Impact of visco-metric profile of composite dough matrices on starch digestibility and firming and retrogradation kinetics of breads thereof: additive and ~~non-additive~~ **interactive** effects of non-wheat flours.

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Abstract The impact of wheat (WT) flour replacement up to 45% (weight basis) by incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) flours on the viscometric pasting and gelling profiles of quaternary blended dough matrices was investigated by applying cooking and cooling cycles to rapid viscoanalyser (RVA) canisters with highly hydrated samples (3.5:25, w:w). Viscometric cooking and cooling parameter trends related to suitable patterns for lower and slower starch hydrolysis, and lower and/or slower firming and starch retrogradation kinetics in blended breads mainly include higher viscosity values for peak viscosity, breakdown on cooking and viscosity of hot (95°C) paste, but lower viscosity values after gelling (50°C). These visco-metric requirements for achieving suitable textural and thermal features in blended breads, were met by adding T/GP/BW to replace 22.5% of WT flour in blended dough formulations. Larger WT flour replacement by 37.5% of the ternary mixture T/GP/BW (7.5/15/15) provided hydrated blends with higher values for viscosity of hot (95°C) paste, and lower viscosity values after gelling (50°C), in good accordance with poorer formation of rapidly digestible starch and total digestible starch, and more prominent formation of resistant starch and slowly digestible starch in breads, respectively.

49	Abbreviations and symbols
50	BW buckwheat
51	C_{∞} maximum starch hydrolysis extent
52	DS digestible starch
53	GP green pea
54	H_{90} starch hydrolysis extent at 90 min
55	ΔH_0 retrogradation enthalpy at 0 time
56	ΔH_{∞} retrogradation enthalpy at ∞
57	ΔH_d enthalpy of dissociation of amylose-lipid complex
58	ΔH_g gelatinization enthalpy
59	k kinetic constant for starch hydrolysis
60	k_f constant of proportion of firming kinetics
61	k_r constant of proportion of retrogradation kinetics
62	n_f Avrami exponent of firming kinetics
63	n_r Avrami exponent of retrogradation kinetics
64	R gelatinization temperature range
65	RDS rapidly digestible starch,
66	RS resistant starch
67	RVA rapid visco analyzer
68	SDS slowly digestible starch

69 TDF total dietary fibre

70 T teff

71 T_{∞} crumb firmness at time ∞

72 T_0 crumb firmness at time 0

73 TS total starch

74 WT wheat

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1. Introduction

Changes in the viscosity of highly hydrated starch-based systems such as doughs during baking are known to affect the viscoelastic behaviour, the texture, keepability and nutritional performance of finished bread (Collar, 2003; Roder et al., 2005; Waterschoot et al., 2015). Pasting performance of flours during cooking and cooling involves many processes such as swelling, deformation, fragmentation, and solubilisation that occur in a very complex media whose viscoelastic properties in the pasted and gelled states are governed primarily by the volume occupied by the swollen particles.

Starch is the major component controlling pasting properties of grain flours and subsequent impact on finished product performance, but **visco-metric** properties are also affected by other components in the system. 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). Inhibition of enzymic digestion of amylose by free fatty acids *in vitro* contributes to resistant starch formation (Crowe et al., 2000). 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, so that, the disruption of the starch matrix and the uneven distribution of water within the matrix takes place due to the competitive hydration tendency of the insoluble dietary fibers. This probably allows starch granules to rupture during heating, hence releasing higher levels of amylopectin into the system, finally giving rise to a highly porous structure of starch with disrupted amylopectin (Yildiz et al., 2013).

The extent of gelatinization and retrogradation are major determinants of the susceptibility of starch to enzymatic digestion and its functional properties for food processing such as stickiness, ability to absorb water and ageing (Wang and Copeland, 2013). In starch blends, gelatinization occurs mostly independently

in excess water, while at intermediate water content more non-additive behavior is recorded. Pasting, rheological, and textural properties show primarily non-additive effects while retrogradation of starch blends occurs mainly in an additive way (Waterschoot et al., 2015). Interactive effects have been attributed to large differences in granule size and swelling power between the starches in a blend leading to uneven moisture distribution during heating of the starch suspension. This results in a different behavior of the blend than what would be expected based on the behavior of the individual starches (Waterschoot et al., 2015).

In blended flour matrices, high wheat flour replacement by non-gluten forming flours from cereals, pseudocereals and legumes, particularly associated mixtures of teff, buckwheat and green pea, have proven to provide technologically viable and acceptable sensory rated multigrain breads with superior nutritional value compared to the 100% wheat flour counterparts (Collar et al., 2014a). Thermal transitions of multicomponent bread matrices baked at restricted water conditions have recently been described (Collar et al., 2015), and some relationships between thermal properties, textural behaviour and the susceptibility of starch to enzymatic digestion in those heterogeneous matrices defined.

This paper is aimed a) at investigating the visco-metric changes that occur during starch gelatinization, pasting and gelling in complex grain flour matrices with unrestricted water availability, b) at knowing the impact of non-breadmaking whole grains (teff, green pea and buckwheat flours), highly replacing wheat-based matrices on the viscometric profiles, and c) at exploring the relationships between visco-metric properties and starch digestibility and firming and retrogradation kinetics of technologically viable and sensorially accepted multigrain bread matrices.

2. Materials and methods

2.1. Materials

Commercial flours from refined common wheat *Triticum aestivum* (WT), and whole teff *Eragrostis tef* (T), green pea *Pisum sativum* (GP), and buckwheat *Fagopyrum esculentum* (BW) were purchased from the Spanish market. Proximate chemical and nutritional composition of flours (Table 1) were determined following the ICC methods (ICC, 2014). 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. Ireks Vollsauer sour dough was from Ireks (Spain); Novamyl 10000 a maltogenic thermostable α -amylase of 10,000 Maltogenase Units (MANU) of activity, from Novozymes (Denmark); and calcium propionate, from Sigma-Aldrich (USA).

2.2. Methods

Bread making of wheat and wheat-based blended flours

Doughs and breads were prepared from WT as control, and wheat-based blended flours (T, GP, BW) by WT replacement from 22.5% up to 45%, and incorporation of ternary blends of T, GP and BW flours according to a Multilevel Factorial Design (Statgraphics Centurion XV, version 15.2.11, Statpoint Technologies, Inc. Warrenton, Virginia, USA) with the following attributes: 3 experimental factors (T, GP and BW flours) at 2 levels, coded 0 (7.5% wheat flour replacement) and 1 (15% wheat flour replacement), and 5 error degrees of freedom. Levels of wheat flour replacement were chosen after performing preliminary trials to set the range of non-wheat flours to be incorporated in associated blends to the formulations in such a way that significant enhancement of bread nutritional properties was achieved without notable deterioration of sensory attributes (Collar et al., 2014a). The model resulted in 8 randomized runs in 1 block. A 3 digit bread sample code was set referring to low (0) and high (1) wheat flour replacement by T (1st digit), GP (2nd digit), and BW (3rd digit) flours in sample formulation, as it follows: 010, 001, 011, 000, 111, 101, 100, 110. Blended flours, water, commercial compressed yeast, salt, margarine, sugar, commercial sour dough, milk powder, Novamyl 10000, and calcium propionate were mixed, and used to make control and blended

breads according to the quantitative formulations and breadmaking procedure described earlier (Collar et al., 2014a). Bread samples were stored in co-extruded polypropylene bags and stored for 1, 3, 6, and 8 days at 25 °C until performance of firming kinetics studies.

Dough measurements

Visco-metric Properties

The pasting profiles (gelatinization, pasting, and setback properties) were obtained with a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) using ICC standard method 162. Freeze-dried composite dough blends and individual flours (3.5 g, 14% moisture basis) were transferred into canisters and $\approx 25 \pm 0.1$ mL of distilled water were added (corrected to compensate for 14% moisture basis). Three replicates were made per sample. The slurry was heated to 50°C and stirred at 160 rpm for 10 sec for thorough dispersion. The slurry was held at 50°C for up to 1 min, and then heated to 95°C over 3 min 42 sec and held at 95°C for 2 min 30 sec, and finally cooled to 50°C over 3 min 48 sec, and held at 50°C for 2 min. The pasting temperature (°C) (when viscosity first increases by at least 25 mPa.s over a 20-s period), peak time (when peak viscosity occurred), peak viscosity (maximum hot paste viscosity), holding strength or trough viscosity (minimum hot paste viscosity), breakdown (peak viscosity minus holding strength or trough viscosity), viscosity at 95°C (viscosity attained at the beginning of the holding period during cooking), viscosity at the end of the 95°C holding period, viscosity at 50°C (viscosity attained at the beginning of the holding period during cooling), final viscosity (end of test after cooling to 50°C and holding at this temperature), setback (final viscosity minus peak viscosity), and total setback (final viscosity minus holding strength) were calculated from the pasting curve using Thermocline v. 2.2 software (Collar, 2003).

Bread measurements

Starch digestibility

In vitro starch hydrolysis kinetics and relevant starch fractions in freeze-dried and ground fresh blended breads was determined following the AACC (2005) method 32-40, adapted by Angioloni & Collar (2011) as previously described (Collar et al., 2014a). Rapidly Digestible Starch (RDS) and Slowly Digestible Starch (SDS) were measured after incubation for 20 min and 120 min, respectively (Englyst et al., 2003). Digestible starch (DS) was calculated by the sum of RDS and SDS. Total starch (TS) was calculated by the sum of DS and RS. A first order kinetic equation $[C = C_{\infty} (1 - e^{-kt})]$ was applied to describe the kinetics of starch hydrolysis, where C , C_{∞} and k were the hydrolysis degree at each time, the maximum hydrolysis extent and the kinetic constant, respectively. Resistant starch (RS) determination was performed according to AOAC Official Method 2002.02 (AOAC, 2000) and AACC Method 32-40 (AACC, 2005), as described by Megazyme International Ireland (2011) in the Resistant Starch Assay Procedure (kit K-RSTAR 08/11).

Textural measurements and firming kinetics

Bread mechanical characteristics (TPA in a double compression cycle) of fresh and stored breads were recorded in a TA-XTplus texture analyser (Stable Micro Systems, Surrey, UK) using a 25 mm diameter cylindrical aluminium probe, a 5 kg load cell, 50% penetration depth at a running speed of 1mm/s, and a 30 s gap between compressions on crust-free slices of 25 mm width (Armero & Collar, 1998). For textural measurements, three slices of two breads were used for each sample. The obtained firming curves during bread storage were modelled using the Avrami equation, and model factors were estimated by fitting experimental data of hardness to the nonlinear regression equation $\theta = \frac{T_{\infty} - T_t}{T_{\infty} - T_0} = e^{-kt^n}$ where θ is the fraction of the recrystallisation still to occur; T_0 , T_{∞} and T_t are crumb firmness at time zero, ∞ and time t , respectively, k is a rate constant, and n is the Avrami exponent.

Thermal measurements and retrogradation kinetics

Thermal properties regarding starch retrogradation of control and blended samples were assessed in a Differential Scanning Calorimeter Perkin-Elmer DSC-7 (Norwalk, USA) as previously reported by Collar et al. (2015). Stored gelatinized dough samples were submitted to a second DSC scan to analyze starch retrogradation at the different storage periods. Scanning conditions included keeping sample pans at 25°C for 1 min, and then heating from 25 to 130°C at a rate of 10°C/min. The enthalpy of amylopectin retrogradation (ΔH_r) was calculated, and T_o , T_p , and T_e for the thermal transitions, identified. All samples were analyzed in duplicate. Modelling of crystallization data was carried out using the Avrami equation, and model factors were estimated by fitting experimental data for melting enthalpies to the nonlinear regression equation $\theta = \frac{H_\infty - H_t}{H_\infty - H_0} = e^{-kt^n}$ where θ is crystallinity, H_∞ is the levelling-off value of melting enthalpy at which the extent of crystallization in starch stopped, H_t is the melting enthalpy at time t , and H_0 is the melting enthalpy at initial time, t is time of crystallization, k is a rate constant, and n is the Avrami exponent.

Statistical analysis

Multivariate analysis of variance and correlation matrix of data 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

The visco-metric transitions (Figure 1) of composite dough matrices are explored first and compared to those of WF flour counterparts (Table 2), prior to assess the single and interactive effects of the non-wheat flours T, GP and BW on the viscosity profile of doughs (Table 3 and Table 4), and to correlate the pasting and gelling parameters with the firming, starch retrogradation and starch hydrolysis kinetics of blended breads (Table 5).

3.1. Visco-metric transitions of WT and non-WT flours.

During the heating and holding stages of the RVA run of a flour suspension, gelatinization, pasting and breakdown take place successively. The gelatinization process usually refers to increased starch swelling, loss of order, and the disruption of hydrogen bonds at the molecular level. The subsequent loss in structural integrity of the granules, leaching of polysaccharide and increased viscosity of starch-water solutions is referred to as pasting (Roder et al., 2005). Subsequent granule disruption as a consequence of the variable tendency of starch to resist shear force during heating with a concomitant loss of viscosity is called breakdown. When gelatinised starch cools, the molecules begin to reassociate into an ordered structure, and undergo retrogradation. Grain flours are natural and practical food system for studying multiple food component interactions (Zhang and Hamaker, 2005). Quantitative visco-metric profiles of non-WT flours -T, GP and BW- were lower during cooking and higher or lower during cooling as compared to those of WT flour counterparts (Figure 1.A). 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 (T) or practically no breakdown of viscosity at the holding period (GP, BW), and a variable typical increase in setback viscosity on cooling caused by the reassociation of constituent starch molecules, mainly amylose, into a more ordered state (Figure 1.A, Table 2). 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).

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 (71.9-78.75°C) compared to WT flour (81.08°C). Higher pasting peak viscosity of WT flour (2681 mPa.s) vs non-WT flours (1050-2394 mPa.s) is attributed to the higher total starch content of WT, because non-starch components (Chung and Liu, 2012), and particularly lipids for smaller starch granules (Jenkins and Donald, 1995) could

253 restrict swelling and gelatinization during cooking. Most pulse starches exhibit a high pasting temperature,
254 the absence of peak viscosity, increasing viscosity during the holding period and a high set-back, mainly
255 associated to strong interaction between starch chains (amylose–amylose and/or amylose–amylopectin)
256 within the native granule and to the orientation of amylose chains relative to one another (Hoover et al.,
257 2010). The GP flour showed a pasting profile characterized by restricted swelling (maximum viscosity: 1050
258 mPa.s), low breakdown (41 mPa.s), and moderate setback (614 mPa.s) during the cooling period (Figure
259 1.A). Differences between pea starch and GP flour might be due to lower starch content in the flour and to
260 the relevant presence of protein (25.12%), and fiber (14.56%) (Table 1), as found/stated earlier (Chung and
261 Liu, 2012). T and BW with intermediate high protein (13.05% and 19.71%) and dietary fibre (12.19% and
262 13.52%) contents, but higher fat levels (5.06% and 3.44%) respectively (Table 1), developed superior
263 maximum peak viscosity (1983mPa.s and 2394mPa.s) than GP flour, but lower than WT flour (Table 2). The
264 reduced paste peak viscosity after pasting cereal starches in presence of added fatty acids was ascribed to
265 interaction with starch granules through a) formation of a layer of amylose–lipid complexes on the granule
266 surface b) formation of a rigid network of intragranular amylose–lipid complex structures or c) formation of a
267 lipid layer on the granule surface which hinders water uptake through increased hydrophobicity (Wokadala
268 et al., 2012).

269 Lower breakdown viscosity indicates the higher stability of the final product. This is especially true for non-
270 WT flours (41-488 mPa.s), while WT flour showed higher breakdown viscosity (899 mPa.s) associated to an
271 increased granule disruption and less tendency of starch to resist shear force during heating
272 (Thirathumthavorn and Charoenrein, 2006). Singh and Singh (2010) determined that breakdown viscosity
273 showed strong negative relationship with protein content. In good accordance, the protein level of each flour
274 was in the order: GP>BW>WT>T (Table 1), and the breakdown on cooking followed WT>T>BW>GP (Table
275 2). The effect of proteins to decrease breakdown was attributed to stabilization of continuous matrix or

strengthening of the links between the dispersed and continuous phase (Fitzgerald, Martin, Ward, Park, & Shead, 2003).

The setback viscosity value that is associated with the retrogradation and reordering of starch molecules is obtained during cooling. Low setback values indicate low rate of starch retrogradation and syneresis (Ragaei & Abdel-Aal, 2006). A notable large increase in setback viscosity over the cooling stage has been observed in wheat, rice, sorghum and millet stored flours as a result of starch interacting with liberated free fatty acids and flour protein, a three component interaction described earlier using a model system (Zhang and Hamaker 2003), and attributed to both starch-lipid complexation and starch-protein-lipid complexation (Zhang and Hamaker, 2005). Formation of the cooling stage viscosity peak on storage is particularly remarkable for BW flour followed by T flour (Table 2), in accordance with their high lipid -3.4% BW, 5.06%T- and protein -19.71%BW- contents (Table 1). It has been stated that stored whole grain sorghum flour, rather quickly (two months) produces sufficient free fatty acids due to lipolysis of triacylglycerols, to dramatically change pasting properties during cooling at 50–70°C. RVA profiles of BW and T flours on the cooling stage were higher than those respectively described earlier (Yilmaz et al., 2015; D'Silva et al., 2011).

3.2. Effects of the non-wheat flours T, GP and BW on the viscosity profile of doughs

Both additive and non-additive pasting behavior has been described for starch (Waterschoot et al., 2015), additive-wheat flour (Collar, 2003), and flour (Collar et al., 2014b) blends. Viscosity development of a flour suspension is determined by starch-dependent factors - concentration, amylose content, granule size, amylose leaching, swelling power and granule rigidity- and by the nature, chemical composition and qualitative and quantitative presence of non-starch components –mainly proteins, fat and dietary fibre-. Blending different flours increases the complexity of the system and makes interactions of flour components possible. Visco-metric properties during cooking and cooling of wheat-based mixed doughs formulated with T, GP and BW non-wheat flours (Figure 1) exhibited delayed and lower viscosity pattern during pasting but in

general higher viscosity profile during gelling, as compared to wheat dough counterparts (Table 2). Higher pasting viscosity profile of the wheat flour control doughs could be attributed to its higher total starch content because non-starch components could restrict swelling during pasting (Table 1), while the higher setback and final viscosity of mixed doughs reflect a higher extent of amylose leaching and complexation, as observed earlier for peas (Chung et al., 2008).

On cooking, single effects of non-wheat flours were particularly significant ($p < 0.05$) for decreased peak viscosity (-8%T, -5%BW), holding strength (-9%T), breakdown (-7%T, -5%GP, -7% BW) and viscosity of the hot paste (-18%T) of composite dough matrices (Table 3). Extent of the effects followed the order $T > GP > BW$. Increasing doses of GP from 7.5% (0) to 15% (1) into dough formulation delayed pasting temperature (+2.2%) and peak time (+6%), encompassing lower breakdown (-5%) and promoted viscosity at 95°C (+29%). A greater amount of protein in GP could induce increased protein–starch interaction, which could cause retardation toward swelling, thereby increasing the pasting temperature, as observed earlier (Chung and Liu, 2012) for pea cultivars. On cooling, higher partial and total setbacks were respectively provided by increasing doses from 7.5% to 15% of T (+67%, +16%) followed by BW (+31%, +6.2%), while GP dose did not greatly impact gelling (Table 3).

Simultaneous presence of two non-wheat flours at different doses significantly ($p < 0.05$) impacted the viscosity profile of dough blends (Table 4), especially for T/GP. In general, higher GP doses, decreased values for both cooking and cooling parameters at lowest T dose. Similar interactions were observed for T/BW blends on pasting, but a general increase during gelling was observed at higher doses of both T and BW. Binary blends of GP/BW resulted in promoted partial setback and final viscosity at low and high respective doses of single flours, and a decrease in viscosity of the cold paste at high doses of the binary blend (Table 4). In starch blends, differences in intrinsic properties such as gelatinization temperature, swelling power, carbohydrate leaching during swelling, and granule size of the blended starches may lead to specific interactions in the blend (Puncha-arnon et al., 2008). A lower peak viscosity than the peak

viscosities of both separate flours was obtained for flour blends of T/GP and T/BW at doses (0)/(1), indicating that T flour at lower dose inhibits swelling of GP and/or BW flour when added at higher dose. In fact, starches in the flour blends are composed of granules differing in size, from small to large: pea (wrinkled) 5–34 μm , pseudocereals <2 μm (Pérez and Bertoft, 2010), teff 2-6 μm (Bultosa and Taylor, 2004). A difference in granule size may well reduce the swelling power of the blend by producing a more optimally packed system in which the small granules fill the voids between the larger granules (Waterschoot et al., 2015). In addition, high protein and dietary fibre contents of GP and BW (Table 1) can interact with starch and compete for water interfering starch swelling. During the cooling phase of the temperature–time profile, mostly non-additive effects were observed for flour blends (Table 4), as described earlier for starch blends (Waterschoot et al., 2015). Cold paste viscosity of most blends was higher than what could be expected based on the cold paste viscosity of the individual flours and their ratios in the blends. It should be noticed the promoted final viscosity in blended samples formulated with higher doses of T/GP (110) and T/BW (111, 101) (Table 2), in good accordance with 2nd order interactions between pairs (Table 4). For isolated starch, viscosity development during cooling highly depends on the state of the starch gel at the end of the holding phase at 95°C (Waterschoot et al., 2015). In this work, a significant ($p < 0.01$) positive correlation between the relative effect on hot and on cold paste viscosity ($r = 0.7754$) was obtained. As for starch mixtures (Zhang et al., 2011), flour blends with a relatively low (or high) hot paste viscosity also showed a relatively low (or high) cold paste viscosity, as it is the case of samples 100 and 110, respectively (Table 2). For most blends with a relatively low cold paste viscosity, also a relatively lower peak viscosity was recorded ($r = 0.7722$) due to reduced swelling in the blend.

3.3. Relationships between visco-metric parameters, firming, retrogradation and starch digestibility of blended breads

Using Pearson correlation analysis, a range of correlation coefficients (r) (from 0.40 to 0.82) was obtained for the relationships between visco-metric parameters and firming, starch retrogradation and starch hydrolysis kinetics (Collar et al., 2014a; 2015) of blended breads (Table 5). Despite r values were discreet, significant ($0.01 < p < 0.05$) interdependences evidenced the impact of the pasting ($0.40 < r < 0.68$) and gelling ($0.40 < r < 0.82$) features on the starch digestibility and techno-functional characteristics of fresh and aged mixed breads, respectively. Visco-metric parameters retrieved during cooking (peak viscosity, breakdown and viscosity at 95°C) significantly negatively correlated with amylopectin retrogradation (ΔH_{∞} , ΔH_0) and firming kinetics (T_{∞} , n_f) over storage, while cooling parameters (viscosity at 50°C, final viscosity and setback) inversely related with starch nutritional fractions (SDS, DS, RS) of fresh breads and firming kinetics (T_0 , k_f). Higher viscosity profiles during cooking and holding corresponded to slower and lower firming kinetics since n_f and T_{∞} negatively correlated with peak viscosity ($r = -0.41$, -0.40 , respectively) and breakdown ($r = -0.44$, -0.41 , respectively), and lower and variable rate of retrogradation kinetics along storage (Table 5). Analogously, significant relationships were established between higher cold paste profiles on gelling and lower amounts of SDS, RS and TS found in fresh breads, ageing/staling quickly. Partial and total setback negatively correlated with RS formation in fresh breads ($r = -0.603$, -0.4696) and with the constant of proportion of retrogradation kinetics k_f ($r = -0.8194$, -0.6978) during ageing, but positively related to the Avrami exponent of firming kinetics n_f (0.5842 , 0.4646). A relatively high cold paste viscosity can result from increased interactions between leached molecules and/or swollen granules of the different starches in flours, whereas a relatively low cold paste viscosity can be explained by a reduction in swelling power and thus carbohydrate leaching of one starch by the other (Puncha-arnon et al., 2008). Also, the observed changes in the setback viscosity values may be associated with the interaction between the fibers and leached amylose at the hydrophobic regions and binding to the side chains of amylopectin through van der Waals forces, which might affect the retrogradation and reassociation during cooling period (Zhu et al., 2009).

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373 4. Conclusions

374 Non-wheat flour incorporation of ternary blends of T, GP and BW from 22.5% up to 45% into
375 unrestricted water-WT flour systems (14%) resulted in delayed and lower viscosity pattern during pasting
376 but, in general, higher viscosity profile during gelling, as compared to WT dough counterparts. Large
377 differences in starch granule size and swelling power, and complex flour composition seem to promote
378 interactions between starches and non-starch components –protein, lipids, dietary fibre- of different flours,
379 leading to non additive behaviour of main visco-metric features during pasting and gelling of flour blends.
380 However, a large difference in pasting temperature of single hydrated flours seems to explain more
381 independent behavior in blends. Higher visco-metric profiles during both cooking and cooling were fulfilled
382 by adding T/GP/BW, at 15/7.5/15 and 15/15/15 doses (%), to replace 37.5% and 45%, respectively, of WT
383 flour in blended dough formulations.

384 Trends for visco-metric transitions related to suitable patterns for a) slow starch hydrolysis, and b)
385 retarded firming kinetics and lower retrogradation in blended breads mainly include $\uparrow$ increased viscosity
386 values for maximum peak, breakdown and hot paste, and decreased values for partial and total setback on
387 cooling and cold paste (lower ΔH_{∞} , n_f , T_{∞} , RDS and DS formation, but higher RS formation). Blends fitting
388 suitable visco-metric patterns for slower starch digestibility and lower and/or slower retrogradation and
389 firming kinetics related the lowest WT flour replacement (22.5%) by T, GP and BW (7.5: 7.5:7.5).

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Table 1.- Proximate chemical and nutritional composition^a of flours (per 100 g flour, d.b.).

<i>Flours</i>	Protein ¹ (g)	Total dietary fibre (g)	Insoluble dietary fibre (g)	Soluble dietary fibre (g)	Fat (g)	Ash (g)	*DC (g)	Moisture (g)
Wheat	14.13±0.05b	2.19±0.12a	1.20±0.09a	0.99±0.25a	1.56±0.09a	0.63±0.09a	81.70	14.32±0.10c
Green pea	25.12±0.04d	14.56±0.95d	8.50±0.15d	6.05±0.27c	1.27±0.15b	2.58±0.12c	56.63	8.17±0.09a
Buckwheat	19.71±0.06c	13.52±0.38c	6.58±0.25b	6.93±0.36d	3.44±0.18c	2.05±0.19b	61.16	11.70±0.18b
Teff	13.05±0.02a	12.19±0.49b	7.40±0.36c	4.80±0.30b	5.06±0.09d	2.21±0.09b	66.97	11.90±0.09b

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

(*) DC: digestible carbohydrates calculated by indirect determination: DC = 100 – [Moisture + Protein + Fat + Ash + Dietary Fibre]

(¹) Conversion Factor from N to protein = 6.25.

Table 2.- Pasting and gelling viscometric parameters of composite dough matrices.

Sample	Pasting ^a																Gelling ^a																											
	Pasting Temperature				Peak viscosity				Peak Time				Holding strength				Breakdown				Viscosity at 95°C				Viscosity at end of 95°C				Setback				Viscosity at 50°C				Final Viscosity				Total Setback			
	°C				mPa.s				min				mPa.s				mPa.s				mPa.s				mPa.s				mPa.s				mPa.s				mPa.s				mPa.s			
Doughs ^b																																												
010	91.33	±	1.17	c	1094	±	70	b	5.57	±	0.05	c	593	±	30	e	501	±	40	bc	131	±	23	bc	658	±	34	cd	223	±	0	c	990	±	62	bc	1317	±	70	ab	724	±	40	a
001	89.63	±	0.04	bc	1094	±	3	bc	5.44	±	0.05	abc	591	±	8	e	503	±	6	c	131	±	12	bc	652	±	8	bcd	258	±	8	d	999	±	6	bcd	1352	±	11	bc	761	±	2	bc
011	90.88	±	0.53	c	1007	±	11	a	5.50	±	0.04	bc	535	±	5	bcd	472	±	6	ab	136	±	7	bc	633	±	19	bcd	264	±	1	d	916	±	0	a	1270	±	10	a	736	±	5	ab
000	87.55	±	2.9	a	1192	±	8	c	5.50	±	0.04	bc	601	±	13	e	591	±	4	d	147	±	16	c	673	±	7	d	189	±	4	bc	1045	±	11	d	1381	±	4	cd	780	±	8	c
111	90.83	±	1.66	c	1004	±	30	a	5.57	±	0.05	c	507	±	21	abc	497	±	9	bc	134	±	6	bc	610	±	31	abc	421	±	29	g	954	±	13	ab	1424	±	1	de	917	±	20	e
101	89.28	±	0.53	ab	1006	±	8	a	5.37	±	0.05	ab	547	±	9	cd	459	±	17	a	108	±	11	b	620	±	11	abc	466	±	28	h	1014	±	20	cd	1472	±	21	e	925	±	11	e
100	89.58	±	1.17	bc	987	±	80	a	5.47	±	0	abc	495	±	71	ab	492	±	9	bc	46	±	58	a	582	±	71	a	295	±	12	e	919	±	59	a	1281	±	68	a	786	±	3	c
110	91.30	±	1.13	c	1047	±	105	ab	5.57	±	0.05	c	563	±	59	de	484	±	46	abc	156	±	51	c	648	±	71	bcd	370	±	15	f	1020	±	81	cd	1416	±	120	de	853	±	61	de
Control	88.50	±	0.64	ab	1289	±	21	d	5.33	±	0	a	481	±	14	a	808	±	35	e	142	±	5	bc	581	±	6	a	-28	±	37	a	963	±	1	ab	1261	±	16	a	780	±	2	c
Flours																																												
Wheat	81.08	±	0.18	d	2681	±	4	d	6.44	±	0.05	b	1817	±	4	c	899	±	7	d	254	±	0	a	2309	±	9	c	622	±	11	c	2534	±	3	c	3297	±	7	c	1476	±	4	c
Teff	74.30	±	0.50	b	1983	±	4	b	7.00	±	0.03	d	1495	±	3	b	488	±	37	c	268	±	2	b	1979	±	4	b	90	±	15	a	2123	±	47	b	2031	±	33	b	537	±	1	a
Green pea	78.75	±	0.64	c	1050	±	4	a	5.47	±	0.09	a	1009	±	4	a	41	±	0	a	711	±	4	d	1020	±	4	a	583	±	4	b	1371	±	3	a	1628	±	8	a	614	±	4	b
Buckwheat	71.90	±	1.27	a	2394	±	7	c	6.87	±	0.00	c	2200	±	10	d	203	±	5	b	431	±	3	c	2402	±	6	d	2005	±	13	d	3553	±	7	d	4409	±	8	d	2219	±	2	d

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

(^b) Three digit bread sample code refers to low (0) and high (1) wheat flour replacement by teff: green pea: buckwheat flours in sample formulation.

Table 3.- Significant single effects of design factors (teff, green pea and buckwheat flours) on the viscometric parameters of composite dough matrices during cooking and cooling cycles. Levels of design factors were: 1 (7.5 g/100 g flour) and 2 (15 g/ 100 g flour).

Parameter ¹	Unit	Level	Overall mean	SE	Design factors					
					Teff	p<0.05	Green pea	p<0.05	Buckwheat	p<0.05
Cooking										
PastingT ^{re}	°C	1	90.07	0.30	ns		89.09	a	ns	
		2					91.05	b		
Peak viscosity	mPa.s	1	1054	11	1097	b	ns		1080	b
		2			1011	a			1028	a
Peak temperature	°C	1	94.99	0.01	ns		ns		ns	
		2								
Peak time	min	1	5.54	0.07	ns		5.38	a	ns	
		2					5.70	b		
Holding strength	mPa.s	1	554	8	580	b	ns		ns	
		2			530	a				
Breakdown	mPa.s	1	500	5	517	b	511	b	517	b
		2			483	a	488	a	483	a
Viscosity at 95°C	mPa.s	1	123	7	136	b	108	a	ns	
		2			111	a	139	b		
Viscosity at end of 95°C	mPa.s	1	634	9	654	b	ns		ns	
		2			615	a				
Cooling										
Setback	mPa.s	1	311	5	233	a	302	a	269	a
		2			388	b	319	b	352	b
Final viscosity	mPa.s	1	1364	13	1330	a	ns		ns	
		2			1398	b				
Total Setback	mPa.s	1	810	7	750	a	ns		786	a
		2			870	b			835	b

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

Table 4.- 2nd order significant interactions ($p < 0.05$) of rate of wheat flour replacement by low (1) and high (2) dose of teff (T), green pea (GP) and buckwheat (BW) –design factors- on the pasting and gelling parameters of composite dough matrices. Levels of design factors were: 1 (7.5 g/100 g flour) and 2 (15 g/ 100 g flour).

Parameter ¹	Unit	Level	Overall mean	SE	T X GP	p<0.05	T X BW	p<0.05	GP X BW	p<0.05
cooking										
Peak viscosity	mPa.s	11	1054	16	1143	c	1143	c	ns	
		12			1050	b	1050	b		
		21			996	a	1017	a		
		22			1025	b	1005	a		
Holding strength	mPa.s	11	554	11	596	c	ns		548	ab
		12			564	b			569	bc
		21			521	a			578	c
		22			535	ab			521	a
Breakdown	mPa.s	11	500	7	547	b	546	b	541	b
		12			486	a	488	a	481	a
		21			475	a	488	a	492	a
		22			490	a	478	a	484	a
Viscosity at 95°C	mPa.s	11	123	11	139	b	ns		ns	
		12			133	b				
		21			77	a				
		22			145	b				
cooling										
Setback	mPa.s	11	311	7	ns		206	a	242	a
		12					261	b	362	d
		21					332	c	296	b
		22					443	d	342	c
Viscosity at 50°C	mPa.s	11	982	14	1022	b	1018	b	982	b
		12			953	a	958	a	1007	b
		21			967	a	969	a	1005	b
		22			987	ab	984	ab	935	a
Final Viscosity	mPa.s	11	1364	18	1349	b	1349	a	1331	a
		12			1311	a	1311	a	1412	b
		21			1349	b	1349	a	1366	ab
		22			1448	c	1448	b	1347	a
Total Setback	mPa.s	11	810	10	770	b	752	a	ns	
		12			730	a	748	a		
		21			856	c	820	b		
		22			885	d	921	c		

SE: standard error.

(¹) Within parameters, mean values with different following letter do differ significantly from each other ($p < 0.05$).

Table 5.- Significant Pearson correlations (p<0.05 *, p<0.01 **) between viscometric and thermal, starch hydrolysis and firming kinetic parameters of composite dough and bread matrices.

	Pasting Temperature	Peak viscosity	Peak Time	Holding strength	Breakdown	Viscosity at 95°C	Viscosity at end of 95°C	Setback	Viscosity at 50°C	Final Viscosity	Total Setback
ΔH_{∞}		-0,3976 *			-0,4034 *	-0,5229 **					
k_r		0,6762 **		0,4655 *	0,6842 **		0,4242 *	-0,4921 *	0,4971 *		
ΔH_0						-0,4595 *					
RDS		-0,4162 *		-0,4424 *		-0,6465 **	-0,4858 *				
SDS										-0,4051 *	
DS		-0,4064 *				-0,5377 **			-0,4856 *		
RS					0,4692 *			-0,603 **		-0,4013 *	-0,4696 *
TS						-0,5269 **			-0,4875 *	-0,395 *	
T_{∞}		-0,4026 *	0,5062 *		-0,4114 *			0,4892 *			
k_f		0,6124 **		0,5043 *	0,5357 **		0,3968 *	-0,8194 **			-0,6978 **
n_f		-0,4125 *			-0,4373 *			0,5842 **			0,4646 **

T_{∞} , T_0 crumb firmness at ∞ and 0 time, respectively, k_f constant of proportion of firming kinetics, n_f Avrami exponent of firming kinetics, ΔH_{∞} , ΔH_0 retrogradation enthalpy at ∞ and 0 time, respectively, k_r constant of proportion of retrogradation kinetics, RDS rapidly digestible starch, SDS slowly digestible starch, DS digestible starch, RS resistant starch, TS total starch.

Figure

Figure 1.- Visco-metric profile of flours (A) and wheat-based mixed doughs formulated with teff (T), greenpea (GP) and buckwheat (BW) flours. B: 7.5% T, 7.5% GP; C: 7.5% T, 15% GP; D: 15% T, 7.5% GP; E: 15% T, 15% GP. Experimental data: control wheat breads (-----), 7.5% BW (.....), 15% BW (---); temperature profile: grey line.