Value-added of heat moisture treated mixed flours in wheat-based matrices: a functional and nutritional approach.

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Abstract The significance of heat moisture treatment (HMT) of non-wheat –teff (T), chestnut (CN) and chickpea (CP) flours on dough viscoelastic and thermal parameters and on the structural and nutritional pattern of breads was investigated in untreated (-) and HMT (+) associated wheat-based (WT) matrices (WT:T:CN:CP, 66.20:7:7, wt. basis). 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+. In addition, a fine and uniformly- sized cell structure with similar cell walls thickness were achieved in crumb samples. The pair T-CN+ enhanced extracted bioaccessible polyphenols, and the pair CN+CP+ synergistically promoted the anti-radical activity in breads. Blended breads can be labelled as high-fibre breads (≥6 g DF/100 g food), and a recommended daily consumption of 250 g of bread fulfilled from 44% (men) to 67% (women) of dietary fibre requirements.

Keywords Heat Moisture Treatment, flour, dough, bread, functionality, nutritional value

1. Introduction

The increasing demand for novel, tasty and healthy foods has boosted a new market in which products traditionally made from wheat are partially replaced or supplemented with alternative nutrient-dense and health promoting non-wheat ingredients. In this context, ancient crops (Angioloni & Collar, 2011), minor cereals (Collar and Angioloni, 2013), pseudocereals (Collar and Angioloni, 2014a), legumes (Angioloni & Collar, 2012), and non-traditional fruit and seed flours (Paciulli et al., 2016) have received much attention over the last years as functional ingredients for potential breadmaking applications.

Chestnut flour contains high quality proteins with essential amino acids, dietary fibre, low amount of fat and also vitamin E, vitamin B group, potassium, phosphorous, and magnesium of nutritional interest on both wheat (Dall'Asta et al., 2013) and gluten-free (Paciulli et al., 2016) breadmaking. It has been reported that wheat breads enriched with the addition of chestnut flour presented an increased quality from both organoleptic and nutritional points of view (Dall'Asta et al., 2013).

Legumes constitute traditional, ubiquitous and wholesome imaged foods providing nutritional (high protein, mineral and fibre contents, low digestible starch), health (protective and therapeutic effects to chronic health conditions) and functional promoting effects (body, texture and taste enhancement) to foods (Angioloni & Collar, 2012). Associated mixtures of grain (chickpea, greenpea) and oilseed (soybean) legumes replacing wheat flour at 42 % provided, highly nutritious breads–meeting viscoelastic restrictions and sensory standards (Collar and Angioloni, 2017).

Teff (*Eragrostis tef*) is a nutritious cereal wheat-type gluten-free grain indigenous to Ethiopia, rich in carbohydrates and fiber, microelements and phytochemicals with superior amounts of iron, calcium and zinc than wheat, barley and sorghum (Abebe et al., 2007). Teff was successfully applied in breadmaking matrices up to 40% of wheat replacement (Ronda, Abebe, Pérez-Quirce, & Collar, 2015).

Despite partial wheat flour replacement by nutrient-dense flours constitutes a plausible simple strategy to create value-added baked goods, making highly replaced wheat flour breads to assure nutritional and health-related benefits, often encompasses a fall in techno-functional bread quality, particularly loaf volume and texture. Technological treatments have been applied with variable success to palliate the adverse effects associated to gluten and starch dilution in wheat matrices. High Hydrostatic Pressure treatment has been recently used as an alternative to hydrocolloids/gluten addition for the structure rearrangement of legume batters and consequently for their incorporation, in high amount, in breadmaking systems (Collar & Angioloni, 2017). Heat moisture treatment (HMT) constitutes a clean label alternative to chemical modification for altering the gelatinization and retrogradation properties of starches (Gunaratne and Hoover, 2002), flours and doughs (Collar & Armero, 2018), and the aggregation/disaggregation equilibrium of proteins (Mann et al., 2013). Microscopic observations by confocal laser scanning microscopy and light microscopy revealed that HMT caused the clumping of starch granules and the aggregation of denatured protein (Chen et al., 2015). HMT of flours has successfully been applied to wheat (Cetiner et al., 2017), sorghum (Marston, Khouryieh, & Aramouni, 2016), composite oat-wheat (Verdú, Vásquez, Ivorra, Sánchez et al., 2017) and barley-wheat (Collar & Armero, 2018) flours to improve dough functionality, and both volume and textural profile of the resulting breads. Improvement was ascribed mainly to the increase of disulphide cross-linkages of amino acids, and to changes on starch granules conformation, principally in physical reorganization in their structure resulting in higher dough viscosities (Ovando-Martínez et al., 2013).

Significance of HMT on viscoelasticity and functional performance of blended doughs –hydrated wheat/non-wheat flours- has been recently addressed in diluted wheat: barley binary systems (Collar & Armero, 2018). Results pointed out the importance of both the water availability and the heat treatment of compositional flours to obtain a reinforced dough structure with partially restored viscoelasticity.

Although the use of clean label treatments is regaining interest among consumers due to an increasing commitment with the environment, the evaluation of the application of HMT in breadmaking in associated wheat/non-wheat flours is little explored in the literature. This paper is aimed at investigating the potencial of HMT to restore and/or improve bread viscoelasticty and functional performance keeping nutritional value of diluted wheat matrices with incorporation of nutrient-dense value-added non-gluten forming flours (teff, chestnut, chickpea), by studying the changes induced in treated blended bread matrices at both functional and nutritional levels.

2. Materials and methods

2.1. Materials

Commercial flours from refined common wheat *Triticum aestivum* (WT), teff *Eragrostis tef* (T), chestnut *Castanea sativa* (CN), and whole decorticated chickpea *Cicer arietinum* (CP) were obtained from the Spanish market (Navarro, Valencia, Spain). 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 acquired from Copenhagen Pectin (Denmark), and commercial wheat sour dough *Pie* was kindly supplied by Ireks (Spain).

2.2. Methods

2.2.1. Chemical and nutritional composition of flours

Chemical and nutritional composition of native WT, T, CN and CP flours (Table 1) was determined following the ICC methods (ICC, 2014) regarding moisture, protein, and fat contents. Total, soluble and insoluble dietary fibre contents were determined according to the AOAC method 991.43

(AOAC, 1991). Three replicates were made for each analysis. Digestible carbohydrates were calculated by indirect determination as 100 − [Moisture + Protein + Fat + Dietary Fibre] (FAO, 2003).

2.2.2. Heat-moisture treatment (HMT)

HMT conditions (15% moisture content, 1 h and 120ºC) were selected based on previous experiments (Collar and Armero, 2018), 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 placed into screw-capped cylindrical glass containers (150 mm □, 250 mm height). Small amount of distilled water was added slowly with frequent stirring until moisture levels (w/w) of the total mixture reached 15%, and equilibrated for 24 h at room temperature. Hydrated samples that occupied 13 mm height in containers were kept for 1h at 120 ºC in a convection oven (P-Selecta, Barcelona, Spain). Samples took 20 min to reach the pre-set temperature, and 30 min to reach the room temperature after heating. Untreated native flours were used as controls. Untreated (-) and HMT (+) single flours were used in quaternary blends (T:CN:CP:WT) in presence of WT- for doughmaking.

2.2.3. Bread making of wheat -based blended flours

Specific flour composition was set after a prospective study on the compositional and functional characteristics of non-wheat flours (native and HMT) was performed. Results pointed out that besides the superior nutritional value as compared to wheat, teff, chestnut and chickpea individual flours were sensitive to HMT in terms of increased water absorption, viscosity after heating-cooling cycles, increased consistency (forward-extrusion test), and acceptable dough handling ability during processing. This behaviour made flours interesting candidates to be integrated in wheat diluted systems with good prediction as dough strengtheners. Percentages of replacement resulted from experimental studies aimed at knowing maximum amount of each flour without significant deleterious effect on dough machinability.

Doughs and breads were prepared from wheat–based blended flours (T, CP, CN) by WT replacement at 34%, and incorporation of ternary blends of T (20%, flour basis), CN (7%, flour basis), and CP (7%, flour basis) flours according to a Multilevel Factorial Design with the following attributes: 3 experimental factors (T, CP and CN flours) at 2 levels, coded 0 (untreated) and 1 (HMT), and 5 error degrees of freedom. The model resulted in 8 randomized runs in 1 block. A 3 digit bread sample code was set referring to no HMT (0) and HMT (1) T (1st digit), CN (2nd digit), and CP (3rd digit) flours in sample formulation, as it follows: 110, 101,100, 000, 001, 111, 010, 011. Blended flours (100 g), water (100%, flour basis), commercial compressed yeast (3%, flour basis), salt (2%, flour basis), commercial sour dough *Pie* (5%, flour basis), CMC (3%, flour basis), and calcium propionate (0.5%, flour basis) were mixed in a 10 kg mixer at 60 revolutions min-1 for 10-13 min up to optimum dough development. Preliminary tests were performed to know the amount of water necessary to avoid stickiness and deleterious effects on dough machinability, and 100% of water absorption was enough for all the formulations to assure dough handling ability during processing. Fermented doughs were obtained after bulk fermentation (10 min at 28ºC), dividing (300 g), rounding, molding, panning and proofing up to maximum volume increment (50 min at 28ºC), and were baked at 225 ºC for 25 min to make blended breads. Two baking trials were conducted per formulation. Bread samples were packaged in co-extruded polypropylene bags, and stored for 1, 3, 6, and 8 days at room temperature and 56% relative humidity to describe firming kinetics.

2.2.4. Dough measurements

2.2.4.1. Texture Profile Analysis (TPA)

TPA of quaternary doughs were performed in absence of yeast by applying a double compression cycle in a TA-XT2 Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a 30 kg-load cell and operating at 10 mm/s head speed, by using a 1 cm diameter probe, 75 s waiting period, and 60% compression. The primary textural properties (hardness, cohesiveness, springiness and resilience) were measured in absence of dough adhesiveness by using a plastic film on the dough surface to avoid the distortion induced by the negative peak of adhesiveness, while dough adhesiveness was measured separately by running a second TPA without the plastic film and disregarding the other parameters. Runs were performed in triplicate per sample.

2.2.4.2. Stress relaxation test

Doughs without yeast were submitted to uniaxial compression in the Texture Analyser using an acrylic probe (37-mm diameter) to a 10% strain and the change in force with time was measured for 300 s. A pretest speed of 5 mm/s and test speed of 0.5 mm/s were used. The obtained stress relaxation curves were normalized and linearized according to the Peleg model: $F_0 t/(F_0-F(t))=k_1 + k_2 t$, where F_0 is the initial force, $F(t)$ is the momentary force at time (t) and k_1(s), k_2 are constants related to stress decay rate and to residual stress at the end of the experiment, respectively. In addition, percent stress relaxation (% SR= $(F_0-F_{300}).100/F_0$), $1/k_1$ (initial rate of relaxation), $1/k_2$ (extent of relaxation) and relaxation time (RT) as the time required for F_0 to drop to 36.8% of its values, respectively, were compared for the different samples. Tests were performed in duplicate per sample.

2.2.4.3. Thermal analysis

The thermal properties of native and HMT flours were determined by using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT). Dough samples were prepared by mixing flour blends with excess water (1:3) to avoid partial gelatinization of samples due to water restrictions.

For DSC analysis, 50–70 mg of dough samples were weighed in large volume pre-weighed, sealed stainless-steel pans. An empty pan was used as a reference. Simulation of the temperature profile in the center of the bread crumb during baking was performed in the calorimeter under the following scanning conditions: samples were kept at 30°C for 2 min, then heated from 30 to 110°C at a rate of 11.7°C/min, kept at 110°C for 5 min, and finally cooled from 110 to 30°C at a rate of 50°C/min. Thermal transitions of samples for gelatinization were characterised by T_o (onset temperature), T_p (peak temperature), T_e (end temperature), and ΔH_g (enthalpy of gelatinization). The enthalpy calculations were based on a dry-flour weight. The samples were analyzed three times, and the data were calculated with a Pyris software (Perkin-Elmer, Norwarlk, CT).

2.2.5. Bread measurements

2.2.5.1. Physico-chemical determinations

Loaf volume was determined using the rapeseed displacement method as in AACC (2005). Specific loaf volume was calculated dividing the loaf volume by the corresponding loaf weight.

Colour determinations were carried out on bread crumbs using a Photoshop system according to the method previously described by Angioloni & Collar (2009), 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-, and WI -whiteness index-. All measurements were made in triplicate.

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 40 mm × 40 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. In addition, area distribution and cell number distribution were counted, and percentage of cell area and cell number were calculated according to pre-set cell size ranges: <1mm^2, 1-5mm^2, 5-50mm^2, >50mm^2.

Bread mechanical characteristics (TPA in a double compression cycle) of fresh and stored breads were recorded in a TA-XT2 Texture Analyser using a 10 mm diameter probe, a 30 kg load cell, 50% penetration depth and a 30 s gap between compressions on slices of 15 mm width (Armero & Collar, 1998). For textural measurements, three slices of two breads were used for each sample. The obtained firming curves during bread storage were modelled using the Avrami equation, and model factors were estimated by fitting experimental data of hardness 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.

The stress relaxation data were collected by applying an instantaneous strain to the sample and the force required to maintain the formed deformation was observed as a function of time. Samples from the centre of the crumb slices were cut into cylinders (27 mm diameter x 15 mm thick) and were compressed using a TA-XT2 Texture Analyser with a load cell of 30 kg. For compression, a cylindrical upper die of 25 mm diameter was used at a cross speed of 0.5 mm/s and a pretest speed of 5 mm/s. Samples were placed in a semi-close cabinet to prevent moisture loss. The strain used was 20% and the whole relaxation experiment lasted for 10 min. The obtained stress relaxation curves were normalized and linearized according to Peleg model as described for doughs, and previously applied by Angioloni and Collar (2009) for breads. In addition, percent of stress relaxation (% SR= $(F_0-F_{600}).100/F_0$), $1/k_1$ (initial rate of relaxation), $1/k_2$ (extent of relaxation) and relaxation time (*RT* as the time required for F_0 to drop to 60% of its values), were compared for the different bread samples. Stress relaxation tests were replicated two times.

2.2.5.2. Nutritional parameters

Bioaccessible phenol determinations were carried out by conducting an “in vitro” digestive enzymatic mild extraction that mimics the conditions in the gastrointestinal tract according to the procedure of Glahn et al. (1998) and adapted by Angioloni and Collar (2011) for breads. Calibration curve was performed using gallic acid and therefore obtained amounts of phenolics were expressed as gallic acid equivalents.

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical was used to measure the radical scavenging capacity of the bioaccessible polyphenol enzymatic extracts of bread samples according to the DPPH• method modified by Sánchez-Moreno et al. (1998) and adapted by Collar et al. (2014). Readings were taken from two replicates per sample. Plots of μmol DPPH *vs.* time (min) were drawn, and calculations were made to know the antiradical activity (AR). AR= $[([DPPH]_{INITIAL} - [DPPH]_{PLATEAU}) \times 100]/ [DPPH]_{INITIAL}$.

2.2.6. Statistical analysis

Multivariate analysis of variance of data and non-linear regression analysis were performed by using Statgraphics V.7.1 program (Bitstream, Cambridge, MN). Multiple range test (Fisher’s least significant differences, LSD) for analytical variables was applied to know the difference between each pair of means.

3. Results and discussion

3.1. Significance of HMT of non-wheat flours on the functional performance of blended doughs

Functional performance of untreated and HMT blended doughs were characterized at macroscopic and structural levels in terms of their viscoelastic, mechanical and thermal profiles (Table 2), and the significance of HMT of non-wheat flours on the functional parameters of blended matrices was determined (Table 3).

Significant differences ($p<0.05$) were found in the physico-chemical patterns of doughs from untreated and HMT blended flours (Table 2). Dough viscoelasticity parameters ranged from 0.89 N (000) to 1.73N (001, 100) for the initial force F_0, from 11.39s (110) to 19.50s (000) for k_1, and from 1.36 (110) to 1.52 (000, 111.011) for k_2, providing relaxation times *RT* that varied from 53 (110) to 367s (000), and extent of relaxation *SR* ranging from 63% (000) to 71% (110). HMT of single flours in blended doughs, particularly significant for T and CN, provided a fall in the elastic-like nature of doughs. This led to a concomitant promotion of the viscous nature of doughs evidenced by the decreased values of both k_1 and k_2. As a consequence, increased values for the reciprocal $1/k_1$ and $1/k_2$ related to the initial stress decay rate and the asymptotic level of stress not relaxed at long times, respectively were obtained. In accordance, effects of HMT led to shorter *RT* and higher extent of *SR*, particularly for T, which values changed from 181s to 101s (*RT*) and from 66% to 68% (*SR*), respectively (Table 3). In the current work, simultaneous presence of thermally treated T+ and CP+ magnified the above mentioned changes, while treating the pair CN+ and CP+ flours reduced the extent of dough weakening leading to values for dough viscoelasticity near those of untreated flours (Table 3). Observations are in agreement with previous changes of the rheological properties at small and large deformations described for doughs made of heat-treated wheat flour (Mann et al., 2013). Changes comprise dough reinforcement by increase of the dynamic moduli, an easier destruction of the dough network by increase of protein solubility, and an unequal change in loss and storage moduli, leading to irreversible changes in flour. These changes can be ascribed assuming protein aggregation (leading to a weakened protein network and possibly acting as additional filler particles) and starch surface modifications (leading to changed starch-protein and starch-starch) interactions (Mann et al., 2013). Later, it was observed that HMT favoured the strengthening of arrowroot starch gels determined by oscillatory rheological tets, and gave them greater resistance, particularly to acidification stress (Pepe, Moraes, Albano, Telis & Franco, 2015).

Dough mechanical profile of blended flours underwent little changes with HMT in terms of cohesiveness (0.788-0.858), springiness (0.822-0.884) and resilience (0.095-0.108) values (Table 2). On the contrary, hardness and adhesiveness depended on the thermal treatment of the pairs T/CN and T/CP (Table 3). Increase of dough hardness and adhesiveness by thermal treatment of individual flours was observed earlier for treated sweet potato starch with HMT (Collado & Corke, 1999). The observed increase was counteracted after HMT of both T+ and CP+ (4.75N *vs* 6.22N) and after HMT of either T/CN (35.75N.s *vs* 42.37N.s) or T/CP (31.06N.s *vs* 43.27N.s), respectively (Table 3). It was found that modification by HMT resulted in a reduction of gel hardness of both starch and flour rice samples (Puncha-arnon & Uttapap, 2013). In the case of HMT flours, a sharp decrease in gel hardness was affected by components other than starch. Protein layers formed on the starch surface, as well as lipid complexes formed during HMT, could both inhibit the swelling of starch granules. Hamaker & Griffin (1993) earlier explained that proteins with intact disulfide bonds made the swollen granules less susceptible to breakdown, either by imparting strength to the swollen granules or by reducing the degree of swelling. The flours used in this study are rich in both protein and lipids, particularly CP (Table 1), and these components may favour the formation of protein layers and starch-lipid complexes on HMT, boosting the inhibition of starch granules swelling and the dough softening .

During gelatinization, at high water concentration (>66 wt% or water/starch ratio >1.5), a single symmetrical endothermic transition appears in a temperature range of 60–80ºC in the DSC profiles (called endotherm G), as reported by Donovan (1979). In the present research, blended dough samples contain high water concentration (75 wt%), and only one peak was defined during the DSC scan of all samples. The corresponding thermal transitions for the peak occurred at close temperature intervals (ºC) of 61.23-62.24 (T_0), 69.30-70.50 (Tp), and 78.47-80.39 (Te), and similar temperature and enthalpy (J/g, dry flour) ranges of 17.18-18.09 and 5.548-6.170, respectively (Table 2). According to the different models, the endotherm G (peak 1) was suggested to result from a) plasticization in amorphous regions b) swelling-driven crystalline disruption and/or c) melting of the less stable crystallites in sufficient water, and d) associated with the smectic–nematic/isotropic transition (Wang and Copeland, 2013). The differences in gelatinization temperatures among doughs can be attributed mainly to differences in size, form and distribution of starch granules in the blended flours, and to the internal arrangement of starch fractions within the granule, as stated earlier for legume flours (Kaur & Singh, 2005). In fact, starches in the flour blends are composed of granules differing in size, from small to large: pea (wrinkled) 5–34 µm, small wheat granules 2–3 µm (Zhou et al. 2004), large wheat granules 22–36 µm, teff 2-6 µm (Bultosa and Taylor, 2004), and chestnut 2.9-21.4 µm (Demiate, Oetterer, & Wosiacki, 2001) that exhibit irregular oval shaped granules of broad size range. In addition, despite HMT did not alter the size or shape of the starch granules of some cereals, roots and legumes (Hoover, 2010), the thermal treatment may affect the aggregation of starch granules as observed for oat starch. Starch granules that were aggregated in the native state were less compactly packed after heat treatment (Hoover & Vasanthan, 1994). Significant single effect of HMT of T flour revealed a discreet broadening of the gelatinization-temperature range (R) and a shifting of the endothermal transition towards higher temperatures (Table 3), being values 17.34ºC *vs* 17.87ºC (R), 61.54 *vs* 62.28ºC (T_0), 69.60 *vs* 70.50ºC (T_p), 78.87 *vs* 80.15ºC (T_e). Previous studies on tuber, legume, and cereal (normal, waxy, high amylose) starches (Hoover, 2010) and flours (Satmalee & Matsuki, 2011) ascribed the observations to amylose-amylose, amylose-amylopectin and amylose-lipid interactions, and as well as other chemical bonding/interactions that occur during HMT (Watcharatewinkul et al., 2009). Authors stated that interactions suppress the mobility of starch chains in the amorphous regions. Consequently, the amorphous regions would require a higher temperature to incur swelling that could contribute to the disruption of the crystalline regions. Lin et al. (2001) proposed that the increase in gelatinization temperature was caused by the transformation of the inter-crystalline amorphous regions to amorphous phases, which may provide the short chains in the crystalline structure more freedom. Thus, the crystalline micelles undergo a structural transformation towards an increased thermodynamic stability. Besides, it was probably due to the restriction of water penetration into the granules by the new superficial protein layer formed by HMT, thus retarding the granule swelling (Chen, He, Fu, & Huang, 2015).

In addition, T significantly interacted with CP, modifying in small extent the values of the gelatinization enthalpies. HMT of either T or CP resulted in a small decrease of ΔH_g compared to values observed for the untreated pair of flours (5.64 *vs* 6.12 J/g), while HMT of both flours led to intermediate enthalpy values (5.88 J/g). Enthalpy change reflects the melting of imperfect amylopectin-based crystals, with potential contributions from crystal-packing and helix melting enthalpies (Lopez-Rubio et al., 2008). The decrease in ΔH_g on HMT reflects disruption of double helices present in crystalline and non-crystalline regions of the granule (Gunaratne & Hoover, 2002). In addition, most of the semi-crystalline structure and a considerable extent of the concentrated crystalline region were destroyed during HMT.

3.2. Significance of HMT of non-wheat flours on the physical and nutritional profiles of blended breads

In general, mixed HMT breads were visibly similar in colour (Figure 1), and exhibited low-medium lightness *L* (54.8-60.4), >0 *a* (2.3-3.4) and *b* (10.3-12.3) values, resulting in low WI crumbs

(53.6-58.6) (Table 4). Colour coordinates of breads were not dependent on the thermal treatment of any compositional flour, so that all sample crumbs were characterized by a dark orange-brown colour (Figure 1).

Common crumb grain features of breads (Table 4, Figure 1) evidenced main small cells <1 mm^2 accounting for 81% - 92% of total cells, intermediate medium size (1-50 mm^2) cells (7-19%), and marginal big cell (>50 mm^2) proportion (0-0.2%). Mean cell area (mm^2) ranged from 0.43 to 0.82, and cell area distribution covered by cells sized 1-50 mm^2 varied from 63% (001) to 87% (100). Cell density (cells/cm^2) ranged from 48 (111) to 107 (100), being cell/wall ratio from 38/62 (000, 111, 011) to 50/50 (110). Except for the samples 110, 101, and 100 that exhibited heterogeneous and/or highly packed crumb structure, a fine and uniformly-sized cell structure with similar cell walls thickness were achieved in crumb samples (Figure 1). Improvement of the internal grain structure with heat treatment was reported previously for substandard wheat flour breads (Gélinas, McKinnon, Rodriguez, & Montpetit, 2001) and for sorghum breads (Marston et al., 2016). The high fibre content of chestnut flour was reported to enhance the viscoelastic properties and to reduce dough expansibility due to the entanglement of fibres, entrap more air bubbles, and produce pores with a small cell area when added with a relatively high amount of this flour (Demirkesen, Mert, Sumnu, & Sahin, 2010). Positive effects of HMT of CN and/or CP on bread crumb grain have not been described so far.

Macrostructural properties of HMT breads were characterized in terms of specific volume, mechanical/ textural behaviour, stress relaxation pattern and firming kinetics on ageing (Table 4), and the significant ($p<0.05$) single and interactive effects of thermal treatment of compositional flours were determined (Table 5). Compared to untreated blended breads (000), HMT breads exhibited in general from smaller to larger specific volumes (2.59-3.28 mL/g *vs* 3.07 mL/g), similar percent of stress relaxation (43-51%), slightly variable stress decay rate k_1 (45.67-66.58s *vs* 57.24s) and residual stress k_2 (1.86-2.19 *vs* 1.98), similar or higher cohesiveness (0.460-0.568 *vs* 0.485) and resilience (0.186-

0.245 *vs* 0.200), variable fresh crumb firmness (7.89-10.58N *vs* 9.44N) and rate of staling kinetics n_f (0.390-1.817 *vs* 1.097) (Table 4). HMT of single flours T, CN or CP significantly ($p<0.05$) affected bread physical characteristics, being the trend and extent of the changes dependent on the treated flour. CN+ and CP+ increased bread specific volume by 4% and 9%, respectively, while T+ led to loaves with 8% lower specific volume, compared to untreated flours. Effects of CN+ and CP+ on bread viscoelasticity were of similar extent but followed an opposite trend. CP+ decreased the rate of initial stress decay rate $1/k_1$ by 11% with a concomitant increase in the relaxation time of 33%, while CN+ promoted the decay rate by 12% (Table 5). HMT of T provided a fall in the elastic-like nature of doughs, leading to increased initial stress decay rate and level of stress not relaxed at long times. This encompasses higher extent of stress relaxation, and dough weakening. Resulting doughs encompassed lower viscoelasticity and gas retention ability, and reduced specific volume after fermentation and baking. Although legume and chestnut proteins, are generally low in methionine, cysteine and tryptophan, aggregation of proteins can take place on HMT, leading to a reinforced dough structure in some extent, able to retain carbon dioxide and leading to increased specific volume, as previously observed for sorghum breads (Marston et al., 2016). Entanglement/disaggregation of fibres, particularly present in high amount in chickpea flour (Table 1), in the denatured protein network can also play a role. Impact of HMT flours on firming kinetics parameters were relevant (Figure 2). Both initial T_0 and final T_∞ bread firmness were lowered (-7.5%) or promoted (+17-23%) by the respective addition of CP+ or T+ to the formulation. In addition, the rate of ageing n_f was drastically reduced (-26 %) or increased (+78%) by either CP+ or CN+, and k_f decreased by 72% with T+ (Table 5). Simultaneous presence of native and treated flours provided variable impact on the physical profile of blended breads, particularly for the pairs T/CN and T/CP. 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 (Table 5) 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 colour features (Figure 1). Caution should be applied to the pair T+/CP+ because of the adverse effect on the firming of the initial texture of the crumb T_0, and on the rate of staling denoted by increased n_f. It is the case of samples 101 and 111. In fact, comparing staling kinetics parameters (averaged values) in samples T-CP+ *vs* T+CP+ (Table 5), values were 7.97N *vs* 10.84N (T_0), and 0.405 *vs* 1.269 (n_f). Experimental values (T_0, n_f) for bread samples 001 (7.89N, 0.420) and 011 (8.00N, 0.390) *vs* 101 (10.54N, 0.758) and 111 (11.25N, 1.775) are in good accordance with the statistical trends (Figure 2, Table 5).

Blended bread samples (100 g) contain 6,71g of dietary fibre DF, while regular white breads account for 1,67g DF, so that blended breads can be labelled as high-fibre breads (6 g DF/100 g food), according to Nutritional Claims for DF foods (Regulation EC, 2006). Formulations based on WT:T:CN:CP flours, 66.20:7:7 fulfilled from 44% (men) to 67% (women) of dietary fibre daily requirements (Otten, Hellwig, & Meyers, 2006), when a daily consumption of 250 g of bread is accomplished, following the WHO bread intake recommendation.

Chestnut flour is an important source of antioxidant compounds, mainly containing ellagic acid (Dall'Asta et al., 2013). Teff is generally assumed to contain substantial amounts of phenolics (Dykes and Rooney 2007), particularly that ferulic acid, and chickpeas exhibited total polyphenol content ranging from 0.78 to 2.3 mg/ g-1 (Bravo, 1998). Bioaccessible polyphenol content (mg gallic acid/100 g flour, as is) of blended breads in the current work varied from 417 mg to 494 mg (Figure 3). Amount of bioaccessible polyphenols is dependent on several factors. Mechanical input during mixing and thermal treatment of flours (HMT) and doughs (baking) may induce depolymerization of constituents, mainly fibre, and hence may favour bread accessibility to solvents, acids and enzymes and the subsequent release and extraction of fibre-associated polyphenols. Concomitantly, dietary fibre and other compounds of proven resistance to the action of digestive enzymes, such as resistant starch, resistant protein, Maillard compounds and other associated compounds, may reduce the bread phenol bioaccessibility (Saura-Calixto et al., 2000). Extensive depolymerization of fibre constituents can be applied to the increase of bioaccessible polyphenols determined in HMT blended breads compared to the untreated sample 000, since compositional non-wheat flours flours accounted for >10% dietary fibre content (Table 1). Despite HMT may favour the formation of polymeric aggregates that hinder enzyme accessibility and further attack (Mann et al., 2013; Watcharatewinkul et al., 2009), it is apparent that HMT makes bioaccessible polyphenol extraction available, in some cases. In fact, T+ decreased the amount of bioaccessible polyphenols by 4%, but the pair T/CN modulated polyphenol extractability (Table 5). T-/CN- and T+/CN+ gave similar amount of extracted polyphenols at about 425 mg, while T+/CN- led to intermediate amount of 456 mg, and the pair T-CN+ maximized extracted bioaccessible polyphenols (491 mg). In accordance, samples 010 and 011 exhibited the highest amount of bioaccessible polyphenols (≈490 mg) while samples 110, 111, 000, and 001 explicited the lowest values (420-450 mg), and samples 101 and 100 showed intermediate mean values (450-460 mg) (Figure 3).

Anti-radical activity was determined by the extent of the reduction of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical. Results expressed correspond to the remaining unreacted DPPH• amount when 0.494 µmols of the free radical are initially available to react with pepsin/ pancreatin extracts from 2.4-2.6mg freeze-dried breads. Despite different kinetics of the reduction of DPPH• were shown among untreated and treated samples (Figure 4), discreet differences in anti-radical activity of breads (38-45%) were observed. T+ and CN+ respectively induced a depletion (-7%) and an increase (+3%) of the anti-radical activity, while the simultaneous presence of the pair CN+/CP+ synergistically promoted the reduction of DPPH• (Table 5). In good accordance samples 011, 010 and 111 gave the higher values and the more rapid kinetics in terms of anti-radical activity (Figure 4).

3.3. Correlations between dough physical parameters and bread physical and nutritional parameters of blended matrices

Using Pearson correlation analysis, a range of correlation coefficients (r) (from -0.4760 to 0.7352) were obtained for the relationships between viscoelastic, mechanical and starch gelatinization transition parameters of doughs, and physical and nutritional parameters of mixed breads from untreated and HMT matrices (Table 6). Despite r values were discreet in most correlations, significant ($0.01<p<0.05$) interdependences between the extent of stress relaxation ($1/k_2$), starch gelatinization transition (T_p, T_e, ΔH_g) of doughs, and physical (specific volume, n_f) and nutritional (%ARA) parameters of breads were found (Table 6). In accordance, larger specific volume in breads exhibiting higher anti-radical activity corresponded to higher elastic-like doughs with smaller initial decay rate $1/k_1$ and lower extent of stress relaxation $1/k_2$, %SR. Clumping of starch granules and aggregation of denatured protein on HMT were reported (Chen et al., 2015). Crosslinking by oxidizing the free sulfhydryl groups on HMT result in stronger doughs with a greater resistance to mechanical shock, improved oven spring, and larger loaf volume, as described for sorghum breads (Marston et al., 2016). In addition, lower temperatures for starch gelatinization T_0, T_p, T_e in hydrated flours led to breads with higher specific volume (r -0.6132, -0.6293, -0.6299), while smaller energy for the thermal transition ΔH_g was connected with softer fresh breads (r 0.5696) and slower crumb firming rate on ageing (r 0.718). Modifications of starch surface interactions take place on HMT (Mann et al., 2013). Most of the semi-crystalline structure and a considerable extent of the concentrated crystalline region were destroyed during the thermal treatment (Gunaratne & Hoover, 2002) leading to less compact and rigid structures (Hoover & Vasanthan, 1994). Resulting doughs are able to expand during fermentation, and provide breads with more aerated structure and softer crumb, undergoing slower staling kinetics.

Conclusions

HMT of flours modified viscoelasticity and thermal transitions of doughs and techno-functional and nutritional profiles of breads from diluted breadmaking wheat matrices made at 34% of wheat flour replacement by teff (20%), chestnut (7%) and chickpea (7%) flours. The trend and extent of the changes are mainly dependent on the simultaneous presence of specific untreated and thermally treated flours, particularly for the pairs T/CN and T/CP. 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+, Resulting breads showed all homogeneous and fine crumb grain and appealing colour features. The pair T-CN+ maximized extracted bioaccessible polyphenols, and the pair CN+CP+ synergistically promoted the anti-radical activity in breads. Caution should be applied to the pair T+CP+ because of the adverse effect on the firming of the fresh crumb and on the rate of staling.

HMT of associated non-wheat flours appears as a clean label simple strategy to create added value to breads from highly diluted wheat flour matrices, provided single and interactive effects of the thermal treatment of blended flours on the structural features of breads are known.

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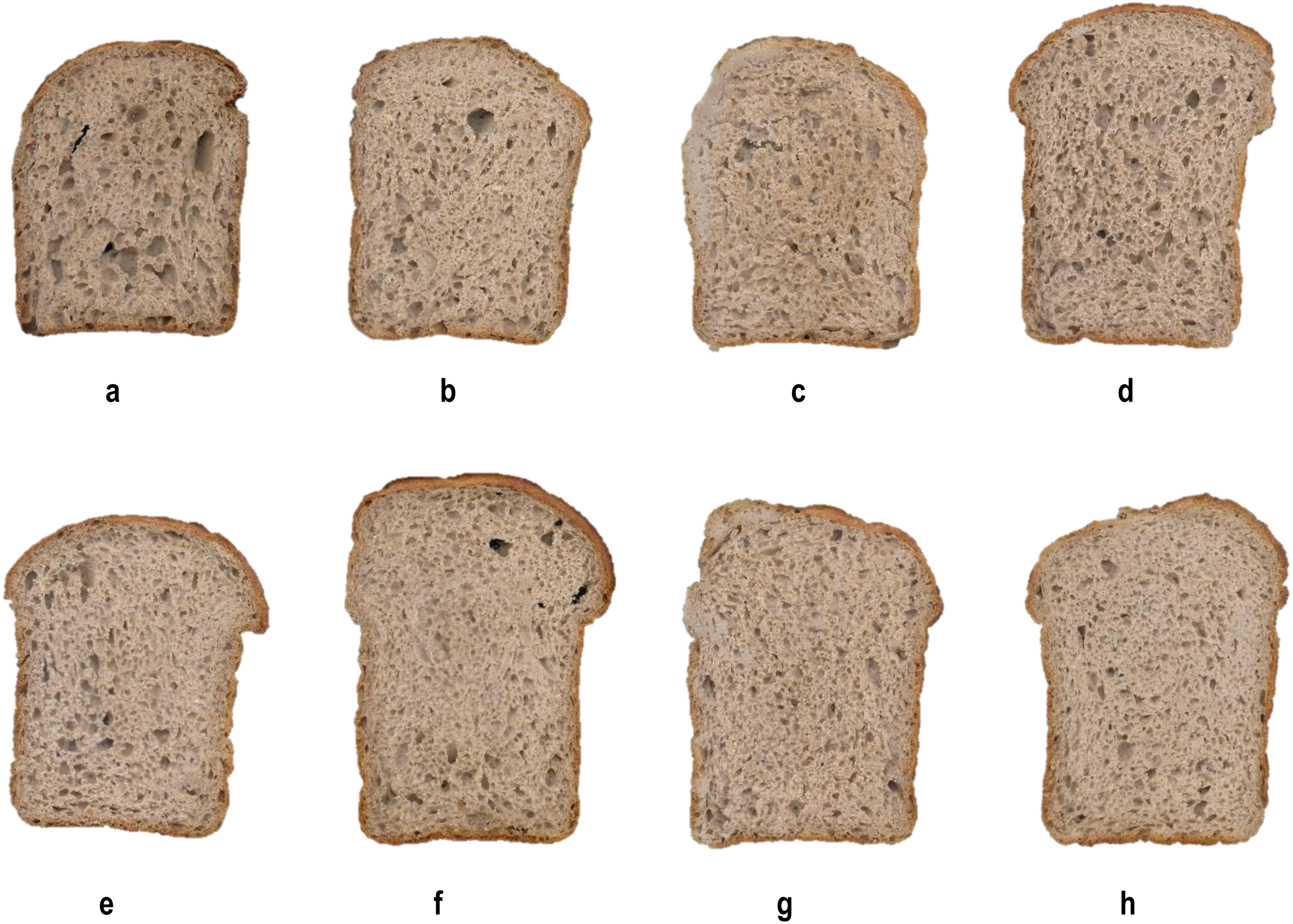

Figure 1.- Digitalized images of central slices of wheat-based blended breads formulated with teff (T), chestnut (CN), and chickpea (CP) flours. Three digit code refers to untreated (0) and heat-moisture treated (1) T:CN:CP mixed flours replacing wheat flour in sample formulation. a: 110, b: 101, c: 100, d: 000, e: 001, f: 111, g: 010, h: 011.

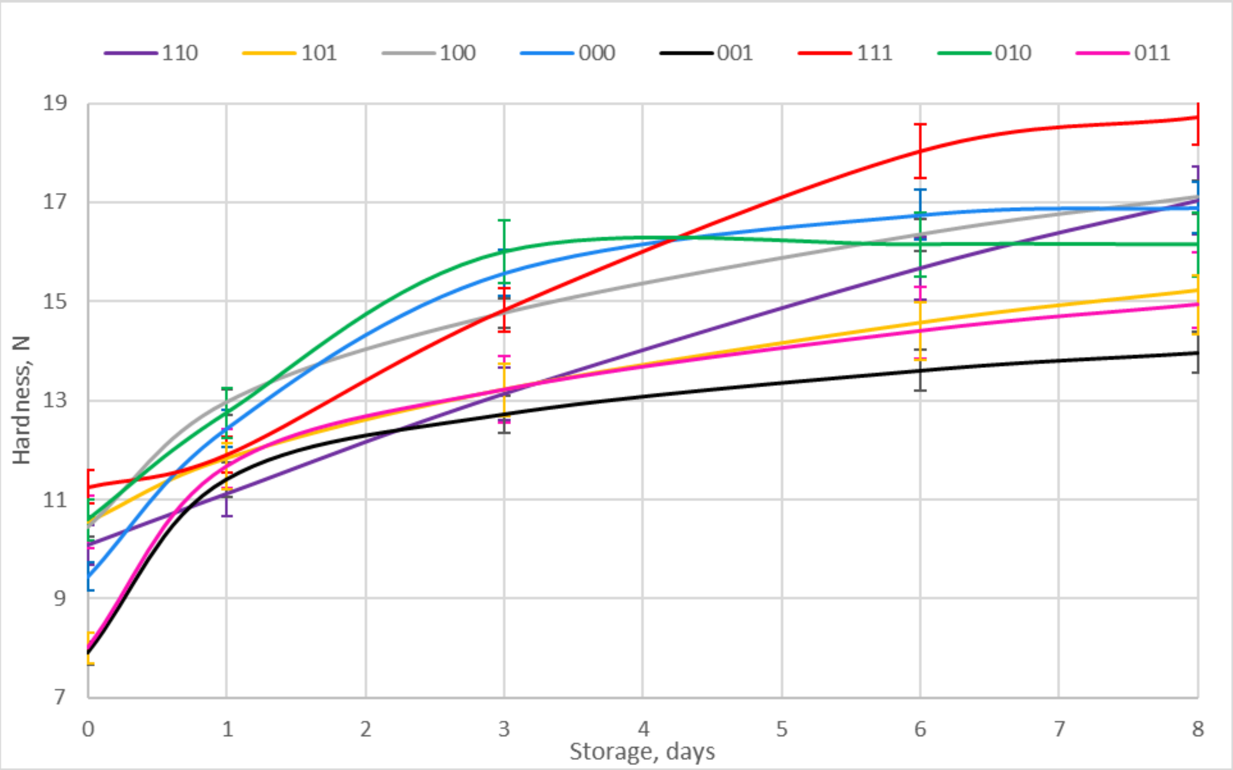

Figure 2.- Evolution of crumb firming during ageing (adjusted mean data and error bars) of wheat-based blended breads formulated with teff (T), chestnut (CN), and chickpea (CP) flours. Three digit code refers to untreated (0) and heat-moisture treated (1) T:CN:CP flours replacing wheat flour in sample formulation.

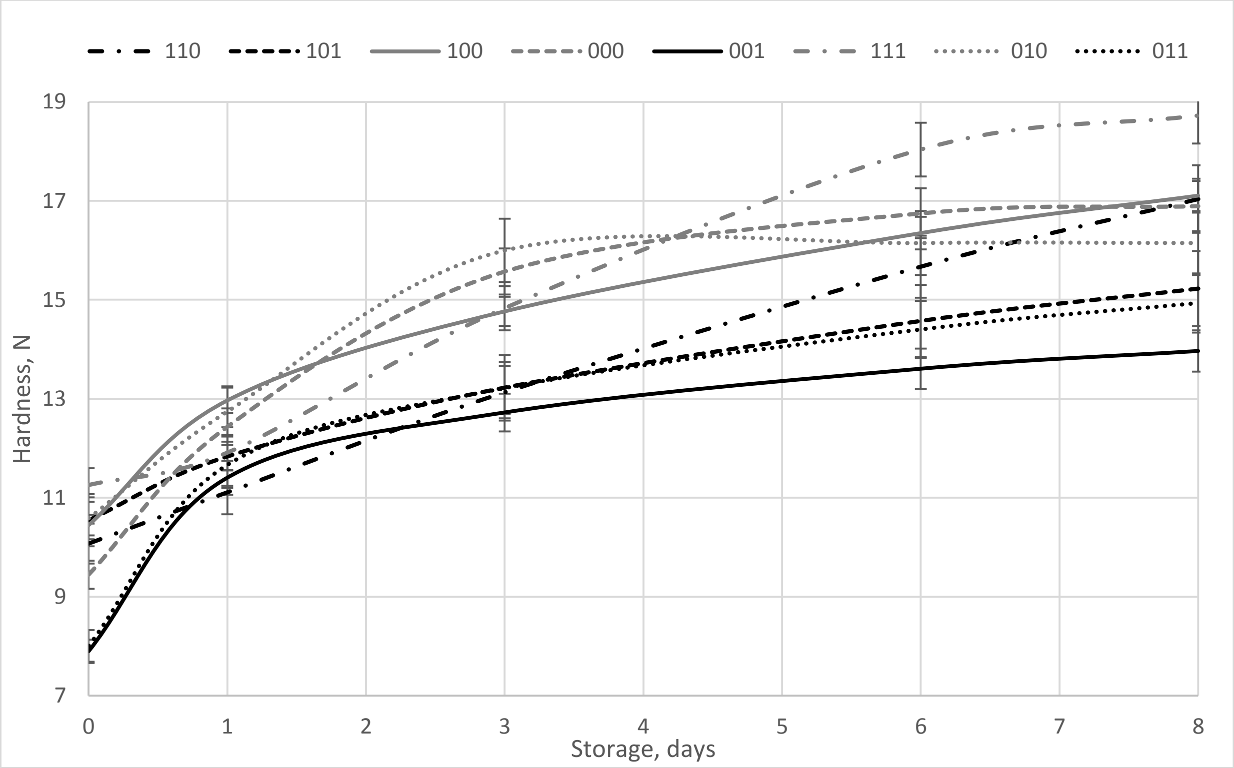

Figure 2.- Evolution of crumb firming during ageing (adjusted mean data and error bars) of wheat-based blended breads formulated with teff (T), chestnut (CN), and chickpea (CP) flours. Three digit code refers to untreated (0) and heat-moisture treated (1) T:CN:CP flours replacing wheat flour in sample formulation.

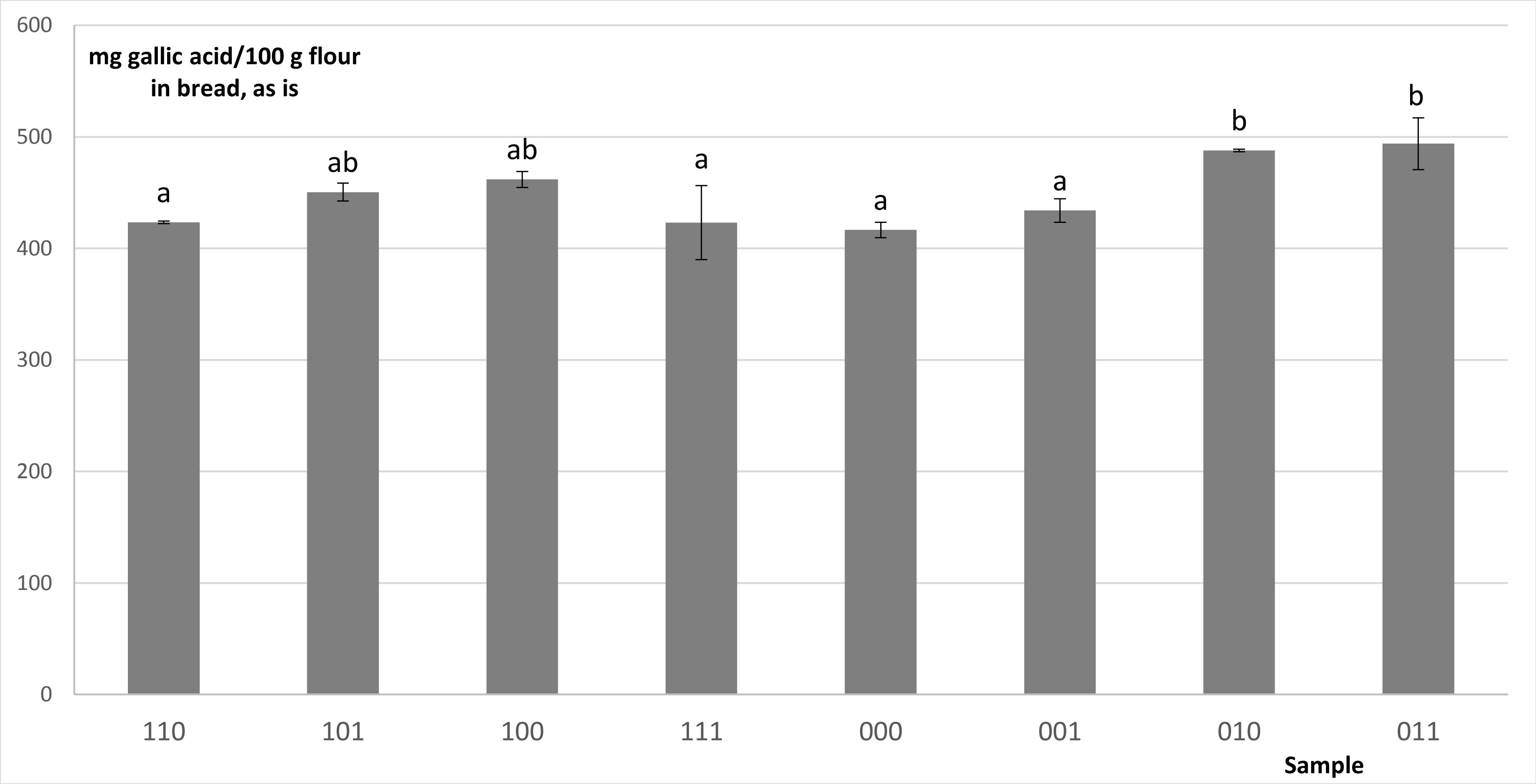

Figure 3.- Bioaccessible polyphenol content of wheat-based blended breads formulated with teff (T), chestnut (CN), and chickpea (CP) flours. Three digit code refers to untreated (0) and heat-moisture treated (1) T:CN:CP flours replacing wheat flour in sample formulation.

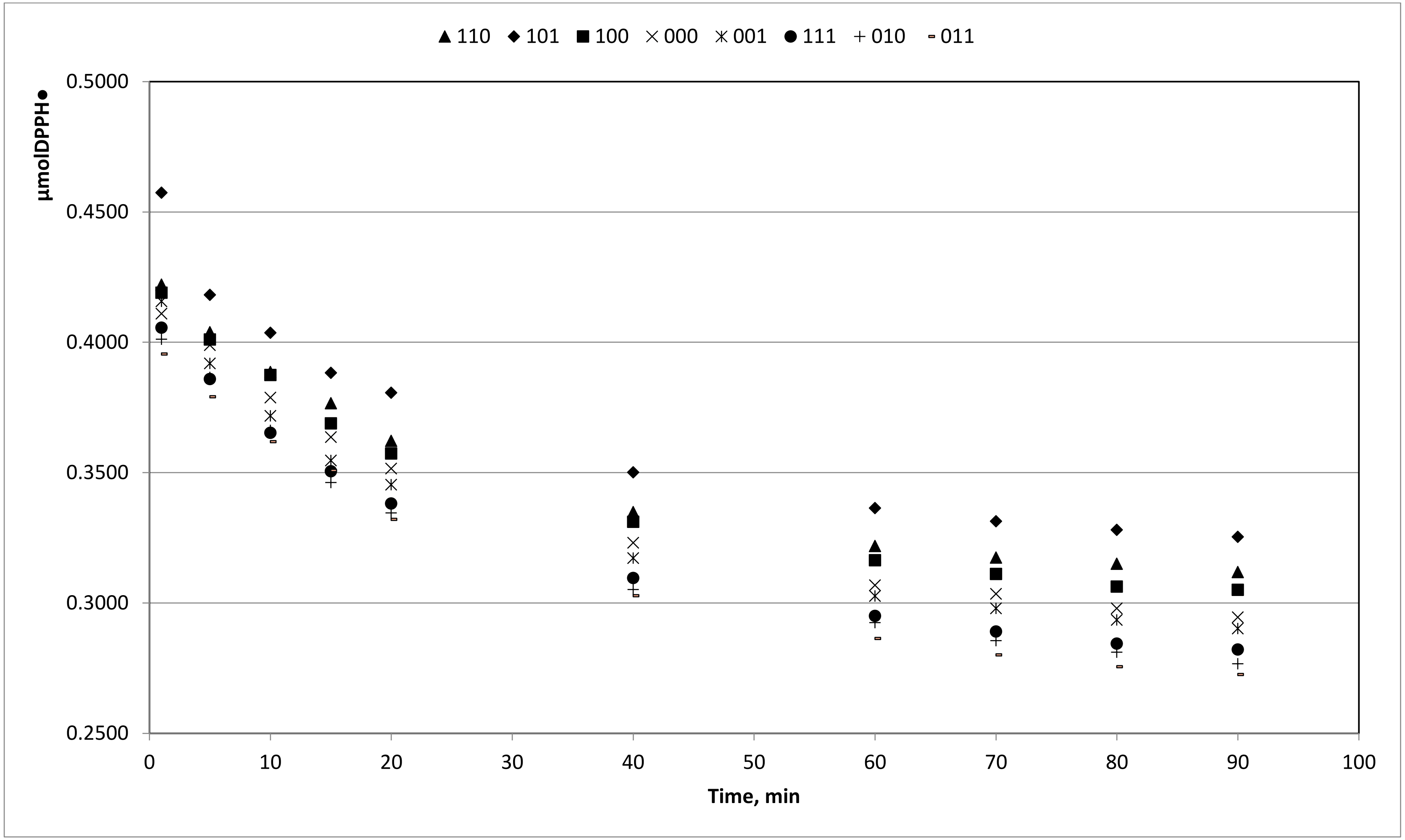

Figure 4.- Kinetics of consumption of 0.494 µmols DPPH by 2.8 mg freeze-dried wheat-based blended breads formulated with teff (T), chestnut (CN) and chickpea (CP) flours. Three digit code refers to untreated (0) and heat-moisture treated (1) T:CN:CP flours replacing wheat flour in sample formulation.

Table 1.- Chemical and nutritional composition[a] of flours

Parameter (g/ 100 g flour, d. b.)	Teff	Chestnut	Chickpea	Wheat
Moisture	12.62±0.13^{c}	6.90±0.05^{a}	11.88±0.09^{b}	14.30±0.12^{d}
Protein	14.08±0.46^{b}	6.44±0.20^{a}	18.82±0.60^{c}	14.12±0.28^{b}
Fat	4.69±0.29^{c}	4.08±0.18^{b}	6.96±0.12^{d}	1.56±0.11^{a}
Digestible carbohydrates	68.7±4.5^{b}	79.5±3.2^{c}	48.8±1.1^{a}	81.7±2.3^{c}
Total Dietary Fibre	12.31±1.42^{c}	9.67±1.03^{b}	25.13±2.33^{d}	2.56±0.23^{a}
Soluble Dietary Fibre	4.84±0.55^{b}	-	6.55±0.42^{c}	1.06±0.11^{a}
Insoluble Dietary Fibre	7.46±1.10^{b}	-	18.62±1.40^{c}	1.49±0.28^{a}

[a] Mean values ± standard deviation. Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other (p> 0.05).

Table 2.- Physicochemical parameters of blended doughs.

Parameter	Dough sample[a, b]							
	110	101	100	000	001	111	010	011
Viscoelasticity								
F_0, N	1.16±0.02b	1.03±0.02a	1.73±0.04d	0.89±0.04a	1.68±0.07d	1.25±0.08b	1.49±0.07c	1.14±0.05b
k_1, s	11.39±0.43a	13.81±0.13b	16.58±0.30e	19.50±0.35f	14.56±0.30c	15.64±0.25d	15.42±0.30cd	15.05±0.04c
k_2	1.36±0.01a	1.41±0.01b	1.44±0.02c	1.52±0.02d	1.42±0.01b	1.47±0.02d	1.44±0.02c	1.48±0.01d
$1/k_1$, s^{-1}	0.087±0.006f	0.072±0.002e	0.060±0.001b	0.051±0.001a	0.068±0.001e	0.063±0.003bc	0.064±0.004cd	0.066±0.003cd
$1/k_2$	0.731±0.001e	0.706±0.002d	0.690±0.001c	0.653±0.011a	0.702±0.005d	0.678±0.006ab	0.691±0.008bc	0.674±0.003ab
F_{RT} (36,8%F_0), N	0.43±0.01b	0.38±0.01a	0.64±0.02d	0.33±0.02a	0.62±0.03d	0.46±0.02b	0.55±0.03c	0.42±0.02b
RT, s	53±2a	83±2b	123±4cd	367±23f	91±3b	144±5de	114±7c	151±8e
%SR	71±2d	68±1c	67±0abc	63±1a	68±2cd	66±2bc	67±2bc	65±0ab
Texture								
Hardness, N	5.88±0.41ab	5.64±0.55ab	6.45±1.54b	4.38±0.58ab	6.30±1.22b	3.85±0.21a	5.91±1.11ab	6.14±1.05b
Cohesiveness	0.788±0.045a	0.79±0.051a	0.791±0.083a	0.854±0.002a	0.811±0.061a	0.858±0.001a	0.797±0.068a	0.800±0.053a
Springiness	0.884±0.098h	0.868±0.076f	0.847±0.096e	0.843±0.063c	0.844±0.094d	0.822±0.076a	0.831±0.085b	0.879±0.096g
Resilience	0.108±0.008ab	0.099±0.013a	0.095±0.005a	0.118±0.011b	0.101±0.002ab	0.101±0.005ab	0.103±0.006ab	0.103±0.005ab
Adhesiveness, N.s	39.19±1.00bc	37.87±2.99bc	46.88±9.46c	26.25±1.51ab	45.25±7.68c	24.26±0.99a	43.24±7.50c	41.30±6.98c
Gelatinization								
T_o, ºC	62.24±0.67ab	62.30±0.47b	61.93±0.16ab	61.64±0.02ab	61.77±0.21ab	61.42±0.13ab	61.50±0.16ab	61.23±0.88a
T_p, ºC	70.50±0.71b	70.50±0.71b	70.10±0.14ab	69.80±0.00ab	69.70±0.14ab	69.50±0.14ab	69.60±0.28ab	69.30±0.99a
T_e, ºC	80.31±1.11b	80.39±1.06ab	79.60±0.49ab	79.15±0.10ab	78.95±0.14ab	78.70±0.04ab	78.93±0.40ab	78.47±1.61a
ΔH_g, J/g	5.548±0.064a	5.643±0.004a	5.709±0.117ab	6.071±0.181c	5.694±0.193a	5.996±0.063bc	6.17±0.139c	5.587±0.132a
R, ºC	18.07±0.44ab	18.09±0.59b	17.68±0.32ab	17.51±0.12ab	17.18±0.07a	17.29±0.09ab	17.43±0.23ab	17.24±0.73ab

[a] Mean values ± standard deviation. Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$). ([b]) Three digit bread sample code refers to untreated (0) and HMT (1) teff: chestnut: chickpea flours in sample formulation.

F_0 is the initial force, k_1(s), k_2 are constants related to stress decay rate and to residual stress at the end of the experiment, respectively, RT as the time required for F_0 to drop to 36.8% to its values, F_{RT}, as the force corresponding to RT, % SR= $((F_0-F_{300})/F_0)100$. T_o: onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization entalpy, R gelatinization temperature range

Table 3.- Significant ($p<0.05$) single effects and 2nd order interactions of HMT compositional flours on dough physical properties.

Single effects										Second order interactions						
Parameter		Level	Overall mean	Teff	*	Chestnut	*	Chickpea	*	Level	Teff x Chestnut	*	Teff x Chickpea	*	Chestnut x Chickpea	*
Stress Relaxation																
k_1	s	0	15.25	16.14	b	16.12	b	15.72	b	00	ns		17.46	c	18,04	d
		1		14.36	a	14.38	a	14.77	a	01			14.81	b	14,19	b
										10			13.98	a	13,41	a
										11			14.73	b	15,35	c
k_2		0	1.45	1.47	b	1.45	b	ns		00	ns		1.49	d	1,49	d
		1		1.43	a	1.44	a			01			1.45	c	1,42	b
										10			1.41	a	1,41	a
										11			1.44	b	1,48	c
$1/k_1$	s-1	0	0.067	0.063	a	0.063	a	ns		00	ns		0.058	a	0,056	a
		1		0.071	b	0.071	b			01			0.068	b	0,071	c
										10			0.074	d	0,076	d
										11			0.068	c	0,065	b
$1/k_2$		0	0.692	0.681	a	0.689	a	0.692	b	00	0.678	a	0.673	a	0,672	a
		1		0.702	b	0.694	b	0.691	a	01	0.683	b	0.689	b	0,705	b
										10	0.699	c	0.711	d	0,711	c
										11	0.705	d	0.693	c	0,677	a
RT	s	0	141	181	b	166	b	164	b	00	229	b	241	c	245	c
		1		101	a	115	a	117	a	01	133	a	121	b	87	a
										10	103	a	88	a	83	a
										11	98	a	113	a	147	b
%SR		0	67	66	a	66	a	ns		00	ns		65	a	65	a
		1		68	b	67	b			01			67	b	68	b
										10			69	c	69	b
										11			67	b	65	a
Texture Profile Analysis																
Hardness	N	0	5.57	ns		ns		ns		00	ns		5.14	ab	ns	
		1								01			6.22	b		
										10			6.17	ab		
										11			4.75	a		
Adhesiveness	N.s	0	38.03	ns		ns		ns		00	35.75	ab	34.74	ab	ns	
		1								01	42.27	b	43.27	b		
										10	42.37	b	43.03	b		
										11	31.72	a	31.06	a		
Gelatinization																
T_o	ºC	0	61.91	61.54	a	ns		ns			ns		ns		ns	
		1		62.28	b											
T_p	ºC	0	70.05	69.6	a	ns		ns			ns		ns		ns	
		1		70.5	b											
T_e	ºC	0	79.51	78.87	a	ns		ns			ns		ns		ns	
		1		80.15	b											
ΔH_g	J/g	0	5.82	ns		ns		ns		00	ns		6.12	b	ns	
		1								01			5.64	a		
										10			5.63	a		
										11			5.88	ab		
R	ºC	0	17.6	17.34	a	ns		ns			ns		ns		ns	
		1		17.87	b											

k_1(s), k_2 are constants related to stress decay rate and to residual stress at the end of the experiment, respectively, RT as the time required for F_0 to drop to 36.8% to its values, % SR= ((F_0-F_{300}) /F_0)100. T_o: onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization enthalpy, R gelatinization temperature range. Level 0: untreated, level 1: HMT.

Table 4.- Physical profile of wheat-based blended breads formulated with untreated and HMT teff (T), chestnut (CN) and chickpea (CP) flours.

Parameter	Bread sample[a]							
	110	101	100	000	001	111	010	011
Volume								
Specific volume, mL/g	2.59±0.09a	2.72±0.09a	2.67±0.06a	3.07±0.01c	3.01±0.08bc	3.28±0.01d	2.89±0.05b	3.18±0.11cd
Stress relaxation								
F_0, *N*	8.01±0.10b	8.62±0.22c	8.46±0.31c	7.92±0.24b	6.99±0.18a	10.72±0.29d	11.31±0.34e	7.07±0.23a
k_1, *s*	56.70±1.24c	63.04±1.89d	51.94±2.03b	57.24±1.32c	66.58±2.56e	50.97±1.95b	45.67±0.97a	55.64±0.99c
k_2	2.06±0.03c	1.98±0.09abc	2.01±0.06ab	1.98±0.11bc	2.19±0.08d	1.96±0.06abc	1.86±0.05a	2.04±0.07bc
F_{RT} (60%F_0), N	4.81±0.09	5.17±0.16	5.08±0.21	4.75±0.42	4.20±0.32	6.43±0.53	6.79±0.78	4.24±0.21
RT, s	132±5f	121±3e	107±4c	111±9d	220±12g	96±8b	72±7a	123±14e
%SR	46±5a	48±3a	48±3a	48±5a	43±2a	49±6a	51±4a	47±2a
Mechanical properties								
Cohesiveness	0.568±0.058c	0.460±0.021a	0.484±0.012a	0.485±0.027a	0.532±0.037bc	0.481±0.012a	0.495±0.027ab	0.501±0.019ab
Springiness	0.853±0.080a	0.875±0.050a	0.898±0.052a	0.879±0.052a	0.899±0.075a	0.895±0.032a	0.906±0.026a	0.872±0.055a
Chewiness, N	4.81±0.95ab	4.21±0.80a	4.55±0.62a	4.03±0.48a	5.83±1.03bc	4.86±0.45ab	6.53±0.22c	4.58±0.86a
Resilience	0.245±0.004b	0.186±0.019a	0.202±0.007a	0.200±0.022a	0.231±0.027b	0.196±0.004a	0.197±0.013a	0.207±0.015a
Firming kinetics								
T_∞, N	23.30e	19.16c	25.30f	16.94b	16.17a	18.93c	16.15a	22.11d
k_f	0.0814a	0.161c	0.185d	0.508g	0.551h	0.089b	0.490f	0.300e
n_f	1.065e	0.758d	0.559c	1.097f	0.420b	1.775g	1.817h	0.390a
T_0, N	10.07c	10.54d	10.44cd	9.44b	7.89a	11.25e	10.58de	8.00a
Crumb grain								
Mean cell area, mm^2	0.59b	0.45a	0.46a	0.43a	0.74c	0.82d	0.77c	0.47a
Cell area distribution, %								
<1mm^2	13a	17b	13a	15ab	12a	16b	13a	18b
1-5mm^2	20a	36c	29b	30b	28b	41c	34c	37c
5-50mm^2	47b	47b	58c	45b	35a	43b	46b	45b
>50mm^2	20c	0a	0a	10b	25c	0a	7b	0a
Cell number distribution, %								
<1mm2	92b	91b	92b	92b	88ab	81a	85a	89ab
1-5mm2	5a	7a	6a	6a	9a	16b	12b	9a
5-50mm2	2a	2a	2a	2a	3a	3a	3a	2a
>50mm2	0.1a	0a	0a	0.1a	0.2a	0a	0.1a	0a
Cell density, cells/cm^2	86c	91c	107d	89c	56b	48a	60b	84c
Cell to total area ratio	50b	41a	49b	38a	41a	39a	46b	39a
Wall to total area ratio	50a	59b	51a	62b	59b	61b	54a	61b
Colour								
L	54.8±a	59.2±e	56.1±b	57.4±c	58.9±de	60.4±f	58.2±d	60.1±f
a	2.4±0.1a	2.8±0.1b	3.4±0.2d	2.5±0.1a	2.5±0.1a	2.5±0.1a	3.0±0.1c	2.3±a
b	10.3±0.3a	11.5±0.1d	12.3±0.1e	10.6±0.1ab	10.4±0.2a	10.7±0.1ab	11.0±0.1c	10.7±ab
Whiteness Index	53.6	57.5	54.3	56.0	57.5	58.9	56.7	58.6

[a] Three digit code refers to untreated (0) and heat-moisture treated (1) T:CN:CP blended breads.

F_0 is the initial force, k_1(s), k_2 are constants related to stress decay rate and to residual stress at the end of the experiment, respectively, RT as the time required for F_0 to drop to 60% to its values, F_{RT}, as the force corresponding to RT, % SR= ((F_0-F_{600}) /F_0)100. k_f constant of proportion of firming kinetics, n_f Avrami exponent of firming kinetics, T_∞, T_0 crumb firmness at ∞ and 0 time.

Table 5.- Significant (p<0.05) single effects and 2nd order interactions of HMT compositional flours on the physical and nutritional profiles of wheat-based blended breads formulated with untreated (0) and HMT (1) teff (T), chestnut (CN) and chickpea (CP) flours.

		Single effects								2nd order interactions						
Parameter	Unit	Overall mean	Level	Teff	*	Chestnut	*	Chickpea	*	Level	Teff x Chestnut	*	Teff x Chickpea	*	Chestnut x Chickpea	*
Volume																
SV	mL/g	2.92	0	3.04	b	2.87	a	2.8	a	00	3.04	b	2.98	b	2.87	b
			1	2.81	a	2.98	b	3.05	b	01	3.03	b	3.10	b	2.87	b
										10	2.69	a	2.63	a	2.74	a
										11	2.94	b	3.00	b	3.23	c
Viscoelastic																
F_0, N		8.59	0	ns		7.97	a	ns		00	ns		9.51	b	ns	
			1			9.21	b			01			7.00	a		
										10			8.24	ab		
										11			9.61	b		
k_1	s-1	55.91	0	ns		59.70	b	52.73	a	00	62.45	b	51.15	a	54.25	a
			1			52.13	a	59.10	b	01	50.65	a	61.95	b	65.15	b
										10	56.95	ab	54.30	a	51.20	a
										11	53.60	a	56.25	ab	53.05	a
RT		122	0	131	b	140	b	105	a	00	165	c	91	a	109	b
			1	114	a	105	a	140	b	01	97	a	171	d	171	c
										10	114	b	119	c	101	a
										11	113	b	108	b	109	b
%SR		47	0	ns		47	a	48	b	00	45	a	50	d	ns	
			1			48	b	46	a	01	49	c	44	a		
										10	48	b	47	b		
										11	47	b	48	c		
Textural																
Cohesiveness		0,503	0	ns		ns		ns		00	0.515	b	0.475	a	ns	
			1							01	0.500	ab	0.540	b		
										10	0.459	a	0.534	b		
										11	0.538	c	0.462	a		
Resilience		0.207	0	ns		0.201	a	ns		00	0.216	bc	0.188	a	0.192	a
			1			0.214	b			01	0.206	b	0.234	b	0.209	b
										10	0.185	a	0.224	b	0.220	b
										11	0.222	c	0.183	a	0.208	b
Chewiness	N	4.59	0	ns		4.11	a	ns			ns		ns		ns	
			1			5.06	b									
Firming kinetics																
T_∞	N	19.69	0	17.67	a	ns		20.46	b	00	16.53	a	16.52	a	21.06	c
			1	21.71	b			18.93	a	01	18.82	b	18.82	b	17.59	a
										10	22.12	c	24.40	c	19.86	b
										11	21.31	c	19.03	b	20.26	bc
k_f		0.297	0	0.463	b	0.353	b	ns			ns		ns		ns	
			1	0.131	a	0.241	a									
n_f		0.986	0	ns		0.709	a	1.135	b	00	ns		1.458	c	ns	
			1			1.263	b	0.837	a	01			0.405	a		
										10			0.813	b		
										11			1.269	bc		
T_0	N	9.77	0	8.99	a	ns		10.14	b	00	ns		10.01	b	ns	
			1	10.55	b			9.41	a	01			7.97	a		
										10			10.26	b		
										11			10.84	b		
Nutritional																
BPP		449	0	458	b	ns		ns		00	425	a	ns		ns	
mg gallic acid/100 g flour			1	440	a					01	491	c				
										10	456	b				
										11	424	a				
ARA	%	42	0	44	b	42	a	ns		00	ns		ns		43	b
			1	41	a	43	b			01					40	a
										10					43	b
										11					44	c

F_0 : initial force, k_1(s), k_2 are constants related to stress decay rate and to residual stress at the end of the experiment, respectively, RT: time required for F_0 to drop to 36.8% (dough) and 60% (bread) to its values, % SR= ((F_0-$F_{300/600}$) /F_0)100, T_0: onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization entalpy, T_∞ crumb firmness at ∞ time, k_f constant of proportion of firming kinetics, n_f Avrami exponent of firming kinetics, BPP bioaccessible polyphenols, ARA anti-radical activity.

Table 6.- Significant Pearson correlations ($p<0.05$ *, $p<0.01$ **) between dough viscoelastic, mechanical, and thermal parameters and bread textural, nutritional, and firming kinetic characteristics from untreated and heat moisture treated blended matrices.

	Bread											
Dough	**SV, ml/g**	**Hardness, N**	**Cohesiveness**	**Resilience**	**F_0, N**	**RT, s**	**SR, %**	**L**	**a**	**% ARA**	**T_∞, N**	**n_f**
F_0, N									0,5382 *			
k_1, s			-0.4975 *	-0.4873 *						0.5365 *		
k_2	0.7352 **							0.4832 *		0.7334 **		
$1/k_1$, s^{-1}	-0.476 *		0.5074 *	0.5343 *						-0.5034 *		
$1/k_2$	-0.7341 **		0.4993 *					-0.5205 *		-0.7221 **		
RT, s										0.5731 *		
SR, %	-0.5436 *		0.5671 *							-0.6011 *		
Springiness		-0.4896 *			-0.6481 **						0.536 *	-0.5938 *
T_o, ºC	-0.6132 *									-0.4835 *		
T_p, ºC	-0.6293 **							-0.487 *				
T_e, ºC	-0.6299 **											
$\triangle H_g$, J/g		0.5696 *			0.6498 **	-0.4886 *	0.5774 *				-0.5772 *	0.718 **

F_0 : initial force, k_1(s), k_2 are constants related to stress decay rate and to residual stress at the end of the experiment, respectively, RT: time required for F_0 to drop to 36.8% (dough) and 60% (bread) to its values, % SR= $((F_0\text{-}F_{300/600})/F_0)100$, T_o: onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization entalpy, T_∞ crumb firmness at ∞ time, n_f Avrami exponent of firming kinetics, SV specific volume, % ARA percent of anti-radical activity.

Abstract The significance of heat moisture treatment (HMT) of non-wheat –teff (T), chestnut (CN) and chickpea (CP) flours on dough viscoelastic and thermal parameters and on the structural and nutritional pattern of breads was investigated in untreated (-) and HMT (+) associated wheat-based (WT) matrices (WT:T:CN:CP, 66.20:7:7, wt. basis). 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+. In addition, a fine and uniformly- sized cell structure with similar cell walls thickness were achieved in crumb samples. The pair T-CN+ enhanced extracted bioaccessible polyphenols, and the pair CN+CP+ synergistically promoted the anti-radical activity in breads. Blended breads can be labelled as high-fibre breads (≥6 g DF/100 g food), and a recommended daily consumption of 250 g of bread fulfilled from 44% (men) to 67% (women) of dietary fibre requirements.