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Impact of Heat Moisture Treatment and hydration level on physico-chemical and viscoelastic properties of doughs from wheat-barley composite flours.

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1 **Impact of Heat Moisture Treatment and hydration level on physico-chemical and viscoelastic**
2 **properties of doughs from wheat-barley composite flours.**

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Abstract The impact of heat moisture treatment (HMT) on the thermoviscous, viscoelastic and mechanical properties of binary flour matrices (wheat:barley, 60:40, wt:wt) was investigated in untreated and HMT (15% moisture content, 1 h heating time at 120°C) hydrated samples to assess the potential of HMT to modify dough viscoelasticity and doughmaking functionality in diluted breadmaking wheat matrices. HMT significance was tackled a) in excess of water, by applying successive cooking and cooling cycles to hydrated samples (14%, w:w), determination of viscometric parameters, and subsequent determination of textural (compression test) and viscoelastic parameters (stress-relaxation test) in pasted and gelled hydrated flours, and b) under water restrictions by assessing the consistency (forward extrusion test), the primary and secondary mechanical properties (Texture Profile Analysis), and the viscoelastic behaviour (stress-relaxation test) of untreated and HMT mixed doughs made at different flour hydration levels (63% and 70%). In highly hydrated blends, HMT barley flour provided enhanced viscosity patterns regardless the presence of native or HMT wheat flour, and harder gels with larger initial stress to reach a defined deformation, particularly in presence of HMT wheat flour. Under restricted water availability, doughs made at 70% hydration level when compared to their counterparts made at 63% explicated lower stress-relaxation curves with higher values for both initial decay rate and extent of the decay, shorter relaxation times and higher percent of stress relaxation, giving softer and more cohesive doughs. The most elastic-like dough blends were those prepared with HMT wheat and barley flours at 63% hydration, while the most viscous-like doughs were those from native flours made at 70% hydration.

Keywords Heat Moisture Treatment, blended flours, pasting, gelling, viscoelasticity, texture.

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3	49 Abbreviations and symbols
4	
5	
6	50 <i>a</i> greenness / redness
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8	51 <i>b</i> blueness / yellowness
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10	52 CB- native commercial barley flour
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12	53 CB commercial barley
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14	54 CB+ commercial barley flour treated at 15% moisture for 1 h at 120°C .
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16	55 DY dough yield
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18	56 <i>F</i> ₀ initial stress (g force, N)
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20	57 HMT Heat Moisture Treatment
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22	58 <i>k</i> ₁ constant related to stress decay rate (s)
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24	59 <i>k</i> ₂ constant related to residual stress at the end of the experiment
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26	60 <i>L</i> lightness
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28	61 %SR percent of stress relaxation
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30	62 RT relaxation time (min)
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32	63 RVA Rapid Visco Analyser
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34	64 SP swelling power, (g/g)
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36	65 SRC solvent retention capacity, (%)
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38	66 WAI water absorption index, (g water/g flour, d. b.)
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40	67 WSI water solubility index, (% solids, flour,d. b.)
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42	68 WT- native wheat flour
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44	69 WT wheat
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46	70 WT+ wheat flour treated at 15% moisture for 1 h at 120°C
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48	71 WT+CB- blend of wheat flour treated at 15% moisture for 1 h at 120°C and native commercial barley flour, mixed at
49	72 60:40, wt:wt
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51	73 WT+CB+ blend of wheat and commercial barley flours individually treated at 15% moisture for 1 h at 120°C and mixed at
52	74 60:40, wt:wt
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54	75 WT-CB- native wheat and commercial barley flours mixed at 60:40, wt:wt
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56	76 WT-CB+ blend of native wheat flour and commercial barley flour treated at 15% moisture for 1 h at 120°C, mixed at
57	77 60:40, wt:wt
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1. Introduction

Barley is increasingly incorporated to baked goods either as a whole grain or as a food ingredient mainly due to its superior nutritional profile and associated health promoting effects. The presence of β -glucan and phenolic compounds in barley flour have shown to lower cholesterol and blood glucose levels [1], and barley proteins have been recognized as a rich source of some essential amino acids [2].

Mixed breads obtained by 40% replacement of wheat flour by commercial barley flour are more nutritious in terms of elevated intake of important nutrients such as dietary fibre fractions (soluble, insoluble, resistant starch and β -glucans), slowly digestible starch subfraction and bioaccessible polyphenols providing higher antiradical activity with health-promoting effects, compared to their wheat flour counterparts [3]. Concomitantly to nutritional and health-related benefits, an impairment of bread quality of high-barley breads particularly loaf volume and texture of composite breads was observed, ascribed to both the dilution of wheat dough biopolymers –gluten and starch- and the mechanical interference with gluten network formation by insoluble dietary fibre [3]. In breadmaking applications, replacement of wheat flour by significant amounts of non-gluten forming flours such as barley, can seriously constrain both dough viscoelasticity and gas retention capability of blended dough matrices that limits final bread performance.

Heat moisture treatment (HMT) constitutes an environmentally friendly technique, of interest to make low glycaemic index foods without any chemical residue, and a clean label alternative to chemical modification for altering the gelatinization and retrogradation properties of starches [4] and flours [5], and the aggregation/disaggregation equilibrium of proteins [6]. HMT is a physical modification that allows control of molecular mobility at high temperatures by limiting the amount of water. HMT causes the rearrangement of starch polymers chains, and may modify its X-ray pattern, crystallinity, swelling power, amylose leaching, pasting, and gelatinization properties, as well as its susceptibility

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104 to enzymatic or acidic hydrolysis, which affect the starch rheological properties [7]. HMT can render
105 granule's surface hydrophobic [8], and can confer to starch granules more rigidity and resistance to
106 the quick heating by altering the swelling behavior [9]. Gluten proteins are responsible for the unique
107 viscoelastic properties of wheat dough. Upon water addition and mixing, they unfold and build a
108 transient network comprised of mainly disulphide bonds, the most favourable arrangement of
109 hydrogen bonds, hydrophobic interactions and entanglements. If the conformation of those proteins is
110 altered by thermal treatments, their ability to take part in these processes is also changed and
111 therefore the dough formation process is greatly modified. During HMT, protein solubility decreases
112 associated to gluten proteins denaturation together with aggregation in wheat systems. Protein
113 bodies were deformed and denatured, and interactions between denatured proteins, and between
114 proteins and starch granules, caused the association of the protein networks with the surfaces of
115 starch granules. Protein layers, in cooperation with the increased hydrophobicity, retarded the
116 swelling of HMT starch granules in wheat flour [10]. Microscopic observations by confocal laser
117 scanning microscopy and light microscopy revealed that HMT caused the clumping of starch
118 granules and the aggregation of denatured protein [10]. In wheat dough, observed rheological
119 changes for small and large deformations can be described assuming protein aggregation (leading to
120 a weakened protein network and possibly acting as additional filler particles) and starch surface
121 modifications (leading to changed starch-protein and starch-starch) interactions [6].
122 Main bulk of research has been devoted to the significance of hydrothermal treatment on starches,
123 being information about the effect of HMT on cereal and grain flours very limited, and the impact of
124 the treatment on dough performance restricted to hydrated wheat flours of different baking quality
125 [11]. Significance of HMT on functional performance of blended doughs –hydrated wheat/non-wheat
126 flours- has not been addressed so far.

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3 127 This paper is aimed at exploring the potencial of HMT to restore/ improve dough viscoelasticity in
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5 128 diluted wheat matrices with incorporation of non-gluten forming flours with added nutritional value
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8 129 (barley) by a) investigating the viscosity changes induced by HMT that occur during starch
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10 130 gelatinization, pasting and gelling in single (wheat, barley) and blended flours (wheat/barley) matrices
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12 131 with surplus water, b) at knowing the impact of HMT on the viscoelastic and textural/mechanical
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14 132 behaviour of blended doughs under water restrictions.
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18 19 20 134 **2. Materials and methods**

21 22 135 23 24 136 **2.1. Materials**

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26 137 Commercial flours from refined common wheat *Triticum aestivum* (WT), and whole barley *Hordeum*
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28 138 *vulgare* L. (CB), were purchased from the Spanish market. Refined WT (70% extraction rate) of 200 x
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30 139 10⁻⁴ J energy of deformation W, 0.6 curve configuration ratio P/L, and 57% water absorption in
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32 140 Brabender Farinograph, was used.
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36 37 38 142 **2.2. Methods**

39 40 143 *Chemical and nutritional composition of flours*

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42 144 Moisture, protein, ash and fat contents of native commercial flours were determined following the ICC
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44 145 methods [12]. Total, soluble and insoluble dietary fibre contents were determined according to the
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46 146 AOAC method 991.43 [13]. Resistant starch determination was performed according to AOAC Official
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48 147 Method 2002.02 [14] by using Megazyme kit K-RSTAR 08/11. β -glucan content (Megazyme kit K-
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50 148 BGLU 07/11) was determined following the ICC Standard Method No. 166. Amylose/amylopectin
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52 149 ratio (Megazyme kit K-AMYL 07/11) was estimated by using a modification of a Con A method with
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55 150 lipid removal prior to analysis. Three replicates were made for each analysis. Digestible
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3 151 carbohydrates were calculated by indirect determination as $100 - [\text{Moisture} + \text{Protein} + \text{Fat} + \text{Ash} +$
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5 152 Dietary Fibre] [15].
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10 154 *Heat-moisture treatment (HMT)*
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12 155 Single BL and WT flour samples were weighed and placed into screw-capped glass containers. Small
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14 156 amount of distilled water was added slowly with frequent stirring until moisture levels (w/w) of the total
15
16 157 mixture reached 15% and 25%, respectively, and equilibrated for 24 h at room temperature. Hydrated
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18 158 samples were kept for 1h at 120 °C in a convection oven (P-Selecta, Barcelona, Spain).After cooling
19
20 159 to room temperature, the samples were ~~dried at 40 °C overnight to a constant weight, and then~~
21
22 160 passed through 100-mesh sieve for further analysis. ~~The moisture content was measured before and~~
23
24 161 ~~after HMT using a moisture analyzer (DBS60-3, Kern, Balingen, Germany).~~ Untreated native flours
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26 162 were used as controls. Untreated (-) and HMT (+) flours were used singly (WT, CB) and in binary
27
28 163 blends (WT:CB, 60:40, w:w) for analysis. Blends were hydrated with 63% and 70% of water (flour
29
30 164 basis) to make doughs of 163 and 170 of dough yield (DY), respectively. Four different blended
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32 165 doughs were obtained per DY: WT-CB-, WT-CB+, WT+CB-, WT+CB+.
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34 166 HMT conditions (15% moisture content, 1 h and 120°C) were selected on the basis of previous
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36 167 experiments in which maximization of viscometric profile and minimization of loss of hydration
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38 168 properties of hydrated flour samples were applied as criteria.
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48 170 *Functional properties of single and blended wheat - barley flours*
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50 171 Functional characteristics of untreated and HMT single WT and CB, and blended WT/CB flours, were
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52 172 assessed as it follows. Solvent-Retention Capacity (SRC) was determined according the AACC
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54 173 method 56-11 [16] to quantify potential contributions to water holding capacity by other flour
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56 174 components having water-uptake capabilities [17]. The solvents used were water, sucrose (50% w/v),
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3 175 sodium bicarbonate (5% w/v), and lactic acid (5% v/v). 25 mL of prepared solvent were added to 5 g
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6 176 of flour in 30 mL centrifuge bottles. Centrifugation at 1,239•g (3,000 rpm) was performed for 15 min.
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8 177 After decanting, a gel remained. Gels were weighed and the SRC value (%) calculated as % SRC =
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10 178 $[(\text{gel wt/flour wt}) \cdot (86/(100 - \% \text{ flour moisture})) - 1] \cdot 100$ for each solvent.
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13 179 Bulk density was calculated as weight of sample per unit volume of sample (g/ml). The flour samples
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15 180 were gently filled into 10 ml graduated cylinders, previously tared. The bottom of the cylinder was gently
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17 181 tapped on a laboratory bench several times until there was no further diminution of the sample level after
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19 182 filling to the 10 ml mark. Measurements were made in triplicate [18].
20
21
22 183 Water absorption index (WAI) and water solubility index (WSI) were determined as described by Singh et
23
24 184 al. [19] with modifications of the heating conditions as follows. The ground flour samples (2.5g) were
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26 185 mixed with 30 ml distilled water, using a glass rod, and heated at 30 and 60°C for 15 min in a water bath.
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28 186 The cooked paste was cooled to room temperature and transferred to centrifuge tubes and centrifuged at
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30 187 3000g for 10 min. WAI and WSI were calculated by using the expressions:
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33 188 $\text{WAI} = \text{Weight of sediment/weight of dry solids}$
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35 189 $\text{WSI} = (\text{Weight of dissolved solids in supernatant/weight of dry solids}) \times 100$
36
37 190 $\text{SP} = \text{WAI}/[1 - \text{WSI}/100]$
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39
40 191 Colour measurements were determined on flours using a Photoshop system following the method
41
42 192 previously described by Angioloni and Collar [20], and the results were expressed in accordance to the
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44 193 Hunter Lab colour space. The Photoshop (PS Adobe Photoshop CS5 extended) system (L, a, b colour
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46 194 coordinates) was calibrated using colour sheets from Pantone®Formula Guide (Pantone, Inc., USA).
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48 195 Images were acquired at 300 pixel resolution with a ScanJet II cx flatbed scanner (Hewlett- Packard,
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50 196 USA). Parameters determined were L (L = 0 [black] and L = 100[white]), a (-a = greenness and +a =
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52 197 redness), b (-b = blueness and +b = yellowness), as described earlier [20]. Hunter Lab colour space
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54 198 parameters from Minolta colorimeter were calculated from the calibration linear equation Colorimeter vs

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8 201 *Rheological assessment of hydrated flours, gels and doughs*
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12 203 **Hydrated flours**
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15 204 *Thermoviscous test*
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17 205 Viscometric profiles (gelatinization, pasting, and setback properties) of 14% (w/v) hydrated single
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19 206 (WT, CB) and blended (WT/CB, 60/40, w/w) untreated (-) and HMT (+) flours were obtained with a
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21 207 Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) using ICC Standard 162.
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23 208 Samples (3.5 g, 14% moisture basis) were transferred into canisters and $\approx 25 \pm 0.1$ mL of distilled
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25 209 water were added (corrected to compensate for 14% moisture basis). The slurry was heated to 50°C
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27 210 and stirred at 160 rpm for 10 s for thorough dispersion. The slurry was held at 50°C for up to 1 min,
28
29 211 and then heated to 95°C over 3 min 42 s and held at 95°C for 2 min 30 s, and finally cooled to 50°C
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31 212 over 3 min 48 s, and held at 50°C for 2 min. The pasting temperature (when viscosity first increases
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33 213 by at least 25 cP over a 20 s period), peak time (when peak viscosity occurred), peak viscosity
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35 214 (maximum hot paste viscosity), holding strength or trough viscosity (minimum hot paste viscosity),
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37 215 breakdown (peak viscosity minus holding strength or trough viscosity), viscosity at 95°C, viscosity at
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39 216 the end of the 95°C holding period, viscosity at 50°C, final viscosity (end of test after cooling to 50°C
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41 217 and holding at this temperature), setback (final viscosity minus peak viscosity), and total setback
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43 218 (final viscosity minus holding strength) were calculated from the pasting curve [21] using Thermocline
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45 219 v. 2.2 software. For each viscometric measurement, 3 samples were used.
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55 221 **Gels**
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57 222 *Gel texture*
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After RVA testing, canisters containing flour pastes were covered with paraffin film and kept at 4 °C for 24 h. Texture of gels in canisters (with a dimension of 20 mm in height and 38 mm in diameter) were determined in a TA- XT2 Texture Analyzer by compression to a distance of 15 mm at a speed of 2.0 mm/s using a cylindrical Perspex probe of 25 mm in diameter. The peak force measured in duplicate was reported as gel hardness.

Stress relaxation test

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 [23]. Stress relaxation tests were applied on the gels obtained from RVA experiments after storage at 4 °C for 24 h. The stress–relaxation tests were conducted using a texture analyzer mentioned in the previous paragraph fitted with a load cell of 30 kg and a P/25 cylindrical probe and the relaxation data were obtained under this configuration. The gel samples in the canisters were directly placed under a cylindrical plate and compressed to 30% a crosshead speed of 0.5 mm/s for 300 s at room temperature (25 °C). In order to minimize the frictional effects, samples, base and probe were lubricated with liquid paraffin [24]. Stress relaxation tests were replicated two times. The obtained stress relaxation curves were normalized and linearized according to the Peleg [25], and Peleg and Pollak [26] model, as $F_0 t / (F_0 - F(t)) = k_1 + k_2 t$, 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= $(F_0 - F_{300}) \cdot 100 / F_0$), $1/k_1$ (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), representing viscoelastic behavior, were compared for the different samples.

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248 *Forward extrusion test*

249 Forward extrusion assays of untreated and HMT binary WT/CB doughs made at DY of 160 and 170

250 were performed in a TA-XT2 texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 30

251 kg-load cell and operating at 10 mm/s head speed. The test measures the compression force

252 required for a piston disc to extrude the dough through a specific size outlet (10 mm) in the base of

253 the sample container. The extrusion cell and the compression plunger were 5 and 4.9 cm in diameter,

254 respectively. Samples (35 cm³ volume) were carefully scooped into acrylic cylindrical containers with

255 help of spatula. The complete sample container was located into a centralising insert fitted into the

256 Heavy Duty Platform, and the plunger was attached to the load cell using a probe adaptor. 14 cm³ of

257 samples were extruded by compressing a fixed distance of 7 mm. Compression force-time curve

258 allowed evaluating maximum force, determined as the force at which the slope changed. The change

259 of slope was visually detected, and the force at this point calculated using the Texture Analyser

260 software. The curve plateau representing the force necessary to continue with the extrusion process

261 and the area under the curve were both used to define the sample consistency. All measurements

262 were performed in triplicate.

263

264 **Doughs**

265 *Texture Profile Analysis (TPA)*

266 TPA of untreated and HMT binary WT/CB doughs made at DY of 160 and 170 were performed by

267 applying a double compression cycle in a TA-XT2 texture analyser using a 1 cm diameter probe, 75 s

268 waiting period, and 60% compression, as described previously [22]. The primary textural properties

269 were measured in absence of dough adhesiveness by using a plastic film on the dough surface to

270 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.

Stress relaxation test

~~Relaxation experiments are related to structural changes and are restricted to ideal deformation. Stress relaxation tests were applied on the gels obtained from RVA experiments after storage at 4 °C for 24 h. The stress relaxation tests were conducted using the texture analyzer mentioned above fitted with a load cell of 30 kg and a P/25 cylindrical probe and the relaxation data were obtained under this configuration. The gel samples in the canisters were directly placed under a cylindrical plate and compressed to 30% a crosshead speed of 0.5 mm/s for 300 s at room temperature (25 °C). In order to minimize the frictional effects, samples, base and probe were lubricated with liquid paraffin [24]. Stress relaxation tests were replicated two times.~~ Doughs were submitted to uniaxial compression 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 [25], and Peleg and Pollak [26] model, as for the gels as previously described. $F_0t/(F_0 - F(t)) = k_1 + k_2t$, 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 = $(F_0 - F_{300}) \cdot 100 / F_0$), $1/k_1$ (initial rate of relaxation), $1/k_2$ (extent of relaxation) and Relaxation time (RT) as the time required for F_0 to drop to 50% of its values, respectively, were compared for the different samples.

Statistical analysis

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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. Functional properties of untreated and HMT single and blended flours

Grain flours constitute natural and practical food systems for studying multiple food component interactions [27], since besides starch, proteins, fat and dietary fibre are included in the chemical and nutritional composition of composite food matrices (Table 1).

Colour parameters, bulk density, and hydration properties -WAI, WSI, SP, and SRC- of untreated and HMT single WT, CB and blended WT/CB flours are compiled in Table 2. The colour of any food product generally changes during heat treatment and thus influences the acceptability [28]. *L*, *a* and *b* values for WT flour samples did not change with HMT, while they showed variable trends for CB flours: a significant decrease in lightness *L* but an increase in both *a* (greenness/redness) and *b* (blueness/yellowness) values, which extent rises with the moisture of CB flour samples. Similar changes previously observed in HMT pearl millet flour (Yadav et al., 2012), were ascribed to polymerisation of phenolics and pigments [29] and to conversion of flavonols in colour pigment intermediate compounds [30], in good accordance with the 30% higher amount of polyphenols in CB flour vs WT flour [3].

HMT of flours at 15% of moisture content had significant rising effect on bulk density of both WT (7%) and CB (4%) samples (Table 2), in agreement with data reported for maize flour [31] and pearl millet flour [28] after hydrothermal treatments. However, decrease in bulk density was observed as

moisture of flours increased from 15% to 25%, particularly for HMTCB flour (-13%). Similar results were associated in wheat corn extruded flour to gelatinisation of starch [32].

The WSI, WAI and SP power of all samples exhibited continuous increase as temperature rose from 30°C to 60°C, particularly for WF flours. Conversely, hydration indexes of flours at 60°C decreased significantly after HMT, decrease being larger with increasing flour moisture. Compared to native flours, WSI of HMT₁₅ and HMT₂₅ flours decreased up to 50% (WT₂₅) and up to 38% (CB₁₅, CB₂₅); WAI declined from 15% (WT₁₅) to 18% (WT₂₅) and by 7% (CB₂₅) at 60°C. Consistently, SP depleted gradually with moisture content in both HMT flours, from 13% to 6% (WT) and from 6% to 4.5% (CB) at 60°C (Table 2). Waduge et al., 2006 [33] proposed that the reduction in solubility and swelling power of barley starch ~~were due to many factors: increased crystallinity, reduced hydration, increased interactions between amylose and amylopectin molecules, strengthened intermolecular bonds, the formation of amylose-lipid complexes and changes in the arrangements of the crystalline regions of starch. As a resulted from the denser granule structure formed after HMT that is partly responsible for the decrease in swelling power~~ [34]. The formation of amylose-lipid complexes within the starch granule was stressed as responsible for the reduction in the swelling capacity and starch solubility in white sorghum starch submitted to HMT [35]. Their results showed that amylose inhibited granule swelling under conditions where amylose-lipid complexes were likely to be formed, as it is the case of flours (Table 1). So that, the decrease in solubility and swelling power were ascribed to the structural rearrangement and/or re-associations of starch chains caused by HMT. In particular, HMT promotes formation of ordered double helices, and consequently, limits starch swelling and solubility, as suggested by Lawal (2005) [36].

The SRC test is a solvation assay for flours based on the enhanced swelling behavior of individual polymer networks in selected single diagnostic solvents— water, 5% w/w lactic acid (LA) in water (for glutenin), 5% w/w sodium carbonate (Na₂CO₃) in water (for damaged starch), and 50% w/w sucrose

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3 342 in water (for pentosans)—which are used to predict the functional contribution of each individual flour
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5 343 component. SRC method is based on a classical thermodynamic determination of the solubility
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7 344 parameter of network-forming polymers. For flour typically used to produce bread by the sponge–
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10 345 dough method, optimal SRC profile values would be ≥ 100 % in 5% w/w lactic acid, ≤ 96 % in 50%
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12 346 w/w sucrose in water, ≤ 72 % in 5% w/w sodium carbonate extracts [37]. In this work, a straight dough
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15 347 breadmaking system was used instead, and some mean values for water retention components of
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17 348 WT and blended wheat–barley flours (WT/CB) were outside the typical range for a sponge–dough
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19 349 bread system in mixed and thermally treated flours, but especially for HMT binary WT/CB samples
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21 350 and water retention of pentosans (124 %) and damaged starch (106 %). Results are consistent with
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23 351 an additional deleterious effects of HMT to the replacement of WT flour by CB, on the optimal SRC
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25 352 profile of mixed flours.
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32 354 **3.2. Impact of HMT on the pasting and gelling of single and blended flours**
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34 355 Pasting properties provide information on intermolecular bonding between densely packed starch
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36 356 granules and the rigidity of swollen starch granules during the heating process, derived parameters
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38 357 being strongly affected by HMT [5]. The mean results pertaining to pasting properties of single (WT,
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40 358 CB) and blended (WT:CB, 60:40, w:w) flours as affected by HMT (15% moisture, 1 h, 120°C) are
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42 359 given in Table 3, and qualitative and quantitative RVA profiles are displayed comparatively in Figure
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45 360 1. HMT flours reached much higher viscosity values during earlier pasting and subsequent gelling
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47 361 than the corresponding native counterparts (Figure 1). Values for peak viscosity and total setback
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49 362 (mPa.s) respectively account for 2683 and 3257 (BL+) vs 224 and 200 (BL-), and 2908 and 1500
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51 363 (WT+) vs 2240 and 1156 (WT-), confirming trends previously found (Collar, 2017). Similar patterns
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53 364 were reported for wheat flour submitted to dry heat and/or mild hydrothermal treatments [11, 38].
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56 365 Authors related the decrease in onset pasting time and increase in peak viscosity in the Amylograph
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profile to the increased hydrophobicity of gluten proteins and to the occurrence of lipophilization of starch granules due to the change of the properties of the proteins on the starch granule surface from hydrophilic to hydrophobic (Ozawa et al., 2009). In addition, changes in the gluten protein structure encompassed a minor swelling of the starch granules that occurred in the presence of the moisture content in the flour, and observed increased retrogradation values compared to the untreated flours indicated a tendency of higher re-association ability of amylose [11] (~~Bucella et al., 2016~~). Analogous increased hydrophobicity of prolamins and glutelins in CB+ could explain the prominent enhanced viscosity profile observed. Both additive and non-additive pasting behaviour has been already found for starch [39], additive-wheat flour [21], and flour blends [40, 41]. In this work, viscosity patterns during both pasting and gelling cycles of blended untreated and HMT flours were much lower than could be expected from its compositional flours (Table 3). Decrease was particularly relevant for blends with untreated CB (WT-CB-, WT+CB-) with viscosity values as low as 27% (peak viscosity), 7% (holding strength), 13% (final viscosity) and 19% (total setback) the expected values for an additive viscometric behaviour of single flours in blends, vs 55%, 30%, 39%, and 46% respectively in blends with treated CB (WT-CB+, WT+CB+). In blended flours, HMT provided enhanced viscometric profiles during both pasting and gelling (Figure 1). Particular higher values of viscosity features were observed for the sample WT+CB+, followed by the sample WT-CB+, while on the contrary, lower profiles were reached by WT-CB- followed by WT+CB- (Figure 1, Table 3) in good agreement with the significant single effect of HMT on the enhancement of CB flour viscometer parameters compared to those of WT counterparts. This is particularly true for peak viscosity (+194% vs +31%), holding strength (710% vs 38%), and viscosity at end of 95°C (+612% vs 37%) during pasting, and total setback (+483% vs +24%), viscosity at 50°C (+489% vs +28%) and final viscosity (+546% vs 29%) during gelling, respectively (Table 3).

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3.3. Impact of HMT on viscoelastic and textural/mechanical behaviour of gels and doughs from single and blended flours

Physical parameters from stress relaxation, compression, forward extrusion and texture profile analysis tests of untreated and HMT blended gel and dough matrices at different DY are compiled in Table 4. Significant single and interactive effects ($p<0.05$) of flour and HMT on viscoelastic and mechanical parameters are shown in Table 5. In excess of water (14% flour concentration), gelatinised and gelled mixed flours in RVA canisters exhibited increased strength to compression with HMT, effect being larger for CB+ (190%) than for WT+ (21%), but lower than the increase provided by the simultaneous presence of WT+/CB+ (230%) in the binary mixture (Table 4). The increase in the final viscosity and the setback (Figure 1) is a sign of the increase of starch paste stability and gel hardness. The starch components have been solubilized during the starch collapse at the breakdown phase, but they were capable of re-association during the cooling process. This phenomenon led to increased retrogradation. During HMT, increase in gel hardness has been attributed to the increased cross-linking between starch chains in the particular amylose portion. These allowed the formation of more junction zone in the continuous phase of the gel, resulting in the increased gel hardness [42]. Results are consistent with those found for early indica rice [43]. Viscoelastic materials exhibit stress relaxation phenomena. In stress relaxation tests, a constant strain is applied and the stress required to maintain the deformation is measured as a function of time. The measured relaxation time shows how fast the material dissipates stress after receiving a sudden deformation. It was reported that relaxation time is the time when the viscoelastic material dissipates its force to about 36.8% of the originally applied force [44]. The relaxation constants are related to viscoelastic characteristics of samples. The constant $1/k_2$ is related to the asymptotic level of stress not relaxed at long times, since the constant $1/k_1$ is related to the initial stress decay rate [45]. At gel level, small variations were observed with HMT in the relaxation parameters, except for the initial

force F_0 that followed the same trend as the gel strength after compression. The lower k_1 values were noticed in both untreated WT-CB- and treated WT+CB+ gels (Table 4). No differences with HMT were gained regarding either the relaxation time or the % of stress relaxation (Table 4). Under restricted water availability, samples such doughs showed differentiated viscoelastic and textural parameters with DY first, and with HMT of flours, secondly (Table 4). In blends, HMT of WT decreased dough consistency, % SR, cohesiveness and resilience, and increased k_2 regardless DY, while HMT of CB increased dough consistency (Table 5). At DY 170, HMT decreased the relaxation time by 12% for CB+ and increased by 35% for WT+, while at DY 163, sharp increases by 93% (WT+) and 73% (CB+) were denoted (Table 5). HMT changed dough hardness, adhesiveness, and cohesiveness in variable extent only for WT+ (32%, -72%, -26%, respectively) when flour is incorporated to doughs of DY 170, and specifically for CB+ (62%, -30%, -17%, respectively) when flour is mixed in doughs of DY 163. HMT of flours decreased dough resilience only for WT, being extent of depletion greater in softer than in harder doughs (-43% vs -31%).

In general, doughs made at DY 170 compared to their counterparts made at DY 163 explicated lower relaxation curves with small values for both k_1 (8.3-9.4 vs 11.8-14.0) and k_2 (1.4-1.5 vs 1.4-1.8), shorter relaxation times (13-19s vs 20-78s) and higher percent of stress relaxation (65-72% vs 54-69%), giving softer (70-117N vs 161-232N), more cohesive (0.51-0.73 vs 0.38-0.63), and adhesive (141-558 vs 72-122g.s) doughs (Table 4).

Obtained results for untreated WT-CB- and WT-CB+ doughs at DY 170 showed lowest values for k_1 and k_2 . This indicates a steeper descent in the relaxation curves toward a lower residual value, which is related to a more viscous-like character of the doughs in agreement with concomitant lower values for F_0 , RT TPA hardness and higher values for cohesiveness and adhesiveness (Table 4). On the contrary, WT+CB+ at DY 163 exhibited the most elastic dough with the smallest depletion in initial

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3 437 rate of stress ($1/k_1$), as well as in the value asymptotically approached by normalized stress ($1/k_2$). It
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5 438 reflects the change in structure of dough, which under water restrictions becomes more elastic.
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10 440 **Conclusions**

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12 441 HMT of flours modified dough viscoelasticity and doughmaking functionality of diluted breadmaking
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14 442 wheat matrices.made with 40% of wheat flour replacement by barley flour. The trend and extent of
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17 443 the changes closely depend on water availability. In excess of water, HMT provided enhanced
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20 444 viscometric profiles during both pasting and gelling in blended flours, particularly for samples with
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22 445 HMT treated barley, while on the contrary, lower profiles were reached by sample blends with native
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24 446 barley flour. The gelling ability of HMT treated barley provided harder gels, and may confer a suitable
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27 447 thickening effect to blended doughs that may improve gas retention in presence of a diluted gluten
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29 448 network. Under water restrictions, flour hydration governs viscoelasticity and mechanical properties of
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31 449 blended doughs, while HMT only modulates dough performance. In general, doughs made at 70% of
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33 450 hydration level compared to their counterparts made at 63% explicated lower relaxation curves with
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36 451 smaller values for both k_1 and k_2 , shorter relaxation times and higher percent of stress relaxation,
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38 452 giving softer and more cohesive doughs. Obtained results for untreated doughs and barley treated
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40 453 blended doughs at 70% hydration showed steeper descent in the relaxation curves toward a lower
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44 455 lower values for initial stress, relaxation time, and dough hardness and higher values for
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47 456 cohesiveness and adhesiveness. On the contrary, blends with both wheat and barley treated flours at
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49 457 63% of hydration exhibited the most elastic dough with the smallest depletion in initial rate of stress,
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51 458 as well as in the value asymptotically approached by normalized stress. It reflects the change in
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53 459 dough structure, which under water restrictions becomes more elastic.
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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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Table 1.- Chemical, biochemical and nutritional composition of flours

Parameter (g/ 100 g flour, as is)	Wheat	Commercial barley
Moisture	14.3±0.1b	12.8±0.1a
Protein	12.10±0.24b	11.27±0.30a
Fat	1.34±0.09a	1.69±0.10b
Ash	0.54±0.03a	1.52±0.06b
Digestible starch	69.8±1.6b	57.5±1.3a
Amylose/amylopectin ratio	23±1/77±1a	29±1/71±1b
Total Dietary Fibre	1.9±0.2a	15.2±1.3b
Soluble Fibre	0.81±0.09a	5.15±0.24b
Insoluble Fibre	1.09±0.24a	10.05±0.95b
Resistant Starch	1.76±0.22a	4.22±1.06b
<u>β-glucans</u>	<u>0.20±0.09a</u>	<u>4.50±0.15b</u>

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

Table 2.- Functional properties of untreated and heat moisture treated (HMT) wheat flour, barley flour and wheat/barley flour blends (60/40, w/w).

Property	Wheat flour (WT)			Barley flour (CB)			WT/CB		
	untreated	HMT ₁₅	HMT ₂₅	untreated	HMT ₁₅	HMT ₂₅	WT/CB	WT/CB _{HMT15}	WT/CB _{HMT25}
<i>L</i>	91.2±0.3 ^a	91.2±0.4 ^a	91.2±0.4 ^a	90.5±2.1 ^b	89.4±3.2 ^b	84.6±5.7 ^a			
<i>a</i>	-2.6±0.0 ^a	-2.6±0.0 ^a	-2.6±0.0 ^a	-2.6±0.0 ^a	-2.6±0.0 ^a	0.2±0.0 ^b			
<i>b</i>	2.9±0.0 ^a	2.9±0.0 ^a	2.9±0.0 ^a	3.9±0.1 ^a	5.4±0.2 ^b	9.2±0.4 ^c			
Bulk density, mL/g	0.82±0.01 ^a	0.88±0.01 ^c	0.84±0.00 ^b	0.73±0.00 ^b	0.76±0.01 ^c	0.66±0.00 ^a			
Swelling power, g/g									
30°C	2.01±0.08 ^a	2.37±0.04 ^b	2.57±0.01 ^c	2.42±0.13 ^a	2.94±0.01 ^b	2.95±0.01 ^b			
60°C	3.70±0.04 ^c	3.21±0.13 ^b	2.99±0.07 ^a	3.33±0.02 ^c	3.18±0.01 ^b	2.99±0.01 ^a			
WAI, g water/g flour, d. b.									
30°C	1.93±0.08 ^a	2.25±0.04 ^b	2.52±0.01 ^c	2.30±0.13 ^a	2.82±0.01 ^b	2.83±0.01 ^b			
60°C	3.55±0.04 ^b	3.02±0.12 ^a	2.90±0.05 ^a	3.06±0.02 ^b	3.02±0.03 ^b	2.84±0.00 ^a			
WSI, % solids, flour,d. b.									
30°C	4±0 ^c	5±0 ^b	2±0 ^a	5±0 ^b	4±0 ^a	4±1 ^{ab}			
60°C	4±1 ^b	6±1 ^b	3±0 ^a	8±0 ^b	5±0 ^a	5±1 ^a			
SRC, %									
water	72±0 ^a	76±2 ^b	74±0 ^b				83±0 ^a	87±1 ^b	83±0 ^a
lactic acid 5%	117±4 ^b	113±2 ^b	98±3 ^a				93±1 ^b	94±1 ^b	88±0 ^a
sucrose 50%	116±2 ^b	117±0 ^b	111±1 ^a				118±1 ^b	124±0 ^c	115±1 ^a
sodium bicarbonate 5%	74±0 ^a	89±1 ^c	83±0 ^b				79±0 ^a	106±1 ^c	100±1 ^b

^aMean values ± standard deviation. Within rows for each single and blended flours respectively, values (mean of three replicates) with the same following letter do not differ significantly from each other (p > 0.05).
HMT₁₅, HMT₂₅: heat moisture treatment at 15% and 25% moisture content, respectively; *L*, *a*, *b*: Hunterlab colour parameters; WAI: water absorption index; WSI: water solubility index; SRC: solvent retention capacity.

Table 3.- Pasting and gelling parameters of untreated (-) and heat-moisture treated (+) single and blended hydrated flour matrices from wheat (WT) and commercial barley (CB) at 60:40, w:w.

Visco-metric property	Single flours ^a				Blended flours ^a			
	WT-	WT+	CB-	CB+	WT-/CB-	WT-/CB+	WT+/CB-	WT+/CB+
Pasting								
Pasting Temperature, °C	74.0±0.0 ^c	72.0±0.0 ^a	78.7±0.6 ^d	73.5±0.0 ^b	80.8±0.0 ^d	71.1±0.0 ^a	75.1±0.0 ^b	79.8±0.0 ^c
Peak viscosity, mPa.s	2240±50 ^b	2908±18 ^d	224±23 ^a	2683±23 ^b	428±42 ^a	1139±56 ^c	488±8 ^b	1558±1 ^d
Peak Time, min	5.9±0.0 ^c	5.7±0.0 ^b	4.8±0.3 ^a	6.0±0.0 ^d	4.5±0.1 ^a	5.2±0.1 ^b	4.5±0.0 ^a	5.4±0.0 ^c
Peak temp, °C	95.0±0.0 ^b	95.0±0.0 ^b	93.8±1.3 ^{abc}	95.1±0.0 ^c	92.0±1.2 ^a	94.9±0.1 ^b	91.9±0.1 ^a	95.0±0.1 ^b
Holding strength, mPa.s	1268±36 ^b	1639±15 ^c	72±9 ^a	1989±39 ^d	66±9 ^a	463±22 ^b	73±18 ^a	655±21 ^c
Breakdown, mPa.s	973±13 ^c	1269±33 ^d	152±14 ^a	695±16 ^b	362±33 ^a	676±34 ^c	415±10 ^b	903±20 ^d
Viscosity at 95°C, mPa.s	356±7 ^c	615±49 ^d	181±10 ^b	88±0 ^a	403±32 ^{bc}	382±43 ^{ab}	453±19 ^{cd}	499±36 ^d
Viscosity at end of 95°C, mPa.s	1554±37 ^b	1928±66 ^c	87±25 ^a	2087±40 ^d	88±7 ^a	541±7 ^b	95±3 ^a	770±25 ^c
Gelling								
Setback, mPa.s	184±25 ^b	231±33 ^c	48±11 ^a	2562±35 ^d	-192±23 ^b	243±7 ^c	-228±0 ^a	265±20 ^c
Viscosity at 50°C, mPa.s	1889±74 ^b	2432±24 ^c	228±35 ^a	4230±21 ^d	193±18 ^a	1032±25 ^b	212±13 ^a	1359±15 ^c
Final Visc, mPa.s	2424±74 ^b	3138±16 ^c	272±12 ^a	5245±13 ^d	236±19 ^a	1382±49 ^b	260±8 ^a	1823±21 ^c
Total Setback, mPa.s	1156±38 ^b	1500±1 ^c	200±3 ^a	3257±52 ^d	170±10 ^a	919±27 ^b	187±10 ^a	1168±0 ^c

^aMean values ± standard deviation. Within rows for single flours and blended flours respectively, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).

Table 4 .- Physical parameters^a from stress relaxation, penetration, forward extrusion and texture profile analysis tests of untreated (-) and heat moisture-treated (+) blended gel and dough matrices from wheat (WT) and commercial barley (CB) at 60:40, w:w.

sample	F_0 , g	k_1 (s)	k_2	$1/k_1$	$1/k_2$	R^2	F_{RT} , g	RT, s	%SR	Firmness/hardness, g	Cohesiveness	Adhesiveness, g.s	Resilience	
gels, 14%							36.8%			Penetration				
WT-/CB-	134±2 ^a	6.17976±0.2051 ^a	1.4059±0.0424 ^a	0.1618±0.0055 ^b	0.7113±0.0229 ^b	99.11	49±1 ^a	35±4 ^a	70±2 ^a	177±13 ^a				
WT-/CB+	503±1 ^c	6.23446±1.0324 ^{ab}	1.4134±0.0144 ^a	0.1604±0.0265 ^{ab}	0.7077±0.0071 ^a	99.34	185±0 ^c	37±9 ^a	70±2 ^a	515±15 ^c	-	-	-	
WT+/CB-	195±8 ^b	6.63628±0.2546 ^b	1.4072±0.0174 ^a	0.1507±0.0055 ^a	0.7106±0.0036 ^b	99.03	72±3 ^b	38±3 ^a	70±2 ^a	215±10 ^b				
WT+/CB+	488±59 ^c	5.67061±0.6081 ^a	1.3898±0.0424 ^a	0.1763±0.0185 ^{bc}	0.7195±0.0220 ^b	99.56	179±22 ^c	29±10 ^a	71±2 ^a	585±9 ^d				
doughs, DY 170							50%			Forward extrusion		TPA		
WT-/CB-	155±19 ^a	8.3797±0.5020 ^a	1.3935±0.0354 ^a	0.1193±0.0072 ^{bc}	0.7176±0.0182 ^c	98.96	77.3±8 ^a	14±0 ^a	70±2 ^{bc}	8645±221 ^b	107±5 ^a	0.7210±0.0057 ^f	543±54 ^d	0.0725±0.0035 ^{de}
WT-/CB+	158±51 ^{ab}	8.2669±1.5203 ^{ab}	1.3615±0.0424 ^a	0.1209±0.0239 ^c	0.7345±0.0226 ^c	98.75	79.0±20 ^a	13±2 ^a	72±2 ^c	11715±956 ^c	98±6 ^a	0.7335±0.0276 ^g	558±46 ^d	0.0670±0.0014 ^d
WT+/CB-	190±14 ^b	9.3777±0.5869 ^{ab}	1.5129±0.0495 ^b	0.1066±0.0067 ^{bc}	0.6610±0.0216 ^b	98.89	95.0±10 ^{ab}	19±1 ^c	65±2 ^b	6965±106 ^a	127±3 ^b	0.5840±0.0396 ^e	163±45 ^c	0.0400±0.0014 ^a
WT+/CB+	214±6 ^b	9.2087±1.0041 ^{ab}	1.4394±0.0566 ^{ab}	0.1086±0.0119 ^{bc}	0.6947±0.0273 ^{bc}	98.92	107±6 ^b	16±0 ^b	68±3 ^{bc}	8282±373 ^b	142±9 ^c	0.5055±0.0813 ^d	141±43 ^{bc}	0.0400±0.0028 ^a
doughs, DY 163														
WT-/CB-	304±30 ^c	12.1934±2.2345 ^b	1.4038±0.0495 ^a	0.0820±0.0154 ^b	0.7124±0.0251 ^e	98.53	152±15 ^{cd}	20±2 ^{cd}	69±2 ^c	20881±2892 ^{ef}	182±9 ^d	0.6335±0.0290 ^e	122±25 ^{bc}	0.0775±0.0021 ^e
WT-/CB+	357±25 ^{cd}	12.4380±1.7964 ^b	1.4366±0.0566 ^{ab}	0.0804±0.0118 ^b	0.696±0.0273 ^{bc}	98.41	178±12 ^d	22±1 ^d	68±3 ^{bc}	23232±2855 ^f	206±26 ^e	0.4900±0.0127 ^c	79±8 ^a	0.0715±0.0092 ^{de}
WT+/CB-	374±24 ^d	11.8359±1.2657 ^b	1.5323±0.0707 ^b	0.0845±0.0090 ^b	0.6526±0.0302 ^b	98.76	187±12 ^{de}	25±1 ^e	64±3 ^b	16076±2687 ^d	101±7 ^a	0.4270±0.0000 ^b	96±9 ^b	0.0465±0.0035 ^{bc}
WT+/CB+	530±4 ^e	14.0082±0.2546 ^c	1.8210±0.1061 ^c	0.0714±0.0013 ^a	0.5491±0.0319 ^a	98.8	265±2 ^f	78±3 ^f	54±3 ^a	19322±496 ^e	252±0 ^f	0.3800±0.0156 ^a	72±8 ^a	0.0500±0.0042 ^c

^aMean values ± standard deviation. Within columns for blended gels and doughs respectively, values (mean of three replicates) with the same following letter do not differ significantly from each other (p > 0.05).
DY: dough yield, 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% (gel) and to 50.0% (dough) to its values, F_{RT} , as the force corresponding to RT, % SR= $((F_0-F_{300})/F_0)100$.

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			1	0.54±0.02 ^b	-1 1	
		Barley	-1	0.65±0.02 ^c	1-1	
			1	0.62±0.02 ^c	1 1	
		163	Wheat	-1	0.56±0.01 ^a	0.63±0.01 ^a
			1	0.40±0.01 ^b	-1 1	0.49±0.01 ^b
			Barley	-1	0.53±0.01 ^a	0.43±0.01 ^c
			1	0.44±0.01 ^b	1 1	0.38±0.01 ^d
Gumminess	N	163	Wheat	-1	1.08±0.04 ^a	115±6 ^a
			1	0.69±0.04 ^b	-1 1	101±6 ^{ac}
			Barley	-1	0.79±0.04 ^b	43±6 ^b
			1	0.98±0.04 ^a	1 1	96±6 ^c
Resilience		170	Wheat	-1	0.070±0.001 ^a	ns
			1	0.040±0.001 ^b	-1 1	
			Barley	-1	0.056±0.001 ^c	
			1	0.053±0.001 ^c	1 1	
		163	Wheat	-1	0.070±0.002 ^a	0.078±0.002 ^a
			1	0.048±0.002 ^b	-1 1	0.063±0.002 ^b
			Barley	-1	0.062±0.002 ^c	0.047±0.002 ^c
			1	0.057±0.002 ^c	1 1	0.050±0.002 ^c

^aLevels single effects: -1 (untreated), +1 (heat moisture treated); interactions: 1st digit (wheat), 2nd digit (barley), -1 (untreated), +1 (heat moisture treated).

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.

^b Mean values ± standard deviation. Within columns for each parameter, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).

Figure 1.- Rapid Visco-Analyzer profiles of untreated (-) and Heat Moisture treated (+) single wheat (WT), commercial barley (CB) and blended (WTCB, 60:40, wt:wt) flours.