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High-legume wheat-based matrices: impact of high-pressure on starch hydrolysis and firming kinetics of composite breads.

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Abstract The significance of high hydrostatic pressure (HHP) processing on the physico-chemical -techno-functional and firming kinetics- parameters and nutritional properties –nutritional composition and “in vitro” starch digestibility- of highly replaced wheat flour breads by chickpea, pea and soybean flours was investigated, and the power/effectiveness of HHP in partially replacing structural agents (gluten and/or hydrocolloids) was discussed. Incorporation of pressured-legume slurries (350Mpa, 10 min) at 42% of wheat replacement into bread formulation provoked a general increase in initial crumb hardness and browning of the crust with a concomitant explicit reduction of moisture, whiteness of the crumb and bread specific volume, but a slower *in vitro* starch digestibility with prominent formation of slowly digestible starch and resistant starch, compared to their counterparts prepared by using a conventional -gluten/carboxymethylcellulose (CMC) – added- breadmaking recipe/process. Pressured breads with no gluten but 3% CMC in the formulation kept higher sensory ratings, softer initial texture and lower firming profiles on ageing than pressured breads with no gluten no CMC. HHP has proven to be an effective technology to partially replace structuring agents (CMC and/or gluten) in high-legume wheat-based matrices providing sensorially acceptable breads with medium physico-chemical quality profile but enhanced formation of nutritionally relevant starch fractions and slower crumb firming kinetics on ageing.

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Keywords Bread · Legumes · Nutritional value · staling *In vitro* starch digestibility ·

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1 Introduction

Legumes that constitute wholesome imaged foods penetrating the modern ingredient market, provide nutritional, health-protective and functional enhancing effects to foods. Legumes deserve a special attention when incorporated into cereal-based goods since high levels of lysine in legumes complement lysine deficiencies in cereal-based diets. The United Nations has dedicated 2016 the International Year of Pulses to heighten public awareness of the nutritional benefits of pulses as part of sustainable food production, aimed towards food security and nutrition (FAO, 2016). Legumes are considered an economical, environmentally sustainable protein source with contents double that of other cereal crops, low starch bioavailability, and high resistant starch content, to potentially enhance the nutritional value of breads, in line with the current suitable dietary trends (Jones, 2009; USDA, 2015). High levels of legumes incorporated into breadmaking products in absence of any structuring agent are cost effective although technologically very challenging to achieve nutritional and health endorsing effects, due to the lack of gluten network to meet dough viscoelastic, fermentative and structure-forming requirements.

High hydrostatic pressure (HHP) processing constitutes an efficient physical tool to modify the structure and function of food biopolymers, such as proteins or starches (Knorr et al., 2006), providing better retention of nutritional and functional ingredients in the processed product than thermal treatments. In breadmaking applications, special emphasis has been placed on the effect of HHP in gluten-free systems: barley and sorghum starches (Vallons and Arendt, 2009), buckwheat, teff and rice (Vallons et al., 2011), sorghum (Vallons et al., 2010) and oat (Hüttner et al., 2009) flours. Also HHP impact on the structural and physical properties of isolated gluten and/or gliadin and glutenin (Kieffer et al., 2007) has been reported. Some more recent studies on wheat flour (Vallons and Arendt, 2010), blended wheat and non-wheat flour systems (Angioloni and Collar, 2012a) and legume batters (Angioloni and Collar, 2013) have been described in terms of starch gelatinization, protein denaturation and structural changes, and promoted dough structure by HHP

1 treatments. HHP effects on breads have been little explored despite available results revealed
2 extended shelf-life in sorghum breads (Vallons et al., 2010), and superior nutritional and sensory
3 profiles in most quality features in binary blends of wheat and oat, millet or sorghum (Angioloni and
4 Collar, 2012a) when HHP treatment was applied to dough batters. In corn tortillas, HHP has proven
5 to alter the structural and molecular properties of water, thereby affecting their quality and microbial
6 stability (Vittadini et al., 2004). Pressure treatment has alluded to delay staling in gluten-free
7 products, particularly in sorghum breads containing 2% of sorghum treated at 600 Mpa (Vallons et
8 al., 2010) and in oat breads containing oat batter treated at 200 Mpa (Hüttner et al. 2009). Also,
9 the effect of HHP on buckwheat nutritional properties has been recently investigated (Zhou et al.,
10 2015) revealing protection of the activities of phytochemicals through the increase of both
11 antioxidant activity and iron chelating capacity at 45 °C compared to untreated sample, and
12 associated with the release of bound phenolic compounds. A higher slowly digestible starch
13 formation in HHP-gelatinized, non-waxy and waxy rice starches than in heat-gelatinized starches
14 was stated (Tian et al., 2014), attributed to the formation of less perfect crystallites and more
15 imperfect crystallites during the HHP and retrogradation treatments. The retardation mechanism of
16 HHP on starch retrogradation is ascribed to less broken granules and lower leached amylose (Hu
17 et al., 2011).

18 Associated mixtures of legumes-wheat-structuring agents (42:52:6, w:w:w) have proven to
19 make highly nutritious breads in terms of promoted dietary fibre fractions, lower and slower starch
20 hydrolysis, decreased rapidly digestible starch and reduced expected glycaemic index. In addition,
21 viscoelastic restrictions and sensory standards are met (Angioloni and Collar, 2012b). In a previous
22 paper (Angioloni and Collar, 2013), it was reported that for legume batters from chickpea, greenpea
23 and/or soybean, HHP provokes changes on the rheology of hydrated samples, particularly in softer
24 batters, leading to an increased solid character. Analysis of proteins extracted in different buffers
25 revealed that pressures of >200MPa induced the formation of urea-insoluble complexes, disulphide
26 bonds and/or other strong protein aggregates. Although the extent of protein modification was

dependent on the applied pressure, the results show that HHP can be used to improve the breadmaking functionality of legume batters.

This paper is aimed at exploring a) the significance of HHP on the physico-chemical - techno-functional and firming kinetics- parameters and nutritional properties –nutritional composition and starch digestibility- of highly replaced wheat flour breads by chickpea, pea and soybean flours, and b) the power of HHP in partially replacing structural agents (gluten and/or hydrocolloids) in high-legume wheat-based bread matrices. For comparative purposes, a refined high grade wheat flour (70% extraction rate) and structuring agents (carboxymethylcellulose and gluten) were used to keep, as much as possible, viscoelasticity and gas retention ability of the basic wheat dough matrix.

Materials and methods

Materials

Commercial flours from chickpea (CP), green split pea flour roasted (GP) and defatted soya flour (SB) were purchased from Trades (Spain). Common refined wheat flour (WT) of 356 x 10⁻⁴ J energy of deformation W, 0.64 curve configuration ratio P/L, 95% Gluten Index, and 62% water absorption in Brabender Farinograph, was from the Spanish market. Gluten (Supra Vital wheat gluten –GL-) and carboxymethylcellulose (Aquasorb® A-500 –CMC-) were acquired from Roquette (Lestrem, France) and Copenhagen Pectin (Denmark), respectively. Chemical and hydration characteristics of commercial gluten were 6.3% moisture, 83% protein, 10% starch, 3.0% fat, 1.0% ash, 6 mL/g swelling, 3.04 g water/g solid water hydration capacity, and 1.89 g water/g solid water binding capacity.

Methods

High hydrostatic pressure (HHP) treatment of flours

Hydrated composite flours CP:GP:SB (20:20:2, 20:14:8, 20:8:14, w:w:w) selected on the basis of their superior functional performance and nutritional profiles from a previous study (Angioloni and Collar, 2012b), were prepared by mixing flours and tap water at a flour-water ratio of 1:1 (w/w). The slurries flours were packed into polyethylene bags (200 x 300 mm), minimising the amount of air entrapped. The slurries were packed into a vacuum bag and vacuum-packed (MULTIVAC Thermosealer) to prevent contact between pressurisation fluid and sample. Samples were transferred to the pressure treatment chamber (High Pressure Food Processor, EPSI, MA, USA). The pressurization liquid (300mL) used was a mixture of water:ethylene glycol (70:30 v/v). The pressure level, pressurization time, and temperature were controlled automatically. After approximately 2 min, the desired pressure was reached and the time course started. The samples were treated for 10 min at 20 °C under a pressure of 350 MPa according to a previous study (Angioloni and Collar, 2013) for maximization of dough reinforcement without excessive dough stiffness. Due to compressive heating, an increase in the temperature of the processing fluid by up to a maximum of 8 °C was observed.

Dough and bread preparation

For the preparation of unpressured and pressured high-legume doughs and breads, wheat flour was replaced by ternary combinations of legume flours CP, GP and SB at 42% of wheat flour substitution (Table 1). Quantitative levels of individual legume flours in the mixture were identified in a previous work (Angioloni and Collar, 2012b) and correspond to highly replaced sensorially accepted legume-wheat samples (overall acceptability >6.5/10) with high recommended dietary allowance coverages. Composite flours (100 g), commercial compressed yeast (8% flour basis), salt (1.5%), and the necessary water to obtain dough of a consistency of 500 Brabender Units in a

Brabender farinograph were mixed in a 10 kg mixer at 60 revolutions per min for 8-10 min up to optimum dough development. Gluten (3-5%) and CMC (3-5%) were used for the preparation of conventional unpressured breads (A, B, C). Doughmaking of pressure-treated (350 MPa) hydrated composite flours optionally included CMC at 3% (AA, BB, CC) or not (AA', BB', CC') (Table 1). Fermented doughs were obtained after bulk fermentation (10 min), dividing (500 g), molding, and proofing up to maximum volume increment (0.5-1 h), and were baked at 210 °C for 30 min in an electric oven to make unpressured and pressured high-legume breads. Wheat breads, as controls, were prepared by using 50% of pressure-treated wheat flour or 100% untreated wheat flour. Two trials were performed per baking test. Bread samples were placed in co-extruded polypropylene bags and stored in a thermostatic cabinet set at 20°C for 1, 3, 6, 8 and 10 days to describe firming kinetics.

Chemical and nutritional composition of flours and breads

Moisture, protein, ash and fat contents of commercial flours, control and high-legume breads (Table 2) were determined following the ICC methods (ICC, 2014). Total, soluble and insoluble dietary fibre contents were determined according to 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 (AA CC, 2005), as described by Megazyme International Ireland (2011) in the Resistant Starch Assay Procedure (kit K-RSTAR 08/11). Samples were incubated in a shaking water bath with pancreatic alpha-amylase and amyloglucosidase (AMG) for 16 hr at 37°C, during which non-resistant starch is solubilised and hydrolyzed to D-glucose by the combined action of the two enzymes. The reaction was terminated by the addition of an equal volume of ethanol, and the RS is recovered as a pellet on centrifugation. This was then washed twice by suspension in

aqueous ethanol (50 % v/v), followed by centrifugation. Free liquid was removed by decantation. RS in the pellet was dissolved in 2 M KOH by vigorously stirring in an ice-water bath over a magnetic stirrer. This solution was neutralized with acetate buffer and the starch quantitatively hydrolyzed to glucose with AMG. D-Glucose was measured with glucose oxidase/peroxidase reagent (GOPOD), and this was a measure of the RS content of the sample. The following formula was used to calculate RS (g/100g sample, d. b.): $RS = \Delta E \times F/W \times 9.27$, where ΔE = absorbance (reaction) read against the reagent blank, F = conversion from absorbance to micrograms and W = dry weight of sample analyzed. Digestible carbohydrates were calculated by indirect determination as $100 - [\text{Moisture} + \text{Protein} + \text{Fat} + \text{Ash} + \text{Dietary Fibre}]$ (FAO, 2003).

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.

Bread colour determination

Color measurements were performed in breads -both crust and crumb- using a colorimeter (Minolta, Osaka, Japan), and the results were expressed in accordance to the Hunter Lab color space. The colorimeter was calibrated before each analysis with white and black standard tiles. Bread color was determined on five points of two pieces of each bread (crust) and central slices (crumb). The primary parameters determined were L (0 black and 100 white), a (greenness and

redness), and b (blueness and yellowness). The browning index $BI=100-L$ (Ramirez-Jimenez et al., 2001) and whiteness index $WI=100-[(100-L)^2+a^2+b^2]^{1/2}$ (Hsu et al., 2003) were calculated in crust and crumb, respectively.

Bread texture and firming kinetics

Bread mechanical characteristics (Texture Profile Analysis 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 (Armero & Collar, 1998).

Sensory analysis

Sensory analysis was performed with a panel of fifty consumers (twenty five males and twenty five females aged 23–53) who scored the overall acceptability of breads. Ratings were given for two loaves of each type of freshly baked unpressured and pressured high-legume breads. A semistructured scale from 0 (dislike extremely) to 10 (like extremely) was used.

Statistical analysis

Statistical analysis of data (one-way analysis of variance, mean comparison of samples - LSD-) was performed by using Statgraphics V.7.1 program (Bitstream, Cambridge, MN).

Results and discussion

A plural physicochemical methodological approach was adopted to assess the technological and nutritional profiles of unpressured and pressure-treated high-legume wheat-based bread samples. Textural pattern, color, crumb grain characteristics, sensory overall acceptance, chemical and nutritional composition, firming and starch digestibility kinetics and starch nutritional fractions were evaluated, and impact of HHP treatment on legume-wheat samples was quantified.

Chemical and nutritional composition of unpressured and pressure-treated high-legume composite breads

Intrinsic characteristics of legume flours, wheat flour replacement by non-wheat flours and structuring agents (gluten and/or CMC) (Table 1), and HHP processing play all relevant roles in the compositional features of high-legume breads. Chemical and nutritional information on unpressured and pressure-treated high-legume wheat-based breads (Table 2) showed superior appealing nutritional quality than wheat breads in terms of lower digestible starch, and higher soluble and insoluble dietary fibre contents. A significant reduction of digestible carbohydrate content (g/100 g bread, as is) with wheat flour substitution at 42% by legumes (32-38 vs 51g) that regulates the total amount of accessible macronutrients takes place, especially for unpressured bread samples (32-35g) that exhibit a concomitant sharp increase in total dietary fibre (7.5-8.8 vs 1.4 g) mainly assigned to the presence of CMC (3-5%, flour basis) in the formulation (Table 1). Application of HHP treatment to hydrated mixed legume flours in dough matrices in presence of 3% CMC led to breads (AA, BB, CC) with a total fibre content of 7.1-7.7g, clearly higher than fibre amounts found in pressure-treated breads with no CMC added (5.4-5.8g, AA', BB', CC'),

particularly for soluble dietary fibre content (3.6-3.8g vs 1.9g), as it was expected. Unpressured and pressured-treated high-legume bread samples contain about three or four times the fibre of the regular white bread (1.4g) regardless the presence of CMC, and breads can be labelled as high-fibre breads (>6 g DF/100 g food, A, B, C, AA, BB, CC) or source of fibre (>3 g DF/100 g food, AA', BB', CC') according to Nutritional Claims for Dietary Fibre Foods (Official Journal of European Community, 2006). Differences were observed in protein contents between unpressured (11.6-14.5 g/100g bread, as is) and pressure-treated samples (9.1-12.3 g/100g bread, as is) associated to the presence of 3-5% gluten in the former (Table 1). Analogously, simultaneous presence of 3-5% gluten and 3-5% CMC in unpressured bread formulations led to promoted moisture contents in samples when compared to pressure-treated counterparts (42.5-44.1 vs 39.5-41.3 g/100g bread, as is), associated to the water binding ability of the structuring agents.

Composite breads made with HHP treated flours and wheat had lower overall protein levels due to the fact that gluten was not added (Table 1). A diluting effect of the main biopolymers by both the moisture content and gluten/CMC addition in untreated conventional samples is observed. The contents of digestible starch in HHP treated samples are in general higher, and no differences in fat levels are observed with pressure (Table 2).

Techno-functional and firming kinetic parameters of unpressured and pressure-treated high-legume composite breads

Bread is a composite solid, comprised, at a macroscopic level, of crumb cells full of air in a viscoelastic solid matrix. In baked products the small holes or voids in the crumb, usually referred to as crumb cell structure or crumb grain, contribute to texture, eating quality, mechanical strength and perceived product freshness (Cauvain et al., 1999) and closely depend on both raw materials and processing conditions (Scanlon and Zghal, 2001) of the bread. Fresh and aged bread crumb mechanical properties dependent on crumb structure are often connected to sensory perception of freshness and elasticity by consumers, and also largely influence subsequent purchase.

Techno-functional and firming kinetic parameters of HHP and conventional (gluten/CMC-added) high-legume breads (Figure 1) evidenced significant differences between samples (Table 3). Incorporation of pressured hydrated flours from CP, GP and SB at 42% into bread formulation provoked a significant general increase in initial crumb hardness (from 15% to 175%) and browning index of the crust (up to 7%) with a concomitant explicit reduction in pressured samples of moisture (up to -9%), whiteness index of the crumb (up to -7%) and bread specific volume (from -9% to -18%), and no significant changes in crumb cohesiveness (≈ 0.80), when compared to their counterparts prepared by using a conventional (gluten/CMC-added) breadmaking recipe/process (Table 3). In general, pressured breads with no gluten but 3% CMC in formulation (AA, BB, CC) kept softer initial texture and larger specific volume reduction than pressured breads with no gluten no CMC (AA', BB', CC'). Values ranged from 7.84N (BB) to 9.71N (AA, CC) and 15.13N (AA') to 18.78N (BB', CC') for initial crumb hardness, and 2.6 ml/g (BB, CC) to 3.1 (AA) and 2.7-2.8 ml/g (AA', BB', CC') for specific volume (Table 3, Figure 1). Despite all high-legume breads deserved overall acceptability scores over 5/10, pressured-breads with no structuring agents in the formulation reached lower consumer ratings (5.0-6.0) than CMC-pressured breads (6.0-7.0), and much lower than CMC/gluten-unpressured breads (7.0-8.0) (Table 3).

Cell size and crumb structure greatly influence how the crumb feels by touch or in the mouth: thin walled, uniformly sized cells yield to a soft and elastic bread texture, properties that are usually welcomed by consumers. In general, HHP breads with denser crumb cell porosity and close crumb structure (Figure 1) corresponding to samples with no added structuring agents (AA', BB', CC') deserved the poorest scores for overall acceptability (5.0/10-6.0/10) and the hardest texture (15.13N-18.78N) (Table 3).

Information on crumb firming kinetics during storage was obtained by modelling the experimental curves according to the Avrami non-linear regression (Figure 2). Values for Avrami kinetic parameters T_{∞} (final crumb firmness), k (rate constant), n (Avrami exponent) and T_0 (crumb firmness of fresh bread) allowed to distinguish different staling kinetics for HHP and conventional

(CMC/gluten-added) breads (Table 3). Higher firming profiles during storage are reached by HHP breads with no CMC/gluten-added formulations, intermediate patterns are exhibited by HHP CMC-breads, and lower firming features are portrayed in unpressured breads (Figure 2). HHP breads showed higher values for both initial and final crumb hardness (T_{∞} values ranging from 32.41 N – CC- to 84.43 N –BB'- vs 16.08N –A- to 24.03 N –B-), but lower Avrami exponent (n values ranging from 0.438 –AA'- to 1.078 –BB'- vs 0.493 –A- to 1.643 –C-) giving initially harder breads with slower staling kinetics.

Starch Hydrolysis kinetics, Expected Glycaemic Index and relevant Starch Nutritional fractions of unpressured and pressure-treated high-legume wheat-based breads.

Starch nutritional fractions determined in unpressured and pressure-treated wheat and high-legume breads by “in vitro” starch digestion included rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and total starch (TS) (Table 4). RDS, SDS and RS contents (g/100g bread, as is) ranged from 32.42% (B) to 37.90% (A, C), from 1.42% (B) to 5.20% (A, C) and at about 1.80-2.40% (A, B, C), in unpressured samples, and from 40.10-43.10% (AA', BB', CC'), from 5.50% (AA', CC') to 7.60% (BB') and $\approx$ 3.0% (AA', BB', CC'), in pressure-treated high-legume samples, respectively. Quantitative data for starch subfractions in control WT samples for unpressured and pressure-treated matrices described a digestibility profile with higher RDS (65.8%, 55.0%), SDS (3.2%, 9%) and similar RS ($\approx$ 2%) contents (Table 4) than high-legume breads, in good accordance with previously reported information (Angioloni and Collar, 2012 a,b). The incorporation of legumes into bread formulation seems to reduce starch hydrolysis, probably because of their lower starch and higher fibre and protein contents (Table 2). The reduced rate and overall reduced starch digestibility of legumes is affected by high content of viscous soluble dietary fibre components (Tharanathan and Mahadevamma, 2003). In addition, high protein content of legumes and oilseed (18.34-55.51%) can promote starch-protein interactions restricting enzyme

attack as pointed out earlier for lentils (Chung et al., 2008). Lower RDS content and both higher SDS and RS contents, which are considered suitable nutritional trends for dietary starch fractions (Englyst et al., 2003), were observed for pressure-treated sample BB' (Table 4), composed by 20CP:14GP:8SB:58WT (Table 1). It appears that the formation of protein network/aggregation and/or intra- and inter-molecular S-S bonds after HHP treatment (Heremans & Smeller, 1998) can lead to a very close and compact structure entrapping components as it was argued for phytochemicals in HHP composite cereal breads (Angioloni and Collar, 2012a), and thus delaying/obstructing both starch swelling and gelatinization and the enzyme accessibility to the biopolymer leading to a slower starch hydrolysis and higher RS content. Another explanation lies in the formation of certain amount of amylose-lipid complex in the starch granules during the HHP process, that could cause a lower susceptibility to the digestive enzymes (Tian et al., 2014), as it was alluded by Zhou et al. (2015) who found that starch digestibility of buckwheat slurries treated with 600 MPa at 45 °C was significantly lower as compared to that of buckwheat treated at room temperature.

Conclusions

Application of HHP to hydrated chickpea, pea and soybean mixed flours significantly change the physico-chemical and nutritional profiles of highly replaced wheat flour breads by legumes. Incorporation of pressured legume slurries at 42% into bread formulation provoked a general increase in initial crumb hardness and browning of the crust with a concomitant explicit reduction of moisture, whiteness of the crumb and bread specific volume, but a slower *in vitro* starch digestibility with prominent formation of slowly digestible starch and resistant starch, compared to their counterparts prepared by using a conventional (gluten/CMC-added) breadmaking recipe/process. Pressured breads with no gluten but 3% CMC in the formulation kept higher sensory ratings, softer initial texture and lower firming profiles on ageing than pressured breads with no gluten no CMC.

HHP has proven to be an effective technology to partially replace structural agents (hydrocolloids and/or gluten) in high-legume wheat-based matrices providing sensorially acceptable breads with medium physico-chemical quality profile but enhanced formation of nutritionally relevant starch fractions and slower crumb firming kinetics on ageing.

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Figure 1.- Crumb slices images of unpressured and pressure-treated high-legume wheat breads based on chickpea, green pea and soybean. A, B, C – unpressured (gluten and carboxymethylcellulose, CMC) high-legume wheat breads. AA, BB, CC – pressure-treated (CMC) high-legume wheat breads. AA', BB', CC' – pressure-treated high-legume wheat breads.

Figure 2 .- Observed (symbols) and fitted (lines) values of crumb hardness during storage for unpressed and pressure treated high legume-wheat breads based on chickpea, green pea and soybean. A, B, C – unpressed (gluten and CMC) high legume-wheat breads. AA, BB, CC – pressure-treated (CMC) high legume-wheat breads. AA', BB', CC' – pressure-treated high legume-wheat breads.

Figure 3.- Digestible starch hydrolysis kinetic curves (mean of three replicates) of unpressed and pressure treated high-legume wheat-based and control breads. A first order kinetic equation $[C = C_{\infty}(1 - e^{-kt})]$ was applied to describe the kinetics of digestible starch hydrolysis. A, B, C : unpressed (gluten and carboxymethylcellulose) high legume-wheat breads; AA', BB', CC': pressure-treated high-legume wheat-based breads; control: unpressed wheat flour bread; HHPcontrol: pressure-treated wheat flour bread.

Table 1.- Design of samples for unpressured and pressure-treated high-legume wheat-based breads.

Sample code ^b	Wheat flour replacement, g/ 100 g flour ^a				
	CP	GP	SB	CMC	GL
A	20	20	2	5	5
B	20	14	8	3	3
C	20	8	14	5	5
AA	20	20	2	3	0
BB	20	14	8	3	0
CC	20	8	14	3	0
AA'	20	20	2	0	0
BB'	20	14	8	0	0
CC'	20	8	14	0	0

CP: chickpea; GP green pea; SB soybean; CMC: carboxymethylcellulose; GL: gluten; A, B, C : unpressured (gluten and carboxymethylcellulose) high-legume wheat-based breads;

AA, BB, CC: pressure-treated (carboxymethylcellulose) high-legume wheat-based breads; AA', BB', CC': pressure-treated high-legume wheat-based breads.

Table 2.- Proximate chemical and nutritional composition^a of flours (per 100 g flour, d.b.) and breads (per 100 g fresh blended bread).

Sample code ^b	Protein ¹ (g)	Total dietary fibre (g)	Insoluble dietary fibre (g)	Soluble dietary fibre (g)	Fat (g)	Ash (g)	*DC (g)	Moisture (g)
<i>Flours</i>								
Wheat	14.1 ^a ±0.1	2.2 ^a ±0.1	1.2 ^a ±0.1	1.0 ^a ±0.2	1.6 ^b ±0.1	0.63 ^a ±0.09	82	14.32 ^d ±0.05
Chickpea	18.4 ^b ±0.0	24.6 ^c ±0.8	18.2 ^d ±1.0	6.4 ^b ±0.9	6.8 ^d ±0.0	2.89 ^c ±0.04	48	9.88 ^b ±0.03
Greenpea	25.1 ^c ±0.1	14.6 ^b ±0.8	8.5 ^b ±1.0	6.1 ^b ±0.9	1.3 ^a ±0.0	2.58 ^b ±0.01	57	8.17 ^a ±0.09
Soybean	55.5 ^d ±0.1	15.2 ^b ±1.2	10.7 ^c ±0.9	4.5 ^b ±1.1	3.6 ^c ±0.2	6.49 ^d ±0.01	19	10.47 ^c ±0.06
<i>Breads</i>								
A	11.6 ^c ±0.1	8.2 ^c ±0.2	3.5 ^a ±0.3	4.7 ^c ±0.4	1.3 ^a ±0.1	-	32	43.8 ^{cd} ±0.7
B	12.3 ^d ±0.2	7.5 ^b ±0.2	3.8 ^a ±0.4	3.7 ^b ±0.2	1.5 ^a ±0.1	-	35	42.5 ^{bc} ±0.6
C	14.5 ^e ±0.2	8.8 ^c ±0.3	3.8 ^a ±0.4	4.9 ^c ±0.5	1.5 ^a ±0.2	-	32	44.1 ^d ±0.8
AA	9.1 ^a ±0.2	7.1 ^b ±0.6	3.5 ^a ±0.5	3.6 ^b ±0.3	1.3 ^a ±0.1	-	35	39.8 ^{ab} ±0.9
BB	12.3 ^d ±0.1	7.6 ^b ±0.4	3.8 ^a ±0.3	3.8 ^b ±0.4	1.5 ^a ±0.3	-	36	39.5 ^a ±0.7
CC	10.7 ^b ±0.2	7.7 ^b ±0.3	3.9 ^a ±0.4	3.8 ^b ±0.2	1.6 ^a ±0.3	-	35	40.9 ^{ab} ±0.8
AA'	9.4 ^a ±0.3	5.4 ^a ±0.2	3.6 ^a ±0.4	1.9 ^a ±0.2	1.4 ^a ±0.2	-	37	39.5 ^a ±0.9
BB'	12.3 ^d ±0.3	5.8 ^a ±0.3	3.8 ^a ±0.3	1.9 ^a ±0.3	1.5 ^a ±0.3	-	38	41.3 ^b ±0.9
CC'	10.9 ^b ±0.3	5.8 ^a ±0.2	4.0 ^a ±0.4	1.9 ^a ±0.3	1.6 ^a ±0.2	-	37	41.3 ^b ±0.4
Control	11.1±0.1	1.4±0.2	0.8±0.4	0.6±0.3	3.4±0.2	-	51	32.9±0.6

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

(^b) A, B, C – unpressured (gluten and carboxymethylcellulose, CMC) high legume-wheat breads; AA, BB, CC – pressure-treated (CMC) high legume-wheat breads;

AA', BB', CC' – pressure-treated high legume-wheat breads.

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

(**) Energy conversion: Protein x 4 Kcal/g; Fat x 9Kcal/g; Digestible Carbohydrates x 4 Kcal/g; Dietary Fibre x 2 Kcal/g

(¹) Conversion Factor from N to protein: wheat, 5.7; legumes, 6.25.

Table 3.- Techno-functional and firming kinetic parameters of unpressured and pressure-treated high-legume wheat-based breads.

Parameter	Blended bread samples ^{a,b}								
	A	AA	AA'	B	BB	BB'	C	CC	CC'
<i>Techno-functional</i>									
Hardness (N)	3.66 ^a ±0.36	9.25 ^d ±0.36	15.13 ^e ±0.25	6.84 ^b ±0.97	7.84 ^c ±0.26	18.78 ^f ±0.48	6.79 ^b ±0.29	9.71 ^d ±0.48	17.83 ^f ±0.69
Cohesiveness	0.78 ^a ±0.08	0.78 ^a ±0.08	0.77 ^a ±0.08	0.79 ^a ±0.04	0.77 ^a ±0.05	0.76 ^a ±0.02	0.80 ^a ±0.03	0.77 ^a ±0.04	0.74 ^a ±0.03
Specific volume (ml/g)	3.4 ^a ±0.2	3.1 ^b ±0.2	2.8 ^a ±0.4	3.2 ^b ±0.4	2.6 ^a ±0.3	2.8 ^a ±0.1	3.4 ^b ±0.2	2.7 ^a ±0.2	2.7 ^a ±0.1
Moisture (%)	43.8 ^a ±0.3	39.8 ^a ±0.3	39.5 ^a ±0.2	42.5 ^c ±0.2	39.5 ^a ±0.3	41.3 ^b ±0.1	44.1 ^c ±0.3	40.9 ^b ±0.3	41.3 ^b ±0.2
Overall acceptability (0-10)	8.0 ^a ±0.3	7.0 ^c ±0.3	6.0 ^b ±0.2	7.0 ^c ±0.3	6.5 ^c ±0.3	5.0 ^a ±0.5	7.5 ^{cd} ±0.3	6.0 ^b ±0.3	5.0 ^a ±0.2
Whiteness Index (crumb)	58.03 ^{bc}	60.17 ^c	54.10 ^a	57.31 ^{bc}	58.46 ^{bc}	54.76 ^a	56.84 ^b	58.77 ^{bc}	54.50 ^a
Browning Index (crust)	67.73 ^a	70.66 ^b	69.84 ^b	66.97 ^a	71.12 ^{bc}	70.61 ^b	67.53 ^a	72.48 ^c	72.80 ^c
Mean cell area, mm ²	0.49 ^b ±0.02	0.46 ^b	0.40 ^a ±0.01	0.43 ^a ±0.02	0.43 ^a ±0.03 ^a	0.41 ^a ±0.01	0.41 ^a ±0.02	0.39 ^a ±0.03	0.36 ^a ±0.03
Cell density, cells/cm ²	57 ^a ±3	60 ^a ±3	67 ^b ±3	71 ^b ±4	72 ^b ±6	73 ^b ±5	64 ^{ab} ±4	70 ^{ab} ±4	82 ^c ±3
Cell to total area ratio, %	28	28	27	31	31	30	26	26	26
Wall to total area ratio, %	72	73	73	69	69	70	74	74	74
<i>Firming kinetics^c</i>									
T_{∞} (N)	16.08 ^a ±1.23	38.46 ^c ±3.41	74.10 ^e ±6.24	24.03 ^b ±3.02	53.53 ^d ±5.26	84.43 ^f ±8.52	22.61 ^b ±3.45	32.41 ^c ±3.26	70.56 ^e ±5.39
k	0.200 ^d ±0.062	0.106 ^b ±0.013	0.126 ^c ±0.013	0.021 ^a ±0.002	0.085 ^b ±0.006	0.013 ^a ±0.009	0.015 ^a ±0.001	0.279 ^d ±0.053	0.136 ^c ±0.013
n	0.493 ^a ±0.091	0.663 ^{ab} ±0.086	0.438 ^a ±0.082	1.491 ^d ±0.011	0.525 ^a ±0.053	1.078 ^c ±0.009	1.643 ^e ±0.021	0.758 ^b ±0.029	0.661 ^{ab} ±0.078
T_0 (N)	3.66 ^a ±0.41	9.29 ^c ±1.87	10.92 ^c ±1.21	6.64 ^a ±0.93	7.92 ^b ±0.83	20.21 ^d ±3.13	7.07 ^b ±0.93	9.79 ^c ±1.83	17.85 ^d ±2.36

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

^(b) A, B, C : unpressured (gluten and carboxymethylcellulose) high-legume wheat based breads; AA, BB, CC: pressure-treated (carboxymethylcellulose) high-legume wheat-based breads; AA', BB', CC': pressure-treated high-legume wheat-based breads.

^(c) T_{∞} , T_0 crumb firmness at ∞ and 0 time, respectively, k constant of proportion of firming kinetics, n Avrami exponent of firming kinetics

Table 4.- Starch Hydrolysis kinetics, Expected Glycaemic Index and relevant Starch Nutritional fractions of unpressured and pressure-treated high-legume wheat-based breads.

Characteristic	High-legume wheat-based bread samples ^{a,b}							
	Unpressured			Pressure treated				
	A	B	C	control	AA'	BB'	CC'	HHPcontrol
<i>Starch Hydrolysis kinetics</i>								
C_{∞}	72.8 ^b ±1.3	59.7 ^a ±0.9	71.5 ^b ±1.5	93.0±2.3	88.9 ^c ±2.3	87.4 ^c ±1.9	87.9 ^c ±1.7	63.5±0.9
k	0.0680 ^a ±0.0031	0.0890 ^a ±0.0091	0.0660 ^a ±0.0031	0.1350±0.0085	0.0726 ^a ±0.0048	0.0610 ^a ±0.0034	0.0711 ^a ±0.0036	0.0695±0.0051
H_{90} , %	72.6 ^b ±1.1	59.7 ^a ±2.1	71.3 ^b ±1.7	93.0±1.1	88.7 ^c ±1.8	87.0 ^c ±1.9	87.8 ^c ±2.1	63.4±0.9
HI , %	77 ^b ±3	63 ^a ±2	77 ^b ±4	100	99 ^c ±3	98 ^c ±3	99 ^c ±3	-
eGI	75 ^b ±3	63 ^a ±4	75 ^b ±3	94±3	94 ^c ±3	93 ^c ±3	94 ^c ±3	-
<i>Starch Nutritional fractions (per 100 g bread, as is)</i>								
RDS, g	34.6 ^b ±0.8	32.4 ^a ±0.7	37.9 ^b ±2.9	65.8±1.9	43.1 ^c ±0.8	40.1 ^c ±1.9	42.8 ^c ±1.8	55.0±1.9
SDS, g	5.2 ^b ±0.9	1.4 ^a ±1.2	5.2 ^b ±0.8	3.2±0.9	5.5 ^b ±1.4	7.6 ^c ±0.9	5.8 ^b ±1.3	9.0±0.9
DS, g	39.8 ^b ±1.2	33.8 ^a ±1.6	43.1 ^c ±1.3	69.1±2.1 ^f	48.6 ^d ±1.9	47.8 ^d ±1.3	48.6 ^d ±2.1	64.0±1.8
RS, g	1.8 ^a ±0.4	2.3 ^a ±0.2	2.4 ^{ab} ±0.5	2.2±0.2	3.0 ^b ±0.3	3.2 ^b ±0.3	3.0 ^b ±0.4	2.0±0.5
TS, g	42	36	46	71	52	51	52	66

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

(^b) A, B, C : unpressured (gluten and carboxymethylcellulose) high-legume wheat based breads; AA', BB', CC': pressure-treated high-legume wheat-based breads; control: unpressured wheat flour bread; HHPcontrol: pressure-treated wheat flour bread. RDS: rapidly digestible starch, SDS: slowly digestible starch, eGI: expected glycaemic index, DS: digestible starch, RS: resistant starch, TS: total starch. C_{∞} : equilibrium concentration, k : kinetic constant, H_{90} : total starch hydrolysis at 90 min, HI : hydrolysis index. A first order kinetic equation [$C = C_{\infty}(1 - e^{-kt})$] was applied to describe the kinetics of starch hydrolysis C concentration at t time; C_{∞} equilibrium concentration; k kinetic constant; t time. TS= DS + RS; DS= RDS + SDS.