Functional and thermal behaviors of heat-moisture treated starch-rich wheat-based blended matrices:
impact of treatment of non-wheat flours.

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Abstract

The significance of heat moisture treatment (HMT, 15% moisture content, 1 h and 120°C) of single common wheat (WT), teff (T), chestnut (CN) and whole chickpea (CP) flours has been investigated a) on main chemical, nutritional and functional flour parameters and b) on visco-metric and thermal behaviors of treated blended breads. Characterization of native and HMT flours evidenced significant ($p < 0.05$) but discreet effects of the thermal treatment of flours on main functional parameters in terms of promotion of the ability of the flour to associate with water and of the formation of water-soluble molecules from small fragments of amylopectin or amylose. Dilution of the basic wheat flour system by accumulative addition of T, CN, and CP flours submitted to partial or total HMT did impact on the disorganization and later reorganization of starch components of the starch-rich blended matrices in excess of water (pasting, gelling) and under water restrictions (retrogradation kinetics). Simultaneous presence of T/CN, T/CP and CN/CP either singly or fully thermally treated provided enhancement of the visco-metric profile on pasting and gelling, and decreased the extent and the rate of amylopectin crystallization on ageing.

Keywords Heat Moisture Treatment, non-wheat flours, thermal properties

1. Introduction

Grains appear as basic, ubiquitous and healthy raw materials that complement one another in multigrain baked goods to enhance desirable functional and nutritional properties, as reported for ancient, minor cereals, pseudocereals, and legumes in wheat-based blended matrices (Collar, 2016a). Highly replaced wheat matrices by non-wheat flours, especially gluten-free flours, exhibit high nutritional and health related characteristics, but poor functional performance due to the significant dilution of main biopolymers –starch and gluten- responsible for the unique viscoelastic behavior of wheat-based systems.

Cereal baked products are composed of interconnected air cells surrounded by cell walls formed during baking. In bread, the microstructure of the cell walls is described as a dispersion of starch granules in a continuous protein network (Gray and BeMiller, 2003), or a bicontinuous structure composed of accumulated swollen starch granules and proteins network (Hug-Iten et al., 2002). Heat moisture treatment (HMT) constitutes a clean label alternative to chemical modification for altering the gelatinization and retrogradation properties of starches (Gunaratne and Hoover, 2003), flours (Collar, 2017), and doughs (Collar and Armero, 2018a), and the aggregation/disaggregation equilibrium of proteins (Mann et al., 2013).

HMT is reported to alter simultaneously multiple aspects of starch granule structure under controlled conditions of temperature and moisture, and to impart concomitant changes to the physical characteristics and properties of starches (BeMiller and Huber, 2015) and starch-based matrices (Collar and Armero, 2018a). The high-temperature, low-moisture treatment conditions of HMT afford increased mobility to starch chains and helical structures, resulting in major structural changes to both crystalline and amorphous regions of starch granules (Zavareze and Dias, 2011), and may induce molecular degradation of starch chains (Lu et al., 1996). Since starch swelling and solubilization are restricted by HMT treatments, a change in the competition for available water between starch gelatinization and protein denaturation processes during heating of a composite food matrix may take place. Consequently, the

structure development and the textural attributes of the end product could be modified (Collar and Armero, 2018b, c).

In starch matrices, HMT increases the gelatinization temperature and its range, the water binding capacity, the stability of starch pastes to heat and shear, and in most cases the granule susceptibility to enzyme-catalyzed hydrolysis of cereals, legumes and tubers (Biliaderis, 2009). HMT also decreases the swelling power and, for most starches, their hot paste consistency and solubility (amylose leaching), and the gelatinization enthalpy (LeBail et al., 1993), being the magnitude of these changes dependent on the moisture content and starch type. An increase in the gelatinization temperature, obtained by hydrothermal treatments, could be an advantage in thermal processing of some foods such as bread, by extending the period of loaf expansion (Biliaderis, 2009).

In complex starch-based matrices such as flours, information on the effects of HMT on the functional performance of both the raw materials and end-products is limited. Thermal treatment of flours has successfully been applied to wheat (Cetiner et al., 2017), sorghum (Marston et al., 2016), barley (Purhagen et al., 2011), and composite oat-wheat (Verdú et al., 2017), and barley-wheat (Collar and Armero, 2018a) flours to improve volume, textural profile and/or keeping behavior of the resulting cakes (Nakamura et al., 2008; Neill et al., 2012) and/or breads (Collar and Armero, 2018b,c).

In authors' previous studies, HMT effects of non-wheat –teff, chestnut and chickpea flours on dough viscoelastic and starch digestibility parameters and on the structural pattern of breads were investigated in associated wheat-based matrices (Collar and Armero, 2018c,d). Suitable trends for the enhancement of the physical characteristics of breads in terms of larger specific volume, higher viscoelastic and textural profiles, with lower and slower staling kinetics on ageing were achieved in breads formulated with HMT flours, as compared to native flours.

However, despite the functional and nutritional benefits of HMT blended matrices, as a wholegrain multigrain initiative, to the authors best knowledge there are not any extensive studies of the effect of the

thermal treatment of flour blends on the visco-metric and thermal behaviors of treated mixed breads. The current paper is aiming at investigating how HMT influenced the disorganization and reorganization of starch components in complex systems in excess water (pasting, gelling) and under water restrictions (retrogradation kinetics) in mixed grain matrices.

2. Materials and methods

2.1. Flours

Commercial flours from refined common wheat *Triticum aestivum* (WT), teff *Eragrostis tef* (T), chestnut *Castanea sativa* (CN), and whole chickpea *Cicer arietinum* (CP) were obtained from the Spanish market. Refined WT (70% extraction rate) of 195 x 10⁻⁴ J energy of deformation W, 0.57 curve configuration ratio P/L, and 58.8% water absorption in Brabender Farinograph, was used. Carboxymethylcellulose Aquasorb® A-500 (CMC) was bought from Copenhagen Pectin (Denmark), and commercial wheat sour dough Pie was kindly supplied by Ireks (Spain).

2.2. Heat-moisture treatment (HMT)

HMT conditions (15% moisture content, 1 h and 120°C) were selected based on previous experiments (Collar and Armero 2018c), in which maximization of viscometric profile and minimization of loss of hydration properties of flour samples were applied as criteria. Single T, CN and CP flour samples were thermally treated. Untreated (-) and HMT (+) single flours were used in quaternary blends (T:CN:CP:WT) in presence of WT- for doughmaking.

2.3. Chemical, nutritional and functional characteristics of native and HMT flours

Moisture, protein, dietary fibre and fat contents (% flour, dry basis) of native (untreated) and HMT single WT, T, CN, and CP flours were determined following the ICC methods (ICC, 1976-1996). Two replicates were made for each analysis. Starch content of flours were determined by using total Starch Assay Kit (Megazyme kit AA/AMG). Amylose/amylopectin ratio (Megazyme kit K-AMYL 07/11) was estimated by

using a modification of a Con A method [23] that uses an ethanol pre-treatment step to remove lipids prior to analysis (Morrison and Laignet, 1983).

Bulk density was calculated as weight of sample per unit volume of sample (g/ml). The flour samples were gently filled into 10 ml graduated cylinders, previously tared. The bottom of the cylinder was gently tapped on a laboratory bench several times until there was no further diminution of the sample level after filling to the 10 ml mark. Measurements were made in triplicate.

Colour measurements were determined on flours using a Photoshop system following the method previously described by Angioloni and Collar (2009), and the results were expressed in accordance to the Hunter Lab colour space. The Photoshop (PS Adobe Photoshop CS5 extended) system (L , a , b colour coordinates) was calibrated using colour sheets from Pantone®Formula Guide (Pantone, Inc., USA). Images were acquired at 300 pixel resolution with a ScanJet II cx flatbed scanner (Hewlett- Packard, USA). Parameters determined were L ($L = 0$ [black] and $L = 100$ [white]), a ($-a$ = greenness and $+a$ = redness), b ($-b$ = blueness and $+b$ = yellowness), as described earlier (Angioloni and Collar, 2009). Hunter Lab colour space parameters from Minolta colorimeter were calculated from the calibration linear equation Colorimeter vs. Photoshop.

Swelling or the volume occupied by a known weight of fibre was evaluated by mixing 5 g (± 0.1 mg) of fibre with 100 mL distilled water and allowed it to hydrate overnight (Nelson, 2001).

Solvent-Retention Capacity (SRC) was determined according the AACC method 56-11 (AACC, 2005) to quantify potential contributions to water holding capacity by other flour components having water-uptake capabilities (Traynham et al., 2007). The solvents used were water, sucrose (50% w/v), sodium bicarbonate (5% w/v), and lactic acid (5% v/v). 25 mL of prepared solvent were added to 5 g of flour in 30 mL centrifuge bottles. Centrifugation at 1,239•g (3,000 rpm) was performed for 15 min. After decanting, a gel remained. Gels were weighed and the SRC value (%) calculated as $\% \text{ SRC} = [((\text{gel wt}/\text{flour wt}) \cdot (86/(100 - \% \text{ flour moisture})) - 1)] \cdot 100$ for each solvent.

119 Water absorption index (WAI) and water solubility index (WSI) were determined as described by Singh et
120 al. (2003) with modifications of the heating conditions as follows. The ground flour samples (2.5g) were
121 mixed with 30 ml distilled water, using a glass rod, and heated at 30 and 60°C for 15 min in a water bath.
122 The cooked paste was cooled to room temperature and transferred to centrifuge tubes and centrifuged at
123 3000g for 10 min. WAI and WSI were calculated by using the expressions:

124 $WAI = \text{Weight of sediment} / \text{weight of dry solids}$

125 $WSI = (\text{Weight of dissolved solids in supernatant} / \text{weight of dry solids}) \times 100$

126 $SP = WAI / [1 - WSI / 100]$

127 **2.4. Bread making of wheat and wheat-based blended flours**

128 Doughs and breads were prepared from wheat-based blended flours (T, CN, CP) by WT replacement at
129 34%, and incorporation of ternary blends of T (20%, flour basis), CN (7%, flour basis), and CP (7%, flour
130 basis) flours according to a Multilevel Factorial Design with the following attributes: 3 experimental factors
131 (T, CN and CP flours) at 2 levels, coded 0 (untreated) and 1 (HMT), and 5 error degrees of freedom. The
132 model resulted in 8 randomized runs in 1 block. A 3 digit bread sample code was set referring to no HMT
133 (0) and HMT (1) T (1st digit), CN (2nd digit), and CP (3rd digit) flours in sample formulation, as it follows:
134 110, 101, 100, 000, 001, 111, 010, 011. Blended flours (100 g), water (100%, flour basis), commercial
135 compressed yeast (3%, flour basis), salt (2%, flour basis), commercial sour dough Pie (5%, flour basis),
136 and CMC (3%, flour basis) were mixed in a 10 kg mixer at 60 revolutions min⁻¹ for 10 min up to optimum
137 dough development. Fermented doughs were obtained after bulk fermentation (10 min at 28°C), dividing
138 (300 g), rounding, molding, panning and proofing up to maximum volume increment (50 min at 28°C), and
139 were baked at 225 °C for 25 min to make blended breads, and stored for 0, 1, 3, 6 and 8 days to study
140 kinetics of thermal transitions on ageing.

141 **2.5. Visco-metric Properties**

The 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. Freeze-dried composite dough blends (3.5 g, 14% moisture basis) were transferred into canisters and $\approx 25 \pm 0.1$ mL of distilled water were added (corrected to compensate for 14% moisture basis). Three replicates were made per sample. The slurry was heated to 50°C and stirred at 160 rpm for 10 sec for thorough dispersion. The slurry was held at 50°C for up to 1 min, and then heated to 95°C over 3 min 42 sec and held at 95°C for 2 min 30 sec, and finally cooled to 50°C over 3 min 48 sec, and held at 50°C for 2 min. The pasting temperature (°C) (when viscosity first increases by at least 25 mPa.s over a 20-s period), peak time (when peak viscosity occurred), peak viscosity (maximum hot paste viscosity), holding strength or trough viscosity (minimum hot paste viscosity), breakdown (peak viscosity minus holding strength or trough viscosity), viscosity at 95°C (viscosity attained at the beginning of the holding period during cooking), viscosity at the end of the 95°C holding period, viscosity at 50°C (viscosity attained at the beginning of the holding period during cooling), final viscosity (end of test after cooling to 50°C and holding at this temperature), setback (final viscosity minus peak viscosity), and total setback (final viscosity minus holding strength) were calculated from the pasting curve using Thermocline v. 2.2 software (Collar, 2016b).

2.6. Thermal properties

Thermal properties regarding starch retrogradation, and amylose-lipid complexation of fresh and stored blended bread samples were assessed in a Differential Scanning Calorimeter Perkin-Elmer DSC-7 (Norwalk, USA) according to the method previously reported by Collar et al. (2015). Sample pans containing about 50-70 mg of bread were scanned at 25°C for 1 min, and then heating from 25 to 130°C at a rate of 10°C/min. An empty stainless steel pan was used as reference. The enthalpy of amylopectin retrogradation (ΔH_r) and the enthalpy for amylose-lipid complex dissociation (ΔH_d) were calculated, and T_o , T_p , and T_e for the different thermal transitions, identified. R was defined as the temperature range $T_p - T_o$ for each transition. Percentage of retrogradation (R%) was calculated as ratio of enthalpy of

gelatinization (Collar and Armero, 2018c) to enthalpy of retrogradation at 8 days (Berski et al., 2018). All samples were analyzed in duplicate. Modelling of crystallization data was carried out using the Avrami equation, and model factors were estimated by fitting experimental data for melting enthalpies to the nonlinear regression equation [33] $\theta = \frac{H_{\infty} - H_t}{H_{\infty} - H_0} = e^{-kt^n}$ where θ is crystallinity, H_{∞} , is the levelling-off value of melting enthalpy at which the extent of crystallization in starch stopped, H_t is the melting enthalpy at time t , and H_0 is the melting enthalpy at initial time, t is time of crystallization, k is a rate constant, and n is the Avrami exponent.

Statistical analysis

Statistical package Statgraphics Plus V 5.1 (Statpoint Technologies, Warrenton, Virginia, USA) was used to perform univariate (One-way analysis of variance ANOVA) and multivariate (two-way analysis of variance MANOVA, Pearson correlation matrix, non-linear regression analysis and factor analysis FA) data analysis. Results were presented as the mean value $\pm$ standard deviation of at least duplicate determinations. Significant differences within pairs of means were assessed by Fisher's least significant differences test LSD at 95% confidence interval ($p < 0.05$) in all cases. FA was carried out using a matrix of normalized correlation to calculate the eigenvalues (loadings), eigenvectors and related components with the original variables. The first two factors using principal components as factoring type were plotted to show factor scores in scatter plots for variables and samples.

3. Results and discussion

3.1. Chemical, nutritional and functional properties of native and HMT flours

Characterization of native and HMT flours evidenced significant ($p < 0.05$) effects of the thermal treatment of flours on main functional parameters while chemical and nutritional features did not significantly ($p > 0.05$) depend on HMT, but on the non-wheat flours used (Table 1).

Flours account for variable protein contents (g/ 100 g flour, d. b.) from 6.44 (CN) up to 18.82 (CP), with cereal flours (WT, T) exhibiting intermediate protein levels at about 14%. Amounts of fat and dietary fibre were the highest (7.41%, 25.13%) in CP, the lowest in WT (1.56%, 2.56%), and reached intermediate values in T and CN (4.08-4.79%, 9.23-12.31%) flours, respectively (Table 1). Total starch content was prominent in WT (77.2%), followed by CN and T (64.1-66.2%), and smaller in CP (at about 47-49%). Amylose content was >18% (WT), and <14.5% in main flours irrespective of the thermal treatment application. Obtained data on the chemical and nutritional composition of grain flours are in general in line with previous reports [Gebremariam et al., 2014; Han and Baik, 2008; Moreira et al., 2012]. Slight/little effects of hydrothermal treatments on main macronutrient contents were reported for millet (Dharmaraj and Malleshi, 2011) and rice (Nakamura et al., 2017) flours, even when submitted to severe treatment conditions of temperature, pressure and/or time.

Bulk density of flours submitted to HMT explicated a discreet increase by 7% in cereal flours, and a decrease of 5-6% in CN and CP (Table 1). Increase in bulk density after hydrothermal treatments ascribed to a more compacted structure were previously reported for maize (Bolade et al., 2002) and millet (Lawal, 2009) flours, whereas decreased bulk density with increase in gelatinization for wheat corn extruded flours were observed (Case et al., 1992).

HMT slightly changed the colour of the flour in cereal samples, except for the significant increase in redness (+300% *a*) and yellowness (+25% *b*) in T (Table 1). Colour of CN and CP flours significantly changed on HMT, particularly *b* values that tripled (CN) and doubled (CP) yellowness, respectively. Increased *a* and *b* colour values have been reported for japonica milled heat moisture treated rice flour (Takahashi et al., 2005), and for hydrothermally treated pearl millet starch (Balasubramanian et al., 2014), and may be attributed to the change in colour caused by Maillard reaction between reducing sugar from the heated starch and the amino group in the proteins during thermal modification (Lorlowhakarn and Naivikul, 2006).

214 The SRC test is a solvation assay for flours based on the enhanced swelling behavior of individual polymer
215 networks in selected single diagnostic solvents— water, 5% w/w lactic acid (LA) in water (for glutenin),
216 5% w/w sodium carbonate (Na_2CO_3) in water (for damaged starch), and 50% w/w sucrose in water (for
217 pentosans)—which are used to predict the functional contribution of each individual flour component. For
218 flour typically used to produce bread by the sponge–dough method, optimal SRC profile values would be
219 ≥ 100 % in 5% w/w lactic acid, ≤ 96 % in 50% w/w sucrose in water, ≤ 72 % in 5% w/w sodium carbonate
220 extracts (Collar and Armero, 2018a). In this work, a straight dough breadmaking system was used instead,
221 and some mean values for water retention components of flours were outside the typical range for a
222 sponge–dough bread system in mixed and thermally treated flours, especially for native and HMT CN and
223 CP flours and water retention of pentosans (126 %) and damaged starch (110 %). Results are consistent
224 with an additional deleterious effects of HMT to the replacement of WT flour by T/CN/CP on the optimal
225 SRC profile of mixed flours (Table 1).

226 The swelling power as the ability of the flour to associate with water, is primarily a property of amylopectin,
227 where amylose is considered a diluent and an inhibitor of swelling (Hoover et al., 2010). In our study, WT
228 flour amylopectin accounted for 58-59%, whereas T and CN flours presented 50-52% of amylopectin
229 content, and CP flour contained at about 35% (Table 1). In addition, soluble dietary fibre content of flours
230 followed the order $\text{WT} < \text{T} < \text{CP}$, and the lipid content followed the order $\text{WT} < \text{CN} < \text{T} < \text{CP}$. T flour shows the
231 highest swelling and WAI values at both 30 and 60°C, while WT exhibits the lowest values only at 30°C.
232 WAI increased significantly ($p < 0.05$) but slightly in HMT flours, particularly for CN (+28% at 30°C, +18%
233 at 60°C), in accordance with previous results on hydrothermally treated maize grains (Bolade et al., 2002)
234 and millet (Balasubramanian et al., 2014) matrices.

235 Solubility is a result of amylose leaching, which dissociates and diffuses out of the starch granule during
236 swelling, and corresponds to both hydrophilicity and amylose content i.e. more the dissociation of inter
237 and intra hydrogen bonds, more will be the amylose leaching and hence solubility (Lawal, 2009). In our
238 study, low levels of soluble material (4–6%) were found in both unprocessed and HMT cereal flours, in

contrast with CN and CP flours that solubilized 20-30% of material (Table 1). HMT leads to the formation of water-soluble molecules from small fragments of amylopectin or amylose that may have leached out through opened starch granules of the flour after hydrothermal treatment, and are captured by the measurement of the water solubility index (WSI). Increase in solubility after hydrothermal treatment has been reported previously by Adebawale et al. (2005) for finger millet starch and by Shobana & Malleshi (2007) for finger millet flour, and by Robin et al., (2015) for whole grain cereals and pseudocereals flours. The increased level of WSI after HMT was found to vary considerably with the grain sources and the severity of the treatment. It has been reported that WSI of amaranth, teff and wheat dramatically changed after extrusion, while lesser changes in WSI levels were shown for quinoa and sorghum, and no changes at all were observed for millet (Robin et al., 2015). The current conditions used for HMT are milder (15% moisture content, 120°C, 1 h), and effects of HMT were discreet except for WT flour that showed an increase by 50% on WSI at 60°C (Table 1), implying a higher flour structure susceptibility to degradation during the thermal treatment as compared to the other flours.

3.2. Pasting and gelling

Visco-metric profiles of native and HMT blended matrices illustrated significant ($p < 0.05$) different behavior during pasting and gelling (Figure 1). Quantitative viscosity patterns of native (000) and fully thermally treated (111) blends were similar and lower during both cooking and cooling as compared to partially processed counterparts, either singly (100, 001, 010,) or binary (110, 101, 011) HMT blends. The RVA profile of HMT blends was characterized by a delayed pasting temperature (88.78-92.05°C), the development of a variable maximum of viscosity in the 95°C holding period (1767-1927 mPa.s) representing starch gelatinization under a constant shear, variable holding strength (1314-1472 mPa.s) leading to similar breakdown of viscosity at the holding period, (440-542 mPa.s) and a slightly variable typical increase in setback viscosity on cooling (1076-1119 mPa.s) ascribed to the reassociation of constituent starch molecules, mainly amylose, into a more ordered state (Figure 1, Table 2). The superior viscosity pattern during both pasting and gelling was particularly explicated by sample 100, and the inferior

profile was observed in sample 111 (Figure 1). Quantitative RVA profile of native blends (000) clearly defined the lowest pasting temperature (89.55°C), maximum viscosity during the holding period (1737 mPa.s), and holding strength (1280 mPa.s), and the subsequent lowest breakdown on cooking (458 mPa.s), followed by the poorest setback on cooling (1037 mPa.s).

Single effects of HMT flours (+) on most pasting and gelling parameters were discreet but significant ($p < 0.05$) (Table 3), as compared with native flours (-). On cooking, T+ led to increased holding strength (+5%), decreased breakdown (-7%) and promoted viscosity at end of 95°C (+16%), while CP+ encompassed a small drop in both peak viscosity (-2%) and breakdown (-5%), and CN+ induced a slight promotion in breakdown values (+3%). On cooling, T+ increased final viscosity (+3%) and viscosity at 50°C (+4%), whereas CN+ promoted total setback values (+3%) (Table 3). Simultaneous presence of 2 flours either native (-) or HMT (+) significantly ($p < 0.05$) impacted the viscosity profile of blends (Table 4), especially for T/CN and T/CP. In general, T+ maximized values for cooking and cooling primary parameters in presence of either native CN- or native CP-. The pairs T+/CN- and T+/CP- correspond to higher viscosity profile blended samples 101, 100, 110 (Figure 1a). They respectively provided maximum values for peak viscosity (1891, 1918 mPa.s), holding strength (1443, 1449 mPa.s), and viscosity of the hot paste (1610, 1617 mPa.s) on pasting, and the greatest values for viscosity at 50°C (2011, 2019 mPa.s) and viscosity of the cold paste (2529, 2554 mPa.s) on gelling (Table 4).

It has been reported that HMT starches compared to native starches almost always exhibit an increased pasting temperature or time (Yadav et al., 2012), indicating that more forces and cross-links are present within the starch granules, along with decreased peak and trough viscosities and breakdown, associated to a loss of ability of the granules to swell freely before their physical breakdown and to more stability during continuous heating and stirring (Adebawale et al., 2005). Significance of HMT on setback and final viscosities vary among literature reports, according to starch botanical source and HMT conditions (Zavareze and Dias, 2011). The increase in viscosity during the cooling period indicates a tendency of various constituents present in the hot paste (swollen granules, fragments of swollen granules, colloiddally-

and molecularly-dispersed starch molecules) to associate or retrograde as the temperature of the paste decreases. Gelation can be characterized as the process by which dispersed starch molecules in an aqueous medium begin to reassociate and form three-dimensional network structures. Interchain associations may involve both short- and long-range order depending on the extent of lateral associations between ordered chain segments. Lan et al. (2008) have shown that retrogradation is influenced by the amount of leached amylose, granule size, and the presence of rigid, non-fragmented swollen granules. Chung et al. (2010) found that HMT reduces amylose leaching from starch granules and that this reduction is more significant in starches containing high levels of amylose. They also reported that HMT promotes additional amylose–amylose and/or amylopectin–amylopectin chain interactions, which reduce amylose leaching and decrease retrogradation.

3.3. Retrogradation

Bread and other baked products are complex heterogeneous systems consisting of starch and gluten/protein polymers in structurally diverse domains with their own distinct sensitivity to water plasticization. Retrogradation is sensitive to the water content of the baked product because of its action as a plasticizer of amorphous regions, with maximum crystallinity taking place in bread crumb at 35–45% water content (Biliaderis, 2009). This is the moisture range of the mixed breads under study (41.1–44.4%), indicating that kinetics of deteriorative physicochemical processes in these products (Collar and Armero, 2018c) is likely to be favored. Crystallization in bread did not occur below 10% and above 80% solids level, but accelerated with increasing water content from 10% to 50%; so that the overall response of retrogradation enthalpy to moisture content has been described to follow a bell-shaped curve (Biliaderis, 2009). At intermediate water contents, formation of B-type crystallites might also reduce the water content of the surrounding amorphous phase, and thereby increase its rigidity as observed in increased crumb firming (Collar and Armero, 2018c).

DSC thermograms of amylopectin retrogradation for blended breads at any time of storage showed a similar qualitative shape regardless the native/HMT flour composition in the blends (Fig. 2a). Quantitative

changes in the ΔH thermal profile for up to 8 days of storage were defined according to the flour blend composition (Fig. 2b). Data of starch retrogradation on ageing were modelled using the Avrami equation, that provides a convenient means to quantify crystallization kinetics and make comparisons among similar starch-based systems submitted to different processing treatments, as previously reported (Collar et al., 2015). Results on the model factors ΔH_0 , ΔH_∞ , n_r , and k_r for the enthalpy of amylopectin retrogradation are compiled in Table 2. Compared to native sample 000, and with some exceptions, HMT blended breads exhibited lower (ΔH_∞ : 2.433-2.928 J/g flour vs 3.358 J/g flour) and variable rate (n_r : 0.4473-1.2912 vs 0.6945; k_r : 0.2238-0.6210 vs 0.4668) of retrogradation kinetics along storage, with lower percentage of retrogradation R% in most treated samples (43-51% vs 53%), denoting lower extent and slower rate of amylopectin recrystallization (Fig. 2b).

Native flour (-) replacement by single HMT (+) T, CN and/or CP significantly ($p < 0.05$) modified amylopectin retrogradation kinetics (Table 5), in terms of H_∞ (-19% T, CP; +11% CN) and n_r (+52% T, +28% CP, -18% CN). Simultaneous presence of T/CN, T/CP and CN/CP either singly treated -/+, +/- or fully thermally treated +/+ provided changes in the extent and the rate of amylopectin crystallization on bread storage (Table 5, Fig. 2b). Suitable trends for meeting the lower retrogradation kinetics were achieved by the pairs T/CP either singly treated (-/+, +/-) or fully treated (+/+), that when incorporated into formulations decreased H_∞ by 27% and 33%, respectively (Table 5). Slower retrogradation kinetics were provided by the pairs T/CN and CN/CP when added singly treated to quaternary blends, associated to a concomitant drop in k_r of 29-32% and 22-24%, respectively (Table 5). In accordance, stored bread samples T+/CN+/CP+ (111) and T+/CN-/CP+ (101) explicated respectively the highest and the lowest DSC profiles on ageing (Fig. 2b), with the greatest (0.6210) and the smallest (0.2238) k_r values (Table 2).

3.4. Amylose-lipid complex dissociation

337 Amylose–lipid complexes constitute a distinct structural domain, as their thermal dissociation occurs in a
338 temperature region ($T_m \approx 100\text{--}120^\circ\text{C}$) different from those of the endothermic transitions of native A- or
339 B-crystallites of amylopectin and retrograded amylopectin ($T_m \approx 40\text{--}55^\circ\text{C}$) or amylose ($T_m \approx 140\text{--}160^\circ\text{C}$)
340 (Biliaderis, 2009). Formation and thermal stability of the thermo-reversible complexes are highly
341 dependent on the nature of the ligand, the chain length of amylose and the conditions (temperature–
342 solvent–time) employed during their preparation, and is also influenced by water content. At high moisture
343 contents, single cooperative transitions are observed, implying zero-entropy production melting (i.e.
344 melting without an apparent change in the metastability of the system on heating). As the water content
345 is decreased ($<70\%$ w/w), however, the peak temperature is elevated and a second endotherm at higher
346 temperatures progressively develops (Copeland et al., 2009). Some amylose-lipid complexes always
347 exhibited a single transition even at intermediate or low water contents (Biliaderis et al., 1985) as it can
348 be the case of blended samples (41.1–44.4% wt/wt).

349 Amylose-lipid complexes can be naturally present in starch or formed upon gelatinization of starch in the
350 presence of lipids (Putseys et al., 2010). Flours used in the present work account for 1.56–7.41 g/100 g
351 flour of lipids (Table 1). Stored untreated and HMT blended bread samples defined a peak corresponding
352 to an endothermal transition phase with T_p ranging from 103.08°C to 111.33°C under a wide range R_d
353 from 19.78°C to 31.91°C , ascribed to the dissociation of amylose-lipid inclusion complex (Table 2, Fig.
354 1a). In agreement with previous results (Collar et al., 2015), no significant change ($p > 0.05$) in the
355 magnitude of the endotherm was observed with ageing (Fig. 1a). The observed values for ΔH_d (0.349–
356 0.947 J/g flour) are low as described earlier for water contents below 50%, and probably reflect the
357 complex effect of several processes such as crystallite melting, helix~coil transition, and recrystallization
358 phenomena that occur simultaneously (Biliaderis et al., 1985). Only HMT of T flour has a significant single
359 effect on the enthalpies for the thermal transition (Table 5), denoted by an averaged decrease in ΔH_d from
360 0.719 to 0.453 J/g flour. Changes may be ascribed to the formation of less thermostable
361 amorphous/crystalline structures, on HMT.

3.5. Distribution of samples according to their thermal characteristics

Factor analysis classified visco-metric and thermal analytical variables of untreated and HMT matrices into two different factors explaining 79.4% of the variability of the results (VE) (Fig. 3a). Factor 1 (56.3% VE) positively correlated ($p < 0.05$) with kinetic parameters describing the rate of retrogradation (n_r , k_r), and with the viscosity at the end of 95°C. Factor 2 (23.1% VE) significantly ($p < 0.05$) and positively depended on breakdown on cooking and total setback on cooling (Fig. 3a). Scores of Factor 1 and Factor 2 clearly differentiated breads with untreated (0) and HMT (1) T flour in the formulation, and segregated those with no HMT (000) and fully HMT treated (111) in a 3rd group (Fig. 3b). HMT T breads (100, 110, 101) vs. untreated T breads (010, 011, 001) were characterized by greater viscosity of the hot paste (1581-1639 mPa.s vs. 1460-1530 mPa.s), higher rate of retrogradation (n_r : 0.9570-1.2912 vs. 0.4473-0.9145), and similar intermediate/ high breakdown (440-482 mPa.s vs. 472-542 mPa.s) and setback on cooling (1076-1114 mPa.s vs. 1069-1119 mPa.s), while untreated and fully HMT samples exhibited intermediate/lower rates for the retrogradation kinetics (n_r : 0.6945, 1.0296), lower viscosity of the hot paste (1440, 1446 mPa.s), breakdown (458, 452 mPa.s) and total setback on cooling (1037, 1077 mPa.s). In a previous paper [12] suitable trends for the enhancement of the physical characteristics of breads in terms of larger specific volume, higher viscoelastic and textural profiles, with lower and slower staling kinetics on ageing were achieved by the pairs T-/CN+, T-/CP+, CN-/CP+ and CN+/CP+, as in 010, 011, 001 samples. These samples also showed homogeneous and fine crumb grain and appealing color features. As well, the lowest value for hydrolysis index corresponded to samples with thermally treated T and CP flours that reached the lowest equilibrium percentage of starch hydrolyzed C_∞ , and hence leading to the lowest expected Glycaemic Index [21].

4. Conclusions

385 HMT of single flours WT, T, CN, and CP did not significantly change the chemical and nutritional
386 composition of macro constituents, promoted a discreet increase of the ability of the flour to associate
387 with water and slightly led to the formation of water-soluble molecules from small fragments of amylopectin
388 or amylose that may leach out through opened starch granules. WT explicated the higher flour structure
389 susceptibility to degradation during the thermal treatment as compared to the other flours. HMT also
390 increased the yellowness of non-cereal flours by promoting Maillard reactions and altered the structure
391 compactness by modifying the bulk density of flours.

392 Dilution of the basic wheat flour system by accumulative addition of T, CN, and CP flours submitted to
393 partial or total HMT did impact on the disorganization and later reorganization of starch components of
394 the starch-rich blended matrices in excess of water (pasting, gelling) and under water restrictions
395 (retrogradation kinetics). Simultaneous presence of T/CN, T/CP and CN/CP either singly or fully thermally
396 treated provided enhancement of the visco-metric profile on pasting and gelling, and decreased the extent
397 and the rate of amylopectin crystallization on ageing. Suitable trends for meeting the lower retrogradation
398 kinetics were achieved by the pairs T/CP either singly or fully treated, that when incorporated into
399 formulations significantly decreased the levelling-off value of melting enthalpy at which the extent of
400 amylopectin crystallization in starch stopped. Slower retrogradation kinetics were provided by the pairs
401 T/CN and CN/CP when added singly treated to quaternary blends, associated to a concomitant drop in
402 the rate constant. In accordance, stored bread samples T+/CN-/CP+ (101) explicated the lowest DSC
403 profile and enthalpy of dissociation of the amylose-lipid inclusion complex on ageing, but conversely one
404 of the higher visco-metric pattern on pasting and gelling.

405 The influence of HMT on the protein and lipid structural features at the interphase starch-lipid-protein
406 remains unexplored in blended wheat-non wheat matrices. The residual protein solubility in different
407 media, the accessibility of thiol groups inside the protein network and the dynamics of lipid binding to the
408 biopolymers may certainly be affected by the hydrothermal treatment, and as a consequence, they may

impact dough viscoelasticity. The research of these (bio)chemical aspects will provide a better understanding of the functionality of the diluted composite wheat matrices submitted to HMT.

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Compliance with Ethical Standards

Conflict of Interest The authors confirm that this article content has no conflict of interest.

5. References

- AACC Approved methods of the American Association of Cereal Chemists, 10th edn. AACC St. Paul, MN: The Association (2005).
- Adebowale, K.O., T.A. Afolabi, B.I. Olu-Owolabi, (2005) Hydrothermal treatments of finger millet (*Eleusine coracana*) starch, Food Hydrocolloids, 19, 974–983.
- Angioloni, A., C. Collar, (2009) Bread crumb quality assessment: a plural physical approach, Eur. Food Res. Technol. 229, 21–30.
- Balasubramanian, S., R. Sharma, J. Kaur, (2014) Characterization of modified pearl millet (*Pennisetum typhoides*) starch. J. Food Sci. Technol. 51, 294-300.
- BeMiller, J. N., K.C. Huber, (2015) Physical Modification of Food Starch Functionalities, Annu Rev Food Sci T 6, 19-69.

430 Berski, W., R. Ziobro, M. Witczak, H. Gambuś, (2018) The retrogradation kinetics of starches of different
 431 botanical origin in the presence of glucose syrup, *Int. J. Biol. Macromol.* 114, 1288–1294.

432 Biliaderis, C. G. (2009) Structural Transitions and Related Physical Properties of Starch, in: *Starch: Chemistry and Technology*, Third Edition. J. BeMiller, R. Whistler (Eds), Academic Press, Elsevier,
 433 Burlington, USA, pp. 293-372.

435 Biliaderis, C.G., C.M. Page, L. Slade, R.R. Sirett, (1985) Thermal Behavior of Amylose-Lipid Complexes.
 436 *Carbohydr. Polym.* 5, 367-389.

437 Bolade, M.K., M.A. Usman, A.A. Rasheed, E.L. Benson, I. Salifou, (2002) Influence of hydrothermal
 438 treatment of maize grains on the quality and acceptability of *tuwon masara* (traditional maize gel), *Food Chemistry* 79, 479–483.

440 Case, S.E., D.D. Hamann, S.J. Schwartz, (1992) Effect of starch gelatinization on physical properties of
 441 wheat and corn based products, *Cereal Chem.* 69, 401–404.

442 Cetiner, B., O. Acar, K. Kahraman, T. Sanal, H. Koksel, (2017) An investigation on the effect of heat-
 443 moisture treatment on baking quality of wheat by using response surface methodology, *J. Cereal Sci.*
 444 74, 103-111.

445 Collar, C. (2016a). Role of Bread on Nutrition and Health Worldwide, in: C.M. Rosell, J. Bajerska, A. F.
 446 El Sheikha (Eds), *Bread and its fortification. Nutritional and health benefits*, CRC Press/ Taylor & Francis
 447 Group, Boca Ratón, FL, pp. 26-52.

448 Collar, C. (2016b) Impact of visco-metric profile of composite dough matrices on starch digestibility and
 449 firming and retrogradation kinetics of breads thereof: Additive and interactive effects of non-wheat flours,
 450 *J. Cereal Sci.* 69, 32-39.

451 Collar, C. (2017) Significance of Heat Moisture Treatment conditions on the pasting and gelling
 452 behaviour of various starch-rich cereal and pseudocereal flours, *Food Sci. Technol. Int.* 23, 623–636.

453 Collar, C., E. Armero, (2018a) Impact of heat moisture treatment and hydration level on
 454 physico-chemical and viscoelastic properties of doughs from wheat-barley composite flours, Eur. Food
 455 Res. Technol. 244, 355–366.

456 Collar, C., E. Armero, (2018b) Significance of Heat Moisture Treatment and hydration level of flours on
 457 the functional and nutritional value of wheat-barley composite breads, Food Bioprocess Tech. 11, 966-
 458 978.

459 Collar, C., E. Armero, (2018c) Value-added dough-based fermented mixed matrices by applying Heat
 460 Moisture Treatment to non-wheat flours: a functional and nutritional approach of blends, Food
 461 Bioprocess Tech. 11, 1536–1551, DOI 10.1007/s11947-018-2125-2.

462 Collar, C., E. Armero, (2018d) Kinetics of *in vitro* starch hydrolysis and relevant starch nutritional
 463 fractions in heat-moisture treated blended wheat-based bread matrices: impact of treatment of non-
 464 wheat flours. Eur. Food Res. Technol. DOI: 10.1007/s00217-018-3109-1.

465 Collar, C., T. Jiménez, P. Conte, A. Piga, (2015) Significance of thermal transitions on starch digestibility
 466 and firming kinetics of restricted water mixed flour bread matrices, Carbohydr. Polym. 122, , 169–179.

467 Copeland, L., J. Blazek, H. Salman, C. M. Tang, (2009) Form and functionality of starch, Food Hydrocoll.
 468 23, 1527–1534.

469 Dharmaraj, U., N. G. Malleshi, (2011) Changes in carbohydrates, proteins and lipids of finger millet after
 470 hydrothermal processing. LWT - Food Sci. Technol. 44, 1636- 1642.

471 Gebremariam, M. M., M. Zarnkow, T. Becker, (2014) (Teff *Eragrostis tef*) as a raw material for malting,
 472 brewing and manufacturing of gluten-free foods and beverages: a review. J. Food Sci. Technol. 51,
 473 2881–2895.

474 Gray, J., J.N. BeMiller, (2003) Bread staling: Molecular basis and control. Comprehensive Reviews in
 475 Food Science and Food Safety 2, 1-21.

476 Gunaratne, A., R. Hoover, (2002) Effect of heat-moisture on the structure and physicochemical
 477 properties of tuber and root starches. Carbohydr. Polym. 49, 425–437.

478 Han, H., B-K. Baik, (2008) Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas
 479 (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*), and their quantitative
 480 changes during processing, Int. J. Food Sci. Technol. 43, 1971–1978.

481 Hoover, R., T. Hughes, H.J. Chung, Q. Liu. (2010) Composition, molecular structure, properties, and
 482 modification of pulse starches: a review. Food Res Int 43, 399–413.

483 Hug-Iten, S., F. Escher, B. Conde-Petit, (2003) Staling of bread: Role of amylose and amylopectin and
 484 influence of starch-degrading enzymes. Cereal Chem. 80, 654-661.

485 ICC, 1976-1996. ICC Standard Methods of the International Association for Cereal Chemistry. 104/1,
 486 105/2, 110/1, 115/1, 136, 162, 166. The Association: Vienna.18.

487 Jouppila, K., J. Kansikas, Y.H. Roos, (1998) Factors affecting crystallization and crystallization kinetics
 488 in amorphous corn starch. Carbohydr. Polym. 36, 143-149.

489 Lan, H., R. Hoover, L. Jayakody, Q. Liu, E. Donner, M. Baga, E.K. Asare, P. Hucl, R.N. Chibbar, (2008)
 490 Impact of annealing on the molecular structure and physicochemical properties of normal, waxy and
 491 high amylose bread wheat starches, Food Chemistry 111, 663–675.

492 Lawal, O.S. (2009) Starch hydroxyalkylation: physicochemical properties and enzymatic digestibility of
 493 native and hydroxypropylated finger millet (*Eleusine coracana*) starch. Food Hydrocoll. 23, 415–425.

494 LeBail, P., H. Bizot, A. Buleon, (1993) 'B' to 'A' type phase transition in short amylose chains, Carbohydr.
 495 Polym. 21, 99-104.

496 Lorlowhakarn, K., O. Naivikul, (2006) Modification of rice flour by heat moisture treatment (HMT) to
 497 produce rice noodles. Kasetsart J. (Natural Science) 40, 135–143.

498 Lu, S., C.Y. Chen, C.Y. Lii, (1996) Gel-chromatography fractionation and thermal characterization of rice
 499 starch affected by hydrothermal treatment, *Cereal Chem.* 73, 5–11.

500 Mann, J., B. Schiedt, A. Baumann, B. Conde-Petit, T.A. Vilgis, (2013) Effect of heat treatment on wheat
 501 dough rheology and wheat protein solubility. *Food Sci. Technol. Int.* 20, 341–351.

502 Marston, K., H. Khouryieh, F. Aramouni, (2016) Effect of heat treatment of sorghum flour on the
 503 functional properties of gluten-free bread and cake, *LWT - Food Sci. Technol.* 65, 637-644.

504 Moreira, R., F. Chenlo, M.D. Torres, D. M. Prieto, (2012) Technological Assessment of Chestnut Flour
 505 Doughs Regarding to Doughs from Other Commercial Flours and Formulations, *Food Bioprocess Tech.*
 506 5, 2301–2310. DOI 10.1007/s11947-011-0524-8.

507 Morrison, W.R. , B. Laignet, (1983) An improved colorimetric procedure for determining apparent and
 508 total amylose in cereal and other starches, *J Cereal Sci* 1, 9–20.

509 Nakamura, C., K. Yoshiki, M. Seguchi, (2008) Increased volume of Kasutera cake by dry heating of
 510 wheat flour, *Food Sci. Technol. Res.* 14, 431–436.

511 Nakamura, S., H. Okumura, M. Sugawara, W. Noro, N. Homma, K. Ohtsubo, (2017) Effects of different
 512 heat–moisture treatments on the physicochemical properties of brown rice flour, *Biosci Biotech Bioch*
 513 81, 2370-2385, DOI: 10.1080/09168451.2017.1387047.

514 Neill, G., A.H. Al-Muhtase, T.R.A. Magee, (2012) Optimisation of time/temperature treatment, for heat
 515 treated soft wheat flour, *J. Food Eng.* 113, 422–426.

516 Nelson, L. (2001) Properties of high-fibre ingredients, *Cereal Foods World* 46, 93–97.

517 Purhagen, J.K. , M.E. Sjöö, A. Eliasson,. (2011) The use of normal and heat-treated barley flour and
 518 waxy barley starch as anti-staling agents in laboratory and industrial baking processes. *J. Food Eng.*
 519 104, 414–421.

520 Putseys, J.A., L. Lamberts, J.A. Delcour, (2010) Review. Amylose-inclusion complexes: Formation,
 521 identity and physico-chemical properties, J. Cereal Sci. 51, 238-247.

522 Robin, F., C. Théoduloz, S. Srichuwong, (2015) Properties of extruded whole grain cereals and
 523 pseudocereals flours. Int. J. Food Sci.Tech. 50, 2152–2159.

524 Shobana, S., N.G. Malleshi, (2007) Preparation and functional properties of decorticated finger millet
 525 (*Eleusine coracana*). J Food Eng. 79, 529–538.

526 Singh, J., N. Singh, T.R. Sharma, S.K. Saxena, (2003) Physicochemical, rheological and cookie making
 527 properties of corn and potato flours, Food Chem. 83, 387–393

528 Takahashi, T., M. Miura, N. Ohisa, K. Mori, S. (2005) Kobayashi Heat treatments of milled rice and
 529 properties of the flours. Cereal Chem. 82, 228–232

530 Traynham, T.L., D.J. Myers, A.L. Carriquiry, L.A. Johnson, (2007) Evaluation of water-holding capacity
 531 for wheat-soy flour blends, J. Am. Oil Chem. Soc. 84, 151–155

532 Verdú, S., F. Vásquez, E. Ivorra, A.J. Sánchez, J.M. Barat, R. Grau, (2017) Hyperspectral image control
 533 of the heat-treatment process of oat flour to model composite bread properties, J. Food Eng. 192, 45-
 534 52.

535 Yadav, N., J. Kaur, T. Anand, A. K. Singh, (2012) Storage stability and pasting properties of
 536 hydrothermally treated pearl millet flour, Int. J. Food Sci.Tech. 47, 2532–2537.

537 Yun, S.H., N.K. Matheson, (1990) Estimation of amylose content of starches after precipitation of
 538 amylopectin with Concavalin A. Starch/Starke 42, 302–305

539 Zavareze, Ed.R., A.R.G. Dias, (2011) Impact of heat-moisture treatment and annealing in starches: a
 540 review. Carbohydr. Polym. 83, 317–328.

Table 1.- Chemical, nutritional and functional characteristics of native and heat-moisture-treated (HMT) wheat, teff, chestnut and chickpea flours^a.

Property ^b	Wheat		Teff		Chestnut		Chickpea	
	Native	HMT	Native	HMT	Native	HMT	Native	HMT
Chemical and Nutritional Composition, %								
Moisture	14.30±0.12 ^d	15.12±0.11 ^e	12.62±0.13 ^c	14.95±0.21 ^e	6.90±0.05 ^a	14.87±0.33 ^e	11.88±0.09 ^b	15.22±0.09 ^e
Protein	14.12±0.28 ^b	13.94±0.21 ^b	14.08±0.46 ^b	13.96±0.56 ^b	6.44±0.20 ^a	6.56±0.34 ^a	18.82±0.60 ^c	18.03±0.51 ^c
Fat	1.56±0.11 ^a	1.62±0.13 ^a	4.69±0.29 ^b	4.79±0.49 ^c	4.08±0.18 ^b	4.21±0.31 ^{bc}	6.96±0.12 ^d	7.41±0.31 ^e
Digestible carbohydrates	81.7±2.3 ^c	81.77±2.51 ^c	68.7±4.5 ^b	69.26±6.1 ^b	79.5±3.2 ^c	80±4.3 ^c	48.8±1.1 ^a	50.3±1.9 ^a
Total starch	77.2±2.1 ^c	76.9±1.9 ^c	65.4±1.3 ^b	66.2±1.6 ^b	64.4±0.9 ^b	64.1±1.7 ^b	48.6±2.1 ^a	47.3±1.8 ^a
Amylose content	18.1±1.4 ^b	18.7±0.9 ^b	14.8±1.8 ^a	14.3±1.3 ^a	13.2±1.5 ^a	14.0±0.9 ^a	13.6±0.8 ^a	12.6±1.3 ^a
Amylopectin content	59.1 ^c	58.2 ^c	50.6 ^b	51.9 ^b	51.2 ^b	50.0 ^b	35.0 ^a	34.7 ^a
Total Dietary Fibre	2.56±0.23 ^a	2.67±0.35 ^a	12.31±1.42 ^c	11.99±1.70 ^c	9.67±1.03 ^b	9.23±1.43 ^b	25.13±2.33 ^d	24.75±3.2 ^d
Soluble Dietary Fibre	1.06±0.11 ^a	0.96±0.21 ^a	4.84±0.55 ^b	4.96±0.66 ^b	-	-	6.55±0.42 ^c	6.39±0.65 ^c
Insoluble Dietary Fibre	1.49±0.28 ^a	1.71±0.33 ^a	7.46±1.10 ^b	7.03±1.23 ^b	-	-	18.62±1.40 ^c	18.36±2.1 ^c
Bulk density, g/mL	0.82±0.01 ^d	0.88±0.01 ^e	0.77±0.00 ^c	0.82±0.01 ^d	0.68±0.00 ^b	0.64±0.00 ^a	0.87±0.01 ^e	0.83±0.00 ^d
Colour								
<i>L</i>	91±0 ^b	91±0 ^b	84±4 ^a	81±5 ^a	91±1 ^b	85±3 ^a	91±1 ^b	90±4 ^{ab}
<i>a</i>	-2.60±0.00 ^d	-2.60±0.00 ^d	0.22±0.00 ^e	0.92±0.01 ^f	-2.57±0.00 ^c	0.92±0.01 ^f	-4.26±0.07 ^b	-5.9±0.1 ^a
<i>b</i>	2.90±0.00 ^a	2.90±0.00 ^a	7.78±0.17 ^c	9.72±0.20 ^d	3.42±0.00 ^b	10.69±0.01 ^e	7.53±0.40 ^c	13.9±0.7 ^f
Swelling, mL/g	2.0±0.0 ^a	2.6±0.1 ^b	4.1±0.1 ^e	4.1±0.1 ^e	3.5±0.1 ^d	2.8±0.1 ^b	3.0±0.0 ^c	3.0±0.0 ^c
Solvent Retention Capacity, %								
water	72±0 ^a	76±2 ^b	129±3 ^g	119±1 ^f	77±2 ^b	89±1 ^c	95±3 ^d	103±0 ^e
lactic acid, 5%	117±4 ^d	113±2 ^d	135±0 ^f	123±0 ^e	79±3 ^a	81±1 ^a	86±3 ^b	103±1 ^c
sucrose, 50%	116±2 ^c	117±0 ^c	165±5 ^f	156±1 ^e	76±0 ^a	123±2 ^d	110±3 ^b	126±3 ^d
sodium bicarbonate, 5%	74±0 ^a	89±1 ^c	129±1 ^f	135±1 ^g	80±2 ^b	97±0 ^d	88±3 ^c	110±1 ^e
Water Absorption Index, g water/g flour, d. b.								
30°C	1.93±0.08 ^a	2.25±0.04 ^b	3.32±0.01 ^e	3.52±0.01 ^f	2.00±0.01 ^a	2.56±0.02 ^c	2.92±0.23 ^d	3.03±0.04 ^d
60°C	3.55±0.04 ^e	3.02±0.12 ^d	4.08±0.21 ^f	4.44±0.01 ^g	2.45±0.08 ^a	2.90±0.03 ^c	2.75±0.22 ^b	3.01±0.05 ^d
Water Solubility Index, % solids, dry flour								
30°C	4±0 ^a	5±0 ^b	6±0 ^c	5±1 ^{bc}	28±6 ^f	32±1 ^g	23±1 ^e	20±1 ^d
60°C	4±0 ^a	6±1 ^{bc}	6±0 ^c	5±0 ^b	28±1 ^e	31±2 ^e	21±1 ^d	21±0 ^d
Swelling Power								
30°C	2.01 ^a	2.37 ^b	3.53 ^d	3.71 ^e	2.78 ^c	3.76 ^e	3.79 ^e	3.79 ^e
60°C	3.70 ^c	3.21 ^a	4.34 ^d	4.67 ^e	3.40 ^b	4.20 ^d	3.48 ^b	3.81 ^c

^a Mean values ± standard deviation. ^b Within rows, mean values with different following letter do differ significantly from each other (p<0.05).

Table 2.- Visco-metric and thermal parameters of wheat based-blended matrices formulated with teff (T), chestnut (CN) and chickpea (CP) flours.

Parameter	Sample ^{a,b}							
	110	101	100	000	001	111	010	011
<i>Pasting</i>								
Pasting temperature, °C	89.60 ± 1.13 ^{ab}	92.05 ± 0.00 ^c	89.60 ± 2.19 ^{ab}	89.55 ± 1.13 ^a	89.95 ± 0.64 ^{ab}	91.18 ± 0.11 ^b	91.13 ± 1.17 ^{bc}	88.78 ± 1.24 ^a
Peak viscosity, mPa.s	1908 ± 2 ^{bc}	1854 ± 4 ^{bc}	1929 ± 19 ^c	1737 ± 57 ^a	1828 ± 7 ^b	1767 ± 40 ^a	1873 ± 22 ^{bc}	1852 ± 39 ^{bc}
Peak time, min	6.13 ± 0.00 ^{abcd}	6.24 ± 0.05 ^d	6.17 ± 0.05 ^{bcd}	6.14 ± 0.09 ^{abcd}	6.20 ± 0.02 ^{cd}	6.00 ± 0.00 ^a	6.00 ± 0.00 ^{ab}	6.04 ± 0.05 ^{abc}
Holding strength, mPa.s	1426 ± 36 ^{cd}	1414 ± 27 ^{cd}	1472 ± 8 ^d	1280 ± 50 ^a	1356 ± 24 ^{abc}	1314 ± 41 ^a	1332 ± 33 ^{ab}	1373 ± 32 ^{bc}
Breackdown, mPa.s	482 ± 38 ^a	440 ± 31 ^a	457 ± 11 ^a	458 ± 6 ^a	472 ± 17 ^a	452 ± 1 ^a	542 ± 11 ^b	479 ± 7 ^a
Viscosity at 95°C, mPa.s	254 ± 6 ^{bc}	230 ± 13 ^{abc}	266 ± 1 ^c	218 ± 1 ^{ab}	219 ± 31 ^{ab}	221 ± 12 ^a	238 ± 18 ^{abc}	238 ± 3 ^{abc}
Viscosity at end 95°C, mPa.s	1594 ± 29 ^{cd}	1581 ± 25 ^{cd}	1639 ± 11 ^d	1440 ± 50 ^{ab}	1530 ± 63 ^{bc}	1446 ± 47 ^a	1460 ± 38 ^{ab}	1512 ± 37 ^{abc}
<i>Gelling</i>								
Viscosity at 50°C, mPa.s	2002 ± 33 ^{cd}	1986 ± 6 ^{cd}	2035 ± 38 ^d	1815 ± 56 ^a	1895 ± 44 ^{abc}	1892 ± 54 ^{ab}	1939 ± 52 ^{bcd}	1969 ± 54 ^{cd}
Setback, mPa.s	632 ± 15 ^b	636 ± 2 ^b	641 ± 26 ^b	579 ± 18 ^a	597 ± 3 ^{ab}	625 ± 16 ^b	577 ± 47 ^a	639 ± 12 ^b
Final viscosity, mPa.s	2539 ± 13 ^{de}	2490 ± 2 ^{cde}	2569 ± 45 ^e	2316 ± 38 ^a	2425 ± 4 ^{bc}	2391 ± 57 ^{ab}	2451 ± 69 ^{bcd}	2490 ± 51 ^{cde}
Total setback, mPa.s	1114 ± 23 ^b	1076 ± 29 ^{ab}	1098 ± 38 ^b	1037 ± 12 ^a	1069 ± 20 ^{ab}	1077 ± 15 ^{ab}	1119 ± 36 ^b	1118 ± 19 ^b
<i>Amylose-lipid complex dissociation</i>								
T_o , °C	91.09±2.43 ^{ab}	91.07± 0.29 ^{ab}	97.63± 3.98 ^b	89.61± 0.04 ^{ab}	89.06± 0.57 ^{ab}	87.17± 0.92 ^a	91.87± 4.88 ^{ab}	91.99± 4.30 ^{ab}
T_p , °C	105.91± 3.88 ^{ab}	105.83±3.06 ^{ab}	111.33±0.94 ^b	109.83±0.94 ^{ab}	105.91±3.88 ^{ab}	103.08±0.11 ^a	109.66±0.47 ^{ab}	109.16±0.94 ^{ab}
T_e , °C	115.16±2.12 ^a	118.08± 2.23 ^a	117.41± 2.94 ^a	115.95± 0.56 ^a	118.66±0.00 ^a	119.08± 0.11 ^a	116.54±0.07 ^a	116.41±0.35 ^a
ΔH_d , J/g flour	0.349±0.049 ^a	0.466±0.039 ^{ab}	0.559±0.291 ^{abc}	0.836±0.027 ^{bc}	0.477±0.109 ^{ab}	0.439±0.004 ^a	0.947±0.124 ^c	0.614±0.009 ^{abc}
R_d , °C	24.07±4.55 ^{ab}	27.01±2.52 ^{ab}	19.78±6.92 ^a	26.33±0.51 ^{ab}	29.60±0.57 ^{ab}	31.91±1.04 ^b	24.67±4.81 ^{ab}	27.21±0.78 ^{ab}
<i>Retrogradation kinetics</i>								
ΔH_0 , J/g flour	0.553±0.063 ^a	1.089±0.008 ^a	0.685±0.075 ^a	0.722±0.081 ^a	0.957±0.099 ^a	0.982±0.089 ^a	0.698±0.072 ^a	0.837±0.064 ^a
ΔH_∞ , J/g flour	2.731±0.352 ^a	2.433±0.290 ^a	2.928±0.342 ^a	3.358±0.417 ^{ab}	2.785±0.274 ^a	2.762±0.320 ^a	4.438±0.510 ^b	2.889±0.296 ^a
k_r	0.4462±0.0562 ^b	0.2238±0.0196 ^a	0.4674±0.0326 ^{bc}	0.4668±0.0415 ^{bc}	0.4828±0.0569 ^{bc}	0.6210±0.0699 ^c	0.2858±0.0496 ^a	0.3738±0.0489 ^{ab}
n_r	0.9570±0.0892 ^c	1.2912±0.0968 ^d	1.0456±0.0885 ^c	0.6945±0.0952 ^b	0.9145±0.0789 ^c	1.0296±0.1210 ^{cd}	0.4473±0.0563 ^a	0.7841±0.0899 ^{bc}
R%	49	43	51	53	49	46	60	51

(^a) Mean values ± standard deviation. Within rows, mean values with different following letter do differ significantly from each other (p<0.05). (^b) Three digit code refers to untreated (0) and heat-moisture treated (1) T:CN:CP flours replacing wheat flour in sample formulation. T_o : onset temperature, T_p : peak temperature, T_e : end temperature, ΔH_d : enthalpy of dissociation of amylose-lipid complex, ΔH_∞ , ΔH_0 : retrogradation enthalpy at ∞ and 0 time, respectively, k_r : constant of proportion of retrogradation kinetics, n_r : Avrami exponent of retrogradation kinetics, R%= percentage of retrogradation = $100.\Delta H/\Delta H_{gel}$.

Table 3: Significant single effects of design factors –teff, chestnut and chickpea flours- on the visco-metric parameters of composite dough matrices during pasting and gelling.

Parameters ^a	Unit	Level	Overall mean	SE	Single effects		
					T	CN	CP
Pasting							
Peak viscosity	mPa.s	0	1840	13	ns	ns	1862 ^b
		1					1818 ^a
Holding strength	mPa.s	0	1368	12	1335 ^a	ns	ns
		1			1402 ^b		
Breackdown	mPa.s	0	472	7	488 ^b	457 ^a	485 ^b
		1			456 ^a	486 ^b	459 ^a
Viscosity at end 95°C	mPa.s	0	1523	14	1485 ^a	ns	ns
		1			1561 ^b		
Gelling							
Setback	mPa.s	0	615	7	598 ^a	ns	ns
		1			633 ^b		
Final viscosity	mPa.s	0	2455	16	2420 ^a	ns	ns
		1			2490 ^b		
Total setback	mPa.s	0	1086	9	ns	1070 ^a	ns
		1				1104 ^b	
Viscosity at 50°C	mPa.s	0	1938	17	1905 ^a	ns	ns
		1			1972 ^b		

Level 0: untreated flours. level 1: heat moisture treated flours at 120°C for 1 h at 15% flour moisture content. SE = standard error.

(^a) Within parameters, mean values with different following letter do differ significantly from each other (p<0.05).

Table 4.- Significant 2nd order interactions (p<0.05) of design factors –teff (T), chestnut (CN) and chickpea (CP) flours- on the visco-metric parameters of composite dough matrices during pasting and gelling.

Parameters ^a	Unit	Level	SE	2 nd order interactions	
				T by CN	T by CP
Peak viscosity	mPa.s	00	18	1783 ^a	1805 ^a
		01		1863 ^{ab}	1840 ^{ab}
		10		1891 ^b	1918 ^b
		11		1823 ^{ab}	1796 ^a
Holding strength	mPa.s	00	18	1318 ^a	1306 ^a
		01		1352 ^a	1364 ^a
		10		1443 ^b	1449 ^b
		11		1360 ^a	1355 ^a
Breakdown	mPa.s	00	10	465 ^a	500 ^b
		01		510 ^b	476 ^{ab}
		10		449 ^a	470 ^{ab}
		11		463 ^a	442 ^a
Viscosity at 95°C	mPa.s	00	9	ns	228 ^{ab}
		01			229 ^{ab}
		10			260 ^b
		11			218 ^a
Viscosity at end 95°C	mPa.s	00	20	1485 ^a	1450 ^a
		01		1486 ^a	1521 ^a
		10		1610 ^b	1617 ^b
		11		1511 ^a	1505 ^a
Viscosity at 50°C	mPa.s	00	24	1855 ^a	1877 ^a
		01		1954 ^{ab}	1932 ^{ab}
		10		2011 ^b	2019 ^b
		11		1934 ^{ab}	1925 ^{ab}
Final viscosity	mPa.s	00	23	2371 ^a	2383 ^a
		01		2470 ^{ab}	2458 ^{ab}
		10		2529 ^b	2554 ^b
		11		2451 ^{ab}	2426 ^a
Total setback	mPa.s	00	13	1053 ^a	ns
		01		1118 ^b	
		10		1087 ^{ab}	
		11		1090 ^{ab}	

Level 0: untreated flours. level 1: heat moisture treated flours at 120°C for 1 h at 15% flour moisture content. SE = standard error.

(^a) Within parameters, mean values with different following letter do differ significantly from each other (p<0.05).

Table 5.- Significant single and interactive effects of design factors (teff, chestnut and chickpea flours) on the amylose-lipid complex dissociation parameters, and on Avrami kinetic parameters for starch retrogradation during storage of blended breads.

Effect	Level	Thermal parameter ^a				
		H_0	H_∞	k_r	n_r	ΔH_d
GRAND MEAN		0.815	3.041	0.4210	0.8955	0.586
Single effects						
Teff		*	**	ns	**	**
	0	0.804 ^a	3.368 ^b		0.7101 ^a	0.719 ^b
	1	0.827 ^b	2.714 ^a		1.0809 ^b	0.453 ^a
Chestnut		**	*	ns	**	ns
	0	0.863 ^b	2.876 ^a		0.9865 ^b	
	1	0.768 ^a	3.205 ^b		0.8045 ^a	
Chickpea		**	**	ns	**	ns
	0	0.665 ^a	3.364 ^b		0.7861 ^a	
	1	0.966 ^b	2.717 ^a		1.0049 ^b	
SE		0.007	0.089	0.0200	0.0171	0.058
Interactive effects						
Teff by Chestnut		*	ns	**	ns	ns
	0 0	0.940 ^c		0.4850 ^b		
	0 1	0.768 ^a		0.3300 ^a		
	1 0	0.887 ^b		0.3460 ^a		
	1 1	0.768 ^a		0.5336 ^b		
Teff by Chickpea		**	**	ns	*	ns
	0 0	0.710 ^b	3.898 ^b		0.5709 ^a	
	0 1	0.897 ^c	2.837 ^a		0.8493 ^b	
	1 0	0.619 ^a	2.830 ^a		1.0013 ^c	
	1 1	1.036 ^d	2.598 ^a		1.1604 ^d	
Chestnut by Chickpea		ns	ns	**	ns	ns
	0 0			0.4671 ^b		
	0 1			0.3533 ^a		
	1 0			0.3660 ^a		
	1 1			0.4974 ^b		
SE		0.010	0.125	0.0290	0.0242	

Level 0: untreated flours. level 1: heat moisture treated flours at 120°C for 1 h at 15% flour moisture content. SE = standard error.

ΔH_d enthalpy of dissociation of amylose-lipid complex, H_∞ , H_0 retrogradation enthalpy at ∞ and 0 time, respectively, k_r constant of proportion of retrogradation kinetics, n_r Avrami exponent of retrogradation kinetics.

(^a) Within parameters, mean values with different following letter do differ significantly from each other ($p < 0.05$).

Figure 1.- Visco-metric profiles of wheat-based blended doughs formulated with teff (T), chestnut (CN) and chickpea (CP) flours. Three digit code refers to untreated (0) and heat-moisture treated (1) T:CN:CP flours replacing wheat flour in sample formulation. a, treated T flour; b, native T flour in blends. Temperature profile: black straight line.

Figure 2.- DSC thermograms of wheat-based mixed bread formulated with heat-moisture treated (HMT) teff (T), chestnut (CN) and chickpea (CP) flours during storage (a), and evolution of retrogradation enthalpy during storage of wheat-based mixed breads formulated with T, CN and CP flours (b). Three digit code refers to untreated (0) and HMT (1) T:CN:CP flours replacing wheat flour in sample formulation.

Figure 3.- Scatterplots from factor analysis (Factor 1 vs Factor 2) of visco-metric and retrogradation thermal parameters of heat-moisture treated (HMT) samples (a), and classification of wheat-based mixed breads formulated with teff (T), chestnut (CN) and chickpea (CP) flours (b). Three digit code refers to untreated (0) and HMT (1) T:CN:CP flours replacing wheat flour in sample formulation.