Value-added of resistant starch maize-based matrices in breadmaking: nutritional and functional assessment.

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Abstract

The ability of white (W) and yellow (Y) maize flours as basic ingredients to make nutritious and healthy breads meeting functional and sensory standards are investigated. Resistant starch (R) and common wheat flour (WF) were incorporated into formulations as single and associated extra ingredients, and dough machinability, bread nutritional and functional profiles, starch hydrolysis kinetics, and keeping behaviour were assessed in blended maize matrices, and compared with the maize and wheat flour counterparts.

Simultaneous replacement of maize flours by R and WF at 40% significantly modified textural profile, crumb grain features and firming kinetics, and free polyphenol pattern of breads thereof compared to the respective Y or W maize counterparts. Bigger specific volume (+28% Y-R-WF, +36% W-R-WF), softer crumb bread (-64% Y-R-WF, W-R-WF), more aerated structure and homogeneous crumb grain, and lower and slower staling kinetics are observed in composite Y and W maize-based breads, respectively. Nutritional information on maize-based blended breads showed most appealing nutritional quality than WF breads, in terms of lower digestible starch (up to -21% in Y-R-WF, W-R-WF, WR) and rapidly digestible starch (up to -37% in W-R-WF), higher slowly digestible starch (up to 3 times in WR), and resistant starch contents (from 5 to 6 times in Y-R-WF, W-R-WF, W-R, Y-R) of medium-high sensorially rated bread matrices. All single and blended maize-based breads can be labelled as high-fibre breads (6 g DF/100 g food). According to health related benefits and prebiotic dosage of resistant starch (Homayouni et al., 2014), a daily intake of 100 g of single Y-R, W-R, W-R-WF, and W-R-WF, provides enough resistant starch to positively affect postprandial glucose and insulin levels, while 170 g cover the amount necessary to enhance health.

Introduction

The general growing demand for novel tasty, nutrient dense and healthy foods together with the increasing number of people suffering from wheat related diseases, have fostered a new increasing market consisting of cereal products made from grains alternative to wheat and rye. In this context, the challenges and opportunities of non-wheat cereals (maize, barley, oat, sorghum and millet) deserve a special attention due to their unique nutritional components –starch, dietary fibre, resistant starch, minerals, vitamins, bioactive compounds- (Angioloni and Collar 2011a, 2011b, 2012; Collar and Angioloni, 2014).

Baked products are excellent carriers for food-grade fractions from grains, legumes, or other non-traditional food sources. In breadmaking applications, the lack of gluten proteins to meet dough viscoelastic and fermentative restrictions has generally constrained the incorporation of substantial amounts of non-wheat cereals into wheat dough systems to achieve dietary and healthy endorsing effects. High levels of grains others than wheat incorporated into baked products is cost effective and nutritionally advantageous but technologically challenging.

Maize (*Zea mays*), also known as corn, is a major cereal grown throughout the world accounting for up to 80% of total carbohydrates, that represents either the major staple or main supplementary staple for many people across the continents (Collar, 2014). Uses of maize flours in cookie-making (Singh et al., 2003), chapati-making (Sandhu et al., 2007), arepa-making (Granfeldt et al., 1995; Schnell et al., 2005), and in general in unleavened breadmaking (Singh, Singh, & Shevkani, 2011) and ethnic goods (Collar, 2014) have been successfully applied. Updated challenges and opportunities for maize in leavened breadmaking have been envisaged scarcely. Few scientific studies on maize broa breadmaking have been reported (Brites et al., 2010; 2011), while major research have been focused on the partial replacement of wheat flour by maize flour (Martínez and el-Dahs 1993), maize starch (Miyazaki & Morita, 2005), maize starch-based formulations (Özboy 2002), heat-moisture treated maize starch (Miyazaki and Morita, 2005) defatted maize germ (Siddiq et al., 2009), and extruded maize flour blends (Yu et al., 2013).

Addition of resistant starch, defined as the starch fraction that escapes digestion in the small gut and can be fermented in the colon by selective microbiota promoting growth of Lactobacilli and Bifidobacteria and enhancing the viability of probiotics, encompasses both technological and health-related benefits (Homayouni et al., 2014). Resistant to *in vitro* hydrolysis by α-amylase treatment after 2 h of incubation (Englyst et al., 1992), resistant starch has a direct impact on glycaemic response. Its consumption prevents the constipation, increasing excretion frequency, and fecal bulk, decreasing production of mutagenic compounds and lowering the colonic pH and ammonia levels (Fuentes-Zaragoza et al., 2011). The unique characteristics of RS -its natural sources, fine particle size, white color, bland flavor, lower water holding and higher water binding capacities than traditional fibers- makes it a valuable supplement in a wide range of functional foods (Homayouni et al., 2014). It has been substantiated that 6–12 g of resistant starch intake at a meal offer positive effects on postprandial glucose and insulin levels (~~Fuentes-Zaragoza et al., 2011;~~ Behall et al., 2006), whereas resistant starch intakes of approximately 20 g/day have been considered necessary to enhance health (e.g., increasing fecal bulk) (Murphy et al., 2008). Also Commonwealth Scientific and Industrial Research Organization of Australia (CSIRO) has recommended that the total intake of RS should be 15–20 g/day to tackle bowel-cancer-in-australia (Baghurst, Baghurst, & Record, 1996).

It can be concluded that the role of RS on the technological quality and health-promoting effects of baked goods is recognized to be dependent on the ingredients and the processing conditions the food matrix is submitted. The role of food matrices to "protect" the RS, added as an ingredient in food formulations, deserves further research.

The paper is aimed at exploring the ability of white and yellow maize flours as basic ingredients to make nutritious and healthy bread meeting functional and sensory standards. Resistant starch and common wheat flour were incorporated into formulations as single and associated extra ingredients, and dough machinability, bread nutritional and functional profiles, starch hydrolysis kinetics, and keeping behaviour were assessed in blended maize matrices, and compared with the wheat flour counterparts.

Materials and methods

Materials

Commercial flours from refined common wheat (*Triticum aestivum*, WT), white (W) and yellow (Y) maize (*Zea mays*), were purchased from the Spanish market. Refined wheat flour (70% extraction rate) of 356 x 10^{-4} J energy of deformation W, 0.64 curve configuration ratio P/L, 95% Gluten Index, 62% water absorption in Brabender Farinograph, was used. Resistant starch (R) produced by ConAgra (USA) under the branded name of HI-MAIZE™260 (natural, food grade high amylose starch that is 56% in dietary fibre, a rich source of resistant starch) was furnished by Ingredion Germany GmbH. Dried dried wheat sourdough (BöCKER F) was provided by BöCKER (Minden, Germany). Standard locust Bean Gum Palgum was purchased from CAROB, S.A. Lbg Palgum™, and Novamyl 10000 a maltogenic thermostable α-amylase was from Novozymes (Denmark).

Methods

Physical, chemical and nutritional composition of wheat and maize flours

Moisture, protein, ash and fat contents of commercial flours WT, Y and W were determined following the ICC methods 110/1, 105/2, 104/1, and 136, respectively (ICC, 1976-1996). Total, soluble and insoluble dietary fibre contents were determined according to the AOAC method 991.43 (AOAC, 1991).Two replicates were made for each flour analysis. Digestible carbohydrates were calculated by difference (FAO, 2003). Amylose/ amylopectin ratio (Megazyme kit K-AMYL 07/11) was estimated by using a modification of a Con A method developed by Yun and Matheson (1990) that uses an ethanol pre-treatment step to remove lipids prior to analysis.

Viscometric properties -dough pasting profiles (gelatinization, pasting, and setback properties)- were obtained with a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) using ICC standard method 162 (Collar, 2003). RVA parameters were calculated from the pasting curve using Thermocline v. 2.2 software.

Colour properties were assessed on flours by using a Minolta colorimeter (Minolta CR- 400, Konica Minolta Sensing, Inc., Osaka, Japan), and results were expressed in accordance with the Hunter Lab colour space. Parameters determined were L (L = 0 [black] and L = 100 [white]), a (-a = greenness and +a = redness), b (-b = blueness and +b = yellowness), ΔE -total colour difference- (Francis and Clydesdale, 1975), and WI -whiteness index- (Hsu et al., 2003). All measurements were made in triplicate.

Dough and Bread making of wheat, maize and maize-based blended flours

Doughs and breads were prepared from a) single flours (WF, Y and W), b) single maize flours replaced by 20% R, flour basis, respectively (Y-R, W-R), c) single maize flours replaced by 20% WF, flour basis, respectively (Y-WF, W-WF), d) single maize flours replaced by both 20% R and 20% WF, flour basis, respectively (Y-R-WF, W-R-WF). 6 different maize-based blended flours were obtained. Blended flours (1200 g), water (110%, flour+R+WF basis), compressed yeast (4%, flour+R+WF basis), BöCKER F (1.5%, flour+R+WF basis), milk powder (5%, flour+R+WF basis), sucrose (3%, flour+R+WF basis), locust bean gum (8%, flour+R+WF basis), salt (1.5%, flour+R+WF basis), vegetable fat (4%, flour+R+WF basis), calcium propionate (0.5%, flour+R+WF basis), and Novamyl (7.5 mg, flour+R+WF basis) were mixed following the procedure described by Brites et al., 2011. Fermented doughs were obtained after bulk fermentation (10 min), dividing (300 g), rounding, hand-shaping, and proofing up to maximum volume increment (30 min), and were baked at 220 ºC for 40 min to make single WF, Y, and W, and maize-based blended breads. Breads were sliced (2 cm) and stored in polypropylene bags for 1, 3, 6, 8 and 10 days at 22ºC until analysis.

Dough rheological measurements

Dough functional behaviour was assessed by either fundamental or empirical dough physical tests. Dough viscoelasticity was determined by dynamic oscillation tests on an RS1 controlled stress rheometer equipped with a Phoenix II circulating bath (Haake, Karlsruhe, Germany) using a 60-mm serrated plate–plate geometry with a 1-mm gap between plates (Angioloni and Collar, 2012). Strain sweep tests were run to identify the linear viscoelastic region. Oscillatory measurements of storage modulus (*G'*) and loss modulus (*G''*) were performed at 25 º C within a frequency range from 0.1 to 10 Hz. Temperature sweeps from 30 °C to 90 °C, at a heating rate of 1.6 °C/min were also achieved at a frequency of 1 Hz (Angioloni and Collar, 2009) for single and blended dough samples. All measurements were made in triplicate. Viscometric properties -dough pasting profiles (gelatinization, pasting, and setback properties)- were obtained with a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) as above described for flour samples.

Bread measurements

Physico-chemical and sensory determinations

Colour determinations were carried out on bread crumb using a Minolta colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan), as described above for flours. Crumb grain characteristics were assessed in bread slices using a digital image analysis system. Images were previously acquired with a ScanJet II cx flatbed scanner (Hewlett-Packard, Palo Alto, CA, USA) supported by a Deskscan II software. The analysis was performed on 20 mm × 20 mm squares taken from the centre of the images. Data were processed using SigmaScan Pro 5 (Jandel Corporation, San Rafael, CA, USA).

The crumb grain features evaluated were mean cell area, cells/cm^2, cell/total area ratio, wall/total area ratio and crumb area/total cell ratio (Collar et al., 2005).

A 8-member trained panel aged 23-55 was recruited for intensity evaluation of selected sensory attributes of fresh breads by using a 15-cm unstructured scale, according to the method of Stone and Sidel (2004), as described by Siddiq et al. (2009). Attributes evaluated were crust color (intensity of ‘‘golden brown’’ color), crumb color (‘‘whitish’’ or ‘‘creamish’’ color), cells, uniformity (uniformity of air cells or porosity), aroma (degree of intensity associated with typical white bread), firmness (resistance experienced by the ‘‘compactness of crumb’’ by finger feel), mouthfeel (intensity of perceived taste of a typical white bread slice), and off-flavor (any off-flavor, e.g. oily, oxidized, rancid, or pulse-like).

Bread primary and secondary mechanical characteristics (TPA in a double compression cycle) of fresh and stored breads were recorded in a TA-XTplus texture analyser (Stable Micro Systems, Surrey, UK) using a 10 mm diameter probe, a 5 kg load cell, 50% penetration depth and a 30 s gap between compressions on slices of 20 mm width (Armero and Collar, 1998). For textural measurements, three slices of two freshly made breads were used for each sample. at different storage periods (0 to 10 days). The obtained firming curves were modelled using the Avrami equation, and model factors were estimated by fitting experimental data to the nonlinear regression equation $\theta = \frac{T_\infty - T_t}{T_\infty - T_0} = e^{-kt^n}$ where Θ is the fraction of the recrystallisation still to occur; T_0, T_∞ and T_t are crumb firmness at time zero, ∞ and time t, respectively, k is a rate constant, and n is the Avrami exponent (Armero and Collar, 1998).

Enzymatic/biochemical determinations

Starch hydrolysis kinetics and relevant starch fractions in single maize and maize-based blended breads was determined following the AACC (2005) method 32-40, adapted as previously described (Angioloni & Collar, 2011b). Each bread sample (100 mg) was incubated with pancreatic α-amylase (10 mg) and amyloglucosidase (12 U) in 4 mL of 0.1 mol/L sodium maleate buffer (pH 6.0) in a shaking water bath (200 strokes/min) at 37 ºC for 0, 0.5, 1, 1.5, 2, 3, 4, and 16 h). After incubation, samples were heated at 100 ºC for 5 min, and ethanol:water (95:5, v:v) was added for enzyme inactivation, prior to centrifugation at 720 g for 10 min. The glucose content of the supernatant was measured using a glucose oxidase/peroxidase (GOPOD) kit. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured after incubation for 30 min and 120 min respectively (Englyst et al., 2003). Total digestible starch (DS) was determined in the supernatant after 16 h of incubation while resistant starch (RS) was determined in the pellet as the starch remaining after 16 h incubation. The digestion kinetics and expected glycaemic index (eGI) of bread were calculated in accordance with the procedure followed by Chung et al. (2008) based on the method established by Goñi et al.(1997). A first order kinetic equation [$C = C_\infty (1-e^{kt})$] was applied to describe the kinetics of starch hydrolysis, where C, C_∞ and k were the hydrolysis degree at each time, the maximum hydrolysis extent and the kinetic constant, respectively. The hydrolysis index (HI) was calculated as the relation between the area under the hydrolysis curve (0-16 h) of blended bread samples and the area of standard material from white bread (control) (Chung et al., 2008). The expected glycaemic index (eGI) was calculated using the equation proposed by Granfeldt, et al. (1992): eGI = 8.198 + 0.862HI.

The extraction of free phenolic compounds was achieved following the procedure described by Zilic et al. (2011). For the quantification of total free phenolics, and polyvinylpolypyrrolidone (PVPP) bound phenolics, extracts were prepared by continuous shaking of 0.3 g of ground air-dried breads in 10 ml of 70% (v/v) aqueous acetone for 30 min at room temperature. After centrifugation (20 min at 15,000 g), the supernatant was used for experiments. For the detection of flavonoids, 1 g of ground air-dried breads was extracted in 10 ml of 40% (v/v) ethanol for 30 min at room temperature. The supernatant, after centrifugation for 20 min at 15,000 g was used in experiments. The total phenolic content was determined according to the Folin-Ciocalteu procedure (Hagerman et al., 2000). Aliquots (0.2 ml) of aqueous acetonic extracts were transferred into test tubes and their volumes made up to 0.5 ml with distilled water. After addition of the

Folin-Ciocalteu reagent (0.25 ml) and 20% aqueous sodium carbonate solution (1.25 ml), tubes were vortexed. After 40 min, the absorbance was recorded at 725 nm against a blank containing an extraction solvent instead of sample. The total phenolic content of each sample was determined by means of a calibration curve prepared using catechin and expressed as mg catechin equivalents (CE) per 100 g of fresh bread. The PVPP bound phenolics were determined according to Makkar et al. (1993). Two milliliters of aqueous acetonic extracts were mixed with 200 mg of insoluble, crosslinked PVPP. After 15 min at 4 ºC, tubes were vortexed and centrifuged for 10 min at 15,000 g. Aliquots of the supernatant (0.2 ml) were transferred into test tubes and non-adsorbed phenolics determined by the same procedure used for total phenolics (Hagerman et al., 2000). The content of PVPP bound phenolics was calculated as the difference between total and non-adsorbed phenolics and expressed in mg CE per 100 g of fresh bread. Total flavonoid content was determined according to Eberhardt et al. (2000). Briefly, 0.075 ml of 5% $NaNO_2$ was mixed with 0.5 ml of the sample (ethanolic extract diluted with 1 ml of water). After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added, and the mixture was allowed to stand for another 5 min. Then, 0.5 ml of 1 M NaOH was added, and the volume was made up to 2.5 ml with distilled water. The absorbance was measured at 510 nm immediately after mixing, against the blank containing the extraction solvent instead of a sample. The results are expressed as mg CE per 100 g of fresh bread.

Statistical analysis

Multivariate analysis of variance (MANOVA) of data was performed by using Statgraphics V.7.1 program (Bitstream, Cambridge, MN).

Results and discussion

Physicochemical, nutritional and techno-functional patterns of single (WF, Y and W) flours (Table 1) and rheological profile of single and blended doughs (Table 2) are investigated prior to compile comparatively the physico-chemical, sensory and nutritional parameters (Table 3), “in vitro” starch hydrolysis parameters and relevant starch nutritional fractions (Table 4), of single and blended maize-based bread matrices.

Physico-chemical, nutritional and functional performance of single flours, and single and blended maize-based doughs

Single WF, Y and W flours exhibited different physico-chemical and nutritional patterns (Table 1). Comparatively (Y, W *vs* WF, per 100g flour basis, d.b), maize flours accounted for higher total dietary fibre (3.55%, 4.31% *vs* 2.34%), and soluble fibre fraction contents (2.55%, 3.54% *vs* 1.05%), similar fat (2.16%, 1.56% *vs* 1.41%) and ash contents (0.66%, 0.56% *vs* 0.68%), and lower protein (5.68%, 5.25% *vs* 11.39%) levels than WF flours. Data are in good accordance with previously reported chemical and nutritional composition of maize flours from India (Sandhu et al., 2007) and Brazil (Uarrota et al., 2013).

Hunter colour parameters (Table 1) evidenced some similarities and significant differences within flour matrices as found before (Sandhu et al., 2007). *L* value of Y and WF flours were similar (L=68) and slightly lower than *L* found in W maize (L=69.7) flour. All flours showed negative *a* values, indicating the presence of green tint, especially for Y maize flour (a=-1.87). The *b* values for flours ranged from 6.35 to 18.7. The highest *b* value of Y flour indicated its yellow colour associated to the presence of a higher amount of carotenoids (Singh et al., 2011). Complex Whiteness Index that depends on *L, a,* and *b* was similar and higher for WF and W maize (67-69) than for Y maize flour, as it was expected. The latter was clearly distinguished by the human eye (ΔE>3) from WF (ΔE=11.4), whereas W maize did not (ΔE=1.9).

Visco-metric profiles of maize flours during both cooking and cooling cycles were systematically higher regardless the variety (Y, W) than patterns shown by WF (Table 1), in line with viscosity trends observed for isolated starches (Waterschoot et al., 2014). Within maize flours, Y flour exhibited higher pasting and lower gelling profiles than W maize flour, in contrast with previously published results (Brites et al., 2010) in which the superior viscosity profile was observed either for white or yellow varieties along both cooking and cooling cycles. The visco-metric profile of dough matrices (Table 2) from Y and W flours is significantly higher than those of flour counterparts (Table 1), due to the viscosity contribution of the auxiliary ingredients, particularly the locust bean gum added at 8%, flour basis to the formulation. In general, the high water binding capability of the galactomannan is responsible for the formation and stabilization of secondary hydrogen bounds in competition with flour for available water, as observed earlier for different hydrocolloids (Collar, 2003; Angioloni and Collar, 2008). Protein-starch linkages established in presence of proteins in flour stabilize starch structure, and hence can delay the gelatinization process (Crockett et al., 2011). Flour replacement by resistant starch (R) and/or wheat flour (WF) on dough viscosity pattern resulted in lower values for pasting and gelling parameters, mainly ascribed to the dilution of the maize starch, effects being greater with the percent of maize flour substitution. During heating, the viscosity increases with increase in temperature due to swelling of the starch granules. This is followed by a decrease in viscosity caused by rupturing and fragmentation of granules. In fact, during pasting peak viscosity values decreased at about 30 % when R was added due to the increases in the insoluble fibre content, and about 22% when WF replaced either Y or W maize flours. Simultaneous replacement of maize flours by R and WF lowered the peak viscosity at about 50% regardeless the maize flour, indicating an additive diluting effect of R and WF on the cooking properties of maize starch. Similar trends were observed for other pasting parameters during cooking such as holding strength, breakdown and viscosity at 95ºC (Table 2). During cooling, the starch molecules re-associate to form gel, wherein amylose molecules aggregate and result in a network, embedding remnants of starch granules. The pasting properties of starch are influenced by the constituents that leached out from the granules during heating and the interactions between the chains. Total setback on cooling observed a depletion by 26-27% with R, by 12-17% with WF, and by 35-39% with binary R/WF, compatible with the additive diluting effect of the pair on the cooling properties of maize starch, as previously observed for pasting parameters and already described for some starch blends (Waterschoot et al., 2014).

Dynamic moduli (storage dynamic modulus G' and loss modulus G'') of individual and blended maize-based doughs showed significant variation among samples when subjected to a frequency sweep ranging from 0.1 to 10 Hz at 25ºC (data not shown). Values for G' and G'' at 1 Hz representing the energy stored in the material and recovered from it (elastic nature of the material), and the energy dissipated or lost per cycle of sinusoidal deformation (viscous nature of the material), respectively (Ferry, 1980) are reported in Table 2. Blended doughs showed lower dynamic moduli values, especially for G', than the individual maize dough counterparts did. This may be attributed to the loss of rigid/solid nature of corn starch granules when blended with R and/or WF that significantly weakened the complex matrix by both a diluting effect and an increase of starch disordered structure. This is especially true for the highly replaced Y-R-WF and W-R-WF samples that exhibited a significant decrease in G'' (65%, 62%) and particularly in G' (73%, 67%), respectively.

Physico-chemical, sensory and nutritional profiles of blended maize-based bread matrices.

~~During mixing, distribution of materials, hydration and energy input for stretching and alignment of protein molecules take place involving shear and extensional deformation. During fermentation, the expansion of the air bubbles previously incorporated during mixing provides the characteristic aerated structure of bread, which is relevant to its appeal. During proof and baking the growth of gas bubbles determines the expansion of the dough and therefore the ultimate volume and texture of the baked product (Collar, 2013). Evaluation of texture at macroscopic level by instrumental and sensory analysis is a key factor for both quality assessment and new product development (Dubost et al., 2003). Instrumental evaluation of bread texture parameters have been shown to correlate well with sensory measurements (Collar et al., 2005; Esteller et al., 2004; Siddiq et al., 2009). At milimeter level, the internal structure of~~

yeast-leavened bread when sliced, commonly referred to as crumb grain, can be described as a complex of interconnected cells in a heat set glutinous-stark matrix (Crowley et al., 2002). Crumb grain or crumb visual texture is an important element of the bread quality, and reflects flour characteristics, dough formulation, and processing (Scanlon and Zghal, 2001).

Single maize-based breads Y and W were sensory rated moderately in terms of crust and crumb colour (8-11/15) and mouthfeel (6-7/15), but deserved medium-high scores regarding aroma (12/15), firmness (12-13/15) and cell uniformity (8-12/15), particularly Y breads (Table 3). In addition, low specific volume (≈2.03mL/g), high hardness values (5.12-5.68 N), medium/high cohesiveness values (0.6) and lightness (63-67), green and yellow tint crumb colour especially for Y samples (a= -2.2, b= 25.8), and high whiteness index especially for W samples (WI= 65) characterized single maize-based breads. At milimeter level, morphological features evidenced significant differences between Y and W matrices: cell area ranged from 0.007 mm^2 to 25 mm^2 (Y) and to 3 mm^2 (W), respectively, with a cell area distribution of 42% of cells from 10 to 30 mm^2 (Y) and of 57% of cells <10 mm^2 (W), respectively. In addition, 92% of the cells were <1 mm^2 in Y samples, while 90% sized from 1 to 10 mm^2 in W breads, so that cell density and cell to wall area ratio were higher in Y (53 cells/cm^2, 22%) than in W (30 cells/cm^2, 9%) samples, providing a denser but more open structure and more homogeneous porosity in Y than in W breads, in line with sensory appreciations for cell uniformity (Table 3). Brescia et al., 2007 reported that computerized image analysis and colour determinations for quantitative assessment of bread crumb attributes give objective and consistent measurements for qualitatively relevant bread crumb features, and the measured features compare well with bread slice visual inspection. Values of Avrami model for crumb firming kinetics evidenced similar initial firming values for fresh breads (T_0=5N), but higher rate and extent of staling/aging for Y (n=0.86; k=0.125; T_∞=31 N) than for W (n=2.6; k=0.043; T_∞=18 N) breads during long term storage. At short term storage (≤ 2 days), W breads that follow a sigmoidal firming curve (Figure not shown), explicited lower hardness values and slower staling kinetics. From the nutritional point of view, polyphenol content was slightly higher in Y (283 mg/100 g bread) than in W (260 mg/100 g bread) bread samples, associated to the greater flavonoid content (274 *vs* 249 mg/100 g bread) of yellow varieties, as it was stated earlier (Singh et al., 2011).

Replacement of either Y or W maize flours by single R at 20% or single WF at 20%, led to respective breads with very similar sensory profile, specific volume, and crumb colour features (Table 3). In addition, R incorporation did not significantly affect the instrumental texture profile and staling kinetics, but modified the crumb morphological features (increasing the % of smaller cells in Y samples and the percentage of bigger cells in W samples cell area), and increased twice the PVPP bound phenolics in detriment of the PVPP free phenolics and free flavonoids. Addition of Hi-Maize260 to wheat flour matrices even in 20% concentration, had no effect neither on dough proofing time, nor on its specific volume. Also it was reported that RS2 did not affect color parameters in crust (Almeida et al., 2013). WF addition to dough formulations significantly decreased initial hardness by 40%, slowed down staling kinetics -particularly for W-WF samples that underwent a decrease of 87 % in the Avrami exponent *n* *vs* a depletion of 7% in Y-WF samples-, and increased twice the PVPP bound phenolics. Simultaneous replacement of maize flours by R and WF at 40% did not change either the sensory appreciations or the individual colour features of the resulting breads compared to the respective Y or W maize counterparts, but significantly modified textural profile, crumb grain features and firming kinetics, and free polyphenol pattern of breads thereof (Table 3). Bigger specific volume (+28% Y-R-WF, +36% W-R-WF), softer crumb bread (-64% Y-R-WF, -62% W-R-WF), more aerated structure –larger cell to crumb ratio in Y-R-WF samples- and homogeneous crumb grain -70-75% of crumb area occupied by cells sized 1-10 mm2-, and lower and slower staling kinetics - lower T_∞, *n* and *k*- are observed in composite Y and W maize-based breads, with respect to their single maize breads counterparts, respectively.

Starch hydrolysis parameters and relevant starch nutritional fractions, of single and blended maize-based bread matrices.

Starch nutritional fractions determined in flours, control WF, single Y and W maize-based, and R and/or WF blended Y and W maize-based breads by ''in vitro'' starch digestion, included rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), based on its digestibility, which is assessed by the rate that glucose is released from the starch and then absorbed (Table 4). Native cereal starches are ideal sources of SDS (>50%), and the slow progressive digestion property is realized by a layer-by-layer inside-outside (radial) digestion process due the presence of surface pores and channels within the granule through a mechanism of side-by-side (tangential) even digestion of amorphous and lamellar semicrystalline layers of starch granules (Zhang et al., 2006a). Our results in WF, Y and W flours are consistent with the previous statement, providing 61.1%, 52.2% and 58.6% of SDS, respectively. Zhang et al., (2006b) demonstrate that the supramolecular A-type crystalline structure, including the distribution and perfection of crystalline regions (both crystalline and amorphous lamellae), determines the slow digestion property of native cereal starches. Mechanical and thermal treatments change the structure and digestibility of starch. Thermal treatments such as the cooking process completely destroys the semicrystalline structure of native starch granules and causes the loss of SDS and RS and increases RDS (Zhang et al., 2006b). Miao et al., 2014 reported that heated maize starch contained 83.5% RDS, 11.1% SDS and 5.4% RS. In the present work, the breadmaking process -mixing, fermentation and baking- that involves mechanical and thermal processes, induced reverse quantitative changes in starch nutritional fractions. From flour to bread, SDS decreases from 61.1 to 7.5% (WF), from 52.2 to 8.5% (Y), and from 58.6 to 12.2% (W); RS falls from 2.5 to 1.8% (WF), from 15.4 to 4.3% (Y), and from 15.5 to 5.5% (W); while RDS promotes from 20.2 to 58.5% (WF), from 25.4 to 49.3% (Y), and from 22.1 to 42.2% (W) (Table 4). Compared to cooked starches (Zhang et al., 2006b), in this work maize breads contained twice (Y) or triple (W) the RS of wheat breads, in line with the lowest "in vitro" starch hydrolysis found in corn arepas compared with wheat bread (Granfeldt et al., 1995). In breads, RDS, SDS and RS contents (g/100 g bread, as is) ranged from 37.1 (Y-R, W-R,W-R-WF) to 58.5% (WF), from 6.2 (WF,Y, Y-WF, Y-R-WF) to 20.9% (W-R) and from 1.80 (WT) to 12.0% (Y-R, W, Y-R-WF, W-R-WF), respectively. Replacement of maize flours by RS increased SDS by 94% (Y-R) and 71% (W-R), doubled (Y-R) or tripled (W-R) the amount of RS, and reduced RDS levels by 22% (Y-R) and 6% (W-R). The ingestion of SDS has been proven to result in a smaller increase and longer sustained rise in plasma glucose (Sands et al., 2009). In fact, RS incorporation slowed down "in vitro" starch hydrolysis kinetics substantially in both Y and W maize blended breads (Fig. 1), giving lower values for C_∞ (21-17%), k (18%) and H_{90} (28-13%), that resulted in eGI 16-13% lower, respectively. R added to formulations is high-amylose corn starch (>50%), while Y and W maize flours have normal levels of amylose (20-25%, Table 1). In R, cooled gels after gelatinization, form retrograded starch crystals that are resistant to enzymes digestion. A particular type of RS2 limits the accessibility of gastrointestinal enzymes because of its compact structure; it is resistant during the preparation of many foods. The RS2 mainly consists of amylose, so it has been called high-amylo maize starch (Homayouni et al., 2014). Normal maize starch is more susceptible to amylolysis compared to high-amylose corn starch, associated to the presence of surface pores and channels in the former that facilitate enzymatic diffusion (Zhang et al., 2006b). The association between amylose chains and their potential for amylose lipid complex formation (Morita et al., 2007), higher crystalline lamella thickness, and a thicker peripheral layer (Jenkins and Donald, 1995) are the factors that make the high-amylose maize starch granules resistant to amylolysis. High-amylose corn starch granules are hydrolyzed predominantly by exocorrosion, whereas normal corn starch is internally hydrolyzed in an "inside-out" pattern (Zhang et al., 2006a). Replacement of maize flours by WF in absence or presence of R did not significantly change neither the starch hydrolysis kinetics (Fig. 1) nor the amount of starch nutritional fractions, giving very similar starch nutritional patterns (Table 4).

Suitable nutritional trends for dietary starch fractions - low RDS content and high SDS and RS contents - (Englyst et al., 2003), were met by blended samples Y-R,W-R,W-R-WF, which showed rather low extent and rate of starch hydrolysis (Fig. 1) with lower values for C_∞ (57-60), k (0.046-0.059) and H_{90} (57-59), and expected Glycaemic Index (71) (Table 4) . Since maltogenic α-amylase is added to formulations, the slow digestion property of starch can be partially attributed to the maltogenic -amylolysis that decreased molecular weight and increased the number of shorter chains (DP < 13) accompanied by a reduction of longer chains (DP > 13), as observed for normal maize starches (Miao et al., 2014). In terms of health related benefits and prebiotic dosage of resistant starch (Homayouni et al., 2014), a daily intake of 100 g of single Y-R, W-R, W-R-WF, W-R-WF, provide enough resistant starch to positively affect postprandial glucose and insulin levels, while 170g cover the amount necessary to enhance health.

Conclusions

Simultaneous replacement of maize flours by R and WF at 40% did not change either the sensory appreciations or the individual colour features of the resulting breads compared to the respective Y or W maize counterparts, but significantly modified textural profile, crumb grain features and firming kinetics, and free polyphenol pattern of breads thereof. Bigger specific volume (+28% Y-R-WF, +36% W-R-WF), softer crumb bread (-64% Y-R-WF, -62% W-R-WF), more aerated structure and homogeneous crumb grain, and lower and slower staling kinetics are observed in composite Y and W maize-based breads, with respect to their single maize breads counterparts, respectively.

Nutritional information on maize-based blended breads showed most appealing nutritional quality than WF breads, in terms of lower digestible starch (up to -21% in Y-R-WF, W-R-WF, W-R) and RDS (up to -37% in W-R-WF), higher SDS (up to 3 times in WR), and resistant starch contents (from 5 to 6 times in Y-R-WF, W-R-WF, W-R, Y-R) of medium-high sensorially rated bread matrices. All single and blended maize-based breads can be labelled as high-fibre breads (6 g DF/100 g food), according to Nutritional Claims for

DF foods (Off J Eur Comm, 2006). ~~In terms of health related benefits and prebiotic dosage of resistant starch (Homayouni et al., 2014), a daily intake of 100 g of single YR, WR, WRWF, WRWF, provide enough resistant starch to positively affect postprandial glucose and insulin levels, while 170g cover the amount necessary to enhance health.~~

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Figure

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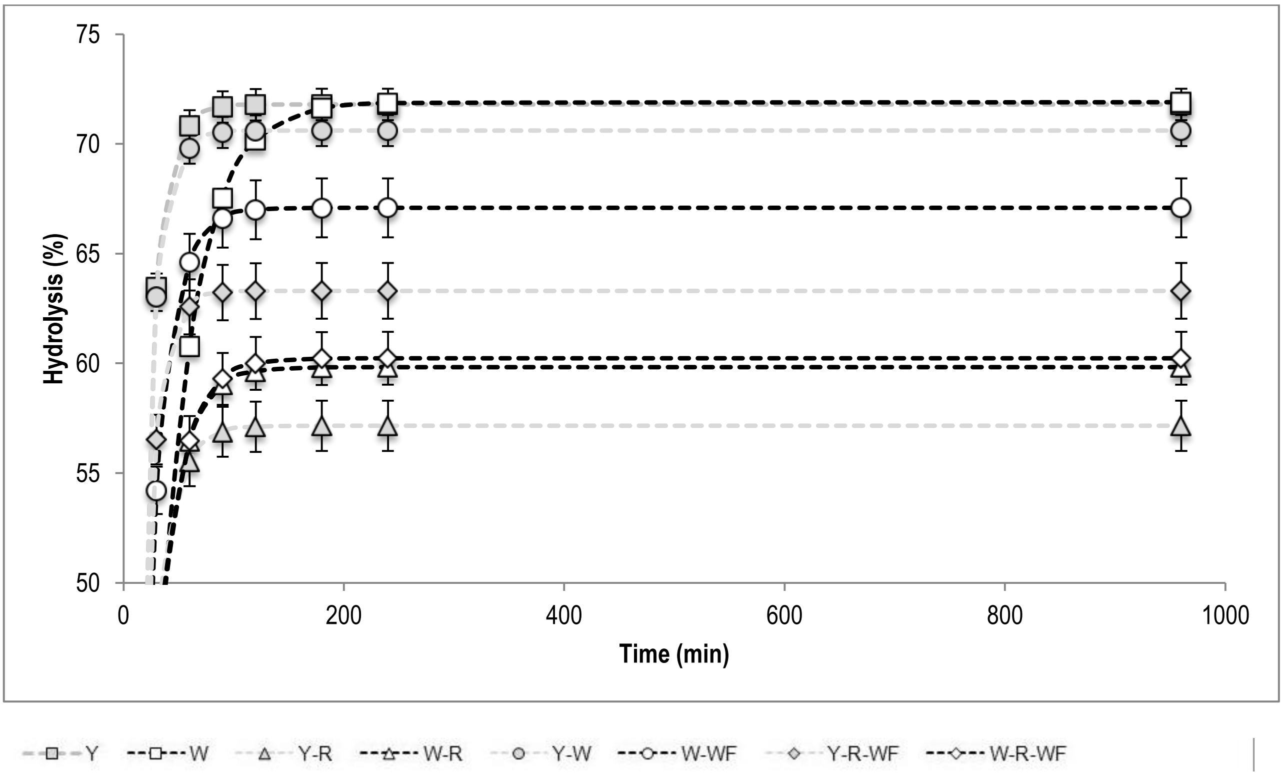

Figure 1.- Total starch hydrolysis curves (mean of three replicates) of yellow (Y) and white (W) maize and maize-based blended breads with resistant starch (R) and/or wheat flour (WF).

.

Table 1.- Physico-chemical and nutritional composition of flours (mean ± standard deviation).

Parameter	Flours		
	Wheat flour	Yellow maize	White maize
Nutritional composition (g/100 gflour, d. b.)			
Moisture	13.38±0.15b	12.99±0.12a	13.54±0.09b
Protein	11.39±0.31b	5.68±0.24a	5.25±0.19a
Fat	1.41±0.23b	2.16±0.24b	1.56±0.36a
Ash	0.68±0.08a	0.66±0.06a	0.56±0.09a
Digestible carbohydrates*	84a	88b	88b
Amylose/amylopectin ratio	23/77	25/75	20/80
Total Dietary Fibre	2.34±0.60a	3.55±0.90b	4.31±1.30b
Soluble Fibre	1.05±0.30a	2.55±0.70b	3.54±1.00b
Insoluble Fibre	1.29±0.11c	0.99±0.08b	0.77±0.03a
Visco-metric parameters			
Peak viscosity, cP	2395±23a	3656±36c	3453±114b
Pasting Temperature, ºC	88.7±0.1c	73.5±0.0a	75.0±0.1b
Holding strength, cP	1209±33a	2324±52b	2326±28b
Breakdown, cP	1187±11a	1322±16b	1127±86a
Visc at 95, cP	2750±5c	2505±45b	1885±64a
Visc at end 95, cP	1537±72a	2500±54b	2513±52b
Visc at 50, cP	2019±6a	3523±15b	3939±75c
Final Visc, cP	2818±1a	5207±37b	5883±101c
Total Setback, cP	1609±34a	2874±15b	3557±73c
Colour parameters			
L	68.1±0.5a	67.9±0.6a	69.7±0.2b
a	-0.73±0.09b	-1.87±0.09a	-0.99±0.06b
b	7.4±0.2a	18.7±1.0b	6.35±0.3a
ΔE	-	11.4	1.9
Whiteness Index	67.2b	62.8a	69b

*Calculated by difference. Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).

Table 2.- Rheological characteristics (mean±standard deviation) of yellow (Y) and white (W) maize and maize-based blended doughs with resistant starch (R) and/or wheat flour (WF).

Property/parameter	Y	W	Y-R	W-R	Y-WF	W-WF	Y-R-WF	W-R-WF
Viscometric								
Peak Viscosity, cP	6593±7d	6814±10d	4535±74b	4606±55b	5132±110c	5264±100c	3422±4a	3371±66a
Pasting Temperature, ºC	74.8±0.5a	75.5±0.5a	76.3±0.6a,b	76.3±0.7a,b	75.6±0.6a	76.7±1.1a,b	76.0±0.6a	77.2±0.6b
Starch gelatinization, ºC	63a	70b	-	-	71b	76c	64a	64a
Holding strength, cP	2859±45e	3034±91f	2403±1c	2640±71d	2379±70c	2721±61d	1900±8a	2038±4b
Breakdown, cP	3734±38g	3780±81g	2132±75d	1967±16c	2754±40f	2543±59e	1522±4b	1333±69a
Viscosity at 95ºC, cP	4679±210g	4228±51f	2578±6d	2208±163c	2936±29e	2439±204cd	1648±39b	1307±108a
Viscosity at end of 95ºC, cP	3157±65f	3335±78g	2484±33c	2794±17e	2628±98d	3006±94f	2017±14a	2161±13b
Viscosity at 50ºC, cP	3826±54f	4322±35g	3182±100c	3581±19e	3379±49d	3821±50f	2692±12a	2910±43b
Final Viscosity, cP	5332±71g	5954±17h	4227±149c	4760±40e	4544±42d	5133±75f	3505±21a	3810±51b
Total setback, cP	2473±26d	2920±74e	1824±150b	2120±31c	2165±28c	2412±14	1605±28a	1773±54b
Visco-elastic								
Storage modulus 1 Hz, Pa	6581d	6385d	3812c	3621c	2826b	2875b	1766a	2090a
Loss modulus 1 Hz, Pa	4669d	4714d	3057c	2929c	2473b	2429b	1621a	1788a

Within row, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).

Table 3.- Physico-chemical, sensory and nutritional characteristics (mean±standard deviation) of yellow (Y) and white (W) maize and maize-based blended breads with resistant starch (R) and/or wheat flour (WF).

Property/parameter	**Y**	**W**	**Y-R**	**W-R**	**Y-WF**	**W-WF**	**Y-R-WF**	**W-R-WF**
Sensory (/15)								
Crust Color	10±1a	11±1a,b	10±1a	12±1b	12±1b	12±2a,b	12±3a,b	12±4a,b
Crumb Color	11±2a,b	8±1a	9±1a	8±2a	12±2b	8±1a	8±2a	8±3a
Cell Uniformity	12±2b	8±1a	11±2b	11±2b	11±2b	11±3a,b	11±2b	11±1b
Aroma	12±1a	12±2a	12±2a	12±1a	12±2a	12±2a	12±1a	12±1a
Firmness	12±2a,b	13±1b	13±1b	13±2b	10±1a	12±2a,b	10±1aa	12±2a,b
Mouth-Feel	7±2a	6±1a	7±1a	7±2a	8±2a	8±2a	8±2a	8±2a
Off-Flavor	1±0a	4±2b	2±1b	2±1b	2±1b	2±1b	2±1b	2±1b
Physic-chemical								
Weight, g	214±15a	214±22a	206±18a	212±12a	212±28a	204±19a	198±14a	210±10a
Specific volume, mL/g	2.03±0.13a	2.03±0.14a	2.16±0.10a	2.00±0.14a	2.26±0.19a	2.28±0.16a	2.60±0.12b	2.76±0.16b
Hardness, N	5.12±0.40c	5.68±0.61c	5.09±0.38c	4.98±0.36c	2.69±0.28a,b	3.08±0.38b	1.85±0.34a	2.16±0.31a
Cohesiveness	0.64±0.08a	0.62±0.02a	0.59±0.04a	0.57±0.02a	0.58±0.04a	0.60±0.05a	0.60±0.00a	0.54±0.03a
L	63a	67c	65b	69c	61a	65b	61a	64b
a	-2,2b	-1,3a	-2,2b	-1,2a	-2,4b	-1,2a	-2,1b	-1,0a
b	25,8b	11,6a	24,8b	12,2a	22,9b	11,5a	21,0b	11,3a
Whiteness Index	55a	65b	57a	66b	55a	63b	55a	63b
ΔE	-	-	2	1	4	2	5	3
Crumb grain								
Mean cell area, mm^2	0.4a	0.3a	0.5a	0.6b	0.5a	0.6b	0.7b	0.4a
Max area, mm^2	25d	3a	5a	14b	12b	12b	16c	10b
Min area, mm^2	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007
Cell area distribution, %								
$<1mm^2$	30	43	70	24	23	31	17	25
$1,0\text{-}10mm^2$	28	57	30	60	65	54	71	75

10-30mm^2	42			16	12	15	13	
Cell number distribution, %								
<1mm^2	92	10	84	86	86	89	82	89
1,0-10mm^2	7	90	16	14	13	10	17	11
10-30mm^2	1			1	1	1	1	
Cell density, cells/cm^2	53c	30a	45b	38a	46b	34a	44b	44b
Cell to wall area ratio	22-78	9-91	22-79	23-78	25-75	19-81	32-68	19-81
Nutritional (per 100 g bread, as is)								
Total free polyphenols, mg	283 b	260 a	292 c	281 b	297 c	366 d	268 a	264 a
PVPP free phenolics, mg	208 c	212 c	128 a	180 b	204 c	249 d	198 b	181b
PVPP bound phenolics, mg	74 c	49 a	164 h	102 f	93 e	117,00 g	69,38 b	83d
Flavonoids, mg	274 e	249 c	201 a	263 d	277 e	334 f	228 b	253c
Crumb firming kinetics								
T_∞ (N)	31±2c	18±2b	19±2b	16±1b	42±2d	44±3d	15±2b	6±1a
k	0.125±0.002d	0.043±0.004b	0.223±0.003f	0.087±0.008c	0.021±0.004a	0.069±0.010c	0.151±0.005e	0.468±0.003g
n	0.860±0.009d	2.600±0.008g	1.320±0.007f	2.380±0.012	0.800±0.021c	0.330±0.011a	0.520±0.015b	1.003±0.009e
T_o (N)	5±1b	6±1b	5±1b	6±1b	3±1a	3±1a	2±1a	2±1a
r^2	0.9651	0.9942	0.9983	0.989	1.00	0.9758	1.00	0.9848

Within row, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$). T_0 initial crumb firmness; T_∞ final crumb firmness; k rate constant; n Avrami exponent; r^2 adjusted squared coefficient for the fitting model.

Table 4.- Values (mean±standard deviation) of starch hydrolysis kinetic parameters, starch nutritional fractions (per 100 g bread, as is) and expected glycaemic index of yellow (Y) and white (W) maize and maize-based blended breads with resistant starch (R) and/or wheat flour (WF).

Bread	WF	Y	W	Y-R	W-R	Y-WF	W-WF	Y-R-WF	W-R-WF
C_∞	81±1	72±1	72±1	57±1a	60±2a,b	71±2	67±1	63±3b	60±2a,b
k	0.072±0.002d	0.072±0.010d	0.031±0.009a	0.059±0.011c	0.048±0.008b	0.074±0.006d	0.055c±0.002	0.074±0.009d	0.046±0.003 b
r^2	0.98	0.99	0.99	0.99	0.98	0.99	0.99	0.99	0.99
H_{90}	81±1e	72±3d	68±2c	57±1a	59±1a	71±2d	67±2c	63±2b	59±1a
HI (%)	100±1d	88±2cd	87±3cd	72±2a	74±1a	89±2cd	86±2c	79±1b	74±2a
RDS (%)	58.5±0.3f	49.3±0.5d	42.2±0.4b	38.7±0.8a	39.5±1.5a	52.3±1.5e	45.9±0.8c	45.9±1.3c	37.1±0.9a
SDS (%)	7.5±0.2a	8.5±1.7a	12.2±0.8b	16.5±1.5c	20.9±2.0d	8.1±0.7a	12.5±1.2b	6.2±1.8a	16.3±2.3c
eGI	94±1	85±2	83±3	71±3	72±2	85±4	82±2	76±1	72±2
DS (%)	66.0±0.3d	57.8±0.8b	63.1±1.9c	55.2±1.7ab	51.8±1.0a	60.5±1.4bc	58.4±1.5b	52.2±1.3a	53.4±0.9a
RS (%)	1.8±0.1a	4.3±0.4b	5.5±0.6c	12.0±1.2d	11.3±0.9d	4.7±0.5bc	5.5±0.3c	11.8±1.1d	11.6±0.9d
TS (%)	67.8±0.2cd	62.1±0.5a	68.6±0.7d	67.2±1.2bc	63.1±1.4ab	65.2±0.9bc	63.9±0.9b	64.0±1.2b	65.0±1.0bc

Within row, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$). C_∞: equilibrium concentration. k: kinetic constant. H_{90}: total starch hydrolysis at 90 min. *HI*: hydrolysis index. r^2 adjusted squared coefficient for the fitting model. RDS: rapidly digestible starch, SDS: slowly digestibly starch, eGI: expected glycaemic index, DS: digestible starch, RS: resistant starch, TS: total starch.