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ORIGINAL ARTICLE

A study on fibre addition to gluten free bread: its effects on bread quality and in vitro digestibility

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Abstract The aim of this study was to assess the impact of fibre addition on gluten-free (GF) dough properties and bread technological quality, and on protein and starch in vitro digestibility. Soluble (Inulin, In) and insoluble fibres (oat fibre, OF, and type IV resistant starch, RSIV) were used at 5 and 10% substitution levels. Dough firmness increased when insoluble fibres were added, and decreased when In was used. Incorporation of insoluble fibres resulted into bread with a low specific volume (SBV) since firmer dough were more difficult to expand during proofing and baking. Staling rate was reduced after fibre addition, with the exception being OF 10%, as its lower SBV may have favoured molecule re-association. In general, protein and starch digestibility increased when fibres were added at 5%, and then decreased after further increasing the level. Fibres may have disrupted bread crumb structure, thus increasing digestibility, although the higher addition may have led to a physical and/or chemical impediment to digestion. Inulin has well-known physiological effects, while RS presented the most important effect on in vitro starch digestibility (GI). These results showed the possibility of adding different fibres to GF bread to decrease the GI and increase protein digestibility, while obtaining an overall high quality end-product.

Keywords Gluten free bread · Soluble and insoluble fibres · Bread technological quality · Protein in vitro digestibility · In vitro glycemic index

Introduction

Celiac patients should avoid the intake of any gluten from wheat, rye or barley and, in some cases, oats should not be ingested. However, obtaining gluten-free (GF) bread with appropriate technological properties is a challenging task, since the viscoelastic properties of gluten give the dough the mechanical properties required to obtain the typical sponge-like structure of the final product.

In previous investigations, Ribotta et al. (2004) and Sciarini et al. (2012a) developed a simple GF bread formulation based on rice flour, active soy flour and cassava starch, which had good technological properties. In these studies, soy flour was added to overcome the general lack of proteins in GF bakery formulations. In this regard, nowadays, a greater emphasis is placed on the nutritional status of celiac patients, given that carbohydrates, proteins and lipids are often consumed in unbalanced proportions, while the intake of some essential nutrients is usually deficient (Thompson 2000; Thompson et al. 2005). Furthermore, dietary fibre (DF) intake, which can modify the rate at which nutrients such as starch and proteins are digested and/or absorbed (Capriles and Arêas 2013), is particularly low for those following a gluten-free diet (Hager et al. 2011).

To classify food items on the basis of their postprandial blood glucose response, Jenkins et al. (1981) introduced the concept of the glycemic index (GI), defined as the

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postprandial incremental glycemic area after a test meal and expressed as the percentage of the corresponding area after intake of an equi-carbohydrate portion of a reference food (glucose or white bread).

The amount of glucose released during digestion is influenced by the food matrix, as it may restrict enzyme accessibility to substrates. In general, it has been established that DF, by increasing digesta viscosity, decreases glucose absorption in the small intestine, thereby decreasing the GI of a given food item and postprandial insulin response.

Despite the nutritional benefits of including DF in GF formulations, the impact of such an addition on the technological properties of the final products has also to be considered. In general, insoluble fibres tend to reduce technological quality, while soluble fibres have a positive impact. In agreement, Korus et al. (2006) reported an increase in loaf volume and lower crumb firmness of a GF bread containing inulin (soluble fibre). Moreover, Martínez et al. (2014) reported that soluble fibres (Nutriose® and polydextrose) decreased dough consistency, favoured volume increase during fermentation, and produced bread with higher specific volumes and a lower firmness, which were more aerated than control bread. On the other hand, cellulose (insoluble fibre)produced bread with poor technological attributes, with a particle size effect being reported.

Cappa et al. (2013) reported that soluble fibre from *Psyllium* may play a central role on GF bread development, dueto its film forming ability and the effective antistaling effect it has as a result of its high water binding capacity. Ronda et al. (2015) studied the fortification of GF bread with soluble β-glucans from oat and barley, which have different average molecular sizes, and found a general decrease in specific volume and a harder crumb after fibre addition, even though both dough and bread properties were shown to be dependent on the molecular weight and the structure of β-glucans.

In view of these above findings, the goals of this study were, first, to assess the effect of adding soluble (inulin) and insoluble fibres (resistant starch and oat insoluble fibre) at two substitution levels (5 and 10%) on the technological quality of gluten free bread and, second, to examine their impact on the nutritional properties of bread (total dietary fibre content, protein, and starch in vitro digestibility).

Materials and methods

Materials

Gluten-free bread was formulated with rice flour (Dimax, Argentina; 7.12% proteins, 0.97% ash, 5.71% lipids, 74.36% carbohydrates, 11.84% moisture), cassava starch (Dimax, Argentina; 0.08% proteins, 0.17% ash, 1.82% lipids, 86.53% carbohydrates, 11.40% moisture), full-fat active soy flour (NICCO, Argentina; 37.80% proteins, 9.80% ash, 26.87% lipids, 17.87% carbohydrates, 7.66% moisture), compressed yeast (Dánica, Argentina), shortening (Dánica, Argentina), salt (Dos Anclas, Argentina), and leavening agent (Dos Anclas, Argentina). The fibres employed were resistant starch type IV (RSIV) (Novelose 480, National Starch, supplied by Gelfix S.A, Argentina; 67.8% dietary fibre content, water holding capacity (WHC): 2.68 g water/g solids), were RSIV is defined as phosphated distarch phosphate, a cross-linked high-amylose maize starch. Oat bran fibre (OF) (Canadian Harves, Oat Fibres 200/58 series, Sunopta, USA; 86.2% dietary fibre content, WHC: 3.99 g water/g solids) and inulin (In) (Orafti HP, Beneo-Orafti Latin America, São Paulo, Brazil, WHC: 0.21 g water/g solids) were supplied by Saporiti S.A., Argentina.

GF breadmaking

The basic bread formulation consisted of 45 g rice flour, 45 g cassava starch, 10 g active soy flour, 2 g salt, 2 g shortening, 3 g compressed yeast and 80 g water, with two levels of flour substitution being used for fibre incorporation (5 and 10%). Ingredients were mixed in a planetary mixer (Arno, Brazil) for 1 min at 156 rpm and 2 min at 214 rpm, and the dough obtained was proofed for 30 min (30 °C and 85% relative humidity), mixed again for 1 min at 156 rpm, poured into aluminium cups (60 g) and proofed again under the same conditions (30 min, 30 °C, and 85% relative humidity). Finally, the dough was baked at 180 °C for 30 min in a forced convection oven (Pauna, Argentina) equipped with a temperature controller, after which the bread was bread cooled for 2 h. Breadmaking was performed in duplicate.

Dough properties

Dough firmness was assessed using a Universal Texture Machine (Instron, USA) equipped with a 50 kg load cell. Samples were prepared as for breadmaking, and 40 g of the resultant dough were weighed in plastic flasks and proofed (60 min, 30 °C, 85% relative humidity), with dough firmness being determined for unfermented and fermented dough. Samples were compressed 40% at 5 mm/s using a 25 mm diameter probe, and the maximum force registered during compression was considered to be the dough firmness. Dough preparation was performed in duplicate, and three determinations were performed for each dough batch.

Bread technological quality

Specific bread volume (SBV)

The volume of each bread loaf was determined by rapeseed displacement according to the AACC method 10-05

(AACC 2000) 2 h after baking, and the specific volume was calculated as the bread volume/bread weight ratio. Three measurements of each breadmaking batch were performed.

Crumb firmness

To assess crumb firmness, bread was longitudinally cut 2 h after baking, and two slices of 15 mm thick were obtained from the centre of each loaf. Firmness was measured using an Instron Universal Texture Machine (Instron, USA) equipped with a 25 mm diameter probe, with the slice being compressed 40% at a rate of 5 mm/s and firmness defined as the maximum force obtained during compression. To obtain the firming rate, the firmness measurements were obtained 2, 24 and 72 h after baking. Between measurements, the bread was stored in sealed plastic bags at 25 °C and the firming rate was calculated as the slope of the straight line obtained from the regression of the three measured points in a force–time plot.

Crumb structure

Digital images from the bread was obtained using a scanner (HP Scanjet G3010, Palo Alto, USA) with a 600 dpi resolution, which were analysed using ImageJ Software 1.41o (National Institutes of Health, USA). The total cell total area (cm^2), cell average size (cm^2) and the number of cells/cm^2 were determined using two slices per loaf, and two loaves from each breadmaking batch were analysed.

Bread nutritional quality

Dietary fibre determination

GF bread was dried at 100 °C overnight and milled for total dietary fibre (TDF) content determination, according to the method 32-05 (AACC 2000). Two replicates from each breadmaking batch were analysed, and results were expressed as the percentage of total dietary fibre on a dry basis.

Protein content and in vitro protein digestion

Nitrogen content in GF bread was determined by the Kjeldahl method according to the AACC method 46-13 (AACC 2000). The percentage of total protein was calculated as Nx6.25. In vitro protein digestion was measured according to Martínez et al. (2016) with slight modifications. Briefly, bread samples (100 mg) were weighed in centrifuge tubes (50 mL) and a chewing process was simulated using a homogenizer plunger after adding 2 mL of distilled water. The plunger was rinsed with 25 mL of phosphate buffer 0.1 M, pH 2.0, and using the same buffer the sample volume was brought up to 30 mL. For protein digestion, 9 mL of pepsin (Sigma P-7000, 975 units/mg protein) solution (5.8 mg of pepsin/mL in 0.1 M phosphate buffer, pH 2.0) were added. Samples were incubated for 2 h at 37 °C in a shaking bath (Vikinf Dufnoff 5002, Argentina) and the digestion reaction was stopped by the addition of 2 mL of 2 N NaOH. Duplicate 1 mL aliquots were taken from the digested samples and centrifuged at 14,000×*g*, 25 °C, for 10 min. Digested protein was determined on 200 µL of supernatants following the Lowry method (Waterborg 2009) by measuring the absorbance at 550 nm (Spectrum SP 2000, China). A standard curve prepared with bovine serum albumin (Sigma 85040C) covering the concentration range of 0.1–2.0 mg/mL was used, with digestibility being expressed as:

$$PD = \frac{(TPC - Protein\ content\ after\ digestion) \times 100}{TPC}$$

PD being Protein digestibility, and TPC Total protein content.

Three replicates of each breadmaking batch were analysed.

Starch analysis

Resistant and digestible starch of the GF bread samples were measured according to the AACC method 32–40 (AACC 2000).

In vitro digestion of GF bread and estimated glycemic index

In vitro digestion was performed using the multi-enzymatic method reported by Bustos et al. (2011). Samples of bread (1 g) were mixed with 20 mL sodium potassium phosphate buffer (pH 6.9) (PBS), after which, the pH was adjusted to 1.5 (using 6 M HCl) and 5 mL pepsin solution (115 U mL^{-1}) was added to the samples, followed by incubation at 37 °C for 30 min. Then, the pH was readjusted to 6.9 with 10% NaOH, the volume completed to 49 mL with PBS, and 1 mL of porcine pancreatic alpha amylase solution (110 U mL^{-1}) was added, with each tube being incubated at 37 °C. Every 30 min for 3 h, aliquots of 1 mL were withdrawn from each tube for analysis of the reducing sugar content, using the 3,5-dinitrosalicylic acid (DNS) method. Maltose was converted into starch by multiplying by 0.9, with a nonlinear model being applied to describe the kinetics of starch hydrolysis and the first order equation being the following formula:

$$C = C\infty\left(1 - e^{-Kt}\right)$$

where C corresponds to the percentage of starch hydrolysed at time t; C_∞ is the equilibrium percentage of starch

hydrolysed after 180 min; K is the kinetic constant; and t is the time (min). The parameter estimation was carried out using ORIGIN PRO software, version 8 (OriginLab Corp., Northampton, MA, USA), and the rate of starch digestion was expressed as the percentage of total starch hydrolysed at different times (30, 60, 90, 120 and 180 min).

The area under the hydrolysis curve (AUC) was calculated, and the hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample (fresh wheat white bread). Then, the expected glycemic index (GI) was estimated using the model

$$GI = 39.21 + 0.803 \times H_{90}$$

where H_{90} is the hydrolysis index after 90 min

Statistical analysis

All measurements were made at least in triplicate. Data were analysed using analysis of variance and Fisher's least significant difference test with a significance level of 0.05, with a correlation test also performed to evaluate the relationship between variables ($p < 0.05$). These tests were carried out using Infostat software (Facultad de Ciencias Agropecuarias, Universidad Nacional de Cordoba, Argentina 2014).

Results and discussion

Dough properties

Dough firmness before and after fermentation is shown in Table 1. It was observed that the addition of fibre increased dough firmness ($r = 0.61$, $p < 0.05$). The effect of fibre addition on this parameter depended on the type and amount of fibre added. OF and RS increased dough firmness at both the 5 and 10% substitution levels, whereas In decreased initial dough firmness. A higher WHC of RS and OF led to an increase in dough viscosity, thus increasing firmness. On the other hand, it is known that soluble fibres, such as inulin, dissolve in the aqueous solution and surround the starch granules, thereby lubricating the final dough, and consequently, the amount of starch available to absorb water is reduced, as well as the dough consistency (Martínez et al. 2014).

As expected, dough firmness decreased after fermentation, although the percentage of this reduction was different from one sample to another. The control dough had a 67% lower firmness after fermentation, with this reduction being 61 and 75% for OF5 and OF10, respectively; 43 and 0% for In5 and In10; and 53 and 56% for RS5 and RS10, respectively. This effect could have been related to the internal structure of the GF dough. As OF has a large particle size of typically around 400 nm (Duta and Culetu 2015), then the presence of such big particles may reduce internal dough cohesion, thereby leading to a weaker structure that is less resistant to a rise in gas pressure during fermentation.

Bread properties

Technological quality

The technological parameters of bread quality are presented in Table 1. As shown, SBV decreased with increasing insoluble fibre (OF and RS) content, whereas it increased with the addition of In. In agreement, it has been found that specific volume of gluten free eggless muffins was also reduced after adding black carrot dietary fibre concentrate (predominantly rich in insoluble fibre) (Singh et al. 2016). This negative effect of insoluble fibres on SBV is mainly related to an increase in dough firmness. If a rather liquid batter is used, an increase in consistency may lead to an increase in SBV, since air entrapment during proofing and baking is favoured. However, an increase in

Table 1 GF dough properties and bread technological parameters after fibre addition

Sample	Dough firmness (g)		Bread properties					
	0 min	60 min	SBV (cm^3/g)	Firmness (g)	Firming rate (g/day)	Total cell area (%)	Cells/cm^2	Cell average size (cm^2)
Control	$89.7 \pm 3.5^{c*}$	29.4 ± 1.1^{a}	2.75 ± 0.04^{bcd}	203 ± 32^{cd}	197.0	48.46 ± 0.36^{b}	7.46 ± 0.02^{c}	0.060 ± 0.003^{ab}
OF5	117.3 ± 1.8^{e}	46.1 ± 3.6^{cd}	2.70 ± 0.01^{bc}	161 ± 13^{b}	170.9	48.94 ± 0.42^{b}	7.26 ± 0.11^{c}	0.064 ± 0.002^{b}
OF10	197.3 ± 0.9^{f}	48.4 ± 1.8^{d}	2.42 ± 0.19^{a}	214 ± 21^{d}	219.5	52.03 ± 0.92^{c}	5.67 ± 0.26^{b}	0.094 ± 0.004^{c}
In5	65.2 ± 0.1^{a}	37.4 ± 3.5^{b}	2.92 ± 0.02^{cd}	121 ± 11^{a}	141.7	50.99 ± 1.24^{bc}	3.57 ± 0.14^{a}	0.143 ± 0.008^{e}
In10	79.9 ± 7.4^{b}	82.4 ± 3.1^{e}	2.98 ± 0.20^{d}	117 ± 23^{a}	169.4	53.48 ± 0.62^{c}	4.89 ± 0.39^{b}	0.116 ± 0.003^{d}
RS5	105.6 ± 4.8^{d}	$42.2 \pm 2.4b^{c}$	2.50 ± 0.09^{ab}	184 ± 29^{bc}	175.2	43.87 ± 0.96^{a}	8.62 ± 0.08^{d}	0.053 ± 0.000^{ab}
RS10	117.9 ± 5.7^{e}	49.2 ± 2.4^{d}	2.36 ± 0.09^{a}	259 ± 19^{e}	163.7	41.59 ± 2.33^{a}	10.50 ± 0.74^{e}	0.048 ± 0.007^{a}

OF5, 5% oat bran fibre; OF10, 10% oat bran fibre; In5, 5% inulin; In10, 10% inulin; RS5, 5% resistant starch; RS10, 10% resistant starch

* Values followed by different letters in the same column are significantly different ($p < 0.05$)

the consistency of a more rigid dough may prevent it from rising during these steps.

In the present study, there was a negative correlation between SBV and TDF ($r = -0.83$; $p < 0.05$), indicating that the higher the dough firmness, the lower the SBV. Martínez et al. (2014) also found that bread obtained from doughs with a lower consistency achieved higher specific volumes, whereas more consistent doughs produced bread with lower specific volumes. Nevertheless, for RS GF bread, factors other than the increase in dough firmness may be involved in the reduced SBV. In this case, a lower bread volume may also result from a low enzymatic susceptibility of these resistant starch granules (Ziobro et al. 2012), resulting in lower fermentation rates.

Regarding crumb structure, fibre incorporation had varying effects. Total cell area increased when In (5 and 10%) and OF (10%) were used. As shown in Fig. 1, bread with In5 presented big holes, probably as a result of cell coalescence during proofing and baking due to low dough viscosity. On the other hand, In10 had the highest cell area, producing bread with high SBV, with its crumb structure being less compact compared to other bread (Fig. 1) and containing bigger cell sizes (Table 1). The internal structure of GF bread with 5% OF (OF5) was similar to that of the control bread, while OF10 presented a higher cell area, with less cells/cm^2 and thicker walls. Bread with RS had a smaller cell area and a more homogeneous overall structure, with a higher number of smaller cells. These results are consistent with the low SBV of RS bread, and are in agreement with those of Ziobro et al. (2012), who also found a decrease in cell average size when using distarch phostate in the formulation of GF bread.

The cell size distribution is mainly governed by dough rheological properties, which in turn affect gas cell expansion during proofing. Moreover, the change in starch structure and enzymatic susceptibility caused by chemical modification can affect the volume of CO_2 produced by yeast (affecting SBV) significantly, and can also shift the

Fig. 1 Representative images of GF bread with fibre addition. OF5, 5% oat bran fibre; OF10, 10% oat bran fibre; In5, 5% inulin; In10, 10% inulin; RS5, 5% resistant starch; RS10, 10% resistant starch

thermal transition temperatures responsible for crumb structure stabilization in the oven (Ziobro et al. 2012).

Regarding crumb texture, the addition of 5% fibre decreased crumb firmness, while bread with 10% fibre presented firmer crumbs, compared to control bread. It is well known that a decrease in SBV is accompanied by an increase in crumb firmness, since the smaller the bread, the more densely the molecules get packed, thereby favouring their interaction. Thus, as expected, crumb firmness was negatively correlated with SBV ($r = -0.86$, $p < 0.05$).

In most cases, the crumb firming rate was slower when fibres were included in the formulation, which could have been related to a decrease in water loss during storage. The exception to this trend was OF10, which had a higher firming rate than control bread. In fact, this bread had a rather low SBV, so closer interactions between molecules were favoured, thus counteracting the effect of reduced water migration.

Nutritional quality

As expected, the total dietary fibre content in bread increased with greater fibre addition. However, it should be made clear here that inulin, as in the case of other dextrins, cannot be accurately determined using this enzymatic method (Table 2). Regarding resistant starch content, this also increased with fibre addition, with it being more evident when RS was used in the GF formulations (Table 2). Protein digestibility ranged from 47.2 to 62.2% of bread total protein, and fibre addition, in general, increased digestibility compared to control bread. The control sample presented a lower digestibility than that found in the literature concerning GF bread, although direct comparisons are rather difficult to make due to the huge variability in the bread formulations used. In addition to this, significant differences have been reported when different enzymatic methods were used (Abdel-Aal 2008), with Shin et al. (2013) observing a 74.4% protein digestibility for bread made with raw soy flour. This high digestibility is probably the result of a less compact structure being more accessible to enzymatic action. Moreover, it has been reported that there is a specific interaction between proteins from active soy flour and cassava starch, which is mainly responsible for this GF bread structure (Sciarini et al. 2012b). It is possible that the presence of fibre partly disrupts the crumb structure by interrupting this interaction, thereby rendering soy proteins more accessible to enzyme digestion. On the other hand, Table 2 shows that protein digestibility was lower when fibre was added at 10% than at 5%. Thus, it is possible that a high fibre level results in a physical or chemical barrier to enzyme hydrolysis.

Table 2 Total dietary fibre (TDF) and resistant starch contents, and protein digestibility of GF bread with fibre addition

Sample	TDF (g/100 g bread)[a]	Resistant starch (g/100 g bread)	Protein in vitro digestibility (g/100 g bread)
Control	3.29 ± 0.45a	0.41 ± 0.07a	51.0 ± 2.3b
OF5	7.57 ± 1.30c	0.99 ± 0.10c	62.5 ± 0.9e
OF10	12.24 ± 0.24d	0.91 ± 0.08bc	58.8 ± 0.7cd
In5	3.49 ± 0.38ab	0.72 ± 0.20b	60.9 ± 0.9de
In10	4.50 ± 0.12b	0.91 ± 0.08bc	47.2 ± 1.3a
RS5	7.56 ± 0.24c	0.99 ± 0.10c	62.2 ± 0.4de
RS10	12.31 ± 0.85d	1.93 ± 0.08d	56.0 ± 2.4c

OF5, 5% oat bran fibre; OF10, 10% oat bran fibre; In5, 5% inulin; In10, 10% inulin; RS5, 5% resistant starch; RS10, 10% resistant starch

* Values followed by different letters in the same column are significantly different ($p < 0.05$)

[a] Inulin is underestimated by using this technique

The glycemic index has been formulated in order to estimate the blood glucose response after food ingestion by humans. It is measured using the postprandial glycemic area of a test meal, which is then expressed as the percentage of the corresponding area of a reference food (fresh wheat white bread). As human subjects would have to be recruited to measure GI, which is time-consuming, invasive, labour-intensive and costly, in vitro testing is commonly used as a faster method to predict in vivo GI (Fardet et al. 1999).

The in vitro hydrolysed starch (%) of gluten-free bread vs. time (min) curves is shown in Fig. 2, where experimental values for each curve were adjusted to a first order kinetic model $C = C_{\infty}(1 - e^{-Kt})$ to calculate the rate of digestion as K (kinetic constant) and the total hydrolysed starch as C_{∞} (equilibrium percentage of starch hydrolysed after 180 min). This model was shown to be suitable for describing in vitro starch digestibility of gluten free samples ($R^2 > 0.97$), with the estimated glycemic index being calculated from the AUC until 90 min (H_{90}) as described in Materials and methods.

The hydrolysis of bread changed depending on which fibre they were enriched with. As a general trend, between 0 and 30 min, the hydrolysis rate increased, until a maximum plateau was reached between 30 and 180 min, with the H_{90} values obtained from the kinetic equation being very similar to experimental values, in all cases (Table 3). The enzymatic susceptibility of starch depends on the spatial organization and structural state of food components. Hence, modifying bread structure through changes in formulation and processing may have an impact on the rate of starch degradation (Petitot et al. 2009). Here, for bread with OF and In, GI increased when fibre was added at 5%, but decreased at 10%. As mentioned above, bread with 5% fibre addition may present a different structure than control

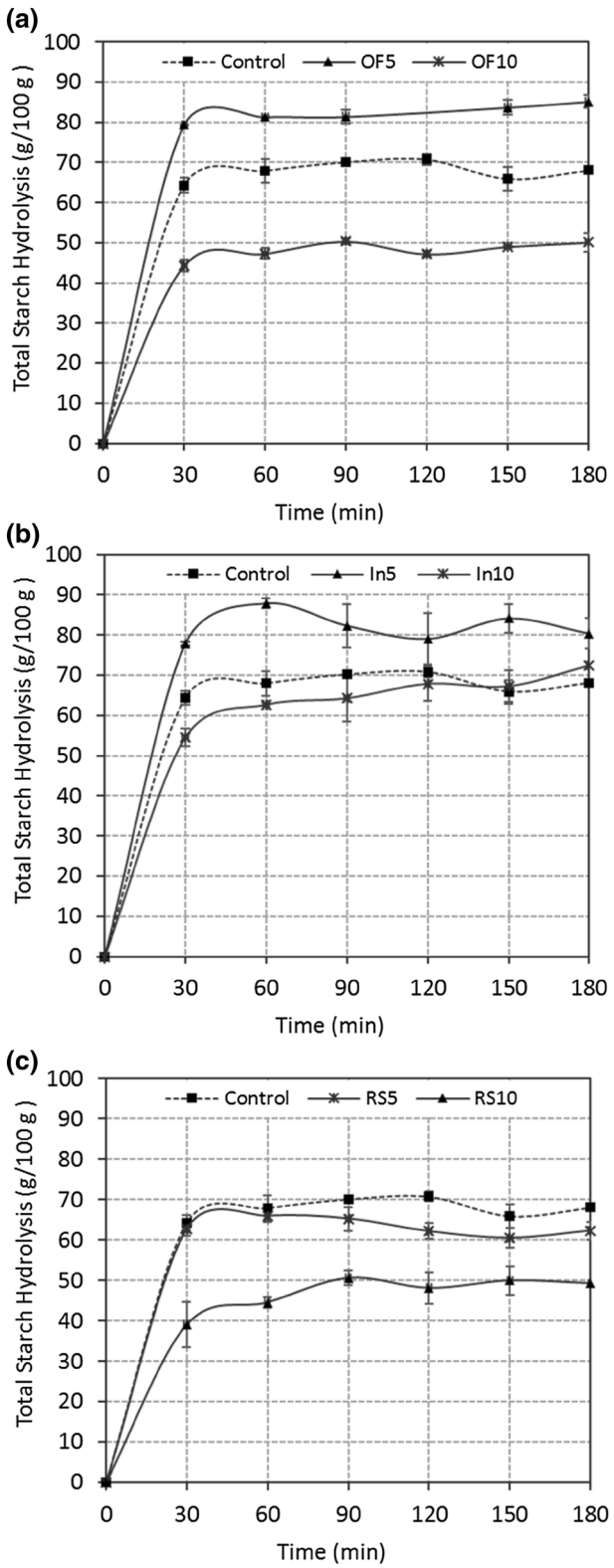

Fig. 2 In vitro starch digestion of GF bread with fibre addition. **a** Oat bran fibre (OF), **b** inulin (In), and **c** resistant starch (RS)

bread (no fibre addition) due to the interruption of the structure-building interaction between proteins and starch. Thus, this change in bread structure could make starch more accessible to enzymatic hydrolysis. However, adding even more fibre may result in a physical or chemical barrier for enzymatic action. In fact, a physical barrier can be seen from different points of view. For example, Englyst et al. (1996) reported a significant inverse correlation between non-starch polysaccharides and in vitro GI for starchy foods, with these authors suggesting that the encapsulation of sugars and starch within a dietary fibre matrix may delay or even prevent their hydrolysis/digestion, leading to lower GI values. In another research, Jenkins et al. (2004) proposed that one of the ways in which fibres may achieve their beneficial metabolic effects is by reducing the rate of absorption, probably as a result of an increased resistance to bulk diffusion due to an increased viscosity of the luminal contents. However, Wood et al. (1990) reported that oat and guar gum had similar effects on the postprandial blood glucose rise measured in vivo, despite differences in apparent viscosity. Related to this, Hardacre et al. (2015) found that fibres with similar viscosities led to differences in in vitro starch digestion, and as a result of these findings, they proposed that some fibres may exert a non-competitive inhibition on certain enzymes (which may be considered as a chemical barrier). In the present study, however, no conclusive evidence was found to support any of these hypotheses.

In a previous work (Juntunen et al. 2003), starch hydrolysis was measured in vitro, while glycemic index was evaluated in vivo, with samples consisting of wheat and rye bread, with different contents of fibre. These authors reported that total dietary fibre was not the only factor affecting starch digestibility, as the structural component was also determinant. The effect of fibres on the glycemic index of different GF bread has been evaluated in various studies (Capriles and Arêas 2013; Giuberti et al. 2016), with fibre presence having been related to a decrease in starch hydrolysis. However, in vitro digestibility was evaluated on dried, ground samples, and no structural effect of the matrix was considered.

For soluble fibres, such as inulin, other phenomena are also possible. For example, solubilized inulin can create a protective layer around the starch granules, thereby limiting their swelling and amylose release, and leading to lower viscosity values. This feature may result in a limited accessibility of starch-degrading enzymes, which could affect starch digestibility in vivo and also the glycemic index (Vázquez-Gutiérrez et al. 2016). Finally, Capriles and Arêas (2013) studied starch digestibility both in vitro

Table 3 Percentage of total starch hydrolysed at 90 min (H_{90}), equilibrium concentration (C_∞), kinetic constant (K), hydrolysis index (HI) and glycemic index (GI) for fibre-enriched GF bread

Sample	H_{90}^{th} (%)[c]	H_{90}^{exp} (%)[c]	C_∞ (%)[b]	K (s^{-1})[b]	HI^{exp} (%)	GI
Control	91.4 ± 3.8c*	88.9 ± 6.5c	68.6 ± 2.8b	0.092 ± 0.001bcd	79.7 ± 3.6c	84.2 ± 1.8c
OF5	112.2 ± 0.1d	107.1 ± 0.3d	82.7 ± 1.3c	0.107 ± 0.015 cd	96.2 ± 1.2d	93.8 ± 0.4d
OF10	63.6 ± 1.1a	62.1 ± 0.2a	48.9 ± 0.1a	0.078 ± 0.008abc	56.2 ± 0.0a	71.1 ± 0.2a
In5	108.2 ± 0.4d	102.8 ± 0.0d	79.1 ± 1.3c	0.114 ± 0.014d	92.1 ± 1.4d	91.7 ± 0.5d
In10	80.9 ± 0.9b	79.4 ± 0.5b	68.6 ± 4.6b	0.052 ± 0.015a	75.9 ± 3.0bc	81.7 ± 1.3b
RS5	89.6 ± 1.8c	85.8 ± 2.5bc	63.2 ± 2.2b	0.162 ± 0.017e	74.3 ± 2.5b	81.9 ± 1.2bc
RS10	58.8 ± 4.4a	58.3 ± 3.3a	49.7 ± 3.0a	0.072 ± 0.008ab	55.0 ± 0.4a	70.2 ± 0.1a

OF5, 5% oat bran fibre; OF10, 10% oat bran fibre; In5, 5% inulin; In10, 10% inulin; RS5, 5% resistant starch; RS10, 10% resistant starch

* Values followed by different letters in the same column are significantly different ($p < 0.05$)

[b] Parameters of the kinetic equation $C = C\infty(1-e^{-K_t})$

[c] H_{90}^{exp} experimental value. H_{90}^{th} theoretical value obtained with the equation

and in vivo of GF bread enriched with inulin type fructans and found that at the 12% addition level in vitro GI was reduced by 10% compared to control GF bread. However, a reduction of 30% in GI measured in vivo was found using the same sample.

Conclusion

The addition of different fibres had varying effects on the GF bread technological quality and nutritional properties. In general, insoluble fibres (OF and RS) decreased bread volume, although this did not necessarily affect technological quality, since crumb firmness was not always increased. Crumb ageing was reduced when adding RS to the formulation. For soluble fibre (In), an improvement in overall bread technological quality was observed, with higher SBV and lower crumb firmness, resulting in a less compact structure which is often desirable for GF bread. In general, the glycemic index was reduced when fibres were added at 10%, but, at the same time, protein digestibility was reduced. However, it is worth highlighting that RS added at 5% reduced GI, and the protein digestibility was increased by 20%. According to these results, GF bread with different characteristics can be obtained by slightly modifying the bread formulation, i.e. fibre type and addition level. Inulin has well-known physiological effects, while RS had the most important effect on in vitro GI. Thus, depending on the objective pursued, a particular bread formulation can be chosen. For example, it is possible to add different fibres to decrease GI and increase the protein digestibility of GF bread, while an overall high technological quality is retained in the final product.

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