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High-Amylose Resistant Starch as a Functional Ingredient in Breads: a Technological and Microstructural Approach

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Abstract

Resistant starches (RS) are important functional fibers with high potential for the development of healthy foods. The technological, nutritional, and commercial possibilities of introducing type 2 RS in white breads were studied. Four levels of maize RS (HM) as wheat flour replacement were evaluated: 0%, 10%, 20%, and 30% (control, HM10, HM20, and HM30, respectively). Thermal transitions experiments were assessed on doughs prior to breadmaking. The bread quality was studied by specific volume, color of crust and crumb, porosity, and texture of the crumb. The microstructure of the crumb was analyzed by environmental scanning electron microscopy (ESEM). Proximate composition and in vitro starch digestibility were performed to characterize the nutritional profile of breads and estimate the glycemic index (GI). Consumer acceptability of breads was also evaluated. Breads with HM showed great performance up to 20% replacement in the specific volume, the crumb porosity, and the texture. Replacement up to 30% caused major damage to those parameters. Differential scanning calorimetry runs demonstrated that HM starch did not gelatinize under the baking conditions, as confirmed by ESEM. The presence of increasing levels of native starch is thought to have the greatest influence on reducing the crust browning, increasing the crumblier texture and decreasing starch digestibility. With respect to the control, a high and progressive reduction in the estimated GI and an outstanding increase of fiber with increasing levels of HM were found. The sensory evaluation of HM20 bread showed that this level of substitution has great consumer acceptance, giving it the chance to become a healthy substitute of white bread.

Keywords Resistant starch · Fiber · Wheat bread quality · Microscopy · In vitro starch digestibility · Estimated glycemic index

Introduction

An excessive glycemic carbohydrate intake is one of the recognized causes in the development of some chronic nontransmissible diseases such as obesity and diabetes mellitus type 2 (Rippe and Angelopoulos 2016; Sami et al. 2017). Many industrialized foods contain appreciable amounts of refined flours and usually have high glycemic indexes. The glycemic index is a way of measuring the rate of absorption of the carbohydrates contained in a certain food and their ability to produce an abrupt glycemic increase in blood. This value is usually obtained through glucose measurements in blood with a group of volunteers after the intake of a particular food (Brouns et al. 2005). Some of the mentioned diseases, such as type 2 diabetes, have been linked not only to the consumption of foods with a high amount of digestible carbohydrates but to the rapid changes in glycemia when these foods are consumed (Augustin et al. 2002; Brand-Miller 2003).

In this scenario, an adequate strategy to cooperate in the prevention of this kind of disease would be the change from a high-digestible carbohydrate diet to a lower one. However, consumers could find some difficulties in the access to these healthy foods due to the current high-processed and high-carbohydrate foods overflowing in the market. Besides, to get an improved adherence to a low-calorie diet, it is necessary to have access to palatable and sensory-acceptable foods with good textural, color and flavor attributes. Bread is one of the highest glycemic index meals (Atkinson et al. 2008) and also difficult to replace or eliminate from the daily diet since it is

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one of the most accepted and consumed foods. Breads with bran or made with whole wheat or non-wheat flours would contribute with a lower calorie content or valuable functional aspects, but their appearance and taste are not always accepted by the consumers (Arvola et al. 2007; Bakke and Vickers 2007; Dhingra and Jood 2004; Duță et al. 2018). When bread quality is analyzed, these facts have to be considered. Thus, the quality of the bread is evaluated from different points of view: the technological quality, which is closely related to the consumer's acceptability, and the nutritional and functional quality, which is associated to the health of the consumer.

For obtaining an acceptable functional food, the combination of both the product (carrier) and the functional ingredient is highly important. Ares and Gámbaro (2007) found that the carrier was the principal factor in the healthiness perception and willingness to try, but also that the carrier × enrichment interaction positively affected the results when the enrichment was an ingredient of the carrier per se, such as cereal-based foods enriched with fibers. Besides, Carrillo et al. (2012) reported that consumers tend to give major importance to some claims than others, "source of fiber", "source of cereals", and "no added sugar" being the most important claims for consumers when they evaluate biscuit products only by observing their packages.

In order to obtain healthy breads, the partial or full replacement of wheat flour by nondigestible carbohydrates (such as dietary fiber) can be performed. Some modified or natural starches called "resistant starches" (RS) are functional ingredients that have been successfully used in many food systems, with particular good results in baked goods (Arp et al. 2017; Baixauli et al. 2008; Sanz et al. 2009). Besides, resistant starches have even been proven to exert a prebiotic effect as well, with good results improving health markers in type 2 diabetes mellitus female population (Gargari et al. 2015; Karimi et al. 2016). In this regard, resistant starches have a good potential for functional food development.

In this work, different aspects of composite breads with wheat flour and different levels of a natural type of resistant starch (high amylose) were studied. The specific aims were (a) to characterize the technological quality of breads with RS; (b) to evaluate the functional properties conferred by the added RS by means of an in vitro approach; (c) to assess the changes in the consumer's perception about the sensory quality of these breads when health claims are used; and (d) to perform an approach of the microstructural changes introduced by the RS in the formulation and their relationship with quality aspects.

Materials and Methods

Materials and Formulation of Composite Flours

Formulations destined to the production of white bread containing resistant starch were prepared using commercial wheat flour (WF) (Molino Campodónico S.A., Argentina), commercial type-2 resistant maize starch Hi-Maize260™ (HM) (Ingredion Inc., United States), NaCl (Celusal, Argentina), fresh yeast (Calsa, Argentina), and distilled water.

The WF characteristics are summarized in Table 1.

Type-2 resistant maize starch provided 60% (dry basis) of insoluble dietary fiber (data supplied by the manufacturer).

The developed formulations were prepared by a replacing methodology, using HM to substitute wheat flour at the following levels: 0% (control), 10% (HM10), 20% (HM20), and 30% (HM30) (w/w). All mixes were prepared with 2% NaCl (flour or flour/HM basis, as appropriate) and 3% of fresh yeast.

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, solid) was purchased from Sigma Argentina S.R.L.

Preparation of Dough for DSC Assays

Doughs were prepared in a planetary mixer (Kenwood, Italy) equipped with a kneading hook as stated in a previous work (Arp et al. 2018). For the control, HM10, HM20, and HM30, the respective water absorption values were 55.4 ± 0.3, 58.2 ± 0.2, 60.3 ± 0.3, and 63.1 ± 0.4 ml/100 g. The farinographic development times (9.8 ± 1.4, 8.6 ± 1.6, 6.8 ± 0.2, and 6.7 ± 0.2 min for the control, HM10, HM20, and HM30, respectively) were used for kneading. No yeast was used in order to avoid changes during the assays.

DSC Measurements

Dough samples were analyzed by differential scanning calorimetry (DSC) for the evaluation of the thermal transitions during the baking step using a Q100 differential scanning

Table 1 Wheat flour characteristics

Protein content (g/100 g, wb)*	13.75 ± 0.02
Alveographic parameters†	
P (mmH_2O)	121
L (mm)	85
P/L	1.42
W (J)	390×10^{-4}
Farinographic parameters	
Water Absorption (ml/100 g)	58.8 ± 0.3
Development Time (min)	9.8 ± 1.3
Stability (min)	21.0 ± 0.9
Gluten	
Wet (g/100 g)	21.4 ± 0.4
Dry (g/100 g)	7.2 ± 0.1

*Mean value ± standard deviation. Measured by Kjeldahl method (nitrogen-protein conversion factor = 5.7)

† Provided by the manufacturer

calorimeter (TA Instruments, USA). Approximately 10.00 mg of each dough sample was weighed in DSC pans and hermetically sealed. Additional runs were performed with suspensions of HM and WF starch in distilled water (1:3 starch:water) to evaluate the thermal profile of each particular starch. Samples were run from 5 to 140 °C with a 10 °C/min heating rate. For thermal stabilization, a 5-min isotherm at 5 °C was employed. Samples were run at least in triplicate.

Fermentation Assays

Fermentation curve assays were performed to optimize the proofing times and prevent the doughs from collapsing while fermenting and/or baking. For this purpose, doughs were prepared as mentioned above but with the addition of 3% fresh yeast and allowing them to rest for 40 min in order to simulate the time elapsed during the dough handling before leavening. The obtained doughs were then divided into three portions of 50 g, and each portion was hand-rounded before placing it into a graduated cylinder. A plunger marker was used to facilitate the volume measurement and a lid for the graduated cylinder to prevent dehydration. The cylinders were placed into a 30 °C chamber (Brito Hermanos, Argentina). The fermentation curves were built by recording the increase in volume (ΔV) over time until reaching a plateau in ΔV (ΔV_{max}). In order to avoid dough collapse by excessive leavening, the time at which the dough reaches 75% of ΔV_{max} was established as the optimum proofing time (Arp et al. 2017). All measurements were made at least with three doughs prepared independently.

Breadmaking

For the preparation of traditional bread, known in Argentina as “French bread”, the dough prepared with fresh yeast was allowed to rest for 10 min and then sheeted four times, with 90° rotation of the dough between successive sheeting steps (Pastafácil, Argentina). Then, the dough was allowed to rest another 10 min and divided into 90-g pieces. Each portion was hand-rounded and then left to rest for 10 min before a sheeter molder (MPZ, Argentina) was used to shape the portions into individual bread loaves. The leavening times obtained in the fermentation assays were used for rising of the bread pieces. Once leavened, the doughs were baked in a convection oven (Ariston, Argentina) at 210 °C for 26 min.

Bread Technological Quality

Bread pieces from a total of eight bakes prepared in two different baking batches were studied. The technological quality was evaluated by the following assays.

Specific Volume

The loaf specific volume, expressed as the displaced rapeseed volume (in ml) per gram of bread, was determined after weighing the pieces. A total of 12 bread pieces per sample were assessed.

Texture Profile Analysis

Bread crumb texture was measured using a TA.XT2i Texture Analyzer (Stable Micro Systems, UK). Two bread slices 2 cm thick were cut at the middle of each bread. Then, a piece of the crumb was cut off from the center of each slice with a mold cutter of 3-cm diameter. A plane cylindrical probe of 75-mm diameter (P/75) was used to compress the bread slices up to 40% of their height in two cycles. At least sixteen slices of each sample were analyzed. The texture characteristics of the crumb were evaluated by calculating, from the obtained profiles, the following parameters: hardness, consistency, cohesiveness, springiness, resilience, and chewiness (hardness × cohesiveness × springiness).

Water Content and Water Activity of Bread Crumb

The water content of crumb samples was determined by water loss at 105 °C until constant weight in an electrical oven (San Jor, Argentina). Measurements of water activity (a_w) at 25 °C were done using an AquaLab Series 4 (Decagon Devices Inc., USA). For each sample, all measures were carried out in triplicate.

Crust and Crumb Color

The CIE-Lab parameters L^*, a^*, and b^* were determined employing a Chroma Meter CR-400C surface colorimeter (Minolta, Osaka, Japan) for evaluation of the color characteristics of bread crust and crumb. The browning index (BI) was also calculated from the CIE-Lab parameters (Salinas et al. 2016).

Crumb Porosity

Image analysis was employed for the evaluation of crumb porosity. An HP Scanjet 4070 scanner was used for the acquisition of a total of sixteen bread slice images per sample. The image processing and analysis were performed using the ImageJ 1.47v software (Wayne Rasband, National Institute of Health, USA). The number of alveoli (N), air fraction (expressed as percentage of the total area occupied by alveoli over the total area of the image), mean alveolar area (MAA), perimeter, and circularity were determined as porosity parameters.

Environmental Scanning Electron Microscopy

Small pieces of bread crumb were taken from the center of bread samples and placed onto a conical concave metallic support for observation in a FEI Quanta 200 environmental scanning electron microscope. The pressures and temperature employed for the observation were 4.14 and 4.41 Torr and 10 °C. Images of different fields were obtained at 500 × and 1500 ×.

Nutritional Value and In Vitro Digestibility of Starch

Proximate Composition

For assessment of the protein, fat, moisture, ash, and dietary fiber contents of bread samples, the corresponding approved AACC methods 46–12.01, 30–10.01, 44–19.01, 08–01.01, and 32–05.01 were performed at least in duplicate (AACC International 2000). The digestible carbohydrate content (different from fiber) was estimated by difference.

In Vitro Digestibility of Starch and Estimated Glycemic Index

The starch digestibility was evaluated by the method described by Goñi et al. (1997) with some modifications. Five hundred milligrams of bread crumb was weighed in duplicate in a 50-ml conical plastic tube and then 10 ml of HCl-KCl buffer (pH 1.5) was added. The tubes were placed in a heat block at 40 °C with shaking (650 rpm). Once the tubes reached the incubation temperature, 200 μl pepsin solution (200 FIT-U/ml) was added to each tube. Samples were left to incubate for 60 min with shaking. Then, they were cooled down to 37 °C, and 15 ml phosphate buffer (pH 6.9) with 20 μl $CaCl_2$ 3 M solution was added before the addition of 5 ml α-amylase solution (10.31 U/ml) prepared with 33 μl $CaCl_2$ 3 M solution. $CaCl_2$ solutions were added separately to avoid Ca^{+2} ion precipitation due to the presence of phosphates. Sample tubes were incubated at 37 °C with shaking (600 rpm) for 180 min. Two hundred microliters were taken at 0, 20, 60, 90, 120, and 180 min, placed in glass tubes and heated at 100 °C for 5 min to inactivate the enzymes. Released free sugars were quantified by the 3,5-dinitrosalicylic acid (DNS) method. Reducing sugars and DNS react after 10 min at 100 °C, and the product absorbance can be read at 530 nm. The hydrolysis ratio was calculated as milligrams of maltose (expressed as starch) per gram of starch by means of a calibration curve (ranging from 0 to 1 mg maltose/ml). For an in vitro estimation of the glycemic index, the ratios between the areas below the curve of breads with HM and the area of control bread (taken as 100%) were calculated.

Sensory Analysis and Nutritional Perception

Samples for sensory analysis were prepared as stated in "Breadmaking" section. An additional factor was included in the tests in order to assess how consumers perceived the nutritional advantages of a healthy substitute of white wheat bread when an undetectable source of fiber, such as HM, was used. For this purpose, the sensory evaluation was performed in two steps. In the first stage, coded slices of breads were presented to the consumers without any information. In the second step, the bread slices were given with a nutritional chart containing information about the recommended daily fiber intake (RDFI) (*Código Alimentario Argentino* 2014) and the fiber contribution of both breads to the RDFI, as well as information about what the HM was and the ingredients employed in breadmaking. Each step of the sensory evaluation was carried out with 24 h in between. Forty consumers (65% females and 35% males, with ages ranging between 24 and 55 years) were recruited for the assay. More than 83% of the evaluators stated they consumed bread products at least once a week. Evaluators had to respond about the appearance, texture, flavor, color, and general acceptability of samples on a balanced hedonic scale ranging from 1 ("I disgust a lot") to 9 ("I like a lot").

Statistical Analysis

The means were compared by analysis of variance (ANOVA) and LSD Fisher's test at a 0.05 significance level. For this purpose, the statistical analysis software OriginPro 8 SR0 v8.0725 (Northampton, USA) was employed. For Pearson's correlation tests and sensory analysis, the Statgraphics Centurion XV version 15.2.06 (StatPoint, Inc.) software was used at a 0.05 significance level. For sensory evaluation, a multifactorial ANOVA was performed. The fixed factors were "samples" and "nutritional chart presence", while the random factor was "consumers."

Results and Discussion

Thermal Transitions of Doughs

The DSC assays performed on bread doughs and starch suspensions give information about the thermal transitions occurring in starch when the dough transforms into crumb.

Endotherms of suspensions of WF starch and HM starch (suspensions 1:3, starch:distilled water) were assessed in order to identify the contributions of each starch to the endotherms of composite doughs (inset Fig. 1). WF starch showed a one-step transition, with a narrow and pronounced gelatinization peak ($T_o = 55.9 \pm 0.0$ °C, $T_p = 61.1 \pm 0.4$ °C, $T_c = 84.8 \pm 0.6$ °C, and $\Delta H_{gel} = 12.4 \pm 0.3$ J/g), and a less prominent

amylose-lipid complex dissociation peak ($T_o = 90.8 \pm 1.1$ °C, $T_p = 100.0 \pm 1.1$ °C, $T_c = 110.3 \pm 0.9$ °C, and $\Delta H_{A-L} = 1.3 \pm 0.3$ J/g). In this case, the starch hydration level was 75% (w. b.). In the case of HM starch, no peak was found between 5 and 200 °C. This means that the HM starch granules did not gelatinize even in excess of water, so they would not contribute to the endotherms observed in composite doughs. In this case, the endotherms observed could be attributed only to the wheat starch gelatinization.

Table 2 lists the thermal parameters obtained from thermograms of the control and composite doughs.

The endotherms showed the typical behavior expected for gelatinization under restricted availability of water, giving thermograms with doublet endotherms corresponding to a process occurring in two steps (Fig. 1, P1 and P2). According to the amounts of starch and water in the evaluated bread doughs, the starch hydration ranged from 44.9 to 46.7 (g H_2O/100 g starch, w. b.) for the control and HM30, respectively. Kovrlija and Rondeau-Mouro (2017) reported similar results when wheat starch suspensions with starch hydration levels ranging from 34.71 to 49.82% (w. b.) were subjected to DSC runs. In a pioneering work, Evans and Haisman (1982) proposed that, in a first step, the weaker crystals interact with the available water and can melt, giving the first peak. Then, the more stable crystals that have less available water for interaction melt at higher temperatures, where the second peak appears. The presence of HM in doughs had a significant effect on their thermal behavior, especially on the second peak of the endotherm (P2 in Fig. 1) which exhibited a decrease in T_{p2}, T_c, and ΔH_{gel} at increasing HM concentration. In the case of the amylose-lipid complex dissociation (A–L in Fig. 1) the decrease was evident in all parameters.

The decrease in T_{p2} and T_c observed in the second peak of the HM doughs would then be due to the increasing amount of water. This increase was caused by the higher water requirements for the preparation of doughs with HM, but also by the augmented proportion of available water that was not utilized for the HM starch for gelatinization. Then, WF starch had more available water and the transition temperatures decreased. On the other hand, the dilution of the gelatinizable WF starch produced by the replacement with HM was responsible for the decrease in enthalpy. In fact, the obtained gelatinization enthalpies negatively ($r = -0.9932$) and significantly ($p < 0.05$) correlated with the replacement levels. It is noteworthy that having non-gelatinized starch in bread pieces is critical for a decrease in digestibility and glycemic index (Burton et al. 2011; Martínez et al. 2018).

Fermentation Assays

For obtaining the optimum fermentation times, the ΔV vs. time data were successfully fitted to the three-parameter Chapman's equation ($r^2 \geq 0.9687$). The fermentation times were then calculated as 75% of the time required by the dough at 30 °C for reaching its maximum ΔV. The values obtained were 59, 77, 97, and 91 min for the control, HM10, HM20,

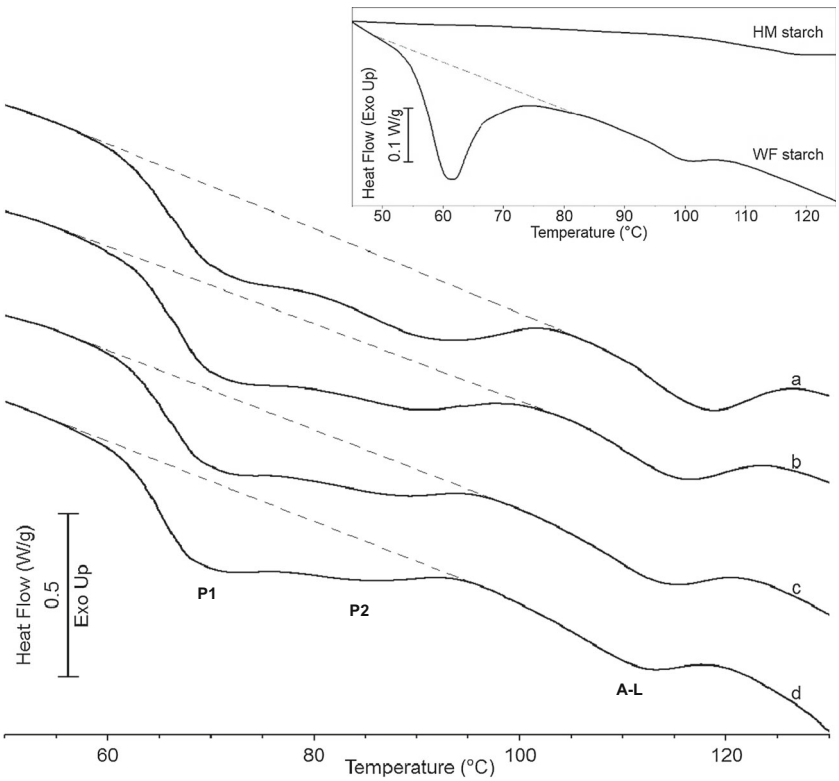

Fig. 1 Thermograms of bread doughs. (**a**) Control. (**b**) HM10. (**c**) HM20. (**d**) HM30. P1 and P2 indicate the first and second peaks of the gelatinization process and A–L the amylose-lipid complex dissociation. Inset: thermogram of isolated WF starch and HM starch

Table 2 Thermal parameters of dough samples by DSC

	Starch gelatinization				
	T_o (°C)	T_{p1} (°C)	T_{p2} (°C)	T_c (°C)	ΔH_{gel} (J/g)*
Control	62.82 ± 0.64^a	70.35 ± 0.30^b	92.36 ± 0.43^d	103.66 ± 0.57^d	8.35 ± 0.23^d
HM10	62.98 ± 0.36^a	69.92 ± 0.49^b	89.97 ± 0.46^c	102.29 ± 0.23^c	7.10 ± 0.47^c
HM20	62.77 ± 0.56^a	69.26 ± 0.08^a	88.19 ± 0.23^b	98.10 ± 0.48^b	6.18 ± 0.24^b
HM30	62.34 ± 0.34^a	69.00 ± 0.15^a	86.28 ± 1.34^a	96.23 ± 0.46^a	5.44 ± 0.21^a
	Amylose-lipid complex dissociation				
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{A-L} (J/g)†	
Control	106.77 ± 0.19^d	117.76 ± 0.42^d	127.72 ± 0.11^d	0.76 ± 0.07^c	
HM10	105.26 ± 0.54^c	115.54 ± 0.40^c	125.82 ± 0.92^c	0.64 ± 0.03^b	
HM20	103.73 ± 0.25^b	113.47 ± 0.16^b	123.19 ± 0.43^b	0.56 ± 0.05^{ab}	
HM30	101.65 ± 1.11^a	111.42 ± 0.42^a	120.82 ± 0.20^a	0.50 ± 0.06^a	

T_o, T_p and T_c correspond to the onset, peak, and conclusion temperatures, respectively

ΔH_{gel} and ΔH_{A-L} correspond to the gelatinization and amylose-lipid complex dissociation enthalpies, respectively

Mean values ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

*Starch basis, dry basis

† Dry basis

and HM30, respectively. The dilution of the wheat starch due to its replacement by HM with a more compact crystalline structure would be responsible for the longer times since yeast could be limited access to fermentable substrates.

Fresh Bread Quality

Specific Volume

The specific volume of the loaves is one of the most important parameters to evaluate the commercial bread quality since it easily gives information on the global characteristics of the piece and the final air retention capacity. Most of the textural and palatability characteristics of bread crumb are related to its volume to some extent (Cauvain 2007). The values of specific volumes for the control and HM breads are shown in Fig. 2. The use of HM did not affect the specific volume at replacement levels up to 20%. Only higher levels of replacement, such as 30%, led to a significant reduction in volume of ca. 30%, with respect to the control. The results demonstrate HM had a great performance in breadmaking even at levels as high as 20% of replacement. However, increasing concentrations of HM would lead to lower volumes likely due to the extent of the gluten protein dilution and a hindrance effect on the gluten network development by the non-gelatinized HM starch granules (Arp et al. 2018).

Crumb Texture

In the case of fresh breads, a spongy, soft, cohesive, and resilient crumb is imperative for good acceptance (Rashidi et al. 2016). In Fig. 3, some of the textural parameters assessed by TPA for bread crumb samples are shown. The hardness increased with the HM concentration with respect to the control, especially for HM30 (Fig. 3a). Besides, cohesiveness was lower at increasing amounts of HM, now again with HM30 showing the lowest value (Fig. 3b). In the case of springiness, small changes were found even with 20% of WF-HM replacement (Fig. 3c). The values for consistency and chewiness followed the same behavior as hardness since these parameters are derived from the latter (data not shown). For resilience, no changes could be noticed at 10% of replacement but they were evident at higher levels (Fig. 3d). HM30 presented the lowest value for both springiness and resilience (Fig. 3c, d). Springiness and resilience both represent elastic recoveries of the crumb, the former related to the recovery capacity once the input force is removed and the latter to the

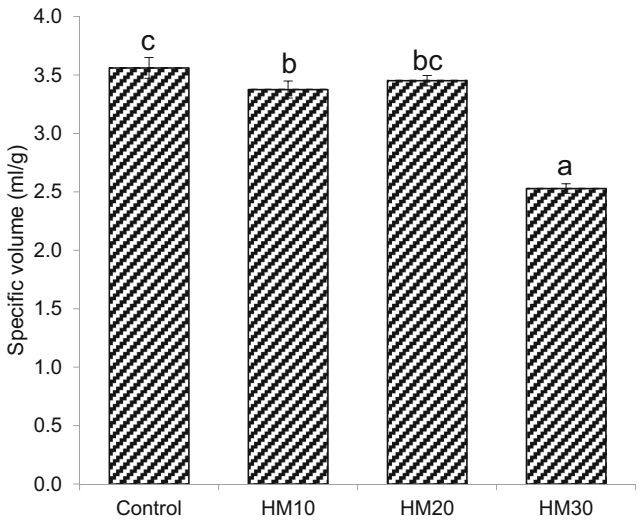

Fig. 2 Specific volume of bread pieces (mean values ± standard error). Different letters indicate significant differences ($p < 0.05$)

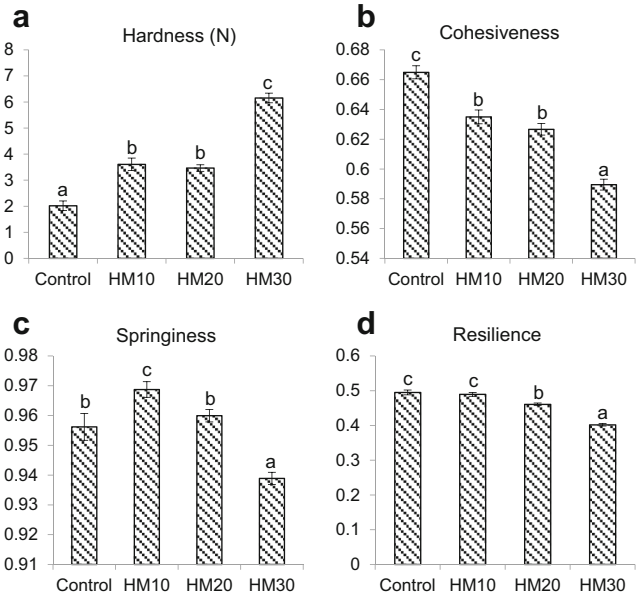

Fig. 3 Crumb texture by TPA (mean values ± standard error). Different letters indicate significant differences ($p < 0.05$)

recovery speed during the first compression cycle. Having a simultaneous decrease in cohesiveness and resilience would result in a crumblier matrix (Armero and Collar 1997; Giannone et al. 2016). This effect was particularly evident for HM30 since its cohesiveness and resilience were the lowest.

Water Content and Water Activity of Bread Crumb

The bread crumb water content and a_w values are shown in Table 3.

The crumb moisture progressively increased with the HM concentration likely due to the augmented amounts of water needed to prepare doughs. However, even though water was added in greater proportion for breads containing HM, its influence on improving texture performance was not evident. Then, the changes seen in texture parameters would be due to the presence of HM starch.

On the other hand, a_w did not show major changes but for HM30, which presented a slightly higher value. This effect could be related to the fact that doughs with HM were prepared with higher water amounts, but it would also reflect a lower capacity of HM to bind water, in accord with DSC assays where HM did not gelatinize.

Table 3 Water content and water activity

	Water content (% w/w, w.b.)	a_w
Control	43.8 ± 0.3^a	0.966 ± 0.001^a
HM10	44.9 ± 0.2^b	0.966 ± 0.003^a
HM20	45.3 ± 0.3^c	0.966 ± 0.002^a
HM30	46.4 ± 0.3^d	0.970 ± 0.001^b

Mean values ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

Crust and Crumb Color

The values of CIE-Lab parameters L^*, a^*, and b^* are listed in Table 4 for bread crust and crumb. With respect to the crust, the increase in HM concentration leads to a rise of L^*, giving breads with whiter appearance. The red-green chromaticity parameter, a^*, showed a progressive reduction in redness with the use of HM. The yellow-blue parameter, b^*, also decreased with the HM concentration except for HM10, which presented the same value as the control. However, the *BI* followed a progressive decrease with the increasing content of HM. The decrease in *BI* would be related to a lower amount of free sugars available for Maillard reaction during baking due to the replacement of WF. Thus, doughs would have less free reducing sugars and breads would have less brown color (Hidalgo and Brandolini 2011).

With respect to the crumb, the effect of HM was not evident since no significant differences were found between HM30 and the control. In this case, the bread crumb of HM breads would be perceived as the same as that of the control.

Crumb Porosity

The analysis of porosity in the bread crumb is useful for evaluating the performance of the fermentative process and the effect of incorporating different ingredients to the formulations since their effects would be reflected in the distribution, size, and shape of the alveoli. The porosity parameters extracted from digital images of bread slices are presented in Table 5.

Samples containing HM always developed a 25% lower *N*. Besides, a reduction in the air fraction was also seen, especially for HM30. These results would explain the differences

Table 4 Crust and crumb color parameters

	L^*	a^*	b^*	*BI*
Crust				
Control	64.4 ± 4.5^a	9.8 ± 1.3^d	28.8 ± 2.0^c	68.7 ± 6.7^d
HM10	72.3 ± 4.4^b	7.0 ± 1.4^c	29.4 ± 1.3^c	61.1 ± 7.1^c
HM20	75.2 ± 3.2^c	3.9 ± 1.9^b	27.7 ± 2.4^b	48.7 ± 8.5^b
HM30	80.9 ± 3.4^d	1.1 ± 1.3^a	23.2 ± 2.8^a	34.0 ± 7.0^a
Crumb				
Control	74.3 ± 4.6^{bc}	− 1.2 ± 0.2^b	13.5 ± 1.0^b	17.9 ± 1.5^a
HM10	75.2 ± 4.1^c	− 1.3 ± 0.1^a	12.9 ± 1.1^a	17.8 ± 1.6^a
HM20	71.1 ± 2.6^a	− 1.3 ± 0.2^{ab}	13.0 ± 0.7^a	18.1 ± 1.2^a
HM30	72.6 ± 3.6^{ab}	− 1.1 ± 0.2^c	13.2 ± 0.8^{ab}	18.3 ± 1.3^a

Mean values ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

Table 5 Crumb porosity

	Number of alveoli in the image (*N*)	Air fraction (%)†	*MAA* (cm^2)‡	Perimeter (cm)	Circularity
Control	208 ± 50[b]	36 ± 3[c]	0.016 ± 0.003[a]	0.57 ± 0.04[b]	0.53 ± 0.03[a]
HM10	157 ± 29[a]	33 ± 4[b]	0.020 ± 0.005[b]	0.61 ± 0.06[c]	0.54 ± 0.03[a]
HM20	151 ± 29[a]	32 ± 3[b]	0.020 ± 0.004[b]	0.59 ± 0.05[bc]	0.55 ± 0.03[ab]
HM30	156 ± 19[a]	27 ± 2[a]	0.016 ± 0.002[a]	0.53 ± 0.03[a]	0.57 ± 0.02[b]

† Calculated as the total area occupied by alveoli over the total area of the image

‡ Mean alveolar area

Mean values ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

found in the specific volume values since less air fraction implies a lower bread volume. However, the *MAA* values were higher for both HM10 and HM20, which would explain the less drastic loss in air fraction and specific volume expected for the lower *N*.

The perimeter and circularity parameters showed minor changes in the shape of alveoli.

Microstructural Analysis by Environmental Scanning Electron Microscopy (ESEM)

Micrographs of internal walls of bread crumb alveoli are presented in Fig. 4. In the images, it is possible to appreciate the starch granules that still kept their granular shape embedded in the coagulated-gluten and lixiviated-amylose structural

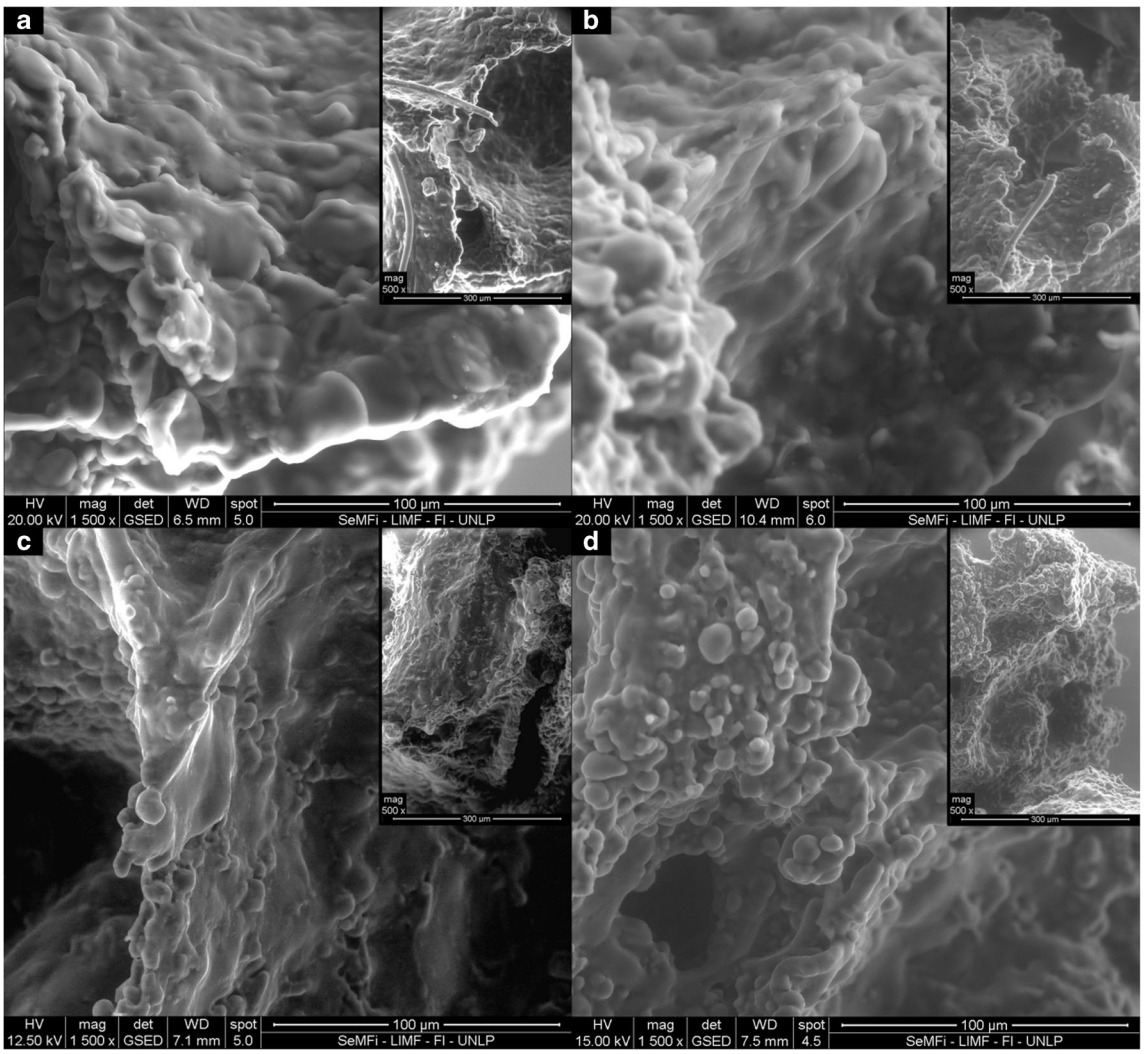

Fig. 4 ESEM images of bread crumb at 1500× and 500× (embedded image). (**a**) Control. (**b**) HM10. (c) HM20. (d) HM30

Table 6 Proximate composition

	Lipids	Ashes	Proteins	Fiber	Carbohydrates*
Control	1.90 ± 0.08^{b}	2.79 ± 0.04^{c}	16.02 ± 0.07^{d}	6.6 ± 0.10^{a}	72.69 ± 0.15
HM10	1.89 ± 0.04^{ab}	2.75 ± 0.03^{bc}	15.14 ± 0.04^{c}	9.5 ± 0.10^{b}	70.68 ± 0.16
HM20	1.82 ± 0.02^{ab}	2.70 ± 0.03^{ab}	13.46 ± 0.06^{b}	17.0 ± 0.70^{c}	65.02 ± 0.70
HM30	1.81 ± 0.02^{a}	2.65 ± 0.03^{a}	11.83 ± 0.08^{a}	26.6 ± 1.80^{d}	57.14 ± 1.83
WF	1.91 ± 0.03	0.68 ± 0.01	15.85 ± 0.02	4.46 ± 0.74	77.11 ± 0.74
HM	1.27 ± 0.05	0.12 ± 0.00	0.78 ± 0.01	58.40 ± 7.76	39.43 ± 7.76

*Carbohydrates different from fiber, obtained by difference

Mean values ± standard deviation, dry basis. Different letters in the same column indicate significant differences ($p < 0.05$)

matrix. The micrographs corresponding to the control and HM10 (Fig. 4a, b) exhibited surfaces with a more regular appearance in contrast with HM20 and HM30 (Fig. 4c, d), the ones that showed a considerable number of small starch granules projecting out of the matrix. The augmented starch/gluten proportion in samples with increasing levels of HM would lead to a saturation of the gluten network by starch granules, so the particles could not remain immersed in the matrix (Arp et al. 2018). Then, beyond the importance of the water content in most of the bread characteristics such as texture, specific volume, and even starch gelatinization, the comparative analysis of the micrographs would indicate that the increasing number of starch particles played a greater role in textural performance than water did. The decrease in cohesiveness and resilience seen in textural assays could be explained by the augmented protruded starch fraction seen at higher HM concentrations since the structural matrix got weakened by the hindrance of non-gelatinized starch granules.

Nutritional Analysis and Digestibility

The proximal composition of the bread samples as well as the WF and the HM is listed in Table 6. As can be seen in the table, HM contributed with much lower amounts of proteins, so the final products obtained with the replacement methodology showed progressive decreases in this component. However, available carbohydrates also exhibited a considerable decrease, this effect having health benefits. Besides, the fiber content (which encompasses HM plus the intrinsic fiber of WF) was 42%, 151%, and 284% higher for HM10, HM20, and HM30 than for the control. This considerable increase in fiber, in addition to the decrease in the available carbohydrate content, leads to a healthier final product.

In order to evaluate the impact of carbohydrate and fiber content on health, starch digestibility assays with pepsin and α-amylase were performed. The results are shown in Fig. 5.

Before the beginning of digestibility (time 0 min), all samples started from the same free sugar amounts, except for HM30, which presented the lowest value. Then, once α-amylase was added, the general behavior exhibited a tendency to higher sugar release by the control, followed by HM10, HM20, and HM30. The last two samples always presented free sugar releases significantly lower than the control and HM10.

The curves were adjusted to an exponential model (Eq. 1):

$$C = C_{\text{inf}} \times \left(1 - e^{-a \times t}\right) \quad (1)$$

where C_{inf} represents the sugar release plateau value reached by the sample, and a could be considered as the hydrolysis rate constant (Goñi et al. 1997). The fitting showed values of $r^2 \geq 0.9285$ in all cases. The parameters obtained are presented in Table 7.

The maximum release of reducing sugars was lower for HM20 and HM30 than for the control, suggesting HM was able to resist the attack of α-amylases even after the baking step of breadmaking. This would be due to the non-gelatinized HM starch granules, as found in DSC.

On the other hand, the control sample exhibited the highest hydrolysis rate, which means that the digestion in this sample progresses faster than in the HM ones. Besides, the estimation of the glycemic index (GI) based on the in vitro test curves showed that all the samples containing HM presented significantly lower values of GI, with a progressive decrease. When the correlation analysis was performed on C_{inf} and GI, a negative and significant correlation was found with the

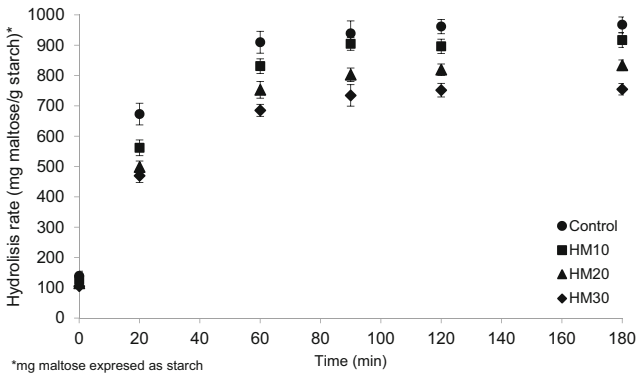

Fig. 5 Hydrolysis rate curves (mean values ± standard error)

Table 7 Digestibility parameters and estimated glycemic index

	C_{inf} (mg maltose/g bread)	a (min^{-1})	Estimated GI
Control	954 ± 57^{c}	0.0601 ± 0.0064^{b}	100^{d}
HM10	907 ± 43^{c}	0.0472 ± 0.0047^{a}	92.4 ± 2.3^{c}
HM20	822 ± 38^{b}	0.0453 ± 0.0033^{a}	83.5 ± 2.3^{b}
HM30	747 ± 43^{a}	0.0480 ± 0.0038^{a}	76.5 ± 0.6^{a}

Mean values ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

replacement level ($r = -0.9938$ and $r = -0.9990$ at $p < 0.05$ for C_{inf} and GI, respectively), and a positive and also significant correlation with gelatinization enthalpy ($r = 0.9752$ and $r = 0.9930$ at $p < 0.05$ for C_{inf} and GI, respectively). This correlation confirms that the decrease in the digestibility parameters was caused by the augmented fractions of non-gelatinizable starch in the HM enriched samples.

In addition, Penn-Marshall et al. (2010) found that ingestion of 12 g of Hi-Maize 260 per day, given as bread, was not enough to improve health markers of type 2 diabetes in the African-American population in comparison to the control bread, and a higher Hi-Maize daily intake must be consumed. However, Gargari et al. (2015) and Karimi et al. (2016) found good results improving health markers in female population with type 2 diabetes with an intake of 10 g/day of Hi-Maize 260. The results of the present work indicate that HM-enriched breads, particularly HM20 and HM30, could improve the fiber intake and would effectively reduce the amount of available carbohydrates. Thus, these breads would be an appropriate daily food that could be included in diets for special nutritional requirements.

Sensory Evaluation and Nutritional Perception

Sensory evaluation of control and HM20 samples was performed. The HM20 sample was selected among the other formulations with resistant starch due to it breadmaking performance. Unlike HM30, the HM20 breads presented a good baking performance and the quality parameters, such as specific volume and crumb texture, were similar and/or not so drastically affected respect to the control, while having a higher fiber content and lower digestibility than the HM10 samples. In general, the majority of the consumers scored the different attributes above 6 for all samples. In fact, no statistical differences were found between samples when the mean score of each attribute was analyzed. The effect of giving nutritional information with the samples did not affect the mean score either. However, some additional information could be extracted from the histograms that show the score responses of the consumers to the texture and overall acceptability attributes of the samples (Fig. 6). In the case of texture, the inclusion of the nutritional chart produced a score displacement from 7 to 8 and 9 for HM20 (Fig. 6b) and in the other hand, the shift was from 8 to 7 for control bread (Fig. 6a). The crust color (data not shown) was the attribute with greater dispersion of the scores since the acceptability for whiter or browner crusts strongly depends on

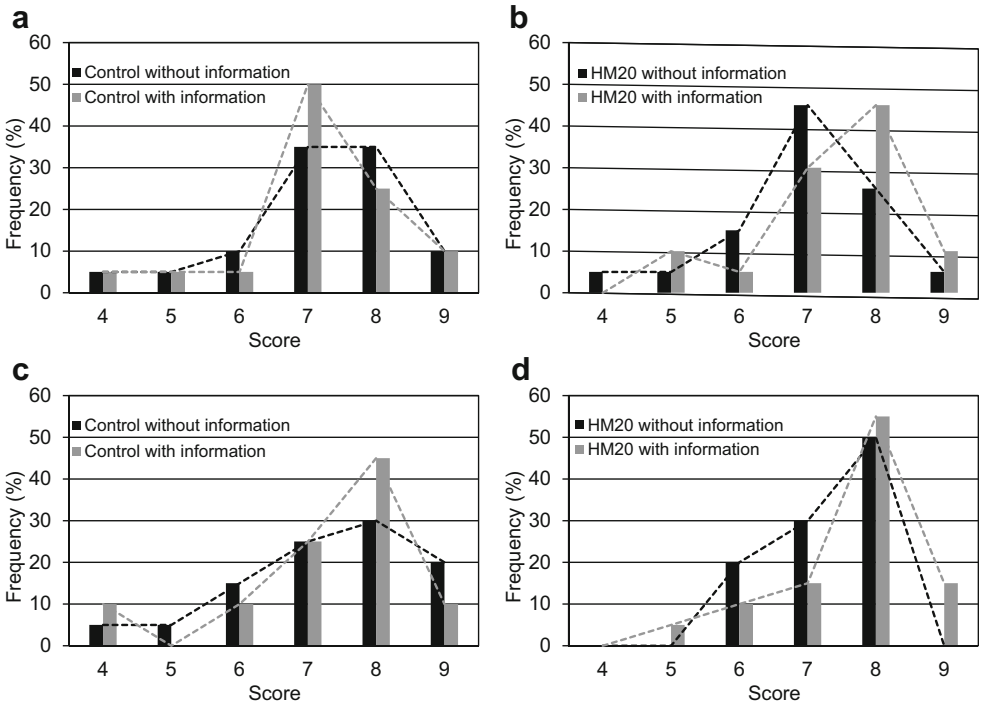

Fig. 6 Sensory evaluation histograms of texture (**a** and **b**) and general acceptability (**c** and **d**)

individual consumer preferences, as evaluators' comments stated. In the case of general acceptability, HM20 was scored more frequently with 8 and 9 compared to the control when the information chart was included (Fig. 6c, d).

These effects could be associated with the kind of product evaluated and the nutritional claim presented. Grunert (2010) found that cereal-based products such as muesli bar and rye bread presented higher purchase intention when the enrichment ingredient was fiber, in contrast to dairy products or fish-based meals where vitamins, fish oils, and omega-3 enrichments were respectively preferred, which suggests consumers tend to be more open to coherently enriched products. All these effects would then influence the positive score of HM20 when information was given. In fact, the effect of including nutritional information was not evident for the control since this sample is not expected to have a healthy role in the diets of the consumers.

The importance of this preliminary sensory study was the checkup of this kind of product as an adequate alternative for the substitution of traditional white wheat bread, since the overall scores indicated that HM20 bread had a good sensory performance and the consumers tend to prefer coherently enriched products, such as cereal-based foods enriched with cereal-based ingredients.

Conclusions

The replacement of wheat flour (WF) by high-amylose resistant starch (HM) used for the development of healthy breads in this work produced final products with lower digestible carbohydrate content and lower estimated glycemic indexes, while preserving acceptable sensory characteristics. Up to 20% replacement, breads presented specific volume values as good as the control ones, and the crumb texture and porosity parameters remained acceptable, as confirmed by sensory analysis. The analysis of the DSC, ESEM, and in vitro starch digestibility experiments suggests that the lower estimated glycemic index found in the samples was due to the inability of the HM starch to gelatinize in the baking conditions. The results of the present work then indicate not only that HM-enriched breads would be an adequate daily food for special nutritional requirements, but that it would be worth making efforts to improve the quality of even higher replacements such as 30%, since the obtained breads also would act as carrier of an important amount of prebiotic dietary fiber.

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