Torres, M. D. (2013). Rheology of Gluten-Free Doughs from Blends of
480 Chestnut and Rice Flours. *Food and Bioprocess Technology, 6*(6), 1476-1485.
481 Moroni, A. V., Dal Bello, F., & Arendt, E. K. (2009). Sourdough in gluten-free bread-making: An ancient
482 technology to solve a novel issue?. *Food Microbiology, 26*(7), 676-684.
483 Niba, L. L., Bokonga, M.M., Jackson, E.L., Schlimme, D.S. & Li, B.W. (2001). Physicochemical
484 properties and starch granular characteristics of flour from various Manihot esculenta (cassava) genotypes
485 *Journal of Food Science, 67(5)*, 1701–1705.
486 Novotni, D., Cukelj, N., Smerdel, B., Bituh, M., Dujmic, F., & Curic, D. (2012). Glycemic index and
487 firming kinetics of partially baked frozen gluten-free bread with sourdough. *Journal of Cereal Science,*
488 *55*(2), 120-125.
489 Omary, M. B., Fong, C., Rothschild, J., & Finney, P. (2012). Effects of Germination on the Nutritional
490 Profile of Gluten-Free Cereals and Pseudocereals: A Review. *Cereal Chemistry, 89*(1), 1-14.
491 Parada, J., & Aguilera, J. M. (2011). Review: Starch matrices and the glycaemic response. *Food Science*
492 *and Technology International, 17*, 187–204.
493 Phimolsiripol, Y., Mukprasirt, A., & Schoenlechner, R. (2012). Quality improvement of rice-based
494 gluten-free bread using different dietary fibre fractions of rice bran. *Journal of Cereal Science, 56*(2),
495 389-395.
496 Protonotariou, S., Mandala, I., & Rosell, C.M. (2015). Jet Milling Effect on Functionality, Quality and In
497 Vitro Digestibility of Whole Wheat Flour and Bread. *Food and Bioprocess Technology, 8*(6), 1319-1329.
498 Protonotariou, S., Drakos, A., Evageliou, V., Ritzoulis, C., & Mandala, I. (2014). Sieving fractionation
499 and jet mill micronization affect the functional properties of wheat flour. *Journal of Food Engineering,*
500 *134*, 24-29
501 Renzetti, S., & Rosell, C.M. (2016). Role of enzymes in improving the functionality of proteins in non-
502 wheat dough systems. *Journal of Cereal Science, 67*, 35–45.
503 Sanguansri, P., & Augustin, M.A. (2006). Nanoscale materials development-a food industry perspective.
504 *Trends in Food Science & Technology, 17*(10), 547–556.

505 Sciarini, L. S., Ribotta, P.D., León, A.E., & Pérez, G.T. (2010). Effect of hydrocolloids on gluten-free
506 batter properties and bread quality. *International Journal of Food Science & Technology, 45*(11), 2306–
507 2312.
508 Sciarini, L. S., Ribotta, P. D., Leon, A. E., & Pérez, G. T. (2012). Incorporation of several additives into
509 gluten free breads: Effect on dough properties and bread quality. *Journal of Food Engineering, 111*(4),
510 590-597.
511 Segura, M. E. M., & Rosell C.M. 2011. Chemical Composition and Starch Digestibility of Different
512 Gluten-free Breads. *Plant Foods for Human Nutrition, 66*(3), 224-230.
513 Storck, C. R., da Rosa Zavareze, E., Gularte, M. A., Elias, M. C., Rosell, C. M., & Dias, A. R. G. (2013).
514 Protein enrichment and its effects on gluten-free bread characteristics. *LWT - Food Science and*
515 *Technology, 53*(1), 346-354.
516 Thompson, T. (2009). The Nutritional Quality of Gluten-Free Foods, in Gluten-Free Food Science and
517 Technology (ed E. Gallagher) (pp 42-51). Wiley-Blackwell, Oxford, UK.
518 doi:10.1002/9781444316209.ch4
519 Trappey, E. F., Khouryieh, H., Aramouni F., & Herald, T. (2015). Effect of sorghum flour composition
520 and particle size on quality properties of gluten-free bread. *Food Science and Technology International,*
521 *21*(3), 188-202.
522 Tsatsaragkou, K., Gounaropoulos, G., & Mandala, I. (2014a). Development of gluten free bread
523 containing carob flour and resistant starch. *LWT- Food Science and Technology, 58*, 124–129.
524 Tsatsaragkou, K., Yiannopoulos, S., Kontogiorgi, A., Poulli, E., Krokida, M., & Mandala, I. (2012).
525 Mathematical approach of structural and textural properties of gluten free bread enriched with carob flour.
526 *Journal of Cereal Science, 56*, 603–609.
527 Tsatsaragkou, K., Yiannopoulos, S., Kontogiorgi, A., Poulli, E., Krokida, M., & Mandala, I. (2014b).
528 Effect of Carob Flour Addition on the Rheological Properties of Gluten-Free Breads. *Food and*
529 *Bioprocess Technology, 7*(3), 868-876.
530 Vallons, K. J. R., Ryan L. A. M., & Arendt, E. K. (2011). Promoting structure formation by high pressure
531 in gluten-free flours. *LWT-Food Science and Technology, 44*(7), 1672-1680.
532 Zhu, K., Huang, S., Peng, W., Qian, H., & Zhou, H. (2010). Effect of ultrafine grinding on hydration and
533 antioxidant properties of wheat bran dietary fiber. *Food Research International, 43*(4), 934–938.

534 Ziobro, R., Korus, J., Witczak, M., & Juszczak, L. (2012). Influence of modified starches on properties of
535 gluten-free dough and bread. Part II: Quality and staling of gluten-free bread. *Food Hydrocolloids, 29*(1),
536 68-74.
537

538 Table 1. Amount of ingredients added for making the different breads.

Bread code	Flour type (g)	Water (g)	Yeast (g)	Egg white powder (g)	Whey protein powder (g)	Shortening (g)	Sugar(g)	Salt (g)	DATEM (g)	LBG (g)
Control	Rice flour 267	320.40	16.02	10.68	10.68	9.34	8.01	5.34	1.33	1.33
BA	Rice /Carob flour 202.00/ 35.65	356.47	14.25	9.50	9.50	8.31	7.12	4.75	1.18	1.18
BB	Rice /Carob flour 209.67/ 37.00	345.33	14.80	9.86	9.86	8.63	7.40	4.93	1.23	1.23
BC	Rice /Carob flour 226.95/ 40.05	320.40	16.02	10.68	10.68	9.34	8.01	5.34	1.33	1.33
BD	Rice /Carob flour 217.95/ 38.46	333.33	15.38	10.25	10.25	8.97	7.69	5.12	1.28	1.28
GF C1	C1 flour mixture 338.54	270.83	13.54	-	-	13.54	10.15	3.38	-	-
GF C2	C2 flour mixture 336.78	269.42	23.57	-	-	20.20	-	-	-	-
GF C3	C3 flour mixture 320.82	282.32	19.24	-	-	21.17	-	6.41	-	-

539

540

541

542

543

544

545

Table 2. Physical and chemical characteristics of flour samples used.

Samples	D_{50} (μm)	Moisture (%)	Protein (% w.b.)	Dietary Fibre (% w.b.)
Carob flour fractions				
FA	258.55 ± 0.68 [a]	9.39 ± 0.01 [a]	14.93 ± 0.24 [a]	65.61 ± 2.34 [c]
FB	174.73 ± 0.45 [b]	8.75 ± 0.05 [a]	22.96 ± 0.74 [c]	51.80 ± 2.67 [b]
FC	126.37 ± 2.10 [c]	9.28 ± 0.07 [a]	25.70 ± 0.03 [d]	43.46 ± 1.45 [a]
FD	80.36 ± 6.38 [d]	9.82 ± 0.06 [b]	18.86 ± 0.09 [b]	53.25 ± 1.64 [b]
Rice /Carob flour blend (85/15)		WHC (ml/g)		
MA		1.73 ± 0.10 [d]	8.5	10.2
MB		1.92 ± 0.06 [e]	9.7	8.1
MC		1.72 ± 0.11 [d]	10.1	6.9
MD		1.65 ± 0.07 [cd]	9.1	8.4
Commercial GF blends				
C1		1.53 ± 0.12 [bc]	2.3	-
C2		0.87 ± 0.04 [a]	5.5	6.3
C3		1.41 ± 0.11 [b]	3.5	4.7

In parentheses standard deviation values. Samples with different letters in the same column differ significantly ($P < 0.05$). D_{50}, moisture, protein, dietary fibre and WHC values are means of duplicates followed by standard deviation.

Table 3 Physical properties of the fresh gluten free breads

Bread code	Specific volume (cm^3/g)	L^*	a^*	b^*	Average cell size (mm^2)	Cell density ($cells/cm^2$)
Control	1.62 ± 0.16^{a}	81.7 ± 0.92^{e}	-4.89 ± 0.36^{a}	14.7 ± 0.38^{b}	0.23 ± 0.07^{a}	64 ± 5^{c}
BA	2.22 ± 0.07^{b}	63.3 ± 2.38^{c}	2.36 ± 0.01^{e}	15.1 ± 1.49^{b}	2.61 ± 1.36^{c}	17 ± 6^{a}
BB	1.70 ± 0.07^{a}	61.7 ± 1.06^{bc}	4.43 ± 0.28^{f}	19.6 ± 0.71^{c}	2.01 ± 0.83^{bc}	21 ± 3^{ab}
BC	1.61 ± 0.12^{a}	61.0 ± 2.05^{b}	4.16 ± 0.48^{f}	19.4 ± 0.66^{c}	1.43 ± 0.21^{b}	25 ± 3^{b}
BD	2.21 ± 0.13^{b}	56.6 ± 2.06^{a}	5.37 ± 0.35^{g}	21.5 ± 0.56^{d}	1.97 ± 0.67^{bc}	19 ± 2^{a}
GF C1	3.37 ± 0.19^{d}	74.4 ± 1.70^{d}	-9.20 ± 0.42^{a}	29.1 ± 0.86^{e}	1.43 ± 0.71^{b}	17 ± 7^{a}
GF C2	2.96 ± 0.03^{c}	76.3 ± 1.13^{d}	-3.06 ± 0.42^{c}	21.1 ± 1.90^{d}	2.65 ± 0.89^{c}	14 ± 3^{a}
GF C3	3.95 ± 0.12^{e}	62.9 ± 2.66^{c}	-2.22 ± 0.15^{d}	12.5 ± 0.60^{a}	2.93 ± 0.93^{c}	14 ± 2^{a}

Samples with different letters in the same column differ significantly ($P < 0.05$). Values are means of quadruplicates followed by standard deviation.

Table 4. Chemical composition (expressed as percentage) of gluten free breads made with different fractions of carob flour, compared to commercial ones.

Sample code	Moisture content	Protein	Insoluble Dietary Fibre	Total Dietary Fibre	Ash
Control	58.27 ± 1.13 ^{b}	9.06 ± 0.05 ^{e}	3.28 ± 0.14 ^{bc}	3.79 (0.00)^{abc}	0.54 ± 0.02 ^{b}
BA	65.36 ± 2.63 ^{de}	7.80 ± 0.00 ^{d}	4.58 ± 1.43 ^{c}	6.26 (0.91)^{c}	0.56 ± 0.03 ^{b}
BB	65.89 ± 0.27 ^{e}	9.79 ± 0.02 ^{f}	4.21 ± 2.02 ^{c}	5.79 (0.49)^{bc}	0.71 ± 0.03 ^{c}
BC	61.69 ± 1.02 ^{c}	9.99 ± 0.05 ^{f}	2.84 ± 0.04 ^{bc}	4.69 (2.56)^{abc}	0.76 ± 0.00 ^{c}
BD	63.11 ± 1.13 ^{cd}	9.06 ± 0.04 ^{e}	3.52 ± 0.00 ^{bc}	3.53 (0.00)^{abc}	0.74 ± 0.05 ^{c}
GF C1	53.30 ± 1.22 ^{a}	2.42 ± 0.13 ^{a}	1.66 ± 0.72 ^{ab}	2.02 (2.11)^{a}	0.44 ± 0.03 ^{a}
GF C2	53.77 ± 0.76 ^{a}	4.74 ± 0.03 ^{c}	0.36 ± 0.10 ^{a}	5.14 (0.69)^{bc}	1.34 ± 0.03 ^{d}
GF C3	54.13 ± 1.98 ^{a}	3.07 ± 0.23 ^{b}	3.06 ± 0.01 ^{bc}	3.21 (0.80)^{ab}	0.73 ± 0.04 ^{c}

Samples with different letters in the same column differ significantly ($P < 0.05$). Values are means of duplicates followed by standard deviation.

Table 5. Crumb firming of different gluten free breads during storage at room temperature.

Sample code	Crumb hardness (N)				Firming rate (N/day)	R^2	Crumb moisture loss (%/day)
	0 day	1st day	2nd day	3rd day			
Control	15.9 ± 1.4 [d]	21.4 ± 0.4	32.4 ± 0.6	41.7 ± 3.0	8.84 ± 0.50 [e]	0.984	3.88
BA	5.1 ± 0.1 [b]	7.2 ± 0.4	8.9 ± 0.6	9.6 ± 0.6	1.54 ± 0.19 [abc]	0.957	1.26
BB	9.4 ± 0.7 [c]	11.1 ± 0.7	12.1 ± 0.1	12.5 ± 0.9	1.02 ± 0.21 [a]	0.931	2.81
BC	16.1 ± 0.1 [d]	17.0 ± 0.8	18.8 ± 0.9	19.7 ± 1.5	1.27 ± 0.48 [ab]	0.974	2.03
BD	5.7 ± 0.6 [bc]	6.1 ± 0.1	7.2 ± 0.6	8.4 ± 0.8	0.94 ± 0.42 [a]	0.949	0.97
GF C1	0.6 ± 0.1 [a]	3.7 ± 0.6	6.1 ± 1.7	9.8 ± 1.1	3.01 ± 0.42 [d]	0.990	3.86
GF C2	3.8 ± 0.6 [ab]	5.7 ± 0.1	8.1 ± 0.8	9.6 ± 0.5	1.95 ± 0.44 [bc]	0.994	3.35
GF C3	0.6 ± 0.2 [a]	3.2 ± 0.6	5.1 ± 0.1	7.4 ± 0.1	2.22 ± 0.06 [cd]	0.994	4.55

Samples with different letters in the same column differ significantly ($P < 0.05$). Values are means of quadruplicates followed by standard deviation.

Table 6. Kinetic parameters of the *in vitro* starch hydrolysis, estimated glycemic index, and *in vitro* starch digestibility of gluten free breads

Sample code	C_∞ (g/100 g)	K (min^{-1})	*eGI*	Free glucose (g/100 g as is)	Resistant starch (g/100 g as is)	Digestible starch (g/100 g as is)
Control	64.92 ± 0.85 ^{cde}	0.019 ± 0.002	119.03 ± 3.25 ^{e}	0.39 ± 0.04 ^{a}	6.36 ± 0.01 ^{b}	36.14 ± 0.99 ^{e}
BA	74.94 ± 15.59 ^{e}	0.009 ± 0.003	92.34 ± 1.97 ^{cd}	0.20 ± 0.04 ^{a}	6.74 ± 0.12 ^{b}	26.99 ± 1.12^{bc}
BB	68.51 ± 5.20 ^{de}	0.013 ± 0.006	104.19 ± 15.08 ^{d}	0.37 ± 0.09 ^{a}	6.45 ± 0.17 ^{b}	28.78 ± 2.45 ^{cd}
BC	52.15 ± 10.91 ^{bcd}	0.016 ± 0.018	62.86 ± 1.87 ^{b}	0.16 ± 0.00 ^{a}	5.97 ± 1.03 ^{ab}	20.61 ± 1.40 ^{a}
BD	30.04 ± 1.01^{a}	0.010 ± 0.007	47.33 ± 0.10 ^{a}	0.25 ± 0.06 ^{a}	4.55 ± 0.50 ^{a}	25.45 ± 1.75 ^{bc}
GF C1	44.54 ± 5.72 ^{abc}	0.019 ± 0.005	82.76 ± 2.64 ^{c}	0.21 ± 0.04 ^{a}	8.97 ± 0.02 ^{c}	31.11 ± 1.51 ^{d}
GF C2	30.13 ± 7.89 ^{abc}	0.009 ± 0.008	55.90 ± 1.13 ^{ab}	1.03 ± 0.30 ^{a}	4.57 ± 1.10 ^{a}	23.3 ± 2.39 ^{ab}
GF C3	35.71 ± 0.93^{ab}	0.009 ± 0.007	60.85 ± 4.38 ^{b}	3.24 ± 0.28 ^{b}	6.20 ± 0.75 ^{b}	26.54 ± 0.99 ^{bc}

Samples with different letters in the same column differ significantly ($P < 0.05$). Values are means of quadruplicates followed by standard deviation.

Table 7. Sensorial characteristics of gluten free breads

Sample code	Appearance	Colour	Flavour (aroma- taste)	Texture	Overall Acceptability
BA	6.23 ± 1.76 [a]	6.23 ± 1.67 [a]	5.23 ± 1.94 [a]	5.73 ± 2.03[a]	5.67 ± 1.82 [a]
BD	6.21 ± 1.63 [a]	6.34 ± 1.59 [a]	5.15 ± 1.82 [a]	5.58 ± 1.91 [a]	5.71 ± 1.64 [a]
GF C1	6.50 ± 1.36 [a]	5.84 ± 1.26 [a]	5.02 ± 1.65 [a]	5.08 ± 1.40 [a]	5.26 ± 1.51 [a]
GF C2	6.82 ± 1.73 [ab]	6.50 ± 1.97 [a]	5.28 ± 1.81 [a]	5.39 ± 1.78 [a]	5.41 ± 1.70 [a]
GF C3	7.23 ± 1.30 [b]	7.47 ± 1.34 [b]	7.21 ± 2.17 [b]	7.60 ± 1.75 [b]	7.26 ± 1.97 [b]

Samples with different letters in the same column differ significantly ($P < 0.05$). Values are means of 50 ratings followed by standard deviation.

Figure 1. Photos of the produced gluten free carob breads and commercial gluten free breads