Running Head: Gluten Free Breads Composition

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Title: Chemical Composition and Starch Digestibility of Different Gluten Free Breads

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Abstract The increasing demand for gluten free products has favoured the design of numerous gluten free bakery products which intended to mimic the quality characteristics of wheat bakery products. The objective of this study was to evaluate the nutritional pattern of gluten free breads representative of the Spanish market for this type of products. The protein, fat and mineral content of the gluten free breads showed great variation, ranging from 0.91g/100g to 15.05g/100g, 2.00g/100g-26.10g/100g and 1.10g/100g to 5.43g/100g, respectively. Gluten free breads had very low contribution to the recommended daily protein intake, with a high contribution to the carbohydrate dietary reference intake. Dietary fiber content also showed great variation varying from 1.30g/100g to 7.20g/100g. *In vitro* enzymatic hydrolysis of starch showed that the most predominant fraction was the rapidly digestible starch that varied from 75.6 g/100g to 92.5g/100g. Overall, gluten free breads show great variation in the nutrient composition, being starchy based foods low in proteins and high in fat content, with high glycaemic index.

Key words Gluten free bread, Nutrient composition, Fibers, Starch digestibility.

Introduction

Bread has been regarded for centuries as one of the most popular and appealing food product both because of its relative high nutritional value and its unique sensory characteristics (texture, taste, and flavor). However, an increasing number of individuals are suffering from celiac disease (CD), the life-long intolerance to the gluten fraction of wheat, rye and barley. In particular, celiac patients are intolerant to some cereal prolamins containing specific toxic oligopeptide sequences. The gliadin fraction of wheat, secalins of rye, hordeins of barley, and possibly avenins of oats are involved in the CD mechanism.

In CD patients, ingestion of gluten leads to inflammation and mucosal damage of the small intestine. The typical lesion in the small intestinal epithelium is villous atrophy with crypt hyperplasia, leading to malabsorption of most nutrients including iron, folic acid, calcium, and fat-soluble vitamins [1]. This can lead to associated diseases such as osteoporosis, anaemia and type I diabetes and skin disorders [2]. An acceptable treatment is strict adherence to a 100g/100g gluten-free diet for life, which results in clinical and mucosal recovery. Nevertheless, the manufacture of bread without gluten results in major problems for bakers, and currently, many gluten free products available on the market are of low quality.

In recent years there has been increasing interest on gluten-free breads. A large number of flour and starches as well as many ingredients such as gums, enzymes, soybean proteins, and have been used to mimic the viscoelastic properties of gluten and contribute to improved structure mouthfeel, acceptability, and shelf life of gluten free breads [3-6]. In such studies various technological parameters and formulations have been extensively investigated for making good quality gluten free bread. However, the nutritional concept of the gluten free baked goods has been scarcely addressed. Some approaches have considered the use of mixed amaranth flours for making gluten free breads and cookies [7] or even blends of plantain and legume flours [8], obtaining gluten free products with high nutritional value and acceptable quality , and also protein enrichment of gluten free breads has been carried out by incorporating soy protein isolates [9].

Historically, nutrition counseling for celiac disease has focused on the foods to avoid in a gluten free diet but they should be advised on the nutritional quality of gluten- free. There are growing concerns over the nutritional adequacy of the GF dietary pattern because it is often characterized by an excessive consumption of proteins, and fats, and a reduced intake of complex carbohydrates, dietary fibre, vitamins and minerals [1,10]. As a consequence, the long life adherence to gluten free products has been associated to undernourished and also minerals deficiencies that could conduct to anemia, osteopenia or osteoporosis [10].

The aim of this work was to evaluate the nutritional pattern of gluten free breads regarding their chemical composition in order to determine their contribution to the daily intake of nutrients. Special emphasis has been addressed to the fiber content of those breads and also to the *in vitro* starch digestibility due to their always high content in starch.

Materials and Methods

Materials

Gluten-free breads (GFB) from the major brands of these specialties were acquired in the Spanish market. Those breads were representative of the most consumed products in Spain. Eleven kinds of gluten-free breads were selected and purchased in general and specialized supermarkets. Duplicates of each sample from different batch were used for the characterization. Information on the ingredient composition, according to the labeling, is given in Table 1. α-Amylase from porcine pancreas (Pancreatin, Cat. No. P-1625, activity

3_USP/g) was 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).

Analytical methods

The chemical composition of GFB samples was determined according to ICC corresponding standard methods [11]. Total carbohydrates were determinate by difference subtracting 100 g minus the sum of protein, ash and fat expressed in grams/100 grams [12]. For the estimation of dietary fiber, samples were finally powdered to pass through a sieve of 250 µm. Total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) contents were determined following the AACC method [13]. Determinations were done in triplicate for obtaining mean values.

In vitro starch digestibility and estimated glycaemic index

Gluten free breads were frozen, freeze-dried and ground in a blender. Starch digestibility of gluten free bread was determined in the powders using AACC methods [13], with the modification reported by Arocha Gularte and Rosell [14]. According to the hydrolysis rate of starch, three different fractions were quantified as suggested Englyst et al. [15]. Rapidly digestible starch (RDS) was referred to the percentage of total starch that was hydrolyzed within 30 min of incubation, slowly digestible starch (SDS) was the percentage of total starch hydrolyzed within 30 and 120 min, and resistant starch (RS) was the starch remaining unhydrolyzed after 16 h of incubation. The percentage of total starch hydrolyzed at 90 minutes (H90) was also calculated.

The *in vitro* digestion kinetics was calculated in accordance with the procedure established by Goñi et al. [16]. A non-linear model following the equation [$C = C_\infty(1 - e^{-kt})$] was applied to describe the kinetics of enzymatic hydrolysis, where C was the concentration at t time, C_∞ was the equilibrium concentration or maximum hydrolysis extent, k was the kinetic constant and t was the time chosen. 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 bread) over the same period of time. The expected glycaemic index (eGI) was calculated using the equation described by Granfeldt et al. [17]: eGI = 8.198 + 0.862HI.

Statistical analysis

The results were expressed as mean values ± standard deviation. Data were analyzed using one-way analysis of variance (ANOVA) to determine whether there was significant difference between gluten-free breads types by using Statgraphics Plus V 7.1 program (Statistical Graphics Corporation, UK). Fisher's least significant differences (LSD) test was used to differentiate means with 95% confidence.

Results and discussion

Chemical Composition

Commercial gluten free breads, according to suppliers' information (Table 1), were based on corn starch, potato starch, or rice flour, either enriched with milk solids, soy protein, eggs or lupine proteins. All of them contained corn starch as main ingredient, with the exception of

GFB4 that was based on potato starch and GFB6 also contained rice flour. Other differences among breads were encountered in the protein source. Eggs were the most common source of proteins, but also caseinate (GFB4), soy (GFB4) or lupine proteins (GFB6) were present. Some types of bread (GFB8, GFB9, GFB10 and GFB11) did not contain any source of proteins among the ingredients. Vegetable oil or margarine was present in the formulations, with exception of GFB8 and GFB9 that did not contain any fat source. Yeast and raising agents were used in combination as leaving agents, with the exception of GFB2, GFB3 and GFB4 that only contained yeast. In addition, salt, emulsifiers, preservatives and a variety of other food grade additives were present in the formulations.

There was important significant differences ($p<0.05$) among the proximate composition of all the GFB samples (Table 2). The protein content of GBF, which ranged from 0.91g/100g to 15.05g/100g, was found to be the highest in GFB4 while GFB9 closely followed by GFB8 showed the lowest values. This increase in the protein content must be associated to the presence of milk and soy proteins in the formulation, since those ingredients are used as protein sources in gluten free breads [9, 18]. GFB8 and GFB9 presented the lowest values of fat content (2.00g/100g), which agrees with the absence of fat ingredient in the formulation. Conversely, GFB10 showed the highest fat value (26.10g/100g), followed by GFB11, GFB2 and GFB3 due to the contribution of the vegetal oil or margarine in these gluten free bread formulations. Large variations were observed in ash contents that ranged from 1.10g/100g to 5.43g/100g. GFB9 had the highest ash content, mainly derived from the level of salt. The total carbohydrate content varied from 68.42g/100g to 92.96g/100g. The different proximal composition of GFB commercial samples studies could be affected by many factors such as the wide range of complex ingredients added and their combinations, besides the additives used to improve the structure, mouthfeel, acceptability and shelf-life of these products [4, 9]. Recently, Yazynima et al. [19] reported the nutritional composition of two kinds of gluten free crispy breads, which contained 3.5-6.0g/100g of proteins, 3.0-6.5g/100g of fats and 80-71g/100g of carbohydrates. The present study shows that marketed gluten free breads are carbohydrate based products. They have great variation in their protein, fat and mineral content, in contrast to the very narrow variation in the proximate composition observed in wheat based bread products [20].

Contribution to dietary reference intakes (DRIs)

Table 3 shows the contribution of macronutrients, protein and carbohydrates intakes (g/100g), to the relevant DRIs consuming an average portion (200g) of gluten free breads. Considering the Dietary Reference Intakes (DRIs) [21] of an adult male and female, an average daily portion of bread (200g) would meet 2.2-39.2g/100g and 2.7- 47.7g/100g of DRIs for proteins, respectively (Table 3). GFB4 showed the highest value of DRIs for proteins on both male (47.7g/100g) and female (39.2g/100g). Only that sample gives a similar protein contribution to that reported for white wheat bread (35.7 g/100g and 43.5g/100g of DRI for male and female when consuming a 200g portion, respectively) [22]. Very low contribution to the recommended daily protein intake could be obtained with the consumption of the other evaluated breads. Regarding the intakes for carbohydrates, the contribution to DRIs ranged from 53.7g/100g to 109.2g/100g, obtaining the highest value with GFB8. Considering that white wheat bread provide an average of 43g/100g of carbohydrate [20] and thus the contribution of a 200 g portion to the carbohydrate DRI will be around 66g/100g, studied gluten free breads are richer in carbohydrates, with the exception of GFB10 and GFB11. Therefore, 200-gram portion of gluten free breads has higher contribution to the carbohydrate dietary reference intake than their wheat containing counterparts.

Soluble, insoluble and total dietary fibre

TDF ranged from 3.60g/100g to 7.20g/100g, except for GFB3 (1.30g/100g) and GFB8 (2.00g/100g) samples, showing that all gluten free breads contained good amount of dietary fiber (>3g/100g) (Figure 1). High values of TDF and SDF were obtained in GFB7, GFB10 and GFB11 samples. In general, gluten free bread samples showed higher amount of soluble dietary fiber than insoluble dietary fraction. The clear exception to the last statement was GFB4 and GFB9, in which 83% and 71% of the total dietary fiber were insoluble, respectively. Values obtained for these gluten free breads slightly differ from those reported by Korus et al. [23], when studied the addition of resistant starch to gluten free formulations as fiber source. Those authors found values of IDF, SDF and TDF in gluten free breads that ranged 2.77- 4.99g/100g, 1.23-1.45g/100g and 3.61-6.30g/100g, respectively. Formulations of GFB usually contain gums or hydrocolloids used as thickeners or stabilizers. Hydrocolloids like xanthan gum, guar gum, carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC), pectin, or varied combinations of those hydrocolloids contained in the formulations might improve the content of TDF, contributing to increase the level of soluble dietary fibers.

Thompson [24] reported values of dietary fiber in commercial gluten free bread samples from 1.2 to 5.6 g/100g, whereas in fiber enriched bread those values varied from 6.1 to 9.6 g/100. Only for comparative purposes, it is worthy to note that white bread contains 0.81g/100g, 3.13g/100g and 3.84g/100g of IDF, SDF and TDF, respectively [25].

Starch digestibility in gluten free breads

The most predominant starch fraction was the RDS that varied from 75.6 g/100g to 92.5g/100g of the total starch (Figure 2). This pattern agrees with the one reported for starchy foods, where starch is highly gelatinised and product structure is very porous, resulting in rapid degradation of starch in small intestine and very rapid rise of blood glucose level (high GI) [26]. SDS and RS of GFB samples ranged between from 2.4g/100g - 21.1g/100g, and 1.0g/100g -2.9g/100g, respectively. GFB9 showed the highest value of SDS content (21.1g/100g), which is more desirable than RDS. SDS is slowly digested in the small intestine and induces gradual increase of postprandial plasma glucose and insulin levels [27], although Englyst et al. [15] reported that the breakdown of solid starchy foods could predict the postprandial response in vivo but SDS has limited effect on the glycaemic response although it is available as sugar.

Kinetic of the *in vitro* starch hydrolysis and expected glycaemic index

Primary and secondary parameters derived from the *in vitro* digestion of the gluten free breads evaluated are listed in Table 4. The maximum hydrolysis, C_{∞}, or hydrolysis degree when the enzymatic reaction reaches a plateau, of gluten free breads was very high, which was associated with the high levels of rapidly hydrolyzed starch. The kinetic constant (*k*), indicative of the hydrolysis rate in the early stage, showed significant differences among the GFBs. The lowest values were observed in GFB9 and GFB11, which were the samples with higher fractions of slowly digested starch. Gelencsér et al. [28] reported values of rate constant comprised between 0.015 and 0.025 (min^{-1}) in pasta products and the addition of resistant starch did not significantly modify that constant. Therefore, higher kinetic constant is obtained for gluten free breads than those determined for pasta, showing the high susceptibility of these starchy products to enzymatic hydrolysis.

The hydrolysis index (HI) of GFBs ranged from 87 to 100 and estimated glycaemic index (eGI) values were between 83.3 and 96.1. All samples showed very high *in vitro* starch digestibility index, being practically hydrolyzed between 60 to 90 min of assay, as indicated the H90. Differences among breads should be attributed to variations in composition (Table 1 and 2). Bernal et al. [29] also observed slightly higher digested starch in gluten free infant cereals. That result was due to the higher starch digestibility of rice and corn (103.98g/100g for rice and 107.05g/100g for corn) compared to white bread (100g/100g) [28]. Therefore, although GFBs are mainly starchy foodstuff, the very complex formulation of those breads might be responsible of the reduction observed in those values. In fact, the glycaemic response to bread varies widely according to the type of bread studied [30]. Low to moderate GI (<70) are considered favorable to health. The glycaemic index could vary from 27 (barley bread with 75g/100g substitution) to 95 (extremely porous French baguette). This extreme variability reflects very different rates of starch digestion. The starch from a French baguette is rapidly digested, leading to glycaemic response close to that of glucose (GI=100), whereas starch from bread containing intact cereal grains is digested more slowly [30]The results obtained in the present study showed that all samples could be considered as food with rapidly digested starch and high glycaemic index. The number and variety of ingredients of gluten free bread can be considered important factors that will determine the starch digestibility.

Conclusions

The nutritional evaluation of different commercial gluten free breads revealed that they are mainly starchy foods with great divergences in fat and protein composition, due to the occasional protein enrichment. In consequence, these products have very low contribution to the recommended daily protein intake, but higher contribution to the carbohydrate dietary reference intake than their gluten containing counterpart. The majority of gluten free breads evaluated contained good amount of dietary fiber (>3g/100g), and in most cases the amount of soluble dietary fiber was higher than the insoluble dietary fraction. The presence of hydrocolloids needed in the formulation of these products could be partially responsible of that pattern. The *in vitro* hydrolysis of the starch of the gluten free breads showed that RDS was the major starch fraction distantly followed by SDS and RS, indicating the high starch digestibility. The estimated glycaemic index of the gluten free breads varied between 83.3 and 96.1, thus all samples could be considered as food with high glycaemic index. Overall, gluten free breads shows great variation in the nutrient composition, being starchy based foods low in proteins and high in fat content.

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References

1. Catassi C, Fasano A (2008) Celiac disease. In Arendt EA, Dal Bello F (eds), Gluten-Free Cereal Products and Beverages. Amsterdam/Boston/Heidelberg/London/New York/Oxford/Paris/San Diego/San Francisco/Singapore/Sydney/Tokyo: Elsevier, pp 1-27.
2. Pruska-Kędzior A, Kędzior Z, Gorący M, Pietrowska K, Przybylska A, Spychalska K (2008) Comparison of rheological, fermentative and baking properties of gluten-free dough formulations. Eur Food Res Technol 227:1523-1536.
3. Gujral H, Rosell CM (2004) Functionality of rice flour modified by microbial transglutaminase. J Cereal Sci 39:225-230.

4. Lazaridou A, Duta D, Papageorgiou M, Belc N, Biliaderis CG (2007) Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. J Food Eng 79:1033-1047.

5. Marco C, Rosell CM (2008a) Functional and rheological properties of protein enriched gluten free composite flours. J Food Eng 88:94-103.

6. Ribotta PD, Ausar SF, Morcillo MH, Pérez GT, Beltrano DM, León AE (2004) Production of gluten-free bread using soybean flour. J Sci Food Agric 84:1969-1974.

7. Calderón de la Barca AM, Rojas-Martínez ME, Islas-Rubio AR, Cabrera-Chávez F (2010) Gluten-free breads and cookies of raw and popped amaranth flours with attractive technological and nutritional qualities. Plant Food Hum Nutr 65:241–246.

8. Akubor PI, Adamolekun FO, Oba CA, Obari H, Abudu IO (2003) Chemical composition and functional properties of cowpea and plantain flour blends for cookie production. Plant Foods Hum Nutr 58:1-9.

9. Marco C, Rosell CM (2008b) Breadmaking performance of protein enriched, gluten-free breads. Eur Food Res Technol 227:1205-1213.

10. Thompson T, Dennis M, Higgins LA, Lee AR, Sharret MK (2005) Gluten-free diet survey: are Americans with celiac disease consuming recommended amounts of fibre, iron, calcium and grain foods? J Hum Nutr Dietetics 18:173–179.

11. ICC (1994) Official Methods of Analysis, Vienna: International Association for Cereal Chemistry.

12. FAO (2003) Food energy-methods of analysis and conversion factors, Rome: Food and Agriculture Organization of the United Nations.

13. AACC International (2000) Approved Methods, 11th edn. St Paul, MN: American Association of Cereal Chemists International.

14. Arocha Gularte M, Rosell CM (2011) Physicochemical properties and enzymatic hydrolysis of different starches in the presence of hydrocolloids. Carbohyd Polym 85:237-244.

15. Englyst HN, Veenstra J, Hudson GJ (1996) Measurement of rapidly available glucose (RAG) in plant foods: a potential in vitro predictor of the glycaemic response. Br J Nutr 75:327-337.

16. Goñi I, Garcia-Alonso A, Saura-Calixto F (1997) A starch hydrolysis procedure to estimate glycemic index. Nutr Res 17:427-437.

17. 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. Eur J Clin Nutr 46:649–660.

18. Moore MM, Schober TJ, Dockery P, Arendt EK (2004) Textural comparisons of gluten-free and wheat-based doughs, batters, and breads. Cereal Chem 81:567-575.

19. Yazynina E, Johansson M, Jägerstad M, Jastrebova J (2008) Low folate content in gluten-free cereal products and their main ingredients. Food Chem 111:236–242.

20. Rosell CM (2011) The science of doughs and bread quality. In Preedy VR, Watson RR, Patel VB (eds), Flour and Breads and Their Fortification in Health and Disease Prevention. London/Burlington/San Diego: Elsevier, pp 3-14.

21. NRC (2001) Dietary reference Intakes table-The complete set http:www.iom.edu/?id=21381.

22. Škrbić B, Milovac S, Dodig D, Filipčev B (2009) Effects of hull-less barley flour and flakes on bread nutritional composition and sensory properties. Food Chem 115:982-988.

23. Korus J, Witczak M, Ziobro R, Juszczak L (2009) The impact of resistant starch on characteristics of gluten-free dough and bread. Food Hydrocolloids 23:988–995.

24. Thompson T (2000) Folate, iron, and dietary fiber contents of the gluten-free diet. J Am Diet Assoc 100:1389-1396.

25. Saura-Calixto F, García-Alonso A, Goñi I, Bravo L (2000) In vitro determination of the indigestible fraction in foods: An alternative to dietary fiber analysis. J Agric Food Chem 48:3342-3347.

26. Poutanen K, Flander L, Katina K (2009) Sourdough and cereal fermentation in a nutritional perspective. Food Microbiol 26:693–699.

27. Jenkins DJA, Wolever TMS, Leeds AR, Gassull MA, Haisman P, Dilawari J, Goff DV, Metz GL, Alberti KM (1978) Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. Br Med J 1:1392-1394.

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

29. Bernal MJ, Periago MJ, Ros G (2002) Effects of processing on dextrin, total starch, dietary fiber and starch digestibility in infant cereals. J Food Sci 67:1249-1254.

30. Fardet A, Leenhardt F, Lioger D, Scalbert A, Rémésy C (2006). Parameters controlling the glycaemic response to breads. Nutr Res Rev 19:18–25.

Figure Captions

Figure 1. Total, soluble and insoluble dietary fiber (TDF, SDF and IDF) content, expressed as gram/100 grams (as is basis), in different gluten free breads (GFBs).

Figure 2. Starch digestibility in different gluten free breads (GFBs) determined by *in vitro* enzymatic hydrolysis. RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch, expressed as gram/100 grams (as is basis).

Table1. Ingredients in gluten free breads (GFBs) according to the producer labelling.

Product	Ingredients
GFB1	Corn starch, water, sucrose, egg, vegetal margarine, acidulant, aromas and colorant, yeast, thickener (xanthan gum), emulsifier, salt, preservative, raising agents, antioxidants.
GFB2	Corn starch, water, vegetal margarine, emulsifiers, salt, acidulant, preservative, antioxidants, aromas and colorant (beta-carotene), egg, sucrose, yeast, thickener (xanthan gum), emulsifiers, dextrose, humidifier, stabilizers (guar gum, pectin, CMC).
GFB3	Corn starch, water, vegetal margarine, acidulant, preservative, antioxidants, aromas and colorant, egg, sucrose, yeast, emulsifier, dextrose, humidifier, stabilizers (guar gum, pectin, CMC) and salt.
GFB4	Potato starch, corn starch, water, casein, sucrose, vegetal oil, corn flour, yeast, soy protein, stabilizers (HPMC, xanthan gum), salt, preservative.
GFB5	Corn starch, water, sucrose, egg, vegetal margarine, acidulant, preservative, aromas and colorant, thickener (xanthan gum), yeast, emulsifiers, salt, raising agents, anis, cinnamon, antioxidant.
GFB6	Corn starch, rice flour, water, vegetal oil, sucrose, thickener (guar gum, HPMC), lupine protein, yeast, salt, vegetal fibre, aroma, emulsifiers.
GFB7	Corn starch, water, sucrose, egg, vegetal margarine, acidulant, preservative, aromas and colorant, yeast, thickener (xanthan gum), emulsifier, salt, raising agents, antioxidants.
GFB8	Corn starch, water, sucrose, yeast, thickeners (xanthan gum, HPMC), salt, raising agent, acidulant, preservative, aromas and colorant.
GFB9	Corn starch, water, sucrose, thickeners (xanthan gum), emulsifier, salt, yeast, preservative, raising agents, antioxidants.
GFB10	Corn starch, vegetal margarine, salt, sucrose, emulsifier, raising agents, antioxidant, thickener (xanthan gum), preservative and yeast.
GFB11	Corn starch, vegetal margarine, salt, sucrose, emulsifier, raising agents, antioxidant, thickener (xanthan gum), preservative and yeast.

Table 2. Chemical composition, expressed as gram/100 gram on dry matter, of eleven types of commercial gluten free breads (GFBs).

Product	Moisture (%)				Protein (%, d.m.)				Fat (%, d.m.)				Mineral (%, d.m)				Total Carbohydrate (%, dm)		
	Mean		SD		Mean		SD		Mean		SD		Mean		SD		Mean		SD
1	29.63	±	0.14	d	3.16	±	0.09	e	8.51	±	0.00	d	2.12	±	0.03	c	86.21	±	0.07
2	31.63	±	0.15	f	6.94	±	0.07	i	16.91	±	0.20	g	1.10	±	0.07	a	75.05	±	0.22
3	29.50	±	0.01	d	7.31	±	0.15	j	16.56	±	0.07	g	1.66	±	0.15	b	74.47	±	0.22
4	27.17	±	0.10	c	15.05	±	0.09	k	7.33	±	0.08	c	1.85	±	0.06	bc	75.76	±	0.06
5	26.27	±	0.03	b	5.13	±	0.03	h	10.64	±	0.06	e	2.01	±	0.15	c	82.22	±	0.19
6	41.66	±	0.21	i	4.92	±	0.07	g	4.86	±	0.03	b	2.03	±	0.02	b	88.18	±	0.12
7	33.60	±	0.08	g	3.96	±	0.00	f	8.28	±	0.05	c	4.53	±	0.00	e	83.22	±	0.03
8	21.10	±	0.01	a	1.01	±	0.02	b	2.00	±	0.10	a	4.03	±	0.01	e	92.96	±	0.11
9	31.33	±	0.04	e	0.91	±	0.02	a	2.03	±	0.37	a	5.43	±	0.33	f	91.63	±	0.04
10	36.13	±	0.07	h	1.91	±	0.00	c	26.10	±	0.05	h	3.57	±	0.04	d	68.42	±	0.14
11	42.03	±	0.04	j	2.80	±	0.02	d	18.32	±	0.00	f	3.98	±	0.02	d	74.91	±	0.03
Mean	*31.82*				*4.83*				*11.05*				*2.94*				*81.18*		
SD	*0.08*				*0.05*				*0.09*				*0.08*				*0.11*		

(*)Total Carbohydrate (d.m) by difference: 100 – (weight in grams [protein + fat + ash] in 100 g of food) (FAO, 2003).

Values are means ± standard deviation (n=3). Different letters within a column mean significant differences ($p<0.05$).

Table 3. Contribution of macronutrient intakes (g/100g) to the relevant DRIs [21] consuming an average portion of 200g of gluten free breads (GFB).

Macronutrient	Gender	DRIs(*) (g/day)	Contribution to DRIs (g/100g) of GFB										
			1	2	3	4	5	6	7	8	9	10	11
Proteins	Female	56	7.9	17.0	18.4	39.2	13.5	10.3	9.4	2.9	2.2	4.3	5.8
	Male	46	9.7	20.6	22.4	47.7	16.4	12.5	11.4	3.5	2.7	5.3	7.1
Carbohydrates	Adults	130	78.4	71.2	77.9	74.6	85.4	71.0	70.6	109.2	86.1	54.6	53.7

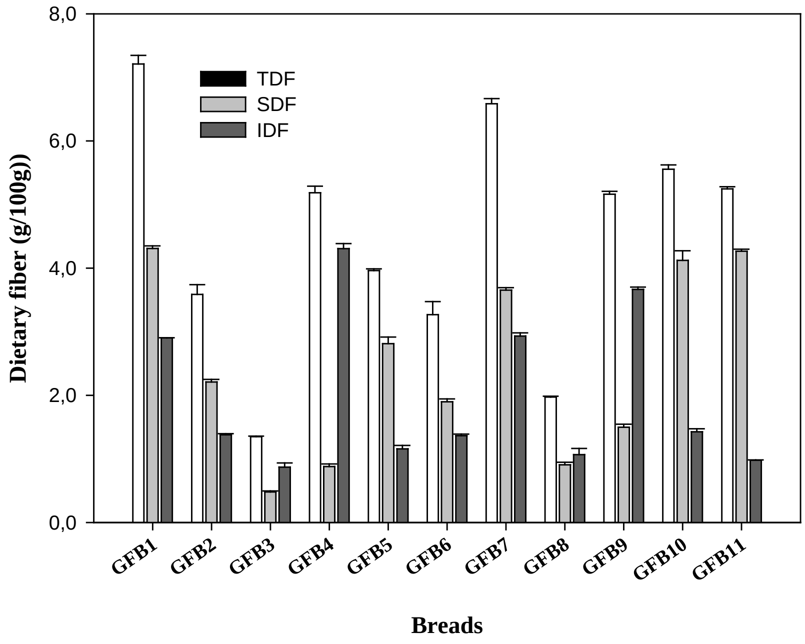
Table 4. Kinetic parameters of the *in vitro* starch hydrolysis and estimated glycaemic index.

Samples	**C∞ (g/100g)**	***k* (min^{-1})**	**AUC 180**	**H90**	**HI**	**eGI**
GFB1	90.7 b	0.0782 c	22345 b	91 b	91 b	87 b
GFB2	94.9 c	0.1218 e	23764 d	95 cd	95 d	90 d
GFB3	86.9 a	0.1458 f	21664 a	86 a	87 a	83 a
GFB4	91.2 b	0.0973 d	22587 b	91 b	91 b	87 b
GFB5	97.8 d	0.0713 b	23740 d	97 e	97 e	91 e
GFB6	96.4 cd	0.0756 c	23653 d	96 e	96 e	91 e
GFB7	95.8 c	0.0723 b	23608 d	96 d	96 d	91 d
GFB8	93.2 b	0.0768 c	23100 c	93 d	93 c	89 c
GFB9	100.1 e	0.0527 a	24732 e	100 f	102 f	96 b
GFB10	94.8 c	0.1232 e	23797 d	95 cd	94 c	89 cd
GFB11	92.0 b	0.0574 a	22127 b	91 b	92 b	87 b

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

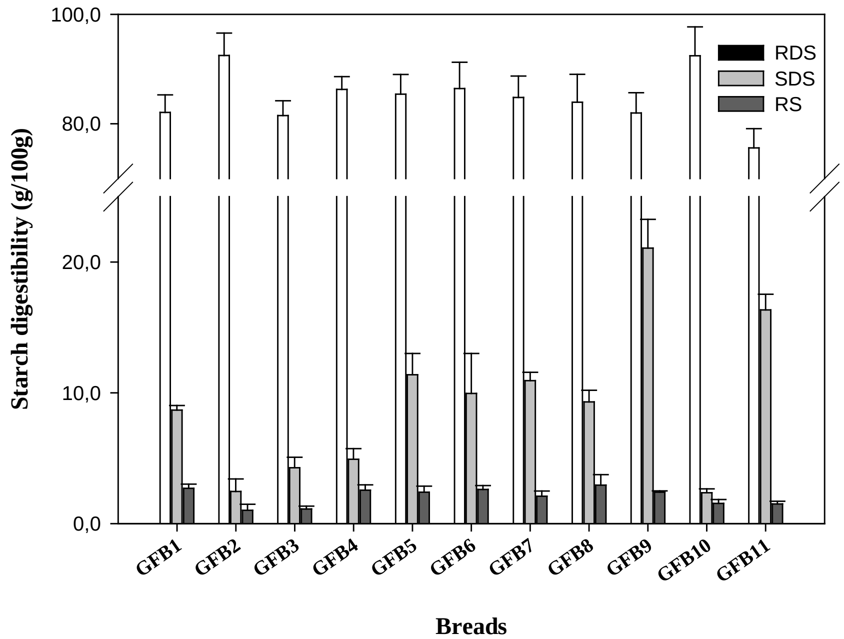
[b] C∞, equilibrium concentration; k, kinetic constant; HI, hydrolysis index; AUC 180, area under curve; eGI, estimated glycaemic index.

Fig 1.



8,0
6,0
4,0
2,0
0,0
TDF
SDF
IDF
Dietary fiber (g/100g))
GFB1
GFB2
GFB3
GFB4
GFB5
GFB6
GFB7
GFB8
GFB9
GFB10
GFB11
Breads

Fig 2.



100,0
80,0
20,0
10,0
0,0
RDS
SDS
RS
Starch digestibility (g/100g)
GFB1
GFB2
GFB3
GFB4
GFB5
GFB6
GFB7
GFB8
GFB9
GFB10
GFB11
Breads