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Original article

Techno-functional properties of wheat flour-resistant starch mixtures applied to breadmaking

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Abstract Resistant starch can be used to reduce the availability of carbohydrates in baked products. In this study, the effect of type 4 resistant wheat starch (RS_4) on wheat flour dough and breads was evaluated. Wheat flour was substituted by RS_4 at 10%, 20% and 30% w/w (RS10, RS20 and RS30, respectively). Rheological and thermal behaviours of dough were evaluated. Besides, bread quality, starch digestibility and bread staling were analysed. All substituted dough exhibited viscoelastic behaviour but lower elastic and viscous moduli. Regarding to bread quality, specific volume and crumb texture were negatively affected in samples with RS_4. However, all samples were technologically acceptable. During storage, crumb hardening was observed in breads without and with RS_4 but amylopectin retrogradation was not particularly affected. The *in vitro* digestibility of bread with RS showed a lower release of reducing sugars and a lower estimated glycaemic index, suggesting a healthier profile for these breads.

Keywords Bread quality, dough rheology, resistant starch, starch digestibility.

Introduction

The term 'resistant starch' (RS) is used to refer to starch and products of starch hydrolysis that are not absorbed in the small intestine of healthy individuals (Asp, 1992). Typically, RS has been classified into four different types: RS_1–RS_4. RS_1 is used to describe starch that is physically inaccessible to digestion, for example, the one that is protected by intact cell walls. In the case of RS_2, starch granules have a type of crystalline structure that protects them from hydrolysis. RS_3 is retrograded starch, and RS_4 refers to chemically modified starch. In RS_4 types, starch has been etherised, esterified or cross-linked. Lately, the amylose–lipid complex has been catalogued as RS_5 as this complex reduces starch susceptibility to hydrolysis (Hasjim *et al.*, 2013). Although RS is not absorbed in the small intestine, it could be fermented in the large bowel by the microflora. Several beneficial physiological effects have been attributed to RS, such as being promoter of a healthier glycaemic and insulinaemic response, a better colonic health and lipid profile, increasing satiety and exerting a prebiotic effect (Nugent, 2005). It has been proposed that many of these effects are mediated through the short-chain fatty acids that are produced when RS is fermented by microflora (Topping *et al.*, 2003).

Diet preparation in the case of those individuals who have to restrict their caloric and carbohydrate intake because of health causes is a difficult subject. The formulation of a popular product like bread with reduced starch availability could contribute to the diet requirements of individuals with special needs, increasing fibre intake and reducing caloric content and carbohydrate availability. This could also help to facilitate the attachment to the diet. Breads prepared with wheat bran present high levels of fibre but have quite different sensorial characteristics from white bread. Thus, the use of RS is one of the strategies for reducing carbohydrate availability in bread products without drastically modifying the sensorial attributes.

In the last years, RS has been used in baked products with wheat flour (Sanz *et al.*, 2008, 2009; Brites *et al.*, 2011; Almeida *et al.*, 2013; Majzoobi *et al.*, 2014) and in gluten-free ones (Korus *et al.*, 2009; Tsatsaragkou *et al.*, 2014). In most of these studies, the focus is placed on the quality of the fresh product, and RS is used at levels not higher than 20%. In

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addition, relatively scarce research has been done on dough performance, the interaction among components inside the matrix and the stability of the products during storage. Besides, the development of bakery products with RS_4 and starch digestibility in these products has not been widely reported. The objectives of this work were: (i) to study the effect of the partial substitution of wheat flour with RS_4 on the rheological, thermal and microstructural characteristics of dough; (ii) to evaluate technological quality of bread; (iii) to analyse *in vitro* digestibility of starch in bread matrix; and (iv) to assess bread stability during storage.

Materials and methods

Materials and formulation of mixtures

Formulations were prepared using commercial wheat flour (Molino Campodónico S.A., Argentina), NaCl (Celusal, Argentina), fresh yeast (Calsa, Argentina) and distilled water. Protein content for wheat flour was 11.92 ± 0.03%, and alveographic parameters P, L and W were 91 mmH_2O, 109 mm and 338×10^{-4} J, respectively (data supplied by the manufacturer). Farinograph water absorption, farinograph development time and stability of the flour employed were 58.9 mL, 9.7 min and 19.1 min, respectively.

Type 4 resistant wheat starch (RS_4) (Fibersym RW, MGP, United States) was used. The chemical modification involves sodium trimetaphosphate as cross-linking agent and sodium tripolyphosphate as substituting agent. The content of phosphorus residues in the product is lower than 0.4%. Starch swelling and solubility were ca. 2.8 w/w and 0.5% at 95 °C, respectively. An 85% (dry basis) of dietary fibre is provided by RS_4 (data supplied by the manufacturer).

Four formulations were prepared by replacing wheat flour with RS_4. Substitution levels were 0% (control), 10% (RS10), 20% (RS20) and 30% (RS30) (w/w). All mixes contained, additionally, 2% NaCl (flour basis).

All the reagents used for composition and *in vitro* digestibility assays were of analytical grade. Enzyme α-amylase type VI-B from porcine pancreas (16 units mg^{-1} solid) was purchased from Sigma.

Farinograph properties of flour and mixtures

Dough mixing properties were investigated with a Brabender farinograph (Duisburg, Germany) according to a modification of the constant flour weight (Variable Dough Weight) Procedure – AACC 54-21.01 (AACC, 2000). The modification consisted on the addition of NaCl 2% to wheat flour and composite flours. Water absorption, development time, stability and softening degree were obtained. Assays were performed in duplicate.

Preparation of dough

Dough was prepared in a planetary mixer (Kenwood, Italy) as stated by Correa *et al.* (2010). Water amount and kneading time were fixed according to absorption value and development time from the farinograph. To avoid changes during rheological measurements, yeast was not added to the formulation.

Oscillatory measurements

Small-amplitude oscillatory testing of dough was carried out using a Haake RS 600 rheometer (Thermo Science, Germany). Dough discs of 30 mm diameter and 2 mm height were assayed using rough surface parallel plates (30 mm diameter) with 1.5 mm gap at 25 °C. Vaseline was spread on sample border to avoid dehydration. For linear viscoelastic range (LVR) determination, dough was subjected to increasing shears at 1 Hz. Frequency sweep tests from 0.005 to 100 Hz were performed at 5 Pa shear within the LVR. All samples were let to rest 15 min before measurement. The elastic and viscous moduli and loss tangent were obtained. Assays were performed in duplicate.

Texture profile analysis (TPA)

Texture profiles were obtained by a two-cycle compression assay using a TA.XT2i Texture Analyzer (Stable Micro Systems, Surrey, United Kingdom) with a cylindrical P/75 probe (75 mm diameter). At least 30 dough discs (30 mm diameter and 10 mm height) were compressed up to 40% of their original height. Textural parameters, such as hardness, adhesiveness, consistency, cohesiveness, springiness, resilience and gumminess, were obtained. Measurements were performed on dough from two independent assays.

Confocal scanning laser microscopy (CSLM)

A Leica TCS SP5 confocal scanning laser microscope (Mannheim, Germany) with an argon and HeNe laser was used. Dough was spread over a slide glass and then dyed with an aqueous mixture of rhodamine B (0.001%) and fluorescein isothiocyanate (FITC) (0.01%). Excitation wavelengths were 488 nm for FITC and 568 nm for rhodamine B, while emission wavelengths were 518 nm and 625 nm for FITC and rhodamine B, respectively. Images were acquired using an HC PL APO CS 10× objective. Leica Application Suite Advanced Fluorescence (LAS AF), version 2.2.1

build 4842 and ImageJ 1.47v software (Wayne Rasband, National Institute of Health, United States) were employed.

Differential scanning calorimetry

A Q100 differential scanning calorimeter (DSC) (TA Instruments, United States) was used to study the thermal transitions of dough samples during the cooking process. For comparison, samples containing only RS, water and salt were run. Samples were heated from 5 °C to 140 °C (10 °C min^{-1}). A 5 min 5 °C isotherm was used for thermal stabilisation. From the thermograms, gelatinisation enthalpy and onset, peak and final temperatures were obtained. Assays were performed at least in duplicate.

Fermentation assays and bread making

Dough was prepared as for rheological assays but with the incorporation of 3% fresh yeast. Dough batches of about 650 g were prepared in a planetary kneader (Kenwood, Italy). Dry ingredients (flour, RS and NaCl) were premixed and the yeast was dissolved in part of the added water. Mixing times were fixed for each formulation according to the development time obtained from farinograph assays. The dough was left to rest for ten 10 min at room temperature, sheeted four times and left to rest again. Then, it was divided into small pieces and each one was rounded by hand.

For fermentation curves, dough was divided into pieces of 50 g and round kneaded portions were introduced into a graduated cylinder with a plunger marker and a lid. Cylinders were put in a chamber (Brito Hermanos, Argentine) at 30 °C. The increase in volume ($\triangle V$) vs. time was recorded using the plunger marker. Measurements were performed until the highest increase in volume ($\triangle V_{max}$) remained constant. The optimum fermentation time was defined as the time it takes to reach 75% of $\triangle V_{max}$ in order to avoid structure collapse by excess of fermentation during the subsequent baking step. Thus, each formulation needed a particular fermentation time. All measurements were made in triplicate.

For French-type bread preparation, dough was divided into 90-g pieces. Each portion was rounded by hand, left to rest for ten 10 min and then shaped into bread loaves by means of a sheeter moulder. These pieces were leavened at 30 °C according to the optimum fermentation time obtained by fermentation assays and baked in a convection oven for 26 min at 210 °C. After at least 3 h, control and RS20 breads were packaged into plastic bags and stored for 1 and 3 days at 20 °C (Correa & Ferrero, 2015). Texture and moisture of crumb and amylopectin retrogradation were evaluated at different storage times.

Fresh bread quality

Bread quality was assessed on fresh pieces from two different bakings. The following assays were performed:

Specific volume
Breads were weighed, and their volume was determined by rapeseed displacement. Specific volume was calculated as the ratio between bread volume and its weight. Eight breads were measured for each baking.

Crumb moisture
It was determined through weight loss up to constant value (by a minimum of 15 h) in an electric drying oven (San Jor, Argentine) at 105 °C. Assays were performed on three samples per replicate.

Crumb porosity
Images of bread slices were obtained with an HP Scanjet 4070 scanner. Eight scans (four from each baking) were employed. The analysis was carried out using ImageJ 1.47v software (Wayne Rasband, National Institute of Health, United States). The parameters determined were: mean alveolar area, perimeter, mode, air fraction, circularity and number of alveoli.

Texture profile analysis
Crumb texture analysis was performed at least 3 h after baking. Two bread slices of 2 cm height were obtained from the central part of each bread piece. Bread slices were subjected to compression in two cycles using a TA.XT2i Texture Analyzer (Stable Micro Systems, Surrey, United Kingdom) with a cylindrical probe of 25 mm diameter (P/25). At least eight slices of each sample were compressed up to 40% of their original height. The textural parameters determined were: hardness, consistency, cohesiveness, springiness, resilience and chewiness.

Nutritional value and in vitro digestibility of starch

Proximate composition. Protein, fat, moisture, ash and dietary fibre contents of bread samples were determined according to AACC methods 46-12.01, 30-10.01, 44-19.01, 08-01.01 and 32-05.01, respectively (AACC, 2000). Protein content was calculated from nitrogen content (Kjeldahl factor = 5.7). Nonfibre carbohydrate content was estimated as: 100 – (% protein – % fat – % moisture – % ash – % dietary fibre). All assays were performed at least in duplicate.

In vitro digestibility of starch
Starch digestibility was evaluated by the method described by Holm *et al.* (1985). An amount of bread crumb corresponding to 500 mg of starch was weighed by duplicate in a beaker, and 50 mL

of phosphate buffer (pH 6.9) was added. The total amount of starch was obtained as the sum of starch provided by flour and the RS added. Beakers were kept at 37 °C under continuous stirring. Aliquots of 0.2 mL were extracted before the addition of α-amylase and at 5, 15, 30 and 60 min after its addition. The amount of reducing sugars was colorimetrically determined with 3,5-dinitrosalicylic acid (DNS) at 530 nm after 10 min of reaction at 100 °C. The hydrolysis ratio was calculated as mg of maltose per gram of starch using a calibration curve (0–2 mg maltose/2 mL). An *in vitro* estimation of the glycaemic index was assessed as the relation between the area below the curve of breads with RS_4 and the corresponding area of control bread.

Amylopectin retrogradation

A Q100 DCS (TA Instruments, United States) was used to evaluate amylopectin retrogradation in crumb during the storage. Samples were subjected to heating at 5 °C min^{-1} from 5 °C to 150 °C. A 5 °C isotherm was employed for 5 min as stabilisation time. The temperature ramp was modulated at 0.5 °C every 20 s. From the thermograms, retrogradation enthalpy and onset, peak and final temperatures were obtained. DSC runs were performed in duplicate.

Statistical analysis

The analysis of variance (ANOVA) and Bonferroni's test were used to discriminate among means at a 0.05 significance level. OriginPro 8 SR0 v8.0725 (Northampton, United States) software was used for this purpose.

Results and discussion

Farinograph properties of flour and mixtures

The addition of RS_4 to wheat flour caused no marked changes in the farinograms. Substitution led to an increase in the peak observed during the first minutes of kneading (data not shown) that has been related to the water absorption process by starch granules (Larsen, 1964). Farinograph water absorption was 56 mL per 100 g of mixture for all samples, while developing time ranged from 7 to 11 min for RS20 and the control, respectively. The lowest stability was found for RS30 (30 min), while the highest was exhibited by the control sample (41 min); the softening degree ranged between 16 FU for the control and 25 FU for RS20. However, no significant differences in any of these parameters were found among samples ($P < 0.05$). The farinograph parameters showed by all mixtures indicate that they are adequate for bread making according to reported data (Lezcano, 2011).

Small-amplitude oscillatory testing

For all dough samples, the dynamic loss modulus G″ was always lower than G′, and both of them depended on the frequency (data not shown), indicating a viscoelastic solid behaviour of dough (Steffe, 1996). In Fig. 1, the values of the G′, G″ and tan δ, evaluated at 1 Hz, are shown. All flour-RS_4 mixtures showed G′ and G″ values lower than those of the control. The effect of RS_4 addition was more pronounced for RS10, which showed a significant decrease in the values of both moduli. For RS20 and RS30, the effect was less marked, and these samples presented similar values of G′ and G″, but lower than those of the control. However, the use of RS_4 resulted in a decrease of tan δ, indicating that G″

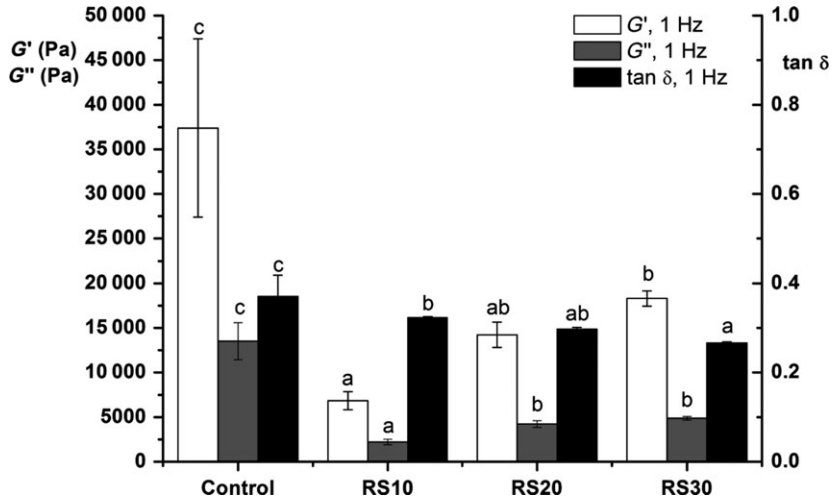

Figure 1 Dough rheological parameters. Elastic modulus (G′, white bar), viscous modulus (G″, grey bar) and tan δ (black bar) at 1 Hz. Different letters on the same parameter indicate statistical differences ($P < 0.05$).

presented a more pronounced decrease than G′. This suggests that an increase in the concentration of RS_4 in dough would lead to a loss of viscous behaviour rather than to a loss of elastic behaviour.

The decrease of G′ has been associated with a lower degree of gluten cross-linking (Khatkar *et al.*, 1995). In this case, the substitution of flour with RS_4 produced a 'dilution' on gluten concentration as protein content decreased from $14.3 \pm 0.2\%$ (control) to $9.8 \pm 0.4\%$ (RS30) (dry basis), and thus, the gluten network would become weaker. However, the effect on G′ could not be explained by this single cause.

The type of RS_4 used in this work is a phosphorylated and cross-linked wheat starch, and this modification could provide net negative charge to starch granules. Thus, RS_4 granules would be able to interact with gluten proteins that are positively charged in dough (pH ca. 5). This interaction could reinforce, at least in part, the gluten network. In samples with 10% of RS_4, this effect could be diminished by a shielding effect of chloride and sodium ions on the starch granules and gluten proteins. However, higher levels of RS_4 in the formulation (and thus, more negative charges) could overcome the shielding effect of ions when the amount of salt is fixed (2%).

Texture profile analysis

The effect of RS_4 on dough texture is shown in Table 1. There were no significant differences in hardness among control dough and RS20 and RS30. However, RS10 sample showed a significantly lower value, which is in good agreement with the results found in small-amplitude oscillatory testing. Consistency was always lower in dough with RS_4 than in the control sample. This parameter exhibited the lowest value for RS10, whereas samples with higher levels of RS_4 showed no significant differences from each other. Cohesiveness showed no significant differences among samples; thus, dough integrity was not negatively affected by RS_4 addition. Adhesiveness and gumminess showed similar behaviour to hardness, the RS10 sample presented significant lowest values of these parameters. In the case of gumminess, the value is strongly marked by hardness as cohesiveness was similar for all samples. Springiness was not significantly affected by RS_4, while resilience, related to dough instant recovery, was lower for samples with RS_4; the lowest value was exhibited by RS20 and RS30 samples.

In general, RS20 and RS30 samples showed no drastic modifications in dough texture with respect to the control.

CSLM

Micrographs of dough with and without RS_4 are shown in Fig. 2. Representative images at 10× were chosen to observe the global structure of dough matrix. Starch granules and gluten network could be seen in all images. RS_4 granules were undistinguishable from native wheat flour starch; the chemical modification of RS_4 did not change granule morphology (data not shown).

Control sample (Fig. 2a) showed gluten network with close parallel strands and starch granules all over the field of view. A similar pattern was observed in RS20 (Fig. 2c) and RS30 (Fig. 2d) samples. A matrix structure with more separated strands was seen for RS10 (Fig. 2b); this more open structure could be attributed to ionic interaction between RS_4 granules and gluten proteins, as mentioned in the previous sections. In this way, the gluten network would exhibit an open structure and RS_4 granules could act as a hindrance for the interaction between gluten strands. However, the amount of ions provided by NaCl could not be enough to shield all the negative charges in RS20 and RS30. In these cases, RS_4 granules could now behave like bridges between gluten strands. This would enhance interactions and would result in a closer structure. This hypothesis also agrees with the results observed in the dynamic oscillatory tests and TPA. In these assays, it was found that RS10 sample always presented a weaker structure, while RS20 and RS30 showed behaviour similar to that of the control.

Table 1 Dough textural parameters

	Control	RS10	RS20	RS30
Hardness (N)	1.5 ± 0.3^{b}	1.1 ± 0.3^{a}	1.4 ± 0.1^{b}	1.6 ± 0.2^{b}
Consistency (N.s)	11 ± 1^{c}	8 ± 1^{a}	9 ± 1^{b}	9 ± 1^{b}
Cohesiveness	0.75 ± 0.07^{a}	0.75 ± 0.02^{a}	0.74 ± 0.02^{a}	0.75 ± 0.01^{a}
Adhesiveness (N.s)	-4.9 ± 0.7^{a}	-3.9 ± 0.7^{b}	-4.6 ± 0.3^{a}	-4.6 ± 0.3^{a}
Springiness	0.90 ± 0.02^{ab}	0.89 ± 0.02^{a}	0.90 ± 0.01^{ab}	0.91 ± 0.01^{b}
Resilience	0.071 ± 0.010^{c}	0.064 ± 0.007^{b}	0.059 ± 0.004^{a}	0.058 ± 0.008^{a}
Gumminess (N)	1.2 ± 0.3^{b}	0.9 ± 0.2^{a}	1.1 ± 0.1^{b}	1.2 ± 0.1^{b}

Values are expressed as mean ± SD.

Different letters in the same row indicate statistical differences ($P < 0.05$).

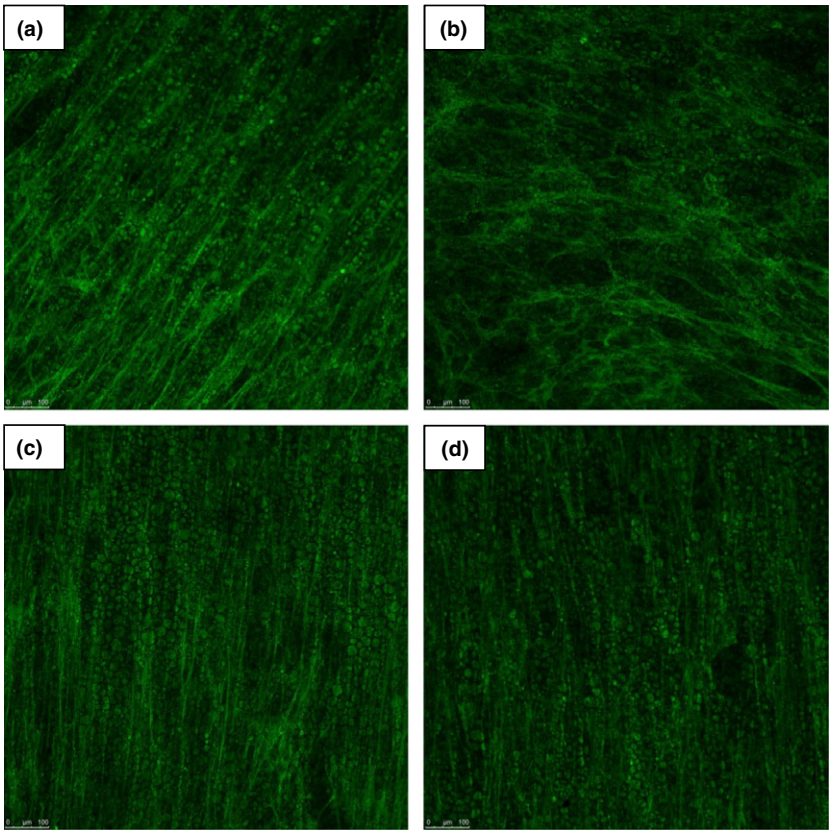

Figure 2 Micrographs of dough dyed with rhodamine B and FITC; captured in FITC channel at 10×. (a) Control, (b) RS10, (c) RS20, (d) RS30.[Colour figure can be viewed at wileyonlinelibrary.com].

DSC

Thermograms of dough showed thermal transitions in the range 61–108 °C, which would correspond to peaks of starch gelatinisation. All samples presented double endotherms indicating that the amount of water in dough was restrictive, so gelatinisation occurs in two steps (Biliaderis, 1983). A third transition was seen at ca. 115 °C, corresponding to dissociation of the endogenous lipid–amylose complex. There were no statistical differences in temperatures of transitions between dough with and without RS_4. The temperature of the first endotherm was between 69 and 71 °C and for the second one, between 90 and 92 °C. However, enthalpies of dough with RS_4 were significantly higher than the control one, possibly due to a greater content of total starch. Thermal profile of a mixture of RS, salt and water presented a single endotherm in the range 78–86 °C, indicating that peaks corresponding to gelatinisation of RS_4 in dough would overlap with the second endotherm of flour starch.

Fermentation curves

The variation of volume ($\triangle V$) with time (t) for all samples was fitted adequately to the three-parameter Chapman model (eqn 1) ($r^2 > 0.9777$), showing an exponential growth followed by a plateau at $\triangle V = \triangle V_{max}$.

$$\Delta V = \Delta V_{max}(1 - \exp^{-bt})^c \quad (1)$$

Fermentation times of RS_4 samples were 55 ± 1, 63 ± 2 and 47 ± 2 min for RS10, RS20 and RS30, respectively. These values were statistically lower than the time needed for the control sample (77 ± 0 min). On the other hand, the values of $\triangle V_{max}$ decreased with the increase in RS_4 level from 101 ± 0 cm^3 (control) to 82 ± 5, 85 ± 2 and 71 ± 1 cm^3 for RS10, RS20 and RS30, respectively.

Bread quality

The use of increasing concentrations of RS_4 led to a decrease in the specific volume of breads, as can be seen in Table 2. This could be related to the lower volumes obtained in the fermentation step which could be related to the effect of RS_4 on the viscoelastic properties of gluten network, as seen above.

Moisture values of fresh crumbs were between 42.9% and 44.2% for the control and breads with RS. Although in some cases statistical differences were found, these values were located within a narrow range.

Table 2 Bread quality

	Control	RS10	RS20	RS30
Sp. volume ($cm^3\ g^{-1}$)	3.6 ± 0.2^{d}	2.9 ± 0.1^{c}	2.5 ± 0.1^{b}	2.1 ± 0.1^{a}
Crumb porosity				
Air fraction (%)	28 ± 1^{b}	28 ± 1^{b}	26 ± 1^{b}	19 ± 1^{a}
Pore area (cm^2)	0.012 ± 0.002^{b}	0.011 ± 0.001^{b}	0.011 ± 0.001^{b}	0.008 ± 0.001^{a}
Number of pores	183 ± 26^{ab}	195 ± 20^{ab}	178 ± 24^{a}	214 ± 9^{b}
Perimeter (cm)	0.66 ± 0.04^{b}	0.63 ± 0.02^{b}	0.71 ± 0.05^{b}	0.52 ± 0.02^{a}
Circularity	0.44 ± 0.01^{a}	0.45 ± 0.01^{a}	0.43 ± 0.01^{a}	0.44 ± 0.01^{a}
Crumb texture				
Hardness (N)	7.4 ± 1.1^{a}	8.0 ± 1.3^{a}	13.0 ± 1.9^{b}	15.5 ± 1.5^{c}
Cohesiveness	0.55 ± 0.01^{c}	0.53 ± 0.01^{c}	0.50 ± 0.01^{b}	0.47 ± 0.01^{a}
Resilience	0.46 ± 0.03^{c}	0.47 ± 0.02^{c}	0.41 ± 0.03^{b}	0.34 ± 0.03^{a}
Springiness	0.97 ± 0.1^{b}	0.97 ± 0.02^{b}	0.94 ± 0.02^{a}	0.92 ± 0.02^{a}

Values are expressed as mean ± SD.
Different letters in the same row indicate statistical differences ($P < 0.05$).
Sp. volume, Specific volume.

When analysing crumb porosity, it was found that substitution at levels of 10% and 20% did not affect the structure (Table 2). Alveolus circularity was not affected in any sample. However, other structural parameters such as air fraction, pore area and perimeter were lower when the level of substitution was 30%. Additionally, the number of pores was significantly higher for this sample. This indicates that RS30 breads presented a higher number of pores with smaller size and higher symmetry. These breads would result in a more compact structure of the crumb, leading to a lower specific volume and foaminess (Zghal *et al.*, 1999).

Crumb quality was affected by RS_4 use as is seen by textural parameters (Table 2). Breads RS20 and RS30 exhibited higher hardness than control sample and lower cohesiveness, resilience and springiness. In the case of RS10, there were found no significant differences respect to control.

Table 3 Nutritional composition and estimated glycaemic index of breads

	Control	RS10	RS20	RS30
Proteins (g/100 g db)	14.3 ± 0.2^{d}	13.0 ± 0.2^{c}	11.6 ± 0.1^{b}	9.8 ± 0.4^{a}
Lipids (g/100 g db)	2.5 ± 0.0^{a}	2.7 ± 0.0^{a}	2.7 ± 0.1^{a}	2.7 ± 0.1^{a}
Ashes (g/100 g db)	2.8 ± 0.0^{a}	2.9 ± 0.2^{a}	2.7 ± 0.1^{a}	3.0 ± 0.2^{a}
Fibre (g/100 g db)	5.4 ± 0.1^{a}	13.9 ± 0.3^{b}	20.7 ± 0.6^{c}	29.2 ± 0.3^{d}
Carbohydrates (by difference)	75.0 ± 0.2	67.5 ± 0.4	62.4 ± 0.6	55.4 ± 0.5
Glycaemic index (%)*	100^{c}	80 ± 4^{b}	29 ± 2^{a}	22 ± 5^{a}

*Control sample defined as IG = 100%.
Different letters in the same row indicate statistical differences ($P < 0.05$).
db, dry basis.

Nutritional value and *in vitro* digestibility of starch

The nutritional composition of breads was evaluated (Table 3). As expected, protein content decreased with the increase in levels of flour substitution and fibre content increased However, lipid and ash contents did not change between formulations. Nonfibre carbohydrates decreased from 41.8% (control) to 31.4% for RS30, and this was reflected in starch digestibility. Samples with RS_4 showed an important decrease in starch hydrolysis compared with control bread (Fig. 3) (RS20 and RS30 samples reached a plateau after 5 min of hydrolysis; at this time, the control and RS10 samples reached much higher values of maltose release. With increasing concentration of RS_4, higher reductions in the value of the plateau were obtained; the lowest value was presented by RS30. All bread samples with RS_4 exhibited a reduction in the estimated glycaemic index respect to control bread (Table 3). These results indicate that the use of RS_4 in bread formulations could be a good strategy to diminish the glycaemic index of common bread. There are clinical trials that have shown that RS_4 suspensions decrease glucose release (Haub *et al.*, 2010) and also insulinaemic response when RS4 is consumed in a cereal bar (Al-Tamimi *et al.*, 2009). Thus, the results obtained in this study could suggest that these features could be maintained in a processed food like bread.

Storage

Bread behaviour during storage at 20 °C was studied for control and RS20. This sample was chosen among

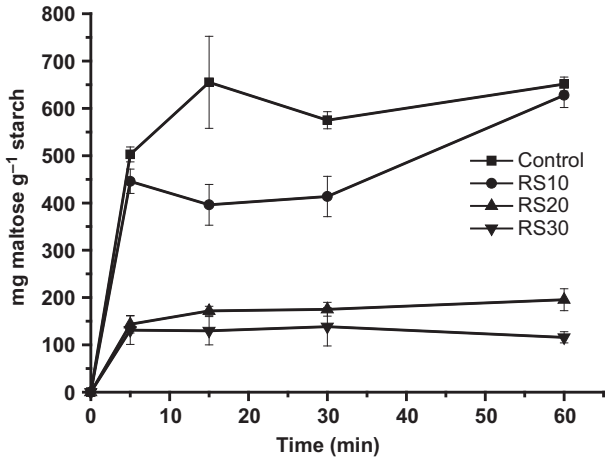

Figure 3 *In vitro* digestibility of starch in bread. -■- Control, -●- RS10, -▲-, RS20, -▼- RS30. Values measured as maltose (mg) delivered per gram of starch as function of time.

others due to its better technological quality with respect to RS10 and RS30. Besides, RS20 sample still provides an important amount of dietary fibre.

An increase in hardness was found for the control and RS20 with storage time (Fig. 4). Additionally, this parameter was significantly lower for control bread than for RS20. However, it was found that the relative percentage increase in hardness between day 0 and day 3 was higher for the control than for RS20. Increase in hardness during staling is not only related to amylopectin retrogradation and moisture losses but also to water redistribution, organisation of polymers within the amorphous region and the porosity of crumb (Davidou *et al.*, 1996).

Cohesiveness, resilience and springiness decreased along the storage period for each sample, and these parameters were always higher for the control than for RS20 at the same day of storage. These results indicate that RS20 would present a crumb with higher hardness and lower elasticity, as well lower cohesiveness, which means a loss of quality. This behaviour could also be related to the 'dilution effect' of gluten proteins by substitution with RS_4, as previously mentioned.

Thermal parameters for amylopectin retrogradation along storage were registered. Immediately after baking, there were no thermal transitions in the range 5–150 °C, suggesting that starch was completely gelatinised. A single endotherm was observed in the range 44–80 °C for the control and RS20 samples after 48-h storage. After 72 h of storage, both samples presented two endotherms. These endotherms exhibited a major peak in the range 44–80 °C, and a minor peak in the range 85–101 °C. In general, there were no statistical differences in transition temperatures (with the exception of T_p) and in enthalpies between samples. The occurrence of two endotherms in bread after storage has been previously reported, but it was not attributed to any dough component (Curti *et al.*, 2014). According to some reports, it could be related to yeast (Santagapita *et al.*, 2007). These results indicate that the addition of RS_4 would not influence the rate of amylopectin retrogradation in relation to the control.

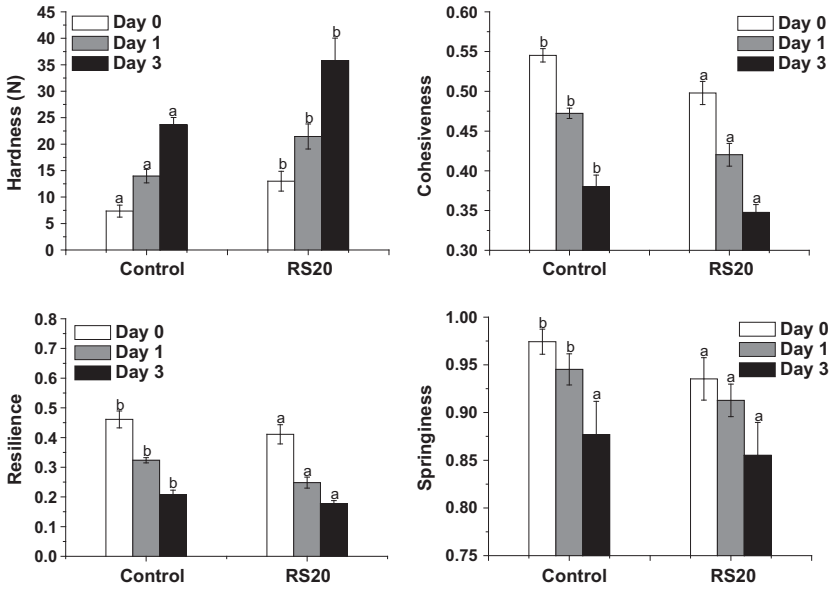

Figure 4 Crumb textural parameters. Control and RS20 fresh breads (white bar), after 24 h. (grey bar) and after 72 h. (black bar) of storage. Different letters indicate significant differences at the same day of storage ($P < 0.05$).

Conclusions

RS_4 was used in the production of French-type breads. Dough rheological behaviour was affected when RS_4 was incorporated, and these modifications caused changes in bread quality parameters, particularly at high levels of substitution. These changes would be related to the combined effect of gluten dilution and differences in gluten network conformation, as assessed by microscopic studies. On the other hand, the degree of starch retrogradation was not significantly affected by the addition of RS_4.

From a functional point of view, breads with increasing concentrations of RS_4 presented lower *in vitro* digestibility of starch and an estimated glycaemic index significantly lower than that of common bread. In this way, these formulations could have potential application in special diets. Further assays with breadmaking improvers are necessary to compensate for the loss of technological quality but maintaining the advantages obtained with flour replacement by RS.

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Conflict of interest

The authors declare no conflict of interest.

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