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Pulse germination as tool for modulating their functionality in wheat flour sourdoughs

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RESEARCH ARTICLE

Abstract

Three different pulses, namely green lentils, broad beans and chickpeas, were subjected to germination under daylight conditions. Germination resulted in changes to proximal composition, protein solubility and protein digestibility, varying according to the investigated legume. Based on chemical scores of raw pulse flours, optimal 70:30 mixtures of wheat and pulse flours were further proposed for potential bread-making applications. The flour mixtures were fermented with lactic bacteria, eventually in admixture with yeast, to obtain sourdoughs. The functionality of the sourdoughs was assessed to estimate the possibility of using germinated pulses for enhancing the nutritional value and modulating the bread-making potential. Fermentation increased the antioxidant activity of sourdoughs, the highest values corresponding to the samples containing germinated pulses. The highest antioxidant activity corresponding to the lowest amount needed to obtain a 50% antioxidant effect (EC50 of 19.83 mg) was registered for samples containing germinated lentil fermented with *Lactobacillus casei* and *Kluyveromyces marxianus*, while control samples (unfermented) presented the lowest antioxidant activity of 206.56 mg. Rheological measurements indicated that wheat flour substitution by raw or germinated pulses flour caused the decrease in sourdough consistency. However, pulse flour addition to the wheat flour and the type of starter culture used to prepare the sourdough exerted no significant influence on the rheological performance of bread batters. Bread specific volume, crumb firmness and colour varied with the type of legume used to substitute the wheat flour and with the sourdough added. Compared to the wheat flour bread, only a slight decrease in the specific volume was observed for samples with broad bean and chickpea flours. The lentil flour bread samples had the lowest specific volume and the highest firmness.

Keywords: germination, digestibility, degree of hydrolysis, pulses

1. Introduction

Pulses are low fat dry legume seeds, and exert beneficial effects on human homeostasis, being recognised as good sources of proteins, dietary fibres, vitamins and minerals (Bassett *et al.*, 2010). They also contain carbohydrates with a low glycaemic load, generating slow and moderate postprandial glucose and insulin response. The germination of legumes is a cheap and easy biotechnological process known to enhance nutritional quality, decrease the content of enzymatic inhibitors and improve nutrient bioavailability.

From the nutritional point of view, the storage proteins of legumes are known to contain relatively low amounts of sulphur-containing amino acids, methionine and cysteine. On the other hand, the proteins from pulses are richer in lysine compared to cereals. Therefore, based on the contents of lysine and sulphur-containing amino acid, legume and cereal proteins can be considered nutritionally complementary, and their combination in the diet ensures the proper ratio of essential amino acids for human nutrition (Duranti, 2006).

The effect of replacing the wheat flour by legume flours on technological functionality of doughs has been previously reported (Giménez *et al.*, 2012; Mohammed *et al.*, 2012). The level of wheat flour substitution by legumes varied from 8 to 54% (Angioloni and Collar, 2012). Most studies focused on wheat-pulses flour mixtures for bread-making applications. Giménez *et al.* (2012) also reported good

texture, flavour and physical-chemical properties of spaghetti obtained by the addition of 30% broad bean to wheat flour dough. The effect of the fermentation with acid lactic bacteria on the functionality of wheat-pulses flour mixtures was less studied. There is an increasing demand for the use of sourdoughs for bread-making, mainly because of the nutritional and physiological advantages resulting from the improvement of dietary fibre properties, mineral bioavailability, vitamin and phytochemical uptake and the decrease in glycaemic response upon bread consumption (Gobbetti *et al.*, 2014). Rizzello *et al.* (2014) reported promising results on the use of sourdough fermentation to improve the functionality of flour blends consisting of wheat and chickpeas, lentils or beans in terms of phytase, antioxidant capacity and hydrolysis index of the bread.

The aim of the present study was to determine the effect of germination on the functionality of lentils, broad beans and chickpeas, and to further test the effect of lactic fermentation on wheat-pulse flour mixtures for potential bread-making applications.

2. Materials and methods

Three different pulses, green lentils, broad beans and chickpeas, together with commercial white wheat flour (total protein – 10.27%; fat – 1.06%; ash – 0.42%; moisture – 7.96%) were purchased from a local market (Galați, Romania). Trypsin (200 FIP-U/g) and pepsin were purchased from Merck, Darmstadt, Germany. Pancreatin (minimal activity: lipase 10,000 U, amylase 8,125 U, protease 536 U) was purchased from Biofarma, Bucharest, Romania.

Pulses germination

All investigated pulses were germinated at 23±1 °C in daylight conditions (~12 h light/12 h dark) for 24 to 72 h, after a preliminary soaking of 7 to 24 h, depending on legume seed size. After germination all samples were dried at 55 °C for 24-30 h in a convection oven (LabTech LDO-030E, Daihan LabTech Co., LTD, Kyonggi-Do, Korea). Germinated broad beans and chickpeas were dehulled prior to drying. Dried seeds were further ground using a laboratory mill (WZ-2, Sadkiewicz Instruments, Bydgoszcz, Poland) to obtain flours with particles size lower than 500 µm. Due to the fact that soaking significantly influences the chemical composition of seeds (López-Amorós *et al.*, 2006), control samples were prepared by soaking, dehulling (in the case of broad bean and chickpeas), and drying at 55 °C before grinding.

Flour mixture obtaining

Taking into account the levels of lysine and sulphur-containing amino acids in pulses and cereal grains, mixtures with different amounts of wheat and pulse flours were obtained, such as to increase the Essential Amino Acid index (EAA). In order to identify the optimal ratio of the two protein sources within blends with targeted balanced amino acid contents, the Amino Acid chemical Score (AAS) of studied flours was first calculated using the equation given by Fouad and Rehab (2015) and considering the whole chicken egg protein as standard protein (WHO/FAO/UNU, 1985).

$$AAS = \frac{\text{Essential amino acid from the studied source}}{\text{Essential amino acid from WHO/FAO protein}} \times 100 \quad (1)$$

Afterwards, the AAS calculated for the proteins from studied raw pulses were plotted against the AAS of the wheat proteins (Figure 1), and the optimal ratio between targeted proteins was considered at the intersection of lysine and methionine. The wheat:pulse proteins ratio varied from 45:55 to 52:48, depending on the pulse sample (Figure 1). Finally, for the sake of comparison, the same optimal ratio of 70:30 between wheat and pulse flours was decided by additionally factoring in the protein content of each flour sample.

EAA was calculated as the geometric mean of the individual amino acid scores being equal to the antilogarithm of the individual scores:

$$EAA = \sqrt[8]{AAS_1 + AAS_2 + ... + AAS_8} \quad (2)$$

The chemical scores for the amino acids of the obtained mixtures were considered based on the selected intersection point (Figure 1).

Sourdough preparation

Three different starter cultures were used for sourdough (SD) preparation: SD1 – fermented at 30 °C with a mixture of *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactobacillus rhamnosus* (DI-PROX MTTX, EDR Ingredients, Romania); SD2 – fermented at 40 °C with a mixture of *Lactobacillus casei* (Nutrish®), and yeast *Kluyveromyces marxianus* subsp. *Marxianus* (LAF4, Chr Hansen, Brasov, Romania); SD3 – fermented at 30 °C with a mixture of *Lactobacillus acidophilus, Bifidobacterium BB12®*, and *Streptococcus thermophilus* (ABT-1, Chr Hansen, Brasov, Romania). Patrascu *et al.* (2016a) reported that these starter cultures are effective for fermenting composite flours consisting of white wheat flour and raw or germinated whole soy flour. The sourdoughs were prepared in large glass beakers by mixing wheat and raw or germinated pulse flours at 70:30 ratio with tap water and inoculum, in order to obtain a 33% flour suspension. Samples were subjected to fermentation for 19/20 h (depending on the starter culture used) in a laboratory incubator (Pol-Eko

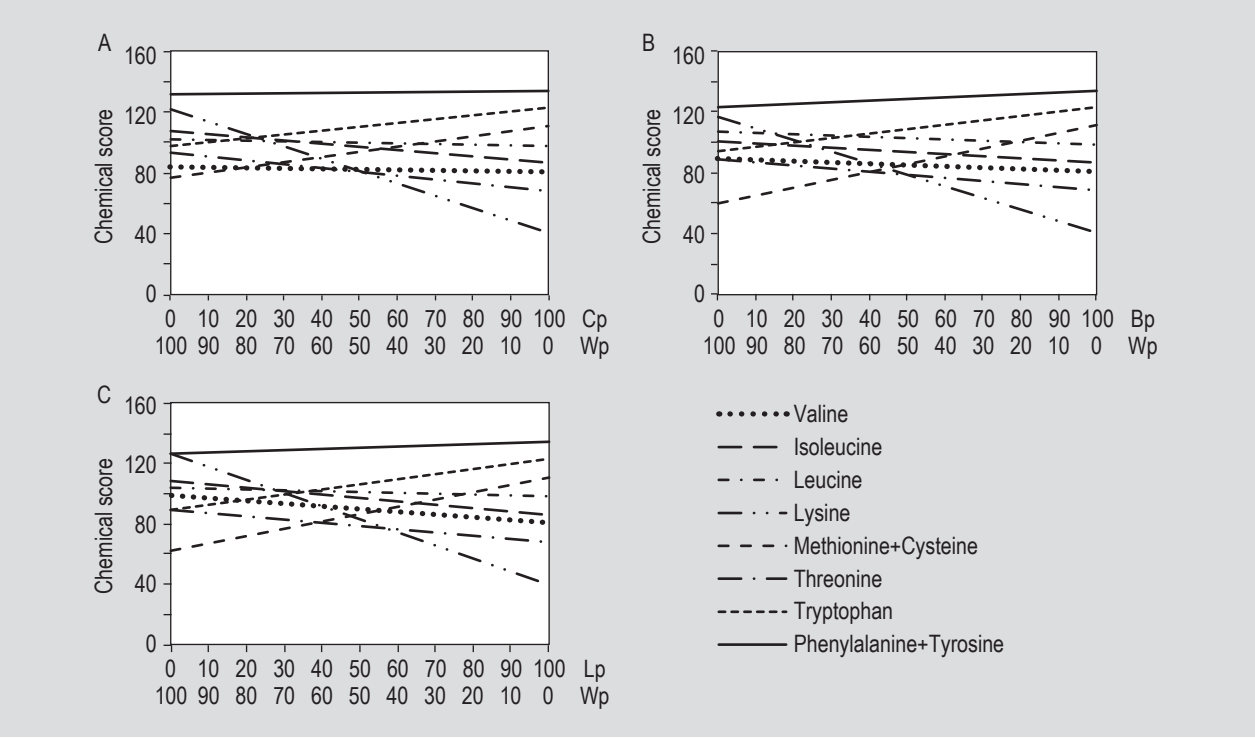

Figure 1. Plots of the chemical scores of essential amino acids from the proteins of the studied flour mixtures. (A) Wheat protein (Wp): chickpea protein (Cp); (B) Wp : broad bean protein (Bp); (C) Wp : lentil protein (Lp).

Aparatura, Wodzisław Śląski, Poland). Control samples were prepared without starter culture addition.

The bread-making procedure

Bread batters were prepared by mixing the following ingredients for 5 min at room temperature: wheat flour or mixtures of wheat flour and raw/germinate pulse flour (80.32%), salt (1.21%), sourdough (16.06%), and fresh yeast (*Saccharomyces cerevisiae;* 2.41%). Water was added in accordance with the water absorption capacity of the flours. The batters were further fermented for 30 min at 30 °C, moulded and placed in the baking trays for additional proofing of 30 min at 30 °C. Baking was performed at 190 °C for 35 min in a preheated oven (Micro 4 T, Mondial Forni, Verona, Italy). For each type of investigated flour mixture, the control batter and bread were prepared without sourdough. Two different batches were prepared for each bread formulation.

Proximate composition

The proximate composition of the studied flours was determined as follows: the moisture content using the AACC 44-51 method (AACC, 2010); the protein content using the semi-micro Kjeldahl method (Raypa Trade, R Espinar, SL, Barcelona, Spain) applying a Nitrogen conversion factor of 6.00; the fat content by extraction with ether using a Soxhlet extractor (SER-148, VELP Scientifica, Usmate Velate, Italy); and the ash content using SR ISO 2171: 2002 method (ASRO, 2008).

Estimation of trypsin inhibitors

The effect of germination on the content of trypsin inhibitors found in studied pulses was estimated by determining the degree of protein hydrolysis (DH) in the presence of trypsin, according to the pH-STAT technique (Navarrete del Toro and García-Carreño, 2002). Samples were equilibrated at 37 °C. Hydrolysis process was initiated with the addition of 1.5 mg Trypsin/g protein (Merck, D-6100 Darmstadt, Germany). The pH value of the mixture was kept constant with 0.01 N NaOH, by using a 702 SM Titrio equipment (Methrom, Herisau, Switzerland). The hydrolysis process was allowed to take place for 3 hours, and the total volume of 0.01 N NaOH used for maintaining the pH value at 7.5 units was recorded. The DH (%) was calculated using Equation 3:

$$DH(\%) = V \times N_{NaOH} \times (1/\alpha) \times (1/M_p) \times (1/h_{tot}) \times 100 \quad (3)$$

where V (ml) is the volume of NaOH used for maintaining the pH value during hydrolysis, N_{NaOH} the normality of NaOH used for titration, M_p (g) the mass of protein being hydrolysed, h_{tot} (meqv/g protein) the total number of peptide bonds in the protein substrate, and α the average degree of dissociation of the α-NH_2 groups, estimated using Equation 4:

$$\alpha = \frac{10^{(pH-pK)}}{1+10^{(pH-pK)}} \quad (4)$$

where pK is the average dissociation value for the α-NH_2 groups liberated during hydrolysis being dependent on temperature, peptide chain length and the nature of the terminal amino acid. At 37 °C, the pK value was calculated as 7.39 (Navarrete del Toro and García-Carreño, 2002). At pH 7.5, 1/α was calculated as 1.776.

The parameter h_{tot} was calculated considering the amino acid content of proteins from raw pulses according to USDA food composition databases (USDA, 2018), by summing the mmoles of each individual amino acid per gram of protein.

In vitro protein digestibility

For determining the *in vitro* protein digestibility of raw and germinated pulse flours, a multi-enzyme assay was used to simulate gastric and small intestinal digestion. Pepsin (15 mg/g protein) was first added to the suspensions consisting of 0.5 g flour samples in 20 ml distilled water, and the pH value was adjusted to 2 units with 0.5 N HCl. Samples were allowed to digest for 2 h at 37 °C. The pH value of the mixture was then adjusted to 7.5 units with 0.5 N NaOH, pancreatin (10 mg/g protein) was added to the samples and was allowed to digest for three additional hours. The remained unhydrolysed protein was precipitated by adding 5 ml of 50% trichloroacetic acid and the nitrogen content was determined using the Kjeldahl method. The *in vitro* protein digestibility was calculated with Equation 5:

In vitro protein digestibility (%) =

$$100 - \frac{\text{protein content after digestion}}{\text{initial protein content}} \times 100 \quad (5)$$

Soluble proteins

In order to determine the soluble protein, 1 g of raw or germinated pulse flours was mixed with 10 ml of 0.01 N HaOH and subjected to extraction by stirring for 1 h at room temperature, using a magnetic stirrer. The mixture was then centrifuged at 4,900×*g* for 10 minutes and the supernatant was collected. The protein content of the supernatant was determined using the method proposed by Ionescu *et al.* (2001).

Determination of free amino acid content

The free amino acid content was determined using a spectrophotometric method, which is based on the formation of a blue colour when ninhydrin reacts with free amino groups of different compounds (Ionescu *et al.*, 2001). Results were expressed as g Tyrosine equivalents/100 g protein.

Extraction for antioxidant activity and total phenolics determination

In order to determine the antioxidant activity and total phenolic contents of flour mixtures before and after fermentation, the extraction was performed using 80% methanol solution while stirring for 2 h at room temperature. Mixtures were then centrifuged at 9,690×*g* for 10 minutes and the supernatant was collected for further readings.

Total phenolic content

The concentration of total phenolic compounds was determined using the Folin-Ciocalteu method. A volume of 0.2 ml of extract was mixed with 1.5 ml of Folin-Ciocalteu reagent (1:10 diluted with water, v/v). After a resting period of 10 min at room temperature, 1.5 ml of Na_2CO_3 (60 g/l) was added. The admixture was allowed to rest for another 90 min and then the absorbance was measured at 725 nm. Results were expressed as mg ferulic acid equivalents (FA)/g sample.

Determination of free radical scavenging activity

The ability to scavenge the free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined by mixing 0.1 ml of extract with 3.9 ml of DPPH solution in methanol (6×10^{-5} M). After a resting period of 30 min in the dark, the absorbance was recorded at 515 nm in order to determine the concentration of remaining DPPH. The DPPH radical scavenging activity was expressed as EC50 values (López-Amorós *et al.*, 2006), meaning that a smaller EC50 value will correspond to a higher antioxidant activity. All samples were prepared and measured separately in duplicate.

Water-holding capacity

The water holding capacity (WHC) of flour mixtures was determined according to the method proposed by Boye *et al.* (2010) and was reported as the maximum amount of water retained by 1 g of flour under centrifugation for 10 minutes at reduced speed (880×*g*).

Rheological behaviour

The rheological measurements were performed using a controlled-stress rheometer (AR2000ex, TA Instruments, Ltd, New Castle, DE USA) equipped with a Peltier temperature control jacket. The geometry used for rheology measurements carried out on wheat-pulse flour-based sourdoughs was a concentric cylinder cup (inner radius of 28.01 mm, cylinder length of 42.01 mm and gap of 5 μm). In the case of the bread batters, a plate geometry with diameter of 40 mm and a closing gap of 2 mm were used.

For all investigated samples the linear viscoelastic region (LVR) with constant values of storage and loss moduli (G' and G'') was determined by running strain sweeps under oscillatory flow in small amplitude conditions. The strain was gradually increased in the 0-100% range (corresponding to 0-2 Pa stress values), while using an oscillation frequency of 1 Hz. The beginning of flow was estimated as the yield point, corresponding to the G'/G'' crossover strain value.

Bread analysis

After two hours of cooling the bread samples were characterised by determining the specific volume (SR 91/2007), crumb colour (lightness – L* and the chromatic components a* and b*) by means of Chroma Meter CR-410 (Konica Minolta Inc, Osaka, Japan) (Angioloni and Collar, 2012) and crumb texture as the maximum compression force required for the 40 mm penetration of the bread slice using the ML-FTA system (Guss, Strand, South Africa) with a 7.9 mm diameter probe.

Statistical analysis

Statistical analysis was performed using Microsoft Excel Software. Two independent germination experiments were conducted and all measurements were performed in duplicate. The results are reported as mean values together with standard deviations. Fisher's least significant difference (LSD) test at 95.0% confidence level was applied using the Statgraphics Centurion XVI.I (Statgraphics Technologies, Inc., The Plains, VA, USA) software so as to determine differences between results.

3. Results and discussion

Effect of germination on the chemical composition of pulses

Proximal composition of raw and germinated pulse flours is presented in Table 1. Pulses germination resulted in a significant increase in total protein content ($P<0.05$), as a consequence of cell constituents and enzymes synthesis, on account of the degrading of other constituents of the cells (Yu-Wei and Wang, 2015). The highest protein content increase of 12-13% was observed in the case of lentils and broad bean germination, while total protein content of chickpeas increased by 7.8%. Significant protein increase in germinated legumes was also reported by Fouad and Rehab (2015) for lentils, and Yu-Wei and Wang (2015) for broad beans.

The soluble protein is an important component of the total protein because it serves as a useful indicator of protein performance in food systems. The soluble proteins were extracted in NaOH solution of pH 11, corresponding to the maximum solubility of globulins, which account for 10-20% of the total protein from pulses (Roy *et al.*, 2010). With regard to the total proteins content of each investigated sample, no significant differences were found among the soluble proteins of the raw pulse flours ($P>0.05$), which ranged from 14.92 to 15.18%. It is known that germination leads to protein breakdown into smaller soluble fractions, such as peptides or even amino acids. For all studied pulses, germination caused no significant increase in the soluble protein content ($P>0.05$). On the other hand, a significant increase in the free amino acid content was observed when germinating lentil and broad bean samples ($P<0.05$). The most significant increase during germination was obtained in the case of lentils (Table 2), this germinated sample having the highest amount of free amino acids of 9.67 g/100 g protein. Not only the process itself, but also

Table 1. The influence of the germination process on proximate composition of studied pulses.[1]

Flour samples	Total protein (g/100 g flour)	Moisture (g/100 g flour)	Fat (g/100 g flour)	Ash (g/100 g flour)	Carbohydrates[2] (g/100 g flour)
Raw lentil	22.43±0.27^{a}	13.03±0.01	1.68±0.02	2.64±0.06^{a}	60.22±0.33
Germinated lentil	25.37±0.15	8.43±0.05^{a}	1.31±0.01	2.75±0.07^{a}	62.15±0.21^{a}
Raw broad bean	29.55±0.19	8.14±0.17	1.16±0.03	3.80±0.20^{b}	57.32±0.03
Germinated broad bean	33.24±0.24	6.69±0.22	0.70±0.01	4.01±0.40^{b}	55.37±0.65
Raw chickpea	21.16±0.06	8.60±0.03^{a}	4.75±0.05	2.47±0.01^{a}	63.02±0.01^{ab}
Germinated chickpea	22.82±0.10^{a}	7.49±0.05	3.93±0.02	2.49±0.08^{a}	63.27±0.03^{b}

[1] Data followed by the same letter within a column denotes statistically insignificant differences at a 95.0% confidence level. Data from columns where no letter was attributed are statistically significantly different at a 95.0% confidence level.
[2] Calculated by difference.

Table 2. The influence of the germination process on protein bioavailability of studied pulses.[1]

Flour samples	h_{tot}	Degree of hydrolysis (%)	Protein digestibility (%)	Soluble protein (g/100 g protein)	Free amino acids (g/100 g protein)
Raw lentil	7.06	7.70±0.26	39.60±0.21	14.92±0.29^{AB}	2.19±0.38
Germinated lentil		8.59±0.38	45.25±0.19	15.50±0.07	9.67±0.10
Raw broad bean	7.14	10.49±0.13^{a}	46.20±0.10	15.07±0.34^{CD}	4.52±0.39
Germinated broad bean		10.94±0.11^{a}	47.30±0.20	16.24±0.07^{ACE}	6.39±0.08^{a}
Raw chickpea	7.41	14.62±0.10	42.51±0.18	15.18±0.24^{EF}	7.12±0.29^{a}
Germinated chickpea		16.48±0.12	43.73±0.21	16.18±0.26^{BDF}	8.22±0.22

[1] Data followed by the same lowercase letter within a column denotes statistically insignificant differences at a 95.0% confidence level. Data followed by the same uppercase letter within a column denotes statistically significant differences at a 95.0% confidence level. Data from columns where no letter was attributed are statistically significantly different at a 95.0% confidence level.

the germination conditions, were reported to influence the accumulation of free amino acids values.

The fat content of all investigated pulses significantly decreased (P<0.05) during the germination process, most probably being used as an energy source (Table 1). Finally, no significant increase in the ash content (P>0.05) was observed during the germination process.

Effect of pulses germination on protein digestibility

The *in vitro* protein digestibility (IVPD) of raw pulse flours was determined by simulating the gastric and small intestinal digestion. Regardless of the investigated pulses, germination caused a significant increase of IVPD (P<0.05) (Table 2). The highest IVPD of 81.14% was obtained for wheat flour, while raw and germinated pulse flours presented significantly lower digestibility, with a minimum of 39.60% for raw lentils and a maximum of 47.30% for germinated broad bean flour. These results can be explained by the high protein content of legumes which can promote starch-protein interactions restricting the enzyme attack. In addition, Carbonaro *et al.* (2012) reported that legume storage proteins, legumin and vicilin, display stability towards gastrointestinal digestion even after heating, due to the presence of disulphide bonds, structural features associated with the oligomeric state and extensive hydrophobicity. However, germination was reported to efficiently increase the IVPD of pulses (Chitra *et al.*, 1996). In particular, germination caused the IVPD increase from 84.20% to 85.15% in the case of lentils (El-Adawy *et al.*, 2003). The increase of the protein digestibility after germination appears as a consequence of the enzyme-assisted hydrolysis of the storage proteins, meant to sustain the early development stages of plant growth. Furthermore, fermentation might also affect the degradation of the proteins from raw and germinated pulses, thus their digestibility.

Protein digestibility and biological value are highly influenced by the cleavage performance of trypsin and therefore by the presence and activity of trypsin inhibitors. The ability of trypsin to recognise and specifically cleave the peptide bonds within proteins varied with the type of studied legume. Trypsin displayed the highest specificity toward chickpea proteins (DH of 14.62%), whereas the less advanced protein hydrolysis (DH of 7.70%) was obtained when testing the lentils. The even lower DH value obtained in the case of wheat flour (4.24%) indicates that trypsin has low hydrolytic efficiency towards wheat gluten. In this respect, when comparing the activity of five different proteases, Kong *et al.* (2007) reported that trypsin displayed the lowest hydrolytic efficiency towards wheat gluten, with only ~4% after 6 h of hydrolysis.

Comparison of the trypsin-assisted hydrolysis of the proteins from raw and germinated pulses highlights the impact of germination on trypsin inhibitors. The presence of trypsin inhibitors is one of the major issues impairing the nutritional value of legume proteins. Lentil germination produced a slight increase in DH, however with no significant differences (P>0.05), while in the case of broad beans DH value remained fairly unchanged after germination (Table 2). These results suggest that the trypsin inhibitors were not substantially degraded during the time period of lentil and broad bean germination. Similar observations were reported by Muzquiz *et al.* (2004). The only significant increase in DH was observed after chickpea germination (P<0.05), suggesting a reduction in the presence of trypsin inhibitors. These results are in agreement with El-Adawy *et al.* (2003) who showed that the germination process was efficient in reducing trypsin inhibitors from lentil seeds.

Functional performance of the wheat-pulses flour blends

Wheat flour fortification with pulses flour is an efficient way of increasing the consumption of pulses. For all investigated pulses, the optimal amount of flour to be blended with the wheat flour was decided based on the protein content and EAA value (Figure 1). The optimal blends consisted of ~70% wheat flour and ~30% pulse flour. Supplementary Table S1 presents the EAA values for raw pulses, wheat flour and obtained flour mixtures. Substituting 30% of wheat flour for pulse flour led to a significant increase in the EAA index, from 87.43 to 94.15-96.15.

In order to assess the potential for using the wheat-pulses flour mixtures for bread-making applications, the ability of the blends to bind and retain water as in the bread batter matrix was determined. WHC is an important functional property of food matrices and gives an indication about protein and carbohydrate hydration. When compared to the wheat flour, significant higher WHC values were determined for the flour mixtures (P<0.05) (Table 3). The addition of germinated pulses flour resulted in slightly higher WHC with respect to the corresponding raw ones, with significant differences in the case of samples containing broad bean (P<0.05). Based on the obtained results the flour to water ratio of 1:2 (w:w) was decided for preparing the sourdoughs. The water content used exceeded the WHC of the flour mixtures, thus ensuring suspensions with flow properties and therefore easy processing and efficient distribution of starter culture. Similar to our observations, Mohammed *et al.* (2012) and Giménez *et al.* (2012) reported the increased water absorption capacity of the doughs due to the addition of chickpea and broad bean, most probably due to a greater water requirement of legumes proteins in order to become hydrated.

Antioxidant properties of the wheat-pulses flour blends and sourdoughs

The flour blends were subjected to fermentation with mixtures of microorganisms including lactic acid bacteria. The obtained sourdoughs were characterised in terms of total phenolic contents and DPPH antiradical activity (Figure 2). Fermentation resulted in a significant increase in the total phenolic contents (P<0.05), except for SD1 samples (Figure 2A). In particular, the highest increase was observed for SD2, closely followed by SD3. Not only the fermentation process but also the addition of pulse flour favoured the increase in phenolic content of wheat flour (P<0.05). The highest values of the total phenol content were obtained for SD2 samples containing germinated lentil (87.05 mg/100 g) and broad bean (89.86 mg/100 g) flours. Similar to our observations, Rizzello *et al.* (2010) reported a significant increase in total phenol content, antioxidant activity, free amino acids and *in vitro* protein digestibility of sourdoughs obtained from wheat germ. There are many studies showing that the germination process is highly efficient in increasing total phenolic content and antioxidant activity of pulse legumes (Gharachorloo *et al.*, 2013). The phenolic compounds are responsible for most of the antioxidant properties of pulses, providing health benefits because of the protective activity against oxidative damage (López-Amorós *et al.*, 2006).

The overall antioxidant activity of the flour blends before and after fermentation was estimated by determining the DPPH antiradical activity. The obtained results indicated the significant contribution of germinated pulse flour addition to the DPPH-RSA of wheat flour (P<0.05). Among the three pulses studied, chickpea had the lowest antioxidant capacity (Figure 2B). Similar to our findings, Zhao *et al.*

Table 3. The effect of the addition of raw and germinated pulses to wheat flour on water-holding capacity of flour mixtures and flow characteristics of obtained sourdoughs (SD).[1,2]

Flour mixtures	Water-holding capacity (g water/g flower)	Control	% γ G'/G'' intersection		
			SD1	SD2	SD3
WFI	0.974±0.01^{ab}	6.91±1.56^{ABCDEF}	1.17±0.14	1.03±0.00^{a}	1.30±0.00^{a}
WFI+L	1.024±0.00^{cde}	1.64±0.80^{A}	2.61±0.31	2.65±0.00^{b}	4.13±0.01^{b}
WFI+Lg	1.049±0.02^{cfg}	2.31±0.54^{B}	1.67±0.01^{a}	2.38±0.10^{b}	3.34±0.04
WFI+B	0.951±0.02^{a}	1.14±0.10^{C}	6.73±0.02	2.34±0.29^{b}	4.20±0.01^{b}
WFI+Bg	1.025±0.01^{dfh}	2.29±1.03^{D}	5.52±0.01	2.74±0.58^{b}	2.34±0.26
WFI+C	0.991±0.00^{bh}	3.25±0.10^{E}	1.65±0.00^{a}	1.29±0.01^{a}	1.80±0.14
WFI+Cg	1.016±0.02^{egh}	1.92±0.10^{F}	2.06±0.00^{a}	1.34±0.02^{a}	1.04±0.00^{a}

[1] B = broad bean; C = chickpea; g = germinated; L = lentil; WFI = wheat flour.

[2] Data followed by the same lowercase letter within a column denotes statistically insignificant differences at a 95.0% confidence level. Data followed by the same uppercase letter within a column denotes statistically significant differences at a 95.0% confidence level.

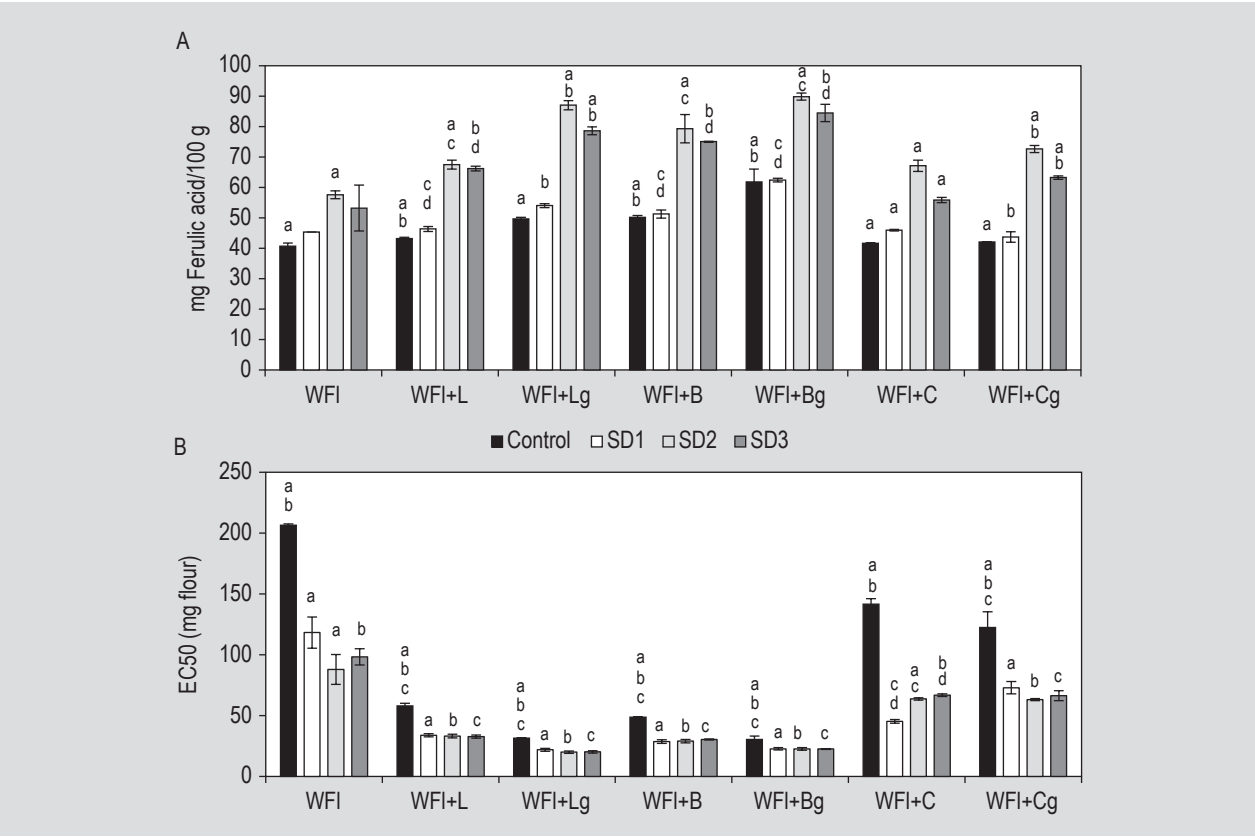

Figure 2. Antioxidant activity in terms of total phenol content (A) and DPPH radical scavenging activity (B) of sourdoughs (SD) obtained from wheat and pulses flour mixtures. WFI = wheat flour; L = lentil; B = broad bean; C =chickpea; g = germinated. Data followed by the same letter within a column set denotes statistically significant differences at a 95.0% confidence level.

(2014) reported significantly lower values for chickpea total phenolic content and DPPH radical scavenging activity in comparison to lentils, which presented the highest values among 10 studied legumes. Regardless of the starter culture and the flour sample subjected to fermentation, all sourdoughs displayed higher DPPH-RSA values (results presented as EC50 in Figure 2B) compared to control samples. These results might be explained by different processes occurring during fermentation, such as the increase in the total phenolic content, the release of diverse compounds with antioxidant activity from the complex vegetal matrix, or the formation of peptides through proteolysis (Coda *et al.*, 2012). Moreover, proteins were reported to contribute to antioxidant activity of food products through different mechanisms such as inactivation of reactive oxygen species, scavenging free radicals, chelation of prooxidative transition metals, and reduction of hydroperoxides (Elias *et al.*, 2008).

Rheological properties of sourdoughs and batters based on wheat-pulses flour blends

Knowing the rheological properties of batter systems allows for an efficient prediction of the processing behaviour and control of the quality of the end-products. The viscoelastic characteristics of the obtained sourdoughs and bread batters were determined by mean of the oscillatory strain sweep test. Figure 3 presents the log-log plot of G* versus strain (%) of wheat-pulses flour suspensions and sourdoughs. G* is the complex modulus, a measure of sample deformation considering both G' and G'' parameters. Among vegetable proteins, gluten presents particular viscoelastic characteristics given by elasticity of glutenin and stickiness and fluidity of gliadin, which assure the formation of a three-dimensional structure, specific to the high-quality bread batters. Therefore, the main technological drawback of wheat flour fortification with non-gluten flours consists in difficulties of CO_2 retention during the fermentation process. Also, it was observed that vegetable proteins can affect water distribution within batters and weaken the interactions between hydrocolloids and starch matrix (Renzetti and Rosell, 2016).

The rheological behaviour of the sourdoughs is presented in Figure 3. When analysing the control samples (Figure 3A), one can see that wheat flour substitution by pulses flour determined the significant reduction in consistency, presenting lower G* values over the entire tested strain domain. Samples containing germinated pulses presented lower G* values compared to the corresponding samples

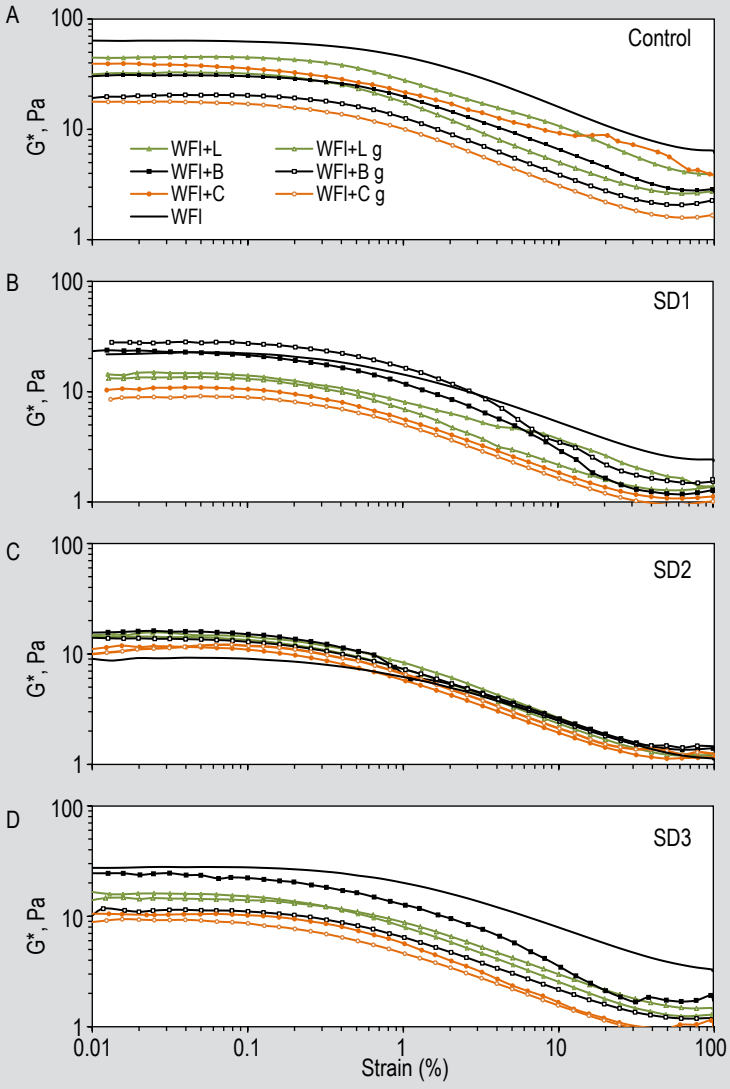

Figure 3. Viscoelastic behaviour of wheat-pulses sourdoughs (A) controls, (B) SD1, (C) SD2, (D) SD3) as a function of the applied strain. WFI = wheat flour; L = lentil; B = broad bean; C = chickpea; g = germinated.

based on raw pulse flours. Pulse flour addition to the wheat flour also resulted in a significant reduction of strain values at G'/G'' cross-over, corresponding to structure transition from solid-like to liquid-like behaviour (Patrașcu *et al.*, 2016b). It was stated that at low stress values the starch-starch interactions are mainly responsible for the rheological behaviour of doughs, whereas the interactions between proteins are visible at higher stress values (Khatkar and Schofield, 2002). The wheat flour suspension presented the transition towards liquid-like behaviour at 6.91% stain value, while for suspensions prepared from mixtures with 30% pulse flours the flowing process initiated at an average stain value of ~2.09% (Table 3). Concerning the rheological behaviour of sourdoughs, it was observed that wheat sourdough resistance to flow suffered a significant reduction (P<0.05) compared to controls, the strain values at G'/G'' intersection reaching those obtained for wheat-pulse flour sourdoughs. Moreover, in the case of sourdoughs based on lentil and broad bean flours the flowing process initiated at significant higher strain values (P<0.05) compared to wheat flour sourdough (Table 3). Overall, fermentation resulted in a significant reduction in sourdough consistency (lower G* values in the LVR) compared to unfermented control samples. The same could be observed in G' and G'' evolution, as both viscoelastic parameters presented a significant decrease after fermentation (Supplementary Table S2). By far, the highest G' and G'' values were registered in the case of wheat-flour samples. A particular behaviour was observed in the case of sourdoughs fermented with yeast containing starter culture (SD2) (Figure 3C), when the lowest consistency values were registered. No significant differences were found among SD2 samples obtained

from different flour mixtures. The lowest G* values in the LVR were observed for the wheat flour SD2 sample. Yeast fermentation is responsible for CO_2 formation, which interferes with the highly cross-linked protein network, being responsible for rheological weakening (Newberry *et al.*, 2002). When comparing the rheological behaviour of sourdoughs obtained with pulse flour addition, it was observed that in all cases, wheat-chickpea flour samples showed the lowest consistency values for the entire considered strain domain.

Regarding the rheological properties of the batters, the G* values were higher for control samples compared to the ones containing sourdoughs (Figure 4). However, the average G' and G'' values registered up to 1% strain showed rather similar results for all batter samples, regardless of the sourdough used for preparing it (P>0.05) (Supplementary Table S3). Partial wheat flour substitution by raw or germinated pulse flours resulted in no significant alteration of the rheological behaviour of batters. No correlations were established between G' and G'' values and flour mixture. Moreover, germination process caused no significant influence on the rheological behaviour of the batters prepared with wheat-pulse flour mixture (P>0.05).

Bread characterisation

The bread samples prepared with three different types of sourdoughs (SD1, SD2 and SD3) and flour blends consisting of 70% wheat and 30% of raw or germinated pulse flour

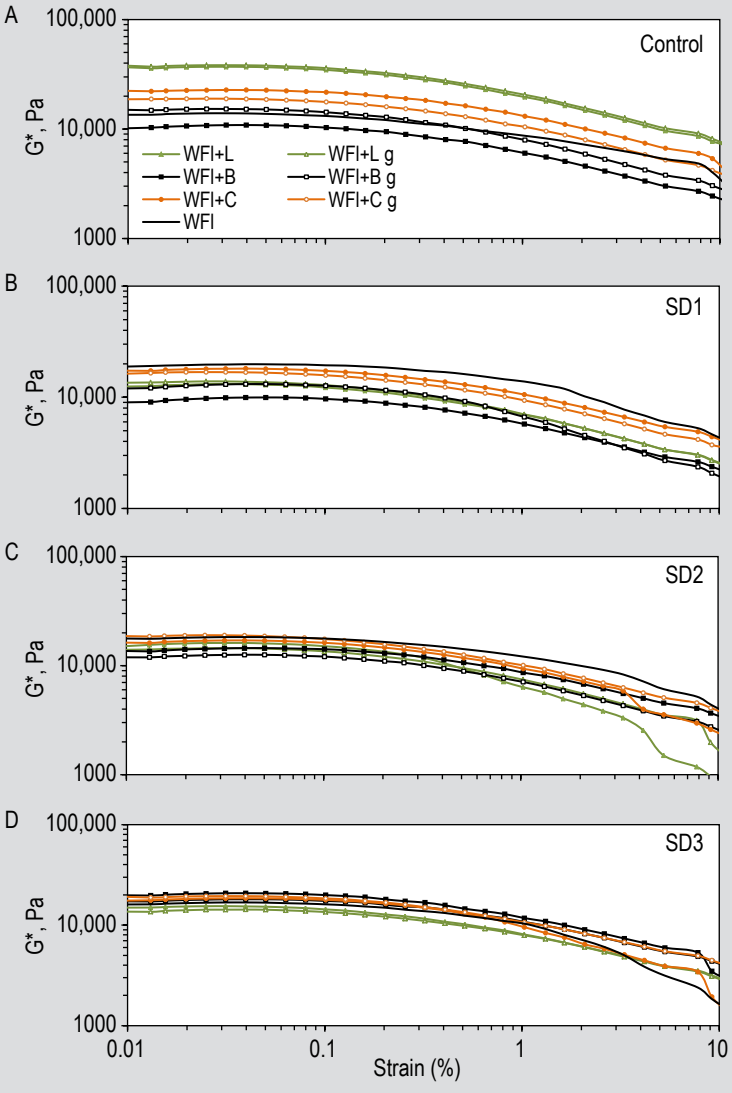

Figure 4. Viscoelastic behaviour of the wheat-pulses bread batters prepared (A) without sourdough (controls) and with sourdoughs (B) SD1, (C) SD2 and (D) SD3 as a function of the applied strain. WFI = wheat flour; L = lentil; B = broad bean; C = chickpea; g = germinated.

were characterised in terms of specific volume of the loaf, crumb firmness and colour (Table 4).

Pulse flour addition to bread formulations with no sourdough (controls) resulted in the alteration of the loaf volumes compared to the wheat flour sample, the decrease being more profound when using germinated pulses in the mixtures. Pulses have a high protein content, but they lack gluten proteins. Therefore, wheat flour substitution by pulse flours resulted in gluten protein dilution, affecting gluten development during batter mixing and quality of the final product. Our results are in line with Baik and Han (2012), who studied the effect of wheat flour fortification with chickpeas, peas, lentils and soybeans treated through cooking, roasting and fermentation, on the characteristics of the batters and breads. They indicated that loaf volume was significantly affected when adding legumes to the wheat flour, the most important decrease being noted for samples with soybeans and lentils. Angioloni and Collar (2012) also reported that the quality of the bread was affected by the addition of mixed legumes, which caused a significant decrease in bread volume and an increase in air cell density.

The effect on the specific volume of adding sourdough to a bread formulation varied with the type of flour and the starter culture used for fermentation. Regardless of pulse germination and sourdough used, the lowest specific volumes were registered for samples with lentil flour. On the other hand, no dramatic changes were noticed in the case of the bread samples containing broad bean and chickpea flour (Table 4). These results comply with the observations of Coda *et al.* (2017), who reported that wheat

Table 4. Quality evaluation of sourdough breads containing raw and germinated pulse flours.[1,2]

Flour sample	Leavening agent	Specific volume (cm^3/100 g)	Firmness (kg)	Crumb colour		
				L	a*	b*
WFl	Control	325.85±0.77	0.70±0.07^{a}	75.33±1.14^{a}	0.17±0.21	18.12±0.17^{a}
WFl+L		294.66±1.68^{a}	1.08±0.09	63.67±0.40	1.66±0.03^{a}	21.59±0.23
WFl+Lg		265.13±1.58	1.15±0.17	60.63±0.63	3.01±0.07	18.43±0.06^{a}
WFl+B		303.29±0.94^{b}	0.76±0.06^{a}	70.96±1.04^{b}	1.19±0.79^{a}	24.79±0.83^{b}
WFl+Bg		299.71±2.43^{ab}	0.79±0.03^{a}	68.54±1.33	1.56±0.35^{a}	24.32±0.16^{b}
WFl+C		301.01±4.00^{ab}	0.70±0.08^{a}	76.09±0.52^{a}	1.11±0.03^{a}	27.76±0.21^{c}
WFl+Cg		280.92±2.01	0.71±0.06^{a}	72.64±0.64^{b}	1.74±0.11^{a}	27.57±0.50^{c}
WFl	SD1	300.03±3.97^{a}	0.37±0.01	78.16±1.92^{a}	1.06±0.00^{a}	18.06±0.19^{a}
WFl+L		253.51±7.30	0.68±0.15^{ab}	58.49±0.19	1.99±0.10^{b}	21.62±0.36
WFl+Lg		226.43±2.18	0.86±0.07^{cd}	55.04±0.79	3.26±0.01	17.82±0.17^{a}
WFl+B		308.81±7.31^{a}	0.58±0.04^{a}	74.90±0.60^{b}	1.15±0.20^{a}	23.78±0.14
WFl+Bg		297.30±0.13^{a}	0.65±0.07^{ab}	71.80±1.73	1.74±0.06^{bc}	24.52±0.19
WFl+C		305.67±1.08^{a}	0.73±0.03^{bc}	75.20±2.24^{b}	1.18±0.45^{a}	28.32±0.81^{b}
WFl+Cg		282.42±3.25^{a}	0.87±0.07^{d}	75.43±0.18^{ab}	1.68±0.25^{c}	28.07±0.05^{b}
WFl	SD2	352.80±1.65	0.27±0.01	77.63±0.91	0.77±0.31^{a}	18.10±0.22^{a}
WFl+L		261.20±8.21^{a}	0.89±0.09^{a}	57.11±0.25	2.27±0.17	21.09±0.12
WFl+Lg		250.78±1.02^{a}	1.07±0.01	53.78±0.35	3.37±0.07	17.72±0.14^{a}
WFl+B		290.26±4.29^{b}	0.69±0.05^{b}	73.97±0.16^{a}	1.17±0.30^{abc}	23.72±0.20^{b}
WFl+Bg		290.51±2.47^{b}	0.69±0.04^{b}	72.17±0.31	1.57±0.04^{b}	23.94±0.10^{b}
WFl+C		318.26±3.03	0.68±0.05^{b}	75.56±0.06^{b}	1.06±0.00^{ac}	29.79±0.15
WFl+Cg		280.33±3.40^{b}	0.90±0.07^{a}	74.53±0.04^{ab}	1.59±0.03^{b}	27.65±0.45
WFl	SD3	295.44±5.02^{a}	0.40±0.06^{a}	76.19±0.93^{a}	1.21±0.37a^{b}	18.03±0.44^{a}
WFl+L		244.55±1.35	0.99±0.09^{b}	57.20±0.56^{b}	2.36±0.16	21.31±0.42
WFl+Lg		222.91±3.05	1.01±0.06^{b}	57.42±1.38^{b}	3.64±0.20	18.27±0.27^{a}
WFl+B		301.20±0.42^{a}	0.52±0.12^{a}	76.68±0.27^{a}	0.80±0.18^{a}	23.69±0.37^{b}
WFl+Bg		298.54±0.77^{a}	0.69±0.12	72.62±0.54	1.49±0.21^{b}	23.86±0.53^{b}
WFl+C		281.64±4.43^{b}	1.14±0.07^{c}	75.20±0.99^{a}	1.33±0.35^{b}	29.33±0.25
WFl+Cg		272.88±0.26^{b}	1.22±0.03^{c}	75.23±0.14^{a}	1.53±0.01^{b}	27.06±0.10

[1] B = broad bean; C = chickpea; g = germinated; L = lentil; WFl = wheat flour; SD = sour dough.

[2] Data followed by the same lowercase letter within a set of data in a column (e.g. Control, SD1, SD2, and SD3) denotes statistically insignificant differences at a 95.0% confidence level.

flour substitution by faba beans, and the use of faba bean sourdough resulted in bread with a slightly lower volume compared to the white bread.

When comparing the control samples with no sourdough addition, one can see that lentil flour addition to the wheat flour caused an increase in bread firmness, whereas the chickpea and broad bean flour caused no significant alteration of the firmness (Table 4). Regardless of the starter culture, the addition of sourdough resulted in breads with lower firmness compared to the controls in the case of the samples obtained with wheat flour, eventually in admixture with raw or germinated lentils and broad beans. On the other hand, of the breads prepared with chickpea flour, the highest firmness was registered for the sample with SD3. These results might be due to the intense proteolytic attack and metabolic activity of the bacterial strains, exerting adverse effects on bread texture (Coda *et al.*, 2017). The weak interactions between the proteins and peptides from the two different flours interfere with the formation of a soft and elastic bread crumb network (Angioloni and Collar, 2012). When comparing the bread samples with germinated and raw pulse flours, one can see that germination resulted in increased firmness, to different extents depending on the pulse type and the starter culture used for preparing the sourdough (Table 4). This trend might be the result of the changes occurring during pulse germination as well as during sourdough fermentation. As indicated by Coda *et al.* (2017), different hydrolytic enzymes act on the macromolecular compounds with a decisive role in bread firmness, such as starch and proteins, and change their intrinsic structure and therefore their behaviour in complex food matrices.

The results of the colour measurements performed on bread crumb are presented in Table 4. The crumb colour usually resembles the colour of the ingredients. Indeed, all samples had positive values for both a* and b* parameters, indicating hues on the red and yellow axis, respectively. The addition of pulses to the wheat flour induced a remarkable increase in both a* and b* values. Among the pulse-containing breads, the lightest crumb colour was registered for bread samples with chickpea flour.

4. Conclusions

The chemical composition of the green lentil, broad bean and chickpea changed during germination. In addition to the total protein content increase, pulses germination resulted in higher amounts of soluble proteins and free amino acids. As a measure of the impact on trypsin inhibitor activity, only a slight improvement in the protein hydrolysis by trypsin was noticed after pulses germination. On the other hand, significant higher *in vitro* protein digestibility values were obtained for all investigated pulses after germination. When using pulses flour to prepare blends with wheat flour for bread-making applications, a significant increase in the Essential Amino Acid Index was obtained. Fermentation of wheat-pulses flour mixtures (70:30) by lactic bacteria, eventually in admixture with *K. marxianus* yeast, produced sourdoughs with higher total phenolic contents and improved antioxidant properties. Fundamental rheological measurements performed on sourdoughs indicated that consistency is affected when substituting 30% of the wheat flour by raw or germinated pulses flour. On the other hand, the rheological performances of batters were unaffected by pulse flour addition or by the starter culture used to prepare the sourdough. Among the investigated pulse-containing sourdough breads, the broad bean based samples presented specific volumes comparable to the wheat bread and rather low firmness values.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/QAS2018.1364.

Table S1. Essential amino acid index for raw pulse flours and wheat-pulse flour mixtures.

Table S2. G' and G'' values registered at 0.01-1% strain domain for sourdoughs (Control, SD1, SD2 and SD3) prepared with wheat-pulse flour mixtures.

Table S3. G' and G'' values registered at 0.01-1% strain domain for bread batters prepared with wheat-pulse flour mixtures without (control) or with sourdoughs (SD1, SD2 and SD3) addition.

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Conflict of interest

The authors declare no conflict of interests.

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