**Significance of thermal transitions on starch digestibility and firming kinetics of restricted water
mixed flour bread matrices**

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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 thermal profiles of quaternary blended dough matrices have been investigated by simulating baking, cooling, and storage in differential scanning calorimeter (DSC) pans. Endothermal transitions related to suitable patterns for low and slow starch hydrolysis, softer crumb and retarded firming kinetics in blended breads include delayed temperatures for starch gelatinization, and for the dissociation of amylose-lipid complex. In addition, a) higher stability for the amylose-lipid inclusion complex, b) lower energy for starch gelatinization, c) lower limiting melting enthalpy and d) slower rate for amylopectin retrogradation meet thermal requirements for achieving suitable textural and starch digestibility features in blended breads, fulfilled by adding T/GP/BW to replace 45% of WT flour in blended dough formulations.

49	Abbreviations and symbols
50	C_{∞} maximum starch hydrolysis extent
51	DS digestible starch
52	H_{90} starch hydrolysis extent at 90 min
53	ΔH_0 retrogradation enthalpy at 0 time
54	ΔH_{∞} retrogradation enthalpy at ∞
55	ΔH_d entalpy of dissociation of amylose-lipid complex
56	ΔH_g gelatinization entalpy
57	k kinetic constant for starch hydrolysis
58	k_f constant of proportion of firming kinetics
59	k_r constant of proportion of retrogradation kinetics
60	n_f Avrami exponent of firming kinetics
61	n_r Avrami exponent of retrogradation kinetics
62	R gelatinization temperature range
63	RDS rapidly digestible starch,
64	RS resistant starch
65	SDS slowly digestible starch
66	$t_{1/2f}$ half-life for firming
67	$t_{1/2r}$ half-life for retrogradation
68	TDF total dietary fibre
69	T_e end temperature
70	T_o onset temperature
71	T_p peak temperature
72	TS total starch
73	
74	

1. Introduction

Bread that exhibits both a multicomponent and multiphase nature, can be viewed as a composite material where amylose, amylopectin, and protein form separate phases due to thermodynamic immiscibility of the polymers in presence of surrounding ingredients (Hug-Iten et al., 2003). The final structure of bread crumb described as a porous material with flexible elastic cell walls, is the result of a water-dependent thermal process related to the number and type of cross-links formed between the nearest neighboring chains of biopolymers (protein network and starch) present in the dough (Biliaderis et al 1986). Starch gelatinization and protein coagulation induce bread crumb formation. After cooling, the higher final water content of the crumb (35–45%) is responsible for the rubbery behavior, which gives structural mobility and smooth bread crumb behavior, and explains the sensitiveness of starch to retrograde during storage (Cuq et al 2003).

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 & Copeland, 2013). The gelatinization degree of starch in baked products depends primarily on the water availability and the amount of heating (Shin et al., 2005). Products (white bread, sugar cookies, pie crust, angel food cake, cake doughnuts and cinnamon rolls) can range from essentially completely gelatinized (97%) to almost native-like conditions (4%) (Lineback and Wongsrikasem, 1980). Other factors influencing gelatinization account for other components in the food matrix competing for water (e.g. sugar and proteins), heat transfer, the presence of lipid/starch complexes or other types of complexes, and they are usually negatively associated with extent of swelling, probably due to increased hydrophobicity. Modifications of water availability by the presence in dough matrices of hydrocolloids (Santos, Rosell & Collar, 2008), low molecular weight dextrans (Miyazaki et al 2004), blended starches of different sources (Waterschoot, Gomand, Fierens & Delcour, 2014) and high damaged starch flours retrogradation (León et al 2006) among other factors changed the thermal behavior of flour-water mixtures

during gelatinization and retrogradation. Mechanisms involved relate restriction of enzyme-substrate contact, interference as a physical barrier to prevent amylopectin chain association during storage, and a viscosity effect that affects mobility within the stored system (Khanna and Tester 2006). All these factors may limit the gelatinization degree constraining the swelling and breakdown of the starch granule structure, thus resulting in less digestible starch (Llorca et al., 2007). In general, any process or condition where the water availability or thermal energy is limited could generate the same effect, this is a lower degree of gelatinization encompassing a lower amorphous structure, and thus a lesser amount of digestible starch (Parada and Aguilera, 2011). In addition, granule size and surface characteristics (for example, pores, grooves or furrows, and surface-associated proteins and lipids), starch damage, amylose content, fine structure of amylopectin, degree of crystallinity and phosphorus content, can all affect digestibility (Wang & Copeland, 2014).

Main studies focused on the effects of gelatinization and retrogradation at higher water content on starch digestibility, but there is scarce information on the effect of retrogradation at low water content on starch digestibility (Wang & Copeland, 2013). The amount of gelatinization, swelling and hydrolysis are intimately controlled by the water content of the system and the temperature, and moderated by the botanical origin and composition of starches in limiting water conditions. According to Tester & Sommerville (2000), gelatinization, swelling and hydrolysis are restricted where crystalline order is retained within starch granules. However, when the water content and temperature profile become sufficient to allow gelatinisation and starch hydrolysis by α -amylase to proceed, swelling may be constrained because starch ability to hydrate and expand is hindered as a consequence of the complex composition, particularly in starch blends of different botanical origin.

Starch retrogradation involves reassociation of starch component molecules into a partially crystalline, ordered structure. Amylopectin recrystallization requires several days. Because firming of bread also develops over several days, most staling models view the changes in amylopectin as the primary

cause for crumb firming (Zobel and Kulp, 1996). The slow crystallization of amylopectin was referred to as a nucleation-limited growth process, which occurred above the glass transition in a mobile, viscoelastic, fringed-micelle network (Roos, 1995). Staling involves hardening of the crumb that is a complex phenomenon in which multiple mechanisms operate, all of them involving amylopectin retrogradation as the main player (Gray & Bemiller, 2003). Water plays a critical role in bread staling. When the retrogradation of amylopectin occurs, water molecules are incorporated into the crystallites and the distribution of water is shifted from gluten to starch/amylopectin, thereby changing the nature of the gluten network (Gray and BeMiller, 2003). Besides the molecular order of starch, water also plays an important role in crumb firmness due to its plasticizing effect on the crumb network (Hug-Iten et al., 2003).

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., 2014). Blended flours of different starch nature are expected to modify the mechanism of water mobility in bread crumb, and concomitantly its thermal properties during gelatinization and ageing due to water restrictions for swelling, gelatinization, and starch hydrolysis. Starch digestibility kinetics and crumb firming evolution during storage of blended breads are both water-dependent processes. Thermal transitions of multicomponent bread matrices baked at restricted water conditions are not well known, and the possible relationships between thermal properties, textural behaviour and the susceptibility of starch to enzymatic digestion in those heterogeneous matrices lack.

This paper is aimed a) at investigating the thermal transitions that occur during starch gelatinization and retrogradation in complex grain flour matrices with restricted 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 transition phases, and c) at exploring the relationships between thermal properties and

starch digestibility and firming 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. Protein, dietary fibre and fat contents (% flour, dry basis) were 14.13%, 2.19%, 1.56 (WT); 25.12%, 14.56%, 1.27 (GP); 19.71%, 13.52%, 3.44% (BW), and 13.05%, 12.19%, 5.06% (T), respectively.

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., 2014). 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, Jiménez, Conte & Fadda, 2014). Bread samples were stored for 1, 3, 6, and 8 days to describe firming kinetics.

Bread measurements

Chemical and nutritional composition of breads

The chemical and nutritional composition of control and blended breads was fully determined in a previous paper (Collar et al., 2014), from where some selected bread characteristics are compiled in Table 1. Moisture, fat and protein contents were determined following the ICC methods (ICC, 2014). Total, soluble and insoluble dietary fibre contents were determined based on the AOAC method 991.43 (AOAC, 1991) as described by Megazyme International Ireland (2012) in the Total Dietary Fibre Assay Procedure (kit K-TDFR 05/12). Three replicates were made for each analysis. 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).

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., 2014). 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.

Physical determinations

Bread texture 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 gelatinization, retrogradation, and amylose-lipid complexation of control and blended samples were assessed in a Differential Scanning Calorimeter Perkin-Elmer DSC-7

(Norwalk, USA) according to the method of León et al (1997), with some modifications as previously reported by Santos et al (2008).

Starch gelatinization. Dough samples were prepared by mixing flour blends and the remaining ingredients with 62% of water. For DSC analysis, 50–70 mg of dough samples were weighed in large volume pre-weighed, sealed stainless-steel pans. An empty pan was used as a reference. Simulation of the temperature profile in the center of the bread crumb during baking was performed in the calorimeter under the following scanning conditions: samples were kept at 30°C for 2 min, then heated from 30 to 110°C at a rate of 11.7°C/min, kept at 110°C for 5 min, and finally cooled from 110 to 30°C at a rate of 50°C/min. Gelatinized samples were stored at 22°C for 0, 1, 3, 6, and 8 days. Thermal transitions of starch samples were defined as T_o (onset), T_p (peak of gelatinization), and T_e (end); the enthalpy associated with starch gelatinization was defined as ΔH_g , and was calculated from the area under the curve defined after scanning, and expressed in J/g of dry sample. The gelatinization temperature range (R) was computed as $(T_e - T_o)$, as described by Vasanthan and Bhatta (1996).

Starch retrogradation and amylose-lipid complexation. Stored gelatinized dough samples were submitted to a second DSC scan to analyze starch retrogradation and amylose-lipid complexation 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) and the enthalpy for amylose-lipid complex dissociation (ΔH_d) were calculated, and T_o , T_p , and T_e for the different thermal transitions, identified. 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 (Jouppila et al., 1998) $\theta = \frac{H_\infty - H_t}{H_\infty - H_0} = e^{-kt^n}$ 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_o is the melting enthalpy at initial time, t is time of crystallization, k is a rate constant, and n is the Avrami exponent.

The values of the constants k and n were used to calculate the value of half-life, $t_{1/2}$, for starch crystallization and bread firming. Half-life was taken as the time required to achieve 50% of the levelling-off extent of crumb crystallinity or firmness as defined as $t_{1/2} = (-\ln 0.5/k)^{1/n}$ by Jouppila et al. (1998) and reported by Ronda and Roos (2011). The shorter the half-life is, the quicker is the amylopectin recrystallization or the hardening of the bread crumb.

Statistical analysis

Multivariate analysis of variance, correlation matrix and non linear multiple regression analysis 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 thermal transitions (Figure 1 and Figure 2) and textural behaviour (Figure 3) during storage of composite breads 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 molecular and macroscopic quality picture of breads (Table 3 and Table 4), and to correlate the thermal parameters with the firming and the starch hydrolysis kinetics of blended breads (Table 5).

3.1. Thermal and textural parameters of composite breads: effects of non-wheat flours on the calorimeter transitions and staling kinetics.

The parameters characterizing endothermal transitions –starch gelatinization, amylose-lipid complex dissociation and melting of amylopectin- and staling kinetics –crumb firming and starch retrogradation- of the different blended doughs and breads thereof are presented in Table 2. The multiple analysis of variance (MANOVA) provided information on the significant ($p<0.05$) single (Table 3) and/or interactive effects (Table 4) of the rate (low and high) of WT replacement by non-wheat flours T, GP and BW in blended breads on thermal transitions and firming (Figure 2) and starch retrogradation kinetics (Figure 3).

Starch gelatinization.-

During gelatinization, DSC thermograms exhibited a biphasic endotherm (Figure 1) associated to water-restricted starch-based systems (Wang and Copeland, 2013). According to Donovan (1979), at high water concentration (>66 wt% or water/starch ratio >1.5), a single symmetrical endothermic transition appears in a temperature range of $60\text{--}80^\circ\text{C}$ in the DSC profiles (called endotherm G). As the water/starch ratio is decreased, the magnitude of this endothermic transition decreases progressively, with a concomitant development of a second high temperature endothermic transition (referred to as endotherm M1). In the present research dough samples contain intermediate water concentration (34 wt%), and 2 peaks were defined during the first DSC scan (Figure 1) named peak 1 (G) and peak 2 (M1), respectively. The thermal transitions (Table 2) for peak 1 were more energetic (ΔH_g : $2.844\text{--}4.232$ J/g flour), occurred at lower and close temperatures (T_p : $75.55\text{--}76.96$ °C) and exhibited a broader gelatinization temperature range (R : $23.67\text{--}33.44^\circ\text{C}$) than those for peak 2 (ΔH_g : $0.821\text{--}1.867$ J/g flour; T_p : $90.78\text{--}93.61$ °C; R : $8.62\text{--}13.88^\circ\text{C}$). The swelling-driven melting theory (Donovan,1979), the crystallite stability model (Evans and Haisman, 1982), the sequential phase transition model (Nakazawa et al.,1984; Slade and Levine,1988), the three-stage phase transition model (Biliaderis et al., 1986) and smectic–nematic/isotropic- helix–coil transitions (Waigh, Gidley, Komanshek, and Donald, 2000) have been proposed so far to interpret experimental observations and the nature of the biphasic endothermic G and M1 transitions of starch–water systems at

medium water content (34–66 wt%). According to the different proposed models to explain the biphasic gelatinization endotherm compiled recently (Wang and Copeland, 2014), the first G endotherm (peak 1) can be suggested to result from a) plasticization in amorphous regions b) swelling-driven crystalline disruption and/or c) melting of the less stable crystallites in sufficient water, and/or d) associated with the smectic–nematic/isotropic transition; while the M1 endotherm (peak 2) represents the melting of the remaining less hydrated and more stable crystallites, encompassing a fast helix–coil transition.

In general, the endothermal transitions peak 1 and peak 2 for gelatinization of blended doughs encompassed higher values for both temperatures and enthalpies but narrower temperature range R than did the control WT doughs, except for ΔH_g (peak 2) that showed the opposite trend (Table 2). When the water concentration is limited, complete gelatinization will not occur at the usual gelatinization temperature range, but the transition temperature as well as the enthalpy of transition increases with decreasing water concentration for a given starch type (Parada & Aguilera, 2011). The higher R values of WT doughs suggest the presence of flour components of varying stability within the different structure domains (amylose/amylopectin ratio) of its starch granules. The differences in gelatinization temperatures among doughs may be attributed mainly to differences in size, form and distribution of starch granules in the blended flours, and to the internal arrangement of starch fractions within the granule, as stated earlier for legume flours (Kaur & Singh, 2005). In fact, starches in the flour blends are composed of granules differing in size, from small to large: pea (wrinkled) 5–34 μm , small wheat granules 2–3 μm (Zhou et al. 2004), large wheat granules 22–36 μm , pseudocereals <2 μm (Pérez and Bertoft, 2010), teff 2–6 μm (Bultosa and Taylor, 2004).

Regarding the complex flour composition, WT flour replacement by T from low (0) to high (1) doses did not affect gelatinization parameters (Table 3), but T significantly interacted with GP and/or BW at different doses (Table 4), modifying in a small extent the thermal parameters. A significant increase on the

gelatinization temperature (1-3°C) of peak 1 and peak 2 were respectively provided by increasing doses of GP and BW (Table 3). In addition, higher doses of GP resulted in a small decrease of ΔH_{g1} . Significant 2nd order interactions GP x BW were denoted (Table 4), leading to the highest values for T_{01} (69.82°C) and the lowest for ΔH_{g1} (3.22 J/gflour) when both flours replaced WT flour at 15%, respectively, in good accordance with the experimental values (Table 2) recorded for samples 011 (T_{01} : 69.62°C, ΔH_{g1} : 3.374 J/gflour) and 111 (T_{01} : 69.45°C, ΔH_{g1} : 2.844 J/gflour).

Amylose-lipid complex dissociation.-

Amylose-lipid complexes can be naturally present in starch (Morrison et al., 1993) or formed upon gelatinization of starch in the presence of lipids (Putseys, Lamberts, & Delcour, 2010). Blended breads used in the present work account for 3.4-3.8 g/100 g fresh bread of lipids (Table 1). Stored gelatinized control and blended dough samples when submitted to a second DSC scan defined a peak corresponding to a endothermal transition phase with T_p ranging from 100.78°C to 106.87°C, and ascribed to the dissociation of amylose-lipid inclusion complex (Table 2). In agreement with previous results (Russell, 1983), no significant change in the magnitude of the endotherm was observed with ageing. Two thermally distinct forms of amylose–lipid complexes have been identified: an amorphous structure with a random distribution of aggregated helices (termed Form I) with an endothermic transition in the DSC near 100 °C, and crystalline structures with DSC transitions at about 115 °C (Form IIa) and 125 °C (Form IIb) (Copeland et al., 2009). Both Forms I and II of amylose–lipid complexes may be present in processed starch-based foods depending on the method of processing, and the length of the available fatty acid to complex. Despite at high moisture levels, melting was highly cooperative and thus a single endothermic transition was shown, some amylose-lipid complexes always exhibited a single transition even at intermediate or low water contents (Biliaderis, Page, Slade & Sirett, 1985) as it can be the case of blended samples (34 wt%). The observed values for ΔH_d (0.317-0.621 J/g flour) are low as described earlier for water contents lower than

50%, and probably reflect the composite effect of several processes such as crystallite melting, helix~coil transition, and recrystallization phenomena that occur simultaneously (Biliaderis et al., 1985). Only BK dose has a significant single effect on the temperatures for the thermal transition as the degree of WT replacement goes from 7.5 to 15% (Table 3), denoted by a decrease in T_0 (-1.5°C), T_p (-1.3°C), and T_e (-2°C). Changes may be ascribed to the formation of less thermostable amorphous/crystalline structures, at low moisture contents. This explanation can also apply to blended samples with the pair T/GP at high/high doses and the pair GP/BW at low/high doses, respectively (Table 4).

Starch retrogradation

Extent of retrogradation is very sensitive to water content of starch gels (Zelevnak & Hosenev, 1986). Crystallization during aging depends on the water content occurring only in gels with starch content of 10–80% with the maximum crystallization taking place in gels with 50–60% starch (Eliasson, 1985). Recrystallization increases with increasing water content up to 45–50% because of progressively more effective plasticization (increased molecular mobility) (Slade & Levine 1987). In this research, a proximate range for starch concentration was 70–76 g/100 g of flour blend (including control) with a concomitant water concentration of 34% in control and blended doughs.

DSC thermograms of amylopectin retrogradation for gelatinized blended doughs at any time of storage showed a similar qualitative shape regardless the quantitative flour composition in the blends (plots not shown). Changes in the ΔH thermal profile for up to 8 days of storage of gelatinized blended doughs were defined according to the flour blend composition (Fig. 2). The kinetics of amylopectin recrystallization on aging of blended doughs were modeled using the Avrami equation as reported previously (Davidou et al 1996; Santos et al., 2008). Results on the model factors ΔH_0 , ΔH_∞ , n , and k for the enthalpy of amylopectin retrogradation are compiled in Table 2. Compared to control WT doughs, and with some exceptions, blended doughs exhibited lower (ΔH_∞ : 0.451-3.860 J/g flour vs 1.302J/g flour) and variable rate (n : 0.7316-

2.5068 vs 0.9075) of retrogradation kinetics along storage, with in some cases much longer half-life $t_{1/2}$ (17 days for 101, 15 days for 100 vs 1.6 days for control WT breads), endorsing slower amylopectin recrystallization (Fig. 2). Increasing WT flour replacement from 7.5% to 15% by single T, GP and/or BW significantly modified amylopectin retrogradation kinetics (Table 3), in terms of a common decrease in the constant of proportion particularly relevant for BW (k_r : -74%). Moreover, main changes were provided by the pair T x GP added at different doses (Table 4, Fig. 2). Suitable trends for achieving the lower and slower retrogradation kinetics were fulfilled by adding T/GP/BW, each at higher dose (15%), to replace 45% of WT flour in blended dough formulations (Fig. 2D). The corresponding composite bread (111) showed the following values of the Avrami model factors for amylopectin retrogradation kinetics: ΔH_0 : 0.000, ΔH_∞ : 0.451, n : 2.5068, and k : 0.0759.

Crumb firming

At macroscopic level, during storage, fresh blended and control breads aged in a variable extent following different firming kinetics (Table 2, Fig. 3). As expected, control WT breads were initially softer (T_0 : 900 g vs 1608-2645 g) and staled less (T_∞ : 5908 g vs 4685-15136 g) and at lower rate (n_f : 0.52 vs 0.50-2.18) than non-wheat flour formulated breads did (Fig.3). Blended breads with the high dose of GP aged in a higher extent than those replaced by the low dose, means values for T_∞ varying from 9741 g to 6509 g (Table 3). The crumb hardening effect of non-wheat flours was particularly relevant in the staling rate k_f by the simultaneous presence at high/low and low/low doses of T/GP and T/BW (Table 4). The staling rate can be minimized when the pairs replaced 30% (T/GP) and 22.5% (T/BW) of WT flour, respectively.

3.2.Relationships between thermal parameters, starch hydrolysis and firming kinetics of composite breads

The retrogradation of amylose in processed foods is considered to contribute to properties relating to stickiness, ability to absorb water, and digestibility, whereas retrogradation of amylopectin is probably a

more important determinant in the staling of bread and cakes (Wang and Copeland, 2013). Cooking and processing increase the susceptibility of starch to enzymatic digestion, which is a function of the degree of starch gelatinization, as shown in several *in vitro* and *in vivo* studies. Breads are processed in limited water conditions, in which starch is only partially gelatinized (Lineback and Wongsrikasem, 1980).

Using Pearson correlation analysis, a range of correlation coefficients (r) (from 0.35 to 0.88) was obtained for the relationships between thermal properties and starch hydrolysis and firming kinetic parameters of multigrain bread matrices (Table 5). Despite r values were discrete, significant ($0.01 < p < 0.05$) interdependences ($0.3600 < r < 0.7773$) between starch gelatinization (T_{pg} , T_{eg}), amylopectin retrogradation (ΔH_{∞} , n_r , $t_{1/2r}$), amylose-lipid complex dissociation parameters (T_{0d} , ΔH_d), and starch digestibility (k , C_{∞} , H_{90} , starch nutritional fractions) were found (Table 5). After cooking, differences in susceptibility of starch to enzymic attack are related more to the products of gelatinization and, more critically, retrogradation (Copeland et al., 2009). In these food systems such as breads, the physico-chemical behaviour (especially swelling and gelatinization) of starch is restricted and subsequently restricts the ease and extent of hydrolysis with amylases during digestion. In fact, the higher the T_{eg} was, the lower the RDS ($r = -0.4056$) and the slower the starch hydrolysis kinetics ($r = -0.4524$) were. As well, both the levelling-off value of melting enthalpy at which the extent of crystallization in starch stopped (ΔH_{∞}) and the half-life time ($t_{1/2r}$) positively correlated with the starch hydrolysis extent ΔH_{∞} ($r = 0.5385$, 0.4196) and with the relevant starch nutritional fractions DS ($r = 0.7773$, 0.7566), and negatively with the starch hydrolysis rate k ($r = -0.6707$, -0.5371). In model systems starch-fatty acid, it has been observed that the hydrolysis rate of the gelatinized starch can be reduced by the complex formation between amylose and fatty acids (Kawai et al., 2012). In this work, the higher the enthalpy of dissociation of amylose-lipid complex ΔH_d , associated to a more stable inclusion complex, the slower the rate (k) of the starch digestibility ($r = -0.5765$), and the higher the amounts of SDS ($r = 0.6395$) and RS ($r = 0.5893$). The presence of some non-starchy substances in breads such as proteins and lipids (Table 1) over the granule

surface may also limit surface accessibility and subsequently the rate and/or extent of enzymatic hydrolysis by blocking the adsorption sites, and therefore influencing enzyme binding (Oates, 1997).

Despite kinetic patterns for crumb firming (Fig. 3) and starch retrogradation (Fig. 2) to assess bread staling/aging at macroscopic and molecular levels, respectively, strongly differed in shape, some correspondence for highly replaced wheat flour breads can be observed in agreement with some results reported in the literature for wheat flour (Russell, 1983; Zobel & Kulp, 1996) and gluten-free matrices (Ronda & Roos, 2011). Significant correlations between T_{eg} vs T_0 ($r=-0.8043$), ΔH_g vs n_f ($r=-0.6061$) and n_r vs T_0 (0.8828) were found (Table 5). This means that softer breads correspond to delayed temperatures and lower associated enthalpy for starch gelatinization and slower rate for amylopectin retrogradation. Solid relationships between parameters characterizing the stability of the amylose-lipid complex and crumb firming kinetics were established in terms of delayed temperatures (T_{0d} , T_{pd} , T_{ed}) for the dissociation of amylose-lipid complex associated to lower initial crumb firmness T_0 ($r=-0.5554-0.7364$) and slower staling rate n_f ($-0.6391-0.7150$) during storage. The presence of lipids during hydrothermal treatments can decrease the swelling capacity of the starch granules, and complex formation has been shown in many studies to retard retrogradation (Wang & Copeland, 2013). ~~In other research no functional relationship between the rate constants obtained from DSC and crumb compressibility measurement was found, when the sample size was significantly increased (Russell, 1983b). Author indicated that, although broadly similar in magnitude, considerable independent variation occurred in rate constants obtained by DSC and crumb compressibility measurement since both techniques examine very different properties, the former a molecular property and the latter a bulk property.~~

4. Conclusions

~~WT flour replacement from 22.5% up to 45% by incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) flours significantly modify the qualitative and quantitative thermal profile of starch~~

~~gelatinization and amylopectin retrogradation kinetics during storage, and impact less on the dissociation of the amylose-lipid complex of the resulting hydrated flour blends assessed by DSC.~~ Non-wheat flour incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) from 22.5% up to 45% into restricted water-WT flour systems delayed endothermic transition temperatures for the bi-phasic gelatinization, and provided variable associated endothermic enthalpies for both gelatinization and retrogradation phenomena. Restricted or delayed swelling of starch granules as a result of the presence of associated flours in limiting water, delayed the T_o for gelatinization in hydrated flour mixtures. Upon melting, unavailable water for the remaining ungelatinized granules, force them to melt at higher temperatures and encompass variable energy to disorganize its structure. With some exceptions, blended doughs exhibited lower and variable rate of retrogradation kinetics along storage, with in some cases much longer half-life endorsing slower amylopectin recrystallization, compared to control WT doughs counterparts. Physical interferences by the presence of proteins and insoluble amylose-lipid complexes in limited water systems can explain hindrance for starch crystallization in blended breads. Suitable trends for achieving the lower and slower retrogradation kinetics were fulfilled by adding T/GP/BW, each at higher dose (15%), to replace 45% of WT flour in blended dough formulations.

Trends for thermal transitions related to suitable patterns for a) low and slow starch hydrolysis, b) initial softer crumb firmness and c) retarded firming kinetics in blended breads include i) delayed temperatures for starch gelatinization (lower RDS contents, slower starch hydrolysis kinetics, and softer bread crumbs), and ii) for the dissociation of amylose-lipid complex (lower initial crumb firmness and slower firming rate), iii) higher enthalpies for the amylose-lipid inclusion complex dissociation (slower rate for starch digestibility, higher amounts of SDS and RS), but iv) lower enthalpy for starch gelatinization (softer bread crumbs), v) lower levelling-off value of melting enthalpy at which the extent of starch crystallization stopped (lower starch hydrolysis extent and lower total DS) and vi) slower rate for amylopectin retrogradation (softer bread crumbs).

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Table 1.- Proximate chemical and nutritional composition of composite breads (per 100 g fresh blended bread).

Characteristic	Blended bread samples ^{1,2}								
	010	001	011	000	111	101	100	110	Control
Moisture, g	33.4±0.8c	32.9±0.6bc	32.5±0.9b	32.3±0.4bc	31.9±0.3b	29.3±0.8a	30.5±0.9a	32.2±0.4bc	32.9±0.6bc
Fat, g	3.5±0.2a	3.6±0.4a	3.6±0.2a	3.6±0.1a	3.8±0.3a	3.8±0.2a	3.7±0.4a	3.7±0.1a	3.4±0.2a
Protein ³ , g	11.9±0.1b	11.7±0.2b	12.3±0.1c	11.6±0.3b	12.2±0.2c	11.7±0.1b	11.7±0.1b	12.2±0.2c	11.1±0.1a
TDF, g	3.3±0.3b	3.3±0.2b	3.9±0.3c	2.9±0.3b	4.3±0.5c	3.8±0.2c	3.3±0.2b	3.8±0.1c	1.4±0.2a
RS, g	2.4±0.1b	2.7±0.3b	2.9±0.5b	2.8±0.4b	2.5±0.2b	2.3±0.2b	2.5±0.2b	2.2±0.2a,b	1.8±0.3a
RDS, g	57.8±0.9b	58.4±1.1b,c	56.4±1.0b	56.2±1.1a	60.0±1.2c	59.6±1.5b,c	62.5±1.4c	54.3±1.0a	68.5±1.1d
SDS, g	8.4±1.1c	5.4±0.7b	17.5±e	5.7±0.6b	2.3±0.6a	11.8±d	12.6±d	4.5±1.1b	7.5±1.0c
C _∞	74.3±1.3c	65.7±0.9a	74.1±0.9c	73.6±1.3c	71.2±1.2b	75.8±0.8c	75.2±0.7c	75.7±0.8c	81.0±0.9d
<i>k</i>	0.0686±	0.0825±	0.0477±	0.0797±	0.1106±	0.0599±	0.0593±	0.0821±	0.0720±
	0.0032c	0.0091c	0.0013a	0.0062c	0.0085d	0.0031b	0.0029b	0.0079c	0.0081c

TDF: total dietary fibre, RS: resistant starch, RDS: rapidly digestible starch, SDS: slowly digestible starch. C_∞ maximum starch hydrolysis extent, *k* kinetic constant for starch hydrolysis.

(¹) Mean values ± standard deviation. Within columns, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).

(²) Three digit bread sample code refers to low (0) and high (1) wheat flour replacement by teff: green pea: buckwheat flours in sample formulation.

(³) Conversion Factor from N to protein = 6.25.

Table 2.- Thermal and textural parameters of composite breads.

Characteristic	Units	Blended bread samples ^{a,b}								
		010	001	011	000	111	101	100	110	Control
Gelatinisation										
peak 1										
T_o	°C	66.99±0.35ab	66.08±1.06ab	69.62±2.74d	67.53±2.16c	69.45±2.08d	66.39±0.93ab	65.84±0.87ab	65.18±1.20a	66.30±0.57abc
T_p	°C	76.65±0.45cd	75.65±0.56a	76.96±1.41d	76.70±0.95cd	76.65±1.95bcd	76.17±0.53abcd	75.74±0.76ab	76.45±1.34abc	75.55±0.40a
T_e	°C	96.60±1.76ab	95.07±3.47ab	96.21±4.71ab	98.94±1.31ab	93.12±5.31a	99.83±0.83b	96.16±4.03ab	98.79±4.41ab	98.61±1.48ab
ΔH_g	J/g flour	3.337±0.270abc	4.232±0.233c	3.374±0.564ab	3.482±0.761abc	2.844±0.772a	3.445±0.565abc	4.007±0.696bc	4.145±0.739abc	3.497±0.571abc
R	°C	29.61	28.99	26.59	31.41	23.67	33.44	30.32	31.89	32.31
peak 2										
T_o	°C	88.08±1.21d	87.83±0.96bc	87.69±0.60d	86.19±1.14b	87.46±0.67cd	86.75±0.50bcd	86.68±0.52bcd	87.25±3.13bcd	83.96±1.10a
T_p	°C	91.81±2.51ab	92.77±0.87ab	93.61±0.63b	91.84±1.14a	93.64±0.90b	92.05±0.68ab	91.10±0.49a	92.42±5.49ab	90.78±0.45a
T_e	°C	96.70±0.76a	98.96±0.90bc	99.43±2.09cd	98.10±1.12abc	99.06±2.28bcd	99.52±0.76cd	97.03±1.49a	100.58±2.00d	97.84±1.21ab
ΔH_g	J/g flour	0.821±0.146a	1.561±0.371bcd	1.138±0.450ab	1.495±0.530bcd	1.258±0.251abc	1.514±0.278bcd	1.203±0.339abc	1.285±0.764cd	1.867±0.765d
R	°C	8.62	11.13	11.74	11.91	11.60	12.77	10.35	13.33	13.88
Amylose-lipid complex dissociation										
T_o	°C	95.66±0.78b	93.45±0.21a	91.47±2.03a	98.10±1.13b	94.18±1.74a	94.88±2.62b	96.21±0.07b	96.64±0.79b	96.38±1.07b
T_p	°C	106.28±1.06bc	104.12±2.71a	100.78±1.77a	105.26±1.67bc	102.70±0.00a	104.26±2.06c	106.60±0.09bc	104.45±0.59ab	106.87±0.47bc
T_e	°C	114.90±3.35d	110.48±2.75ab	105.50±4.29a	113.15±0.68bc	108.03±0.71ab	111.48±2.16bcd	114.18±0.92bcd	113.95±1.30bcd	116.53±0.85cd
ΔH_d	J/g	0.531±0.229a	0.455±0.258a	0.621±0.315a	0.330±0.206a	0.317±0.107a	0.335±0.143a	0.385±0.103a	0.289±0.100a	0.411±0.075a
Retrogradation kinetics										
ΔH_∞	J/g flour	1.136±0.121c	0.575±0.096a	1.672±0.195e	0.915±0.123c	0.451±0.096a	3.860±0.452g	2.980±0.325f	0.754±0.065bc	1.302±0.025d
k_r		0.2798±0.052d	0.1218±0.0094c	0.0379±0.0090a	0.5812±0.0720e	0.0759±0.0082b	0.0552±0.0152a	0.0945±0.0156b	0.2924±0.0458d	0.4578±0.0589e
n_r		1.9609±0.257bc	1.9618±0.324bc	1.7364±0.239b	0.8696±0.0956a	2.5068±0.435c	0.8945±0.0998a	0.7316±0.0965a	1.1611±0.226a	0.9075±0.102a
ΔH_0	J/g flour	0.000	0.023	-0.015	-0.002	0.000	0.034	0.025	0.000	0.003
R^2		99.82	91.34	96.41	95.19	98.36	98.29	98.7	96.22	98.94
$t_{1/2}$	days	1.60±0.90a	2.46±1.02a	5.36±1.59b	1.26±0.92a	2.46±1.02a	16.96±2.96c	15.26±3.56c	2.16±1.00a	1.60±0.85a
Firming kinetics										
T_∞	g force	6174±350b	5131±149a	4685±324a	5050±469a	8921±950c	8280±625c	12472±1900d	15136±1600d	5908±965ab
k_f		0,109±0.031e	0,091±0.006d	0,053±0.010c	0,08±0.009d	0,009±0.002b	0,003±0.000a	0,034±0.009c	0,036±0.010c	0,087±0.008d
n_f		0,86±0.09c	1,03±0.08d	1,75±0.01e	0,64±0.01b	1,93±0.06f	2,18±0.10g	0,50±0.06a	0,72±0.09b	0,52±0.08a
T_0	g force	2374±298c	2156±245c	2529±210c	1608±220b	2645±250c	1822±254b	1945±200b	1899±225b	901±125a
R^2		86,06	91,98	98,15	89,32	95,75	94,89	86,52	94,06	94,1
$t_{1/2}$	days	9±2b	7±2b	4±1a	30±4c	9±2b	12±3b	187±15f	60±9e	55±8d

(^a) Mean values ± standard deviation. (^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.

T_o : onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization enthalpy, ΔH_d enthalpy of dissociation of amylose-lipid complex, ΔH_∞ , ΔH_0 retrogradation enthalpy at ∞ and 0 time, respectively, R gelatinization temperature range, k_r constant of proportion of retrogradation kinetics, k_f constant of proportion of firming kinetics, n_r Avrami exponent of retrogradation kinetics, n_f Avrami exponent of firming kinetics, $t_{1/2r}$, $t_{1/2f}$ half-life for retrogradation and firming, respectively.

Table 3.- Significant single effects of design factors (teff, green pea and buckwheat flours) on the gelatinization and amylose-lipid complex dissociation thermal parameters, and on Avrami kinetic parameters for crumb firming and starch retrogradation during storage of composite breads. Levels of design factors were: 1 (7.5 g/100 g flour) and 2 (15 g/ 100 g flour).

Parameter	Unit	Level	Overall mean	Teff	p<0.05	Green pea	p<0.05	Buckwheat	p<0.05
Gelatinisation									
peak 1									
T_o	°C	0	67.47	ns		66.44±2.00	a	66.92±2.00	a
		1				68.51±2.02	b	68.02±2.01	b
T_p	°C	0	76.47	ns		76.09±1.05	a	ns	
		1				76.85±1.06	b		
T_e	°C	0	96.80	ns		ns		ns	
		1							
ΔH_g	J/g	0	3.62	ns		3.79±0.54	b	ns	
		1				3.44±0.54	a		
peak 2									
T_o	°C	0	86.81	ns		86.37±1.20	a	ns	
		1				87.25±1.20	b		
0	°C	0	92.16	ns		ns		91.28±1.61	a
		1						93.02±1.61	b
1	°C	0	98.50	ns		ns		97.81±1.63	a
		1						99.19±1.61	b
0	J/g	0	1.35	ns		ns		ns	
		1							
1									
T_o	°C	0	95.80	ns		ns		96.58±1.40	b
		1						95.03±1.40	a
0	°C	0	104.99	ns		ns		105.64±1.34	b
		1						104.35±1.34	a
1	°C	0	112.33	ns		ns		113.32±1.87	b
		1						111.33±1.87	a
0	J/g	0	0.379	ns		ns		ns	
		1							
1									
T_∞	g force	0	8125	ns		6509±1499	a	ns	
		1				9741±1585	b		
k_f		0	0.0535	ns		ns		ns	
		1							
n_f		0	1.2008	ns		ns		ns	
		1							
T_0	g force	0	2124	ns		ns		ns	
		1							
$t_{1/2f}$	days	0	40	ns		16±3	a	ns	
		1				64±6	b		
Retrogradation kinetics									
ΔH_∞	J/g flour	0	1.54	1.07±0.14	a	2.08±0.14	b	ns	
		1		2.01±0.14	b	1.00±0.14	a	ns	
k_r		0	0.19240	0.2552±0.0274	b	0.2132±0.0274	b	0.3120±0.0274	b
		1		0.1295±0.0274	a	0.1715±0.0274	a	0.0727±0.0274	a
n_r		0	1.4778	1.6322±0.1733	b	1.1144±0.1733	a	1.1808±0.1733	a
		1		1.3235±0.1733	a	1.8413±0.1733	b	1.7749±0.1733	b
ΔH_{0r}	J/g flour	0	0.008	ns		ns		ns	
		1							
$t_{1/2r}$	days	0	6	ns		4±1	a	ns	
		1				8±2	b		

T_o : onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization enthalpy, ΔH_d enthalpy of dissociation of amylose-lipid complex, 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_{0r} retrogradation enthalpy at ∞ and 0 time, respectively, k_r constant of proportion of retrogradation kinetics, n_r Avrami exponent of retrogradation kinetics, $t_{1/2f}$, $t_{1/2r}$ half-life for firming and retrogradation, respectively.

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 gelatinization and amylose-lipid complex dissociation thermal parameters, and on Avrami kinetic parameters for crumb firming and starch retrogradation during storage of composite breads. Levels of design factors were: 1 (7.5 g/100 g flour) and 2 (15 g/ 100 g flour).

Parameter	Unit	Level	Overall mean	T X GP	p<0.05	T X BW	p<0.05	GP X BW	p<0.05
Gelatinisation									
peak1									
T_o	°C	00	67.47	ns		ns		66.65±2.00	a
		01						66.23±1.99	a
		10						67.19±2.00	a
		11						69.82±2.02	b
ΔH_g	J/g	00	3.62	ns		3.38±0.54	a	3.72±0.54	b
		01				3.83±0.54	b	3.85±0.54	b
		10				4.01±0.54	b	3.66±0.54	b
		11				3.24±0.54	a	3.22±0.54	a
peak2									
T_e	°C	00	98.5	98.53±1.62	ab	ns		ns	
		01		97.79±1.62	a				
		10		98.24±1.62	ab				
		11		99.45±1.62	b				
ΔH_g	J/g	00	1.35	1.46±0.46	b	ns		ns	
		01		1.00±0.46	a				
		10		1.38±0.46	ab				
		11		1.53±0.46	b				
Amylose-lipid complex dissociation									
T_p	°C	00	104.99	104.81±1.34	a	ns		106.08±1.34	c
		01		105.01±1.34	ab			103.74±1.34	a
		10		105.70±1.34	b			105.20±1.34	bc
		11		104.45±1.34	a			104.95±1.34	b
T_e	°C	00	112.33	ns		113.72±1.87	c	ns	
		01				110.77±1.87	a		
		10				112.93±1.87	bc		
		11				111.89±1.87	ab		
Firming kinetics									
k_f		00	0.0535	0.0527±0.0240	ab	0.0795±0.0240	b	ns	
		01		0.0720±0.0240	b	0.0452±0.0240	ab		
		10		0.0668±0.0178	b	0.0358±0.0170	a		
		11		0.0224±0.0205	a	0.0533±0.0213	b		
Retrogradation kinetics									
ΔH_∞	J/g flour	00	1.54	0.74±0.20	a	ns		ns	
		01		1.40±0.20	b				
		10		3.42±0.20	c				
		11		0.60±0.20	a				
k_r		00	0.1924	0.35±0.04	c	0.43±0.04	c	ns	
		01		0.16±0.039	b	0.08±0.04	a		
		10		0.07±0.04	a	0.19±0.04	b		
		11		0.18±0.04	b	0.07±0.04	a		
n_r		00	1.4778	1.42±0.25	bc	ns		ns	
		01		1.85±0.25	c				
		10		0.81±0.25	a				
		11		1.83±0.25	c				
$t_{1/2r}$	days	00	5.89	4.92±1.23	b	ns		ns	
		01		4.71±1.23	b				
		10		2.52±1.13	a				
		11		11.40±1.61	c				

T_o : onset temperature, T_p : peak temperature, T_e : end temperature, ΔH_g : gelatinization enthalpy, k_f : constant of proportion of firming kinetics, ΔH_∞ : retrogradation enthalpy at 0 time, k_r : constant of proportion of retrogradation kinetics, n_r : Avrami exponent of retrogradation kinetics, $t_{1/2r}$: half-life for retrogradation.

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

	T_{0g}	T_{pg}	T_{eg}	ΔH_g	T_{0d}	T_{pd}	T_{ed}	ΔH_d	ΔH_∞	k_r	n_r	ΔH_{0r}	$t_{1/2r}$
C_∞		0.3799 *	0.6052 **		0.3755 *				0.5385 **		-0.5857 **		0.4196 *
k			-0.4524 **					-0.5765 **	-0.6707 **		0.5144 **		-0.5371 **
H_{90}		0.36 *	0.6179 **		0.4508 **		0.3862 *		0.5087 **		-0.5915 **		0.395 *
RDS		-0.5573 **	-0.4056 *						0.5181 **	-0.5292 **		0.6262 **	0.6557 **
SDS					-0.4423 *			0.6395 **	0.6684 **	-0.4248 *			0.5736 **
DS					-0.4076 *			0.5017 **	0.7773 **	-0.5797 **			0.7566 **
RS	0.4431 *				-0.4102 *	-0.4522 **	-0.567 **	0.5893 **				-0.4094 *	
TS					-0.4207 *			0.5213 **	0.7593 **	-0.5699 **			0.7368 **
T_∞				0.3691 *	0.3981 *		0.4296 *	-0.5991 **			-0.3526 *		
k_f								0.5253 **	-0.4733 **	0.4981 **			-0.5841 **
n_f	0.4968 **			-0.6061 **	-0.6391 **	-0.6857 **	-0.715 **			-0.636 **	0.3537 *		
T_0	0.6588 **		-0.8043 **	-0.506 **	-0.7364 **	-0.5554 **	-0.6249 **	0.5441 **		-0.5647 **	0.8828 **	-0.4406 *	

T_0 : onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization enthalpy, ΔH_d enthalpy of dissociation of amylose-lipid complex, 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_{0r} retrogradation enthalpy at ∞ and 0 time, respectively, k_r constant of proportion of retrogradation kinetics, n_r Avrami exponent of retrogradation kinetics, $t_{1/2r}$ half-life for retrogradation, C_∞ maximum starch hydrolysis extent, k kinetic constant for starch hydrolysis, H_{90} starch hydrolysis extent at 90 min, RDS rapidly digestible starch, SDS slowly digestible starch, DS digestible starch, RS resistant starch, TS total starch.

Figure(s)

Figure 1.- DSC thermograms of wheat-based mixed doughs formulated with teff (T), greenpea (GP) and buckwheat (BW) flours during starch gelatinization. Three digit code refers to low (0) and high (1) wheat flour replacement by T:GP:BW flours in sample formulation.

Figure 3.- Evolution of crumb firming during storage of wheat-based mixed breads formulated with teff (T), greenpea (GP) and buckwheat (BW) flours. A: 7.5% T, 7.5% GP; B: 7.5% T, 15% GP; C: 15% T, 7.5% GP; D: 15% T, 15% GP. Control wheat breads (----), 7.5% BW (- - -), 15% BW (....).

Figure 2.- Evolution of retrogradation enthalpy during storage of wheat-based mixed breads formulated with teff (T), greenpea (GP) and buckwheat (BW) flours. A: 7.5% T, 7.5% GP; B: 7.5% T, 15% GP; C: 15% T, 7.5% GP; D: 15% T, 15% GP. Experimental data: control wheat breads (Δ), 7.5% BW ($\bullet$), 15% BW ($\blacksquare$); adjusted data: black lines.