Significance of Heat Moisture Treatment conditions on the pasting and gelling behaviour of various starch-rich cereal and pseudocereal flours.

Concha Collar

Cereals and Cereal-based Products. Food Science Department. Instituto de Agroquímica y Tecnología de Alimentos (CSIC). Avda. Catedrático Agustín Escardino, 7. 46980 Paterna. SPAIN.

Tel.: +34 963 90 00 22; Fax: +34 963 63 63 01

E-mail address: ccollar@iata.csic.es

.

Abstract The impact of heat moisture treatment (HMT) processing conditions (15, 25, and 35% moisture content; 1, 3, and 5 h heating time at 120ºC) on the viscosity pasting and gelling profiles of different grain flours matrices (barley BL, buckwheat BK, sorghum SG, high β-glucan barley ST, and wheat WT) was investigated by applying successive cooking and cooling cycles to rapid visco analyser canisters with highly hydrated samples (3.5:25, w:w). At a milder HMT conditions (15% moisture content, 1 h heating time), except for SG, HMT flours reached much higher viscosity values during earlier pasting and subsequent gelling than the corresponding native counterparts. Besides HMT wheat flour, described behaviour found also for non-wheat treated flours has not been previously reported in the literature. An increased hydrophobicity of prolamins and glutelins in low moisture- short heating time HMT non-wheat flours with high protein content (12.92%-19.95%) could explain the enhanced viscosity profile observed.

Keywords Heat Moisture Treatment, pasting, gelling, gelatinization, grain flours.

Abbreviations and symbols

BL commercial barley

BK buckwheat

FA factor analysis

HMT Heat Moisture Treatment

RVA Rapid Visco Analyser

SG sorghum

ST high β-glucan barley

VE variance explained

WT wheat

XX151 grain flour (BL, BK, SG, ST or WT) treated at 15% moisture for 1 h at 120ºC.

XX153 grain flour (BL, BK, SG, ST or WT) treated at 15% moisture for 3 h at 120ºC.

XX155 grain flour (BL, BK, SG, ST or WT) treated at 15% moisture for 5 h at 120ºC .

XX251 grain flour (BL, BK, SG, ST or WT) treated at 25% moisture for 1 h at 120ºC.

XX253 grain flour (BL, BK, SG, ST or WT) treated at 25% moisture for 3 h at 120ºC .

XX255 grain flour (BL, BK, SG, ST or WT) treated at 25% moisture for 5 h at 120ºC.

XX351 grain flour (BL, BK, SG, ST or WT) treated at 35% moisture for 1 h at 120ºC.

XX353 grain flour (BL, BK, SG, ST or WT) treated at 35% moisture for 3 h at 120ºC.

XX355 grain flour (BL, BK, SG, ST or WT) treated at 35% moisture for 5 h at 120ºC.

1. Introduction

Heat moisture treatment (HMT) is a physical modification that involves low moisture levels, usually in a restricted range of 10–35%, and heating at high temperatures (90–120 ◦C) for a period of time ranging from 15 min to 16 h (Chung et al., 2009), that allows control of molecular mobility at high temperatures by limiting the amount of water. HMT constitutes an environmentally friendly technique, of interest to make low glycaemic index foods without any chemical residue (Ye et al., 2016), and a clean label alternative to chemical modification for altering the gelatinization and retrogradation properties of starches from different sources (Gunaratne and Hoover, 2002). HMT causes the rearrangement of amylose and amylopectin chains in the starch, and therefore may modify its X-ray pattern, crystallinity, swelling power, amylose leaching, pasting, and gelatinization properties, as well as its susceptibility to enzymatic or acidic hydrolysis, which also affect the starch rheological properties (Zavareze and Dias, 2011) of cereal, legume, tuber and root starches. HMT-induced changes in starch are prominent and versatile, and closely dependent on the intrinsic starch characteristics (source, amylose content, amylopectin chain length) and on the processing HMT conditions (moisture content, heating temperature and time) (Lawal, 2005). Tuber starches are more sensitive to HMT than legume or cereal starches (Gunaratne & Hoover, 2002). Depending on the intensity of the process parameters, a reduction of microorganisms, the inactivation of enzymes or the modification of structural, physicochemical and nutritional properties of starches (Jacobs & Delcour, 1998; Chung et al., 2009), and the enhancement of nutritional properties (Satmalee and Matsuki, 2011) and shelf-life extension (Yadav et al., 2012) of starch-rich flours are achieved.

HMT significantly alters the pasting profile of starches from different sources -bambarra groundnut (Adebowale & Lawal, 2002), white sorghum (Olayinka et al., 2008), rice (Zavareze et al., 2010), corn (Chung et al., 2009), and canna starches (Watcharatewinkul et al., 2009)-, resulting in general in increased pasting temperature and decreased peak viscosity, final viscosity, and breakdown. Authors observed that pasting behaviour intensified as the moisture content of the HMT increased (Olayinka et al., 2008), and ascribed that changes to associations between the chains in the amorphous region of the granule as well to changes in crystallinity during hydrothermal treatment. A high paste temperature thus indicates that more forces and cross-links are present within the starch granules, and a reduction in breakdown demonstrates that starches are more stable during continuous heating and agitation (Adebowale et al., 2005). Gelatinized starch gels form thermodynamically unstable structures, which on cooling may result in reassociation of starch molecules leading to a partially crystalline structure, involving both amylose and amylopectin. During gelling, retrogradation is influenced by the amount of leached amylose, granule size, and the presence of rigid, non-fragmented swollen granules. HMT reduces amylose leaching from starch granules particularly in starches containing high levels of amylose, and promotes additional amylose–amylose and/or amylopectin–amylopectin chain interactions, which reduce amylose leaching and decrease retrogradation (Chung et al., 2009).

In contrast to the extensive knowledge on the significance of hydrothermal treatment of starches, only limited information is available about the impact of HMT of cereal and grain flours, despite the application of the treatment is known to improve their food end uses (Ye et al., 2016). The application of steam on wheat flour causes a more significant modification in the structure of wheat components: starch pre-gelatinization occurs and the gluten proteins suffer a nearly total loss of functional properties due to denaturation. Noodle end-product quality can also be improved with the usage of hydrothermally treated rice flour exhibiting gel forming properties and resistance to shear forces (Cham & Suwannaporn, 2010). HMT had a far greater effect on the solubility, swelling power, setback viscosity, through viscosity, enthalpy and crystallinity of sorghum flour than of sorghum starch. The results show that shear stability of the modified sample pastes are improved, the hardness of sorghum starch and sorghum flour gel are increased by HMT, and the retrogradation of the modified sample pastes are weakened by HMT, which are desirable in sorghum food products (Sun et al., 2014).

Despite starch is the major component controlling pasting properties of grain flours and subsequent impact on finished product performance (Collar, 2003; 2016; Waterschoot et al., 2015), viscosity properties as important indexes in determining the cooking and baking qualities of flours are also affected by other components in the system. In fact, compared effects of HMT on starch *vs* flour properties have been described for cowpea (Adebooye and Singh, 2008), rice (Puncha-arnon and Uttapap., 2013), sorghum (Sun et al., 2014) and wheat (Blazek and Copeland, 2008; Chen et al., 2015) revealing different pasting patterns for both matrices. Authors emphasized that components in flours other than starch granules underwent alteration during HMT with proteins playing an important role in change of properties in the modified grain flour samples. Endosperm protein may restrict the starch granules from fully gelatinizing, thereby resulting in lower digestibility, and starch-protein interaction may occur during cooking or cooling that causes gelatinized sorghum starch to be in a less digestible state (Zhang and Hamaker, 2005). The unusual high viscosity peak in the cooling stage of the rapid visco-analyzer (RVA) profile of stored whole grain sorghum flour was the result of starch interacting with liberated free fatty acids and flour protein (Zhang and Hamaker, 2005). Water-insoluble dietary fiber may cause disruption in the structure of amylopectin, resulting in an increase in the swelling power (Yildiz et al., 2013).

This paper is aimed a) at investigating the viscosity changes that occur during starch gelatinization, pasting and gelling in different grain flour (commercial barley, buckwheat, sorghum, high β-glucan barley and wheat) matrices with unrestricted water availability, b) at knowing the impact of HMT processing conditions (moisture content and heating time) on the visco-metric profiles of hydrated grain flours, and c) at classifying HMT flours according to their visco-metric profile during pasting and gelling.

2. Materials and methods

2.1. Materials

Commercial flours from refined common wheat *Triticum aestivum* (WT), and whole barley *Hordeum vulgare* L. (BL), buckwheat *Fagopyrum esculentum* (BK), and sorghum *Sorghum spp.* (SG) were purchased from the Spanish market. High β-glucan barley (ST) produced by ConAgra (USA) under the branded name of Sustagrain® (whole barley flour prepared in the grinding and bolting of varieties of cleaned waxy, hulless barley) was furnished by Ingredion Germany GmbH. Refined WT (70% extraction rate) of 356 x 10^{-4} J energy of deformation W, 0.64 curve configuration ratio P/L, 95% Gluten Index, 62% water absorption in Brabender Farinograph, was used.

2.2. Methods

Chemical and nutritional composition of flours

Moisture, protein, ash and fat contents of untreated and HMTcommercial flours were determined following the ICC methods (ICC, 1976-1996). Total, soluble and insoluble dietary fibre contents were determined according to the AOAC method 991.43 (AOAC, 1991). Resistant starch determination was performed according to AOAC Official Method 2002.02 (AOAC, 2000) by using Megazyme kit K-RSTAR 08/11. β-glucan content (Megazyme kit K-BGLU 07/11) was determined following the ICC Standard Method No. 166. Amylose/amylopectin ratio (Megazyme kit K-AMYL 07/11) was estimated by using a modification of a Con A method with lipid removal prior to analysis. Three replicates were made for each analysis. Digestible carbohydrates were calculated by indirect determination as 100 − [Moisture + Protein + Fat + Ash + Dietary Fibre] (FAO, 2003).

Heat-moisture treatment (HMT)

Single BL, BK, SG, ST and WT flour samples were weighed and placed into screw-capped glass containers. Small amount of distilled water was added slowly with frequent stirring until moisture levels (w/w) of the total mixture reached 15%, 25%, and 35% respectively, and equilibrated for 24 h at room temperature. The moisture content was measured using a moisture analyzer (DBS60-3, Kern, Balingen, Germany). Hydrated samples were kept for 1, 3 or 5 h at 120 ºC in a convection oven. After cooling to room temperature, the samples were dried at 40 ºC overnight to a constant weight, and then passed through 100-mesh sieve for further analysis. Untreated flours were used as controls. A total of 45 different HMT flours were obtained. A 5 digit HMT flours sample code was set referring to coded flours (BL, BK, SG, ST, WT), moisture content (15, 25, 35), and heating time (1, 3, 5). HMT was performed in duplicate.

Viscosity Properties

The pasting profiles (gelatinization, pasting, and setback properties) of untreated and HMT BL, BK, SG, ST and WT flours were obtained with a Rapid Visco Analyser, RVA (RVA-4, Newport Scientific, Warriewood, Australia) using ICC standard method 162. Individual flours (3.5 g, 14% moisture basis) were transferred into canisters and ≈25 ± 0.1 mL of distilled water were added (corrected to compensate for 14% moisture basis). Three replicates were made per sample. The slurry was heated to 50°C and stirred at 160 rpm for 10 sec for thorough dispersion. The slurry was held at 50°C for up to 1 min, and then heated to 95°C over 3 min 42 sec and held at 95°C for 2 min 30 sec, and finally cooled to 50°C over 3 min 48 sec, and held at 50°C for 2 min. The pasting temperature (°C) (when viscosity first increases by at least 25 mPa.s over a 20-s period), peak time (when peak viscosity occurred), peak viscosity (maximum hot paste viscosity), holding strength or trough viscosity (minimum hot paste viscosity), breakdown (peak viscosity minus holding strength or trough viscosity),

viscosity at 95°C (viscosity attained at the beginning of the holding period during cooking), viscosity at the end of the 95°C holding period, viscosity at 50°C (viscosity attained at the beginning of the holding period during cooling) , final viscosity (end of test after cooling to 50°C and holding at this temperature), setback (final viscosity minus peak viscosity), and total setback (final viscosity minus holding strength) were calculated from the pasting curve using Thermocline v. 2.2 software (Collar, 2003).

Statistical analysis

Multivariate analysis of variance of data was 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. Visco-metric transitions of cereal and pseudocereal flours.

Grain flours constitute natural and practical food systems for studying multiple food component interactions (Zhang and Hamaker, 2005), since besides starch, proteins, fat and dietary fibre are included in the chemical and nutritional composition of composite food matrices (Table 1). During the heating and holding stages of the RVA run of a flour suspension, gelatinization, pasting and breakdown take place successively.

Quantitative viscosity profiles of native non-WT flours -BL, BK, SG and ST- were lower (BL, BK, SG) or similar (ST) during cooking, and similar (BK, ST) or lower (BL, SG) during cooling as compared to those of WT flour counterparts (Figure 1). The RVA profile of non-WT flours was characterized by the presence of one maximum in the 95°C holding period representing starch gelatinization under a constant shear, small (BL, ST) or practically no breakdown of viscosity at the holding period (SG, BK), and a variable typical increase in setback viscosity on cooling varying from discrete (BL, BK), medium (ST) to prominent (SG) changes ascribed to the variable reassociation of constituent starch molecules, mainly amylose, into a more ordered state (Figure 1, Table 1). On the opposite, qualitative RVA profile of WT flour clearly defined a maximum viscosity during the holding period, and a subsequent breakdown on cooking, followed by a discreet setback on cooling, as reported earlier (Collar, 2003, 2006).

Minimum temperature required to cook a given sample can be determined by pasting temperature. In this study, the pasting temperature values of the non-WT flours were found to be lower (69.45-77.25ºC) except for BL (79.63ºC) compared to WT flour (78.38ºC), indicating that fewer associate forces and crosslinks are present within the starch granule of non-WT flours. Higher pasting peak viscosity of WT (3839 mPa.s) and ST flours (3651 mPa.s) *vs* non-WT flours BL, BK, and SG (215-2568 mPa.s) is attributed to the higher total digestible carbohydrates content (mainly starch) of WT (82 g/100 g flour, d.b.), and the high β-glucan content of ST (13.30 g/100 g flour, d.b.), respectively (Table 1). Non-starch components, and particularly lipids, proteins and dietary fibre could restrict swelling and gelatinization during cooking attributed, in addition to the diluting effect, to the interaction with starch polymers (lipids, proteins) and to the competition for water (proteins, dietary fibre) interfering starch swelling (Collar, 2016). BL, BK and SG with intermediate/high/low protein (12.92%, 19.71% and 10.34%) and high dietary fibre (17.40, 13.52, and 14.40%) contents, exhibiting higher fat (1.94%, 3.44%, 3.57%) and amylose contents (19%, 15%, 18.2%) respectively, developed significantly poorer maximum peak viscosity than WT flour (Table 1). The drastic differences observed in the pasting properties of sorghum starch and sorghum flour have been attributed to their amylose, lipid and protein contents (Sun et al., 2014). Authors stated that amylose acts both as a diluent and as an inhibitor of swelling, especially in the presence of lipids which can form insoluble complexes with some of the amylose during swelling and gelatinization. Formation of a layer of amylose–lipid complexes on the granule surface, development of a rigid network of intragranular amylose–lipid complex structures or creation of a lipid layer on the granule surface account for the lower water uptake through increased hydrophobicity. It has been claimed that protein components account for the differences in thermal and pasting properties of rice starch and rice flour (Puncha-arnon and Uttapap, 2013), by promoting restriction of the starch granules from fully gelatinizing (Zhang and Hamaker, 2005).

Breakdown as a measure of the ease which the swollen starch granule can be disintegrated, is an indication of the degree of its organization. Lower breakdown viscosity reflects high stability under heat and shear. This is especially true for non-WT flours BL, BK and SG (24-148 mPa.s) followed by ST (1208 mPa.s), while WT flour showed higher breakdown viscosity (1603 mPa.s) associated to an increased granule disruption and less tendency of starch to resist shear force during heating. Singh and Singh (2010) determined that breakdown viscosity showed strong negative relationship with protein content attributed to stabilization of continuous matrix or strengthening of the links between the dispersed and continuous phase. In good accordance except for SG flour, the protein level of each flour was in the order: ST>BK>WT>BL>SG (Table 1), and the relative breakdown on cooking followed BL>WT>ST>BK> SG (Table 1).

The setback viscosity value that is associated with the retrogradation and reordering of starch molecules is obtained during cooling, which appear to be related to the structure of amylose and amylopectin, since small amylose molecules and low-chain amylopectin molecules tend to be retrograded rapidly (Olayinka et al., 2008). Low setback values indicate low rate of starch retrogradation and syneresis. Formation of the cooling stage viscosity peak is particularly remarkable for BK and SG flours (Table 1, Figure 1), in accordance with their high lipid -3.44% BK, 3.57% SG- and protein -19.71% BK- contents (Table 1), that can easily interact resulting in both starch-lipid complexation and starch-protein-lipid complexation (Zhang and Hamaker, 2005). The sorghum protein matrix in the normal sorghum can act as a barrier and retard starch granule expansion (Ezeogu et al., 2008). This protein barrier was presumably the reason for the longer peak time (6.70min) and higher pasting temperature (77.25ºC). RVA profiles of BK and SG flours on the cooling stage were higher than those respectively described earlier (Yilmaz et al., 2015; Sun et al., 2014).

A result that was rather unexpected was that of BL1 (Figure 1, Table 1), it had the lowest viscosity measurements of all the samples. The commercial barley has medium/low rate of β-glucan (5.16%) and high level of fibres (17.40%). Even though it had almost the same amount of starch as BK and SG, no viscosity was created during the RVA, the sample did not gelatinize at all.

3.2. Effects of the flour type and hydrothermal conditions on the viscosity profile of hydrated HMT flours

Viscosity properties during cooking and cooling of HMT non-wheat (BL, BK, SG, ST) hydrated flours (Figure 1) exhibited with some exceptions a delayed and lower viscosity pattern during both pasting and gelling, as compared to native WT flour counterparts. Higher pasting viscosity profile of the wheat flour could be attributed to its higher total starch content because non-starch components could restrict swelling during pasting (Table 1), while the variable setback and final viscosity of HMT non-wheat flours reflect a variable extent of amylose leaching and complexation, since amylose contents are different (19% BL, 15% BK, 18% SG, 5% ST).

Flour type first, and HMT conditions –moisture content and heating time- in second place significantly ($p<0.05$) changed cooking and cooling viscosity parameters as individual design factors (Table 2), being RVA pasting and gelling profiles highly dependent on the interactions between flour type and moisture content (Table 3). An additional dependence of pasting on both flour/ heating time and moisture content/ heating time was observed (Table 3). From all the HMT flours, SG exhibited the highest pasting temperature (93.4ºC) and peak time (6.77 min), and the lower viscosities at both paste and gel states; whereas ST provided the lowest pasting temperature (77.3ºC) and the highest viscosity profile on both cooking and cooling stages (Figure 1, Table 2). The severity of applied HMT conditions affect the viscosity patterns of flours. Increased values of moisture content from 15% to 35% and/or prolonged heating time from 1 to 5 h in HMT flours provoked/encompassed gradual delayed pasting temperature and decreased viscosity values of treated samples at paste and gel states (Figure 1, Table 2). Lower paste viscosity and higher pasting temperature indicated that the starches were strengthened by HMT. The increase in pasting temperature after modification supported the fact that HMT process tended to increase the region of crystallinity due to reorientation of the starch granules (Sun et al., 2014). The compaction of granular matter by vapor pressure force, as well as chemical bonding and the interactions that occur during HMT, might also be factors that influence the stability of starches exposed to HMT (Puncha-arnon and Uttapap, 2013). The reduction in pasting viscosity of the HMT starch was caused by low restricted swelling capacity such that only a small amount of amylose was able to leach into the medium to elevate its viscosity. Breakdown practically disappeared, indicating that the starch became more stable when exposed to heat and mechanical shearing as previously found for different starches modified by HMT (Yadav et al., 2013). Breakdown may be influenced by the presence of rigid non-fragmented swollen granules, the granule size and the amount of leached amylose. Richer amylose flours (BL, SG) may increase amylose leaching extent, thus keeping developed viscosity during heating, and encompassing practically no breakdown (Figure 1).

Chung et al. (2009) and Zavareze et al. (2010) stated that the reduction of setback value because the treatment promoted additional interactions between amylose–amylose and ⁄ or amylopectin–amylopectin chain which reduce leached amylose content and lower setback. Induced changes by HMT processing conditions followed the same trend but in different extent, being those promoted by moisture content greater than those associated to heating time (Table 2). A larger extent of the changes was observed especially for peak viscosity (-78% vs -67%) and breakdown (-81% vs -77%) on pasting, and final viscosity (-67% vs -57%) and total setback (-58% vs -51%) on gelling (Table 2). Changes in RVA profiles associated to applied HMT conditions were flour type dependent, particularly for pasting parameters (Table 3). During HMT, protein bodies were deformed and denatured. Interactions that occurred between denatured proteins, and between proteins and starch granules, consequently caused the association of the protein networks with the surfaces of starch granules (Chen et al., 2015). These protein layers, in cooperation with the increased hydrophobicity, retarded and restricted the swelling of HMT starch granules in treated flours with a particular high protein content like BK and ST. Also, the high amount of dietary fibre, particularly insoluble fibres, in non-wheat flours (9.45-20%) compete for water with starch, leading to additional restriction of starch swelling.

It should be noted that at a milder HMT conditions (15% moisture content, 1 h heating time), except for SG, HMT flours reached much higher viscosity values during earlier pasting and subsequent gelling than the corresponding native counterparts (Figure 1.A.). Values for peak viscosity and total setback (mPa.s) respectively account for 3213 and 3646 *vs* 215 and 202 (BL), 3100 and 1418 *vs* 2568 and 1639 (BK), 5444 and 4052 *vs* 3651 and 2907 (ST), 4614 and 2654 *vs* 3839 and 2137 (WT). Similar pattern was reported by Ozawa et al. (2009) and by Bucsella et al. (2016) for wheat flour submitted to dry heat and/or mild hydrothermal treatments. Authors related the decrease in onset pasting time and increase in peak viscosity in the Amylograph 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 starch granule surface proteins 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 (Bucsella et al., 2016). Besides HMT wheat flour, described behaviour found also for treated BL, BK and ST flours has not been previously reported in the literarure. An analogous increased hydrophobicity of prolamins and glutelins in low moisture- short heating time HMT non wheat flours with high protein content (12.92%-19.95%) could explain the enhanced viscosity profile observed.

During HMT, the rearrangement of molecular chains formed ordered double helical amylopectin clusters. This rigid structure could limit starch swelling. The formation of starch-lipid complexes might be also responsible for the reduction of swelling (Olayinka et al., 2008). This may be the reason for the marked reduction in pasting viscosity for the HMT samples. In addition, the partial gelatinization of starch for 15%-HMT (Figure 1A) and 25%-HMT flours (Figure 1B) at prolonged heating times (3, 5h), particularly for BK and SG was responsible for the low viscosity profiles (Table 3). Viscosities of 35% of moisture (Figure 1C) could not be detected by RVA in BL, BK and SG because 35%-HMT samples did not gelatinize during heating, as previously reported for rice (Puncha-arnon and Uttapap, 2013) and sorghum (Sun et al., 2014). On the contrary, HMT WT and ST flours developed high final viscosites even at 35% of moisture, particularly at 1 h of heating time, supporting a high gelling ability and gel stability associated to an increased amylose leaching and promoted interactions between leached molecules and/or swollen granules (Collar, 2016).

The treatment time mainly affected the pasting properties for HMT samples with 15% and 25% of moisture, in such a way that the longer treatments promote more rearrangement in the starch granules (Figure 1, Table 3).

3.3. Classification of HMT flours according to their visco-metric profile during pasting and gelling

Classification of 90 HMT treated flours (5 flours x 3 moisture levels x 3 heating times made per duplicate) on the basis of their distinctive and significant responses in terms of 11 viscosity parameters during pasting and gelling was achieved per flour by means of multivariate data handling using Factor Analysis (FA). Effects of HMT conditions were better known per flour since the type of flour greatly affected viscosity profiles (Tables 2 and 3), and masked the impact of the moisture level and the heating time on the RVA profiles (Figure 2).

FA grouped visco-metric parameters into different factors with the first two factors explaining from 77% (SG) to 98% (ST) of the variability of the results (Figure 2A). Factor 1, which makes the highest contribution accounting from 59% (SG) to 80% (WT) of the total variance (VE), grouped main parameters during cooking, particularly peak viscosity, viscosity during the holding period, holding strength and pasting temperature, and a few during cooling, significant for samples developing quantitative gelling ability (ST, WT) even at severe HMT conditions (35%, 5 h) (Figure 1). Factor 1 correlated positively with major parameters during pasting and gelling except for the pasting temperature (BL, BK, WT). Factor 2 (11.00% -20%VE) included peak time exhibiting positive (BL, BK, SG) or negative correlations (ST, WT), and pasting temperature only in the case of SG (Figure 2A).

In general, HMT flours with the lower hydration (15%) presenting the higher viscosity values at both pasting and gelling states and the lower pasting temperatures were mainly located across the positive x-axis, following the increasing order SG, BL, BK, WT, and ST (Figure 2). Intermediate (25%) and higher (35%) hydrated samples were positioned around the central and negative x-axis, respectively, exhibiting intermediate and higher values for pasting temperature and intermediate and lower viscosity profiles at pasting and gelling states, respectively (Figure 2). Plots of scores of Factor 1 *vs* Factor 2, illustrating sample location in the scatterplots (Figure 2B) revealed a clear differentiation of HMT samples according to the treatment conditions for flours expliciting a gradual/progressive starch gelatinization with the severity of the HMT conditions. This is the case of

ST and WT treated flours in which the lower the moisture content and the shorter the heating time applied corresponded to the higher pasting and gelling profiles and lower pasting temperatures. With some overlapping, treated flours at 15% moisture were located in the area defined by the positive values of both x and y axes, while those treated at either 25% or 35% moisture were positioned in the area defined by the negative values of both *x* and *y* axes, expliciting poorer viscosity profiles and higher both pasting temperature (WT) and peak time (ST, WT). Visco-metric profiles of WT treated flours were more sensitive to heating time, allowing to distinguish samples at 15 and 25% moisture content on the basis of the duration of heating (Figure 2B).

HMT flours showing intermediate (BL, BK) or faster (SG) starch gelatinization with the intensity of the applied HMT conditions only differentiated within samples with developed quantitative viscosity from the rest: BL151, BL153, BL 155, BL251, BL253 for commercial barley BL; BK151, BK153, BK155, BK251, for buckwheat; and SG151, SG153, SG251, SG253 for sorghum (Figure 1A, 1B; Figure 2B).

4. Conclusions

Heat moisture treatment (HMT) processing conditions (15, 25, and 35% moisture content; 1, 3, and 5 h heating time at 120ºC) applied to different grain flours matrices (BL, BK, SG, ST, and WT), greatly impacted the trend and the extent of the visco-metric pasting and gelling profiles of highly hydrated (3.5:25, w:w), as compared to the respective untreated counterparts. Complex flour composition promotes interactions between starch and non-starch components –protein, lipids, dietary fibre- of different flours, leading to different visco-metric features during pasting and gelling of untreated flours. At a milder HMT conditions (15% moisture content, 1 h heating time), except for SG, HMT flours reached much higher viscosity values during earlier pasting and subsequent gelling than the corresponding native counterparts. Besides HMT wheat flour, described behaviour found also for treated BL, BK and ST flours has not been previously reported in the literature. An analogous increased hydrophobicity of prolamins and glutelins in low moisture- short heating time HMT non wheat flours with high protein content (12.92%-19.95%) could explain the enhanced viscosity profile observed.

Different kinetics of starch gelatinization during HMT of flours defined diverse RVA patterns of treated matrices. A clear differentiation of the visco-metric profiles of treated samples expliciting a gradual/progressive starch gelatinization with the severity of the processing conditions (ST, WT) was evidenced according to the treatment conditions for flours. The lower the moisture content and the shorter the heating time applied corresponded to the higher pasting and gelling profiles and lower pasting temperatures. Visco-metric profiles of WT treated flours were more sensitive to heating time, allowing to distinguish samples at 15 and 25% moisture content on the basis of the duration of heating. HMT flours showing intermediate (BL, BK) or faster (SG) starch gelatinization with the intensity of the applied HMT conditions differentiated in viscosity pattern from the rest only within samples with developed quantitative viscosity: 15% moisture/ 1, 3, 5h heating and 25% moisture/ 1, 3h heating for BL, BK and SG.

Acknowledgements The authors acknowledge the institutions Ministerio de Economía y Competitividad (MINECO) and Federación Europea de Desarrollo Regional (FEDER) for funding the Project AGL2015-63849-C2-1-R.

5. References

Adebooye, O. C., & Singh, V. (2008). Physico-chemical properties of the flours and starches of two cowpea varieties (Vigna unguicu-lata (L.) Walp). Innovative Food Science and Emerging Technologies, 9, 92–100.

Adebowale, K. O., & Lawal, O. S. (2002). Effect of annealing and heat moisture conditioning on the physicochemical characteristics of bambarra groundnut (*Voandzeia subterranea*) starch. Nahrung-Food, 46, 311–316.

Adebowale, K. O., Afolabi, T. A., & Olu-Owolabi, B. I. (2005). Hydrothermal treatments of Finger millet (Eleusine coracana) starch. Food Hydrocolloids, 19, 974–983.

AOAC. (1991). Total, soluble, and insoluble dietary fiber in foods. Association of Official Analytical Chemists.

AOAC. (2000). Official Methods of Analysis 17th Ed., AOAC International.

Blazek, J., Copeland, L., 2008. Pasting and swelling properties of wheat flour and starch in relation to amylose content. Carbohydr. Polym. 71, 380-387.

Bucsella, B., Takács, A., Vizer, V., Schwendener, U., Tömösközi, S. (2016). Comparison of the effects of different heat treatment processes on rheological properties of cake and bread wheat flours. Food Chemistry 190 (2016) 990–996.

Cham, S., Suwannaporn, P. (2010). Effect of hydrothermal treatment of rice flour on various rice noodles quality. Journal of Cereal Science, 51, 284–291.

Chen X, He X, Fu X and Huang Q. (2015). In vitro digestion and physicochemical properties of wheat starch/flour modified by heat-moisture treatment. Journal of Cereal Science 63: 109–115.

Chung, H. J., Liu, Q., & Hoover, R. (2009). Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. Carbohydrate Polymers, 75, 436–447.

Collar, C. (2003). Significance of viscosity profile of pasted and gelled formulated wheat doughs on bread staling. European Food Research and Technology, 216, 505-513.

Collar, C. (2016). Impact of visco-metric profile of composite dough matrices on starch digestibility and firming and retrogradation kinetics of breads thereof: additive and interactive effects of non-wheat flours. Journal of Cereal Science, 69, 32-39.

Ezeogu, L. I., Duodu, K. G., Emmambux, M. N., Taylor, J. R. N., (2008). Influence of cooking conditions on the protein matrix of sorghum and maize endosperm flours. Cereal Chemistry, 85, 397-402.

FAO/WHO (2003) Food energy-methods of analysis and conversion factors. FAO Food and Nutrition Paper 77, Rome.

Gunaratne, A., & Hoover, R. (2002). Effect of heat-moisture on the structure and physicochemical properties of tuber and root starches. Carbohydrate Polymers, 49, 425–437.

ICC (1976-1996). ICC Standard Methods of the International Association for Cereal Science and Technology 104/1, 105/2, 110/1, 115/1, 136, 162, 166. Vienna: The Association.

Jacobs H and Delcour JA. (1998). Hydrothermal modifications of granular starch, with retention of the granular structure:A review. Journal of Agricultural and Food Chemistry 46: 2895–2905.

Lawal OS. (2005). Studies on the hydrothermal modifications of new cocoyam (Xanthosoma sagittifolium) starch. International Journal of Biological Macromolecules 37: 268–277.

Olayinka, O. O., Adebowale, K. O., & Olu-Owolabi, B. I. (2008). Effect of heat moisture treatment on physicochemical properties of white sorghum starch. Food Hydrocolloids, 22, 225–230.

Ozawa, M., Kato, Y., & Seguchi, M. (2009). Investigation of dry-heated hard and soft wheat flour. Starch – Stärke, 61(7), 398–406.

Puncha-arnon, S., Uttapap, D., 2013. Rice starch vs. rice flour: differences in their properties when modified by heat-moisture treatment. Carbohydrate Polymers, 91, 85-91.

Satmalee, P., & Matsuki, J. (2011). Effect of debranching and heat-moisture treatment on the properties of Thai rice flours, rice flour. International Journal of Food Science and Technology, 12, 2628–2633.

Singh, S., Singh, N. (2010). Effect of debranning on the physico-chemical, cooking, pasting and textural properties of common and durum wheat varieties. Food Research International, 43, 2277–2283.

Sun, Q., Han, Z., Wang, L., and Xiong, L. (2014). Physicochemical differences between sorghum starch and sorghum flour modified by heat-moisture treatment. Food Chemistry, 145, 756–764.

Waterschoot, J., Gomand, S. V., Fierens, E., & Delcour, J. A. (2015). Starch blends and their physicochemical properties. Starch/Stärke, 67, 1–13.

Yadav,D. N., Kaur, J., Anand, T., Singh, A. K. (2012). Storage stability and pasting properties of hydrothermally treated pearl millet flour. International Journal of Food Science and Technology, 47, 2532–2537.

Yildiz, O., Yurt, B., Baştürk, A., Toker, O. S., Yilmaz, M. T., Karaman, S., Dağlıoğlu, O. (2013). Pasting properties, texture profile and stress–relaxation behavior of wheat starch/dietary fiber systems. Food Research International 53 (2013) 278–290.

Ye, X., Lu, F.,Yao, T., Gan, R., Sui, Z. (2016). Optimization of reaction conditions for improving nutritional properties in heat moisture treated maize starch. International Journal of Biological Macromolecules, 93, 34–40.

Zavareze, E. R., & Dias, A. R. G. (2011). Impact of heat-moisture treatment and annealing in starches: A review. Carbohydrate Polymers, 83, 317–328.

Zavareze, E. R., Storck, C. R., Castro, L. A. S., Schirmer, M. A., & Dias, A. R. G. (2010). Effect of heat-moisture treatment on rice starch of varying amylose content. Food Chemistry, 121, 358–365.

Zhang, G., Hamaker, B. R. (2005). Sorghum (*Sorghum bicolor* L. Moench) Flour Pasting Properties Influenced by Free Fatty Acids and Protein. Cereal Chemistry, 82, 534–540.

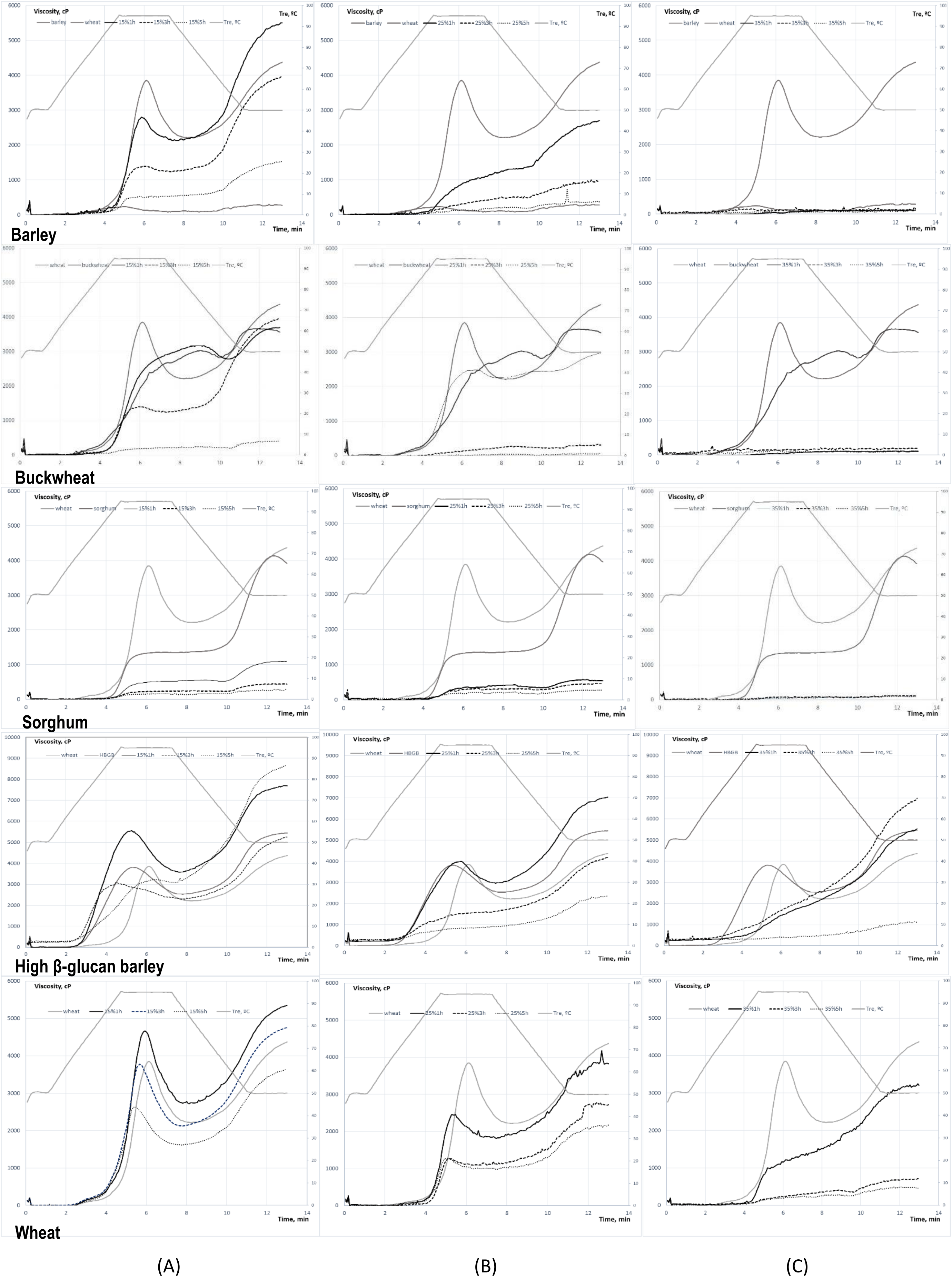
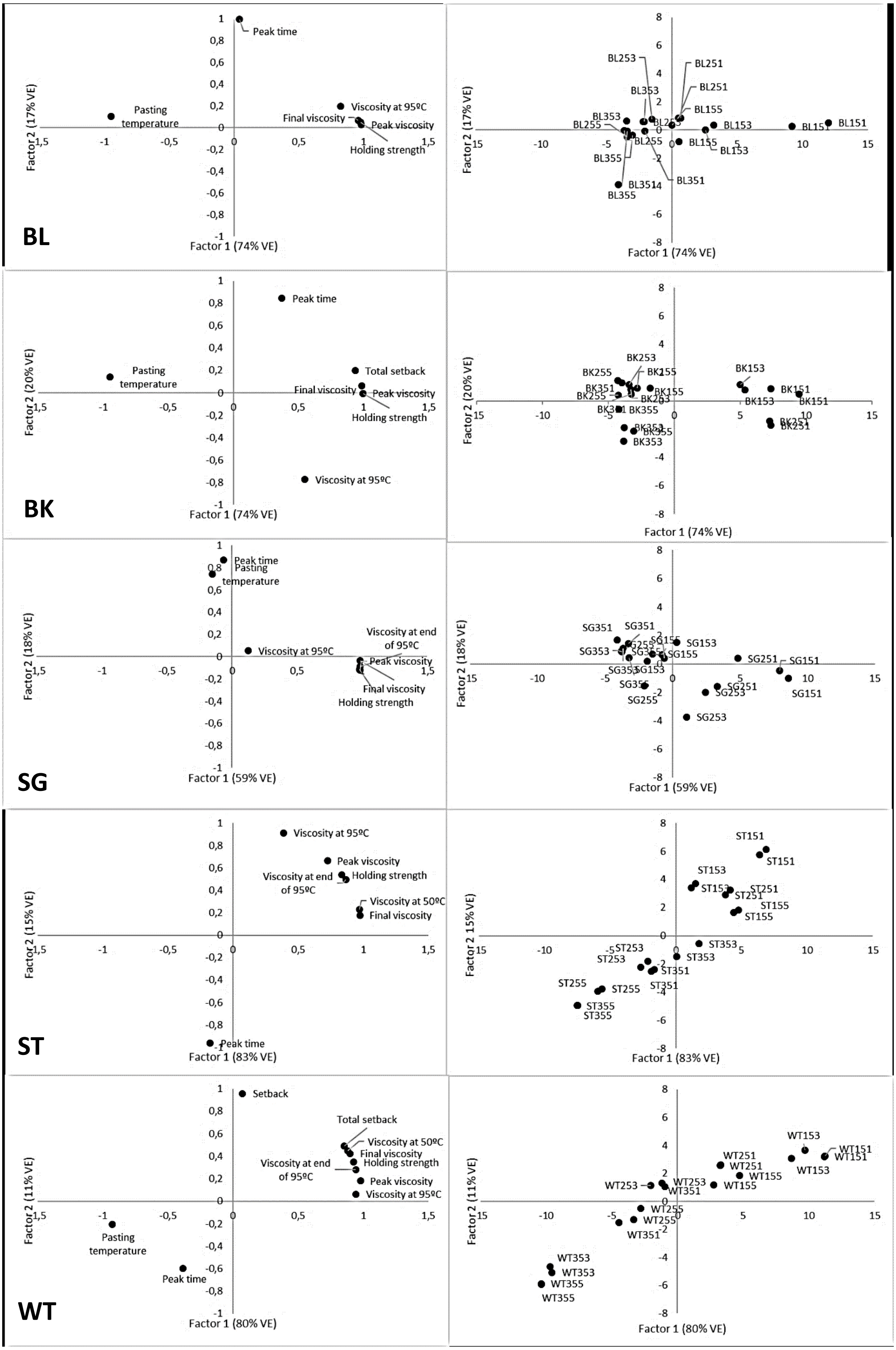


Figure 1.- Comparative visco-metric profiles of untreated barley, buckwheat, sorghum, high β-glucan barley (HBGB) and wheat flours, and flours submitted to HMT at 120ºC for 1, 3 and 5 h at moisture contents of 15% (A), 25% (B) and 35% (C).



(A) (B)

Figure 2.- Scatterplots from factor analysis (Factor 1 *vs* Factor 2) of visco-metric parameters of HMT flours (A) and classification of treated flour samples (B). Coded samples are flours: barley BL, buckwheat BK, sorghum SG, high β-glucan barley ST, and wheat WT; moisture (%): 15, 25, and 35; heating time (h): 1, 3, and 5.

Table 1.- Proximate chemical and nutritional composition and visco-metric parameters of native flours.

Parameter[a]	Flour				
	Barley	Buckwheat	Sorghum	High β-glucan barley	Wheat
Chemical and nutritional (g/ 100 g flour, d. b.)					
Protein[1]	12.92 ± 0.34 b	19.71 ± 0.06 d	10.34 ± 0.07 a	19.95 ± 0.23 d	14.13 ± 0.05 c
Total dietary fibre	17.40 ± 1.50 c	13.52 ± 0.38 b	14.40 ± 0.42 b	35.00 ± 2.60 d	2.19 ± 0.12 a
Insoluble dietary fibre	11.53 ± 1.09 d	6.58 ± 0.25 b	9.45 ± 0.30 c	20.17 ± 1.44 e	1.20 ± 0.09 a
Soluble dietary fibre	5.91 ± 0.28 c	6.93 ± 0.36 d	4.95 ± 0.50 b	14.95 ± 0.33 e	0.99 ± 0.25 a
Resistant starch	4.84 ± 1.22 b	7.83 ± 0.28	7.95 ± 0.06	8.33 ± 1.42 c	2.05 ± 0.26 a
β-glucans	5.16 ± 0.17 b	1.12 ± 0.12	0.059 ± 0.02	13.30 ± 0.71 c	0.23 ± 0.11 a
Fat	1.94 ± 0.11 b	3.44 ± 0.18 c	3.57 ± 0.02 c	5.87 ± 0.09 d	1.56 ± 0.09 a
Ash	1.74 ± 0.07 c	2.05 ± 0.19 d	1.48 ± 0.03 b	2.00 ± 0.08 d	0.63 ± 0.09 a
Digestible carbohydrates*	66	61	70	38 a	82
Amylose/ amylopectin ratio	29/71 d	24/76 bc	26/74 c	14/86 a	23/77 b
Moisture	12.80 ± 0.10 d	11.70 ± 0.30 c	11.10 ± 0.30 b	8.30 ± 0.10 a	14.32 ± 0.10 e
Visco-metric					
Pasting Temperature, ºC	79.6 ± 1.6 d	74.2 ± 0.1 b	77.2 ± 0.0 c	69.5 ± 0.1 a	78.3 ± 0.0 d
Peak viscosity, mPa.s	215 ± 22 a	2568 ± 30 c	1313 ± 60 b	3651 ± 223 d	3839 ± 16 d
Peak Time, min	4.93 ± 0.37 a	7.00 ± 0.00 d	6.70 ± 0.24 c	5.30 ± 0.04 a	6.10 ± 0.04 b
Holding strength, mPa.s	67 ± 10 a	1936 ± 74 c	1289 ± 57 b	2444 ± 120 e	2236 ± 35 d
Breakdown, mPa.s	148 ± 12 b	633 ± 104 c	24 ± 4 a	1208 ± 104 d	1603 ± 51 e
Viscosity at 95ºC, mPa.s	163 ± 32 b	288 ± 30 d	52 ± 11 a	2109 ± 264 e	241 ± 1 c
Viscosity at end of 95ºC, mPa.s	87 ± 18 a	2594 ± 6 c	1313 ± 58 b	2634 ± 117 cd	2673 ± 6 d
Setback, mPa.s	54 ± 14 a	1006 ± 11 c	2455 ± 159 e	1700 ± 94 d	534 ± 18 b
Viscosity at 50ºC, mPa.s	211 ± 38 a	3415 ± 95 c	2909 ± 40 b	4495 ± 211 d	3391 ± 26 c
Final Viscosity, mPa.s	269 ± 9 a	3574 ± 18 b	3767 ± 219 b	5351 ± 129 d	4372 ± 1 c
Total setback, mPa.s	202 ± 5 a	1639 ± 93 b	2478 ± 163 d	2907 ± 10 e	2137 ± 33 c

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

(*) Digestible carbohydrates calculated by indirect determination = 100 − [Moisture + Protein + Fat + Ash + Dietary Fibre]

(1) Conversion Factor from N to protein = 6.25 except for wheat flour = 5.70.

Table 2.- Significant single effects of design factors (flour type, moisture content and heating time) on the viscometric parameters of HMT treated flour matrices during cooking and cooling cycles. Levels of design factors were for moisture (%, flour basis): 15, 25, 35; for heating time (h): 1, 3, 5.

Parameter	Unit	Overall mean	FLOUR			MOISTURE, %			TIME, h		
			Level	mean	p<0.05	Level	mean	p<0.05	Level	mean	p<0.05
Cooking											
PastingTre	ºC	87.1	barley	90.6	c	15	81.4	a	1	84.0	a
			buckwheat	90.0	c	25	86.9	b	3	88.3	b
			sorghum	93.4	d	35	93.2	c	5	89.1	b
			High β-glucan barley	77.3	a						
			wheat	84.4	b						
SE				0.67			0.52			0.52	
Peak viscosity	cP	1305	barley	815	b	15	2292	c	1	2048	c
			buckwheat	985	b	25	1127	b	3	1184	b
			sorghum	227	a	35	496	a	5	683	a
			High β-glucan barley	2547	d						
			wheat	1949	c						
SE				97			75			75	
Holding strength	cP	993	barley	622	b	15	1674	c	1	1508	c
			buckwheat	840	b	25	928	b	3	915	b
			sorghum	196	a	35	377	a	5	555	a
			High β-glucan barley	2009	d						
			wheat	1298	c						
SE				90			70			70	
			barley	5.99	a	15	ns		1	ns	
Peak time	min	6.24	buckwheat	6.17	a	25			3		
			sorghum	6.77	b	35			5		
			High β-glucan barley	6.29	ab						
			wheat	5.98	a						
SE				0.17							
Breakdown	cP	312	barley	194	b	15	618	c	1	541	c
			buckwheat	147	b	25	200	b	3	269	b
			sorghum	31	a	35	118	a	5	126	a
			High β-glucan barley	538	c						
			wheat	649	d						
SE				31			24			24	
Viscosity at 95ºC	cP	386	barley	75	a	15	669	c	1	514	c
			buckwheat	95	a	25	331	b	3	411	b
			sorghum	16	a	35	157	a	5	232	a
			High β-glucan barley	1519	c						
			wheat	224	b						
SE				43			34			34	
Viscosity at end of 95ºC	cP	1089	barley	688	b	15	1811	c	1	1669	c
			buckwheat	960	b	25	983	b	3	1010	b
			sorghum	218	a	35	475	a	5	589	a
			High β-glucan barley	2165	d						
			wheat	1416	c						
SE				96			75			75	
Cooling											
Setback	cP	1058	barley	958	b	15	1480	b	1	1274	b
			buckwheat	305	a	25	935	a	3	1143	ab
			sorghum	155	a	35	761	a	5	758	a
			High β-glucan barley	2930	c						
			wheat	944	b						
SE				186			144			144	
Viscosity at 50ºC	cP	1920	barley	1428	b	15	3054	c	1	2763	c
			buckwheat	1053	b	25	1696	b	3	1824	b
			sorghum	329	a	35	1011	a	5	1174	a
			High β-glucan barley	4380	d						
			wheat	2411	c						
SE				208			161			161	
Final	cP	2364	barley	1773	c	15	3771	c	1	3325	c
			buckwheat	1296	b	25	2065	b	3	2327	b
			sorghum	382	a	35	1254	a	5	1439	a
			High β-glucan barley	5477	e						
viscosity			wheat	2890	d						
SE				257			199			199	
Total	cP	1372	barley	1152	b	15	2098	b	1	1819	c
			buckwheat	458	a	25	1139	a	3	1412	b
			sorghum	187	a	35	879	a	5	884	a
			High β-glucan barley	3468	c						
Setback			wheat	1594	b						
SE				178			138			138	

SE: standard error. ([1]) Within parameters, mean values with different following letter do differ significantly from each other ($p < 0.05$).

Table 3.- Significant ($p<0.05$) 2nd order interactions of design factors -flour type, moisture content and heating time- on the pasting and gelling parameters of HMT treated flour matrices. Levels of design factors were for flour: commercial barley (BL), Buckwheat (Bk), Sorghum (SG), High-β-glucan barley (ST) and Wheat (WT); for moisture (%, flour basis): 15, 25, 35; for heating time (h): 1, 3, 5.

Level	Pasting temperature, ºC	Peak time, min	Peak viscosity, mPa.s	Holding strength, mPa.s	Breakdown, mPa.s	Viscosity at 95ºC, mPa.s	Viscosity at end of 95ºC, mPa.s	Setback, mPa.s	Viscosity at 50ºC, mPa.s	Final viscosity, mPa.s	Total setback, mPa.s
FLOUR X MOISTURE (%)											
BL x 15	82.3 b	5.89 ab	1716 cd	1372 cd	344 b	110 a	1418 c	2147 b	3055 c	3863 d	2491 c
BL x 25	94.4 cd	6.58 b	579 ab	456 ab	123 a	52 a	554 ab	782 a	1127 b	1361 b	905 b
BL x 35	95.0 d	5.49 a	151 a	38 a	114 a	64 a	91 a	-55 a	103 a	97 a	59 a
Bk x 15	86.7 c	6.99 b	1884 cd	1619 cd	265 b	78 a	1891 d	719 a	2105 bc	2602 c	984 b
Bk x 25	88.3 c	6.57 b	901 b	806 b	103 a	103 a	866 b	196 a	904 ab	1114 ab	317 ab
Bk x 35	95.0 d	4.95 a	170 a	97 a	73 a	106 a	122 a	1 a	151 a	171 a	73 a
SG x 15	93.5 cd	6.90 b	300 a	277 ab	23 a	7 a	296 ab	318 a	508 ab	618 a	341 ab
SG x 25	92.3 cd	6.52 b	295 a	253 ab	42 a	32 a	277 ab	121 a	374 ab	416 a	163 ab
SG x 35	94.2 cd	6.89 b	86 a	57 a	29 a	7 a	81 a	27 a	103 ab	113 a	56 a
ST x 15	70.6 a	5.40 a	3944 e	2980 e	964 c	2693 d	3101 f	3300 c	5945 d	7244 e	4265 d
ST x 25	71.9 a	6.47 b	2177 d	1794 d	383 b	1300 c	1863 cd	2378 bc	3622 c	4555 d	2761 c
ST x 35	89.2 c	6.99 b	1521 c	1253 c	268 b	564 b	1532 c	3111 bc	3571 c	4631 d	3378 c
WT x 15	73.6 a	5.68 a	3615 e	2121 d	1493 d	458 a	2349 e	916 a	3655 c	4530 d	2409 c
WT x 25	87.2 c	5.37 a	1682 cd	1331 c	351 b	170 a	1353 c	1196 a	2451 bc	2878 c	1547 bc
WT x 35	92.2 cd	6.89 b	551 ab	441 ab	104 a	44 a	547 ab	720 a	1128 b	1261 ab	827 ab
SE	1.17	0.30	169	157	53	75	167	323	360	444	309
FLOUR X TIME (h)											
BL x 1	87.2 d	5.52 a	1520 c	1112 b	408 b	96 a	1241 bc	ns	ns	ns	ns
BL x 3	92.5 e	6.57 ab	667 ab	542 ab	125 a	73 a	576 ab				
BL x 5	91.9 e	5.87 a	259 a	211 a	48 a	57 a	246 a				
Bk x 1	83.5 cd	6.81 b	1895 c	1657 c	245 ab	150 a	1860 cd				
Bk x 3	92.2 e	5.73 a	874 b	732 b	142 a	85 a	859 b				
Bk x 5	94.2 e	5.96 a	186 a	132 a	54 a	51 a	160 a				
SG x 1	94.0 e	6.79 b	343 a	308 ab	35 a	16 a	341 ab				
SG x 3	93.1 e	6.68 b	192 a	163 a	29 a	1 a	179 a				
SG x 5	93.0 e	6.85 b	146 a	115 a	31 a	30 a	134 a				
ST x 1	73.8 a	5.97 a	3746 e	2653 d	1093 e	2059 d	2880 e				
ST x 3	77.5 b	6.13 ab	2371 d	1941 c	431 b	1670 c	2140 d				
ST x 5	80.4 bc	6.76 b	1524 c	1433 bc	91 a	827 b	1476 c				
WT x 1	81.1 c	6.12 ab	2735 d	1811 c	925 d	247 b	2024 cd				
WT x 3	86.1 d	5.88 a	1816 c	1197 b	618 c	227 a	1294 bc				
WT x 5	85.8 d	5.93 ab	1297 c	885 b	405 b	197 a	931 b				
SE	1.2	0.30	169	157	53	75	167				
MOISTURE (%) X TIME (h)											
15 x 1	ns	ns	3381 d	2349,5 f	1031 e	868 d	2595 d	ns	ns	ns	ns
15 x 3			2149 c	1568,6 e	580 d	730 d	1700 c				
15 x 5			1345 b	1103,1 d	242 b	410 b	1138 b				
25 x 1			2106 c	1680,5 e	429 c	545 bc	1779 c				
25 x 3			793 a	699,7 bc	94 a	275 ab	738 a				
25 x 5			481 a	403,4 ab	78 a	173 a	431 a				
35 x 1			657 a	494,2 ab	162 ab	128 a	634 a				
35 x 3			609 a	476,9 ab	133 ab	229 a	591 a				
35 x 5			221 a	159,7 a	58 a	114 a	199 a				
SE			131	122	41	58	129				

SE: Standard error. (1) Within column parameters of each interaction, mean values with different following letter do differ significantly from each other ($p < 0.05$).