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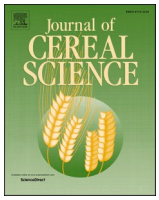

Expanding the application of germinated wheat by examining the impact of varying alpha-amylase levels from grain to bread

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ABSTRACT

Controlled germination is recognized for its potential to enhance both the nutritional profile and functional properties of grains, but there is limited information about the level of wheat germination that promotes functional changes without losing breadmaking potential. This research aims to analyze the physicochemical changes in wheat during germination for 36 h, focusing on evaluating kernel changes, the flour breadmaking functionality, and bread characteristics. Pasting properties progressively decreased as the germination progressed, and apparent viscosity was barely detected after 36 h germination. Initial decline in gluten index was observed at 24 and 36 h germination, but gluten kept its aggregation capabilities. Optimal germination periods of 6–18 h significantly improved flour functionality, evidenced by increased Gluten Performance Index, gluten index, and enhanced dough mixing properties. Further, mini-breads, developed after optimizing breadmaking conditions, displayed increased 2D areas in 24 and 36 h and lower crumb hardness in 24 and 36 h of germinated bread compared to those obtained with sound wheat flour. Significant correlations were found among alpha-amylase activity, Falling number, total and damaged starch content, RVA parameters, gelatinization enthalpy, and breadcrumb texture parameters. Developing mini bread using different levels of germinated flour demonstrates its viability for breadmaking offering a promising innovation within the whole-grain food industry.

1. Introduction

Wheat is a fundamental staple in global food production, and any disruption in wheat production significantly impacts the global economy and food supply. Climate change and unpredictable weather conditions, like heavy rainfalls during harvest, are further straining the wheat supply chain due to the detrimental effects of wheat germination on the flour's bread-making performance, owing to the increasing enzyme activity (Newberry et al., 2018; Hossain et al., 2021). Amylases are among the most active enzymes during sprouting, particularly alpha-amylases (Fannon et al., 1992). Despite the differences between preharvest sprouting, with highly heterogeneous degrees of sprouting and alpha-amylase activity, and controlled germination, with similar kernel characteristics, the latter has been often applied to understand changes triggered by germination Ding et al. (2018); Grassi et al. (2018) and it is the approach of the present research. It is known that germination affects Falling number (FN), pasting and mixing properties of whole wheat for 5 h–24 h (Ding et al., 2018), as well as enzyme activities, starch properties, and gluten aggregation in flours sprouted for 24 to 72 (Grassi et al., 2018).

Despite those changes in the flour, Marti et al. (2018) proposed to enrich (i.e. 15%, 25%, 33%, 50%, 75%, and 100%) wheat flour with its related refined sprouted wheat flour (SWF) (for 48 h at 20 °C). The dough rheological properties did not show significant changes in mixing properties from 25 to 75% of enrichment, and the most favorable outcome, in terms of bread volume and crumb porosity, was observed when incorporating 50% SWF rather than using SWF alone. Similarly, Cardone et al., (2020a) reported that sprouting for 48 h at 20 °C reduced dough development time, stability, and water absorption during mixing and sprouting improved bread height (approx. 20%), specific volume (approx. 15%), and crumb softness by around 200% after 24 h of storage even when whole wheat flour was used. In the case of semolina from sprouted durum, Cardone et al. (2020b) found that samples sprouted from 24 to 38 h had the best breadmaking performance in terms of specific volume while sticky and irregular structure was observed with further increase in the germination time.

Hence, previous studies analyzed the impact of controlled sprouting of wheat, for 24 h and beyond, on the final quality of baked goods,

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dough rheology, and pasting properties. Likewise, Olaerts et al. (2018) reviewed the relationship between FN and alpha-amylase activity and the impact on bread quality. However, there is limited research on how different levels of germination from lower to higher levels progressively affect the kernel, wheat flour and the end-product characteristics made from germinated flours. Additionally, although FN is the reference indicator of sprouting, there is insufficient coverage of values ranging widely from above 300 s–60 s. This highlights the need for studies with larger intervals to fully understand the effects of germination levels on wheat kernels, flour, and dough, and ultimately the characteristics of the final bread. Therefore, the aim of this research was to measure and understand the impact of a wider range of endogenous alpha-amylase activity on the functionality of flour, characteristics of dough, and quality of final breads. Further, examining how different parameters are correlated with one another will deepen our understanding.

2. Materials and methods

2.1. Materials

Wheat kernels belonging to the class of Canada Western Red Spring (CWRS) harvested in 2023 were provided by Cereals Canada (Winnipeg, Canada).

2.2. In-house controlled germination

Wheat grains were cleaned to remove foreign materials and broken kernels. Kernels underwent a controlled germination starting with disinfection process involving soaking in 1% (v/v) sodium hypochlorite solution for 15 min followed by extensive washing with distilled water until neutral pH. For germination, kernels placed in a tray were kept between two mesh papers (kernels: water ratio of 1:1) in an environmental chamber (Caron 6010, Marietta, Ohio, US) at 21 °C with 80% relative humidity as described by Grassi et al. (2018). Preliminary assays were performed germinating kernels for different times, and the Rapid Visco Analyzer (RVA-4, Newport Scientific, Warriewood, Australia) was used to assess their apparent peak viscosity. A maximum germination time of 36 h was identified as the time required to completely decrease the apparent peak viscosity of the wholegrain flour. Then, samples were collected at different times (0, 6, 12, 18, 24, and 36 h which were coded as G0, G6, G12, G18, G24, and G36, respectively) and dried in a hot air oven at 50 °C for 10 h until moisture content dropped below 15%. Finally, wholegrain flours were attained using a laboratory hammer mill (Perten Lab Mill 3100, Perten instruments AB, Huddinge, Sweden) equipped with a 0.5 mm screen. Flour coming from kernels, not subjected to any soaking and germination, represented the sound control, while those obtained after soaking with no germination treatment were designated as soaked control (G0). Germination was performed using three different wheat batches.

2.3. Grain morphology assessment and proximate composition of flours

Images of the whole grains were captured at 1200 dpi by using a Perfection V39 flatbed scanner (Seiko Epson Corp., Nagano, Japan) as described by (Beltrao Martins et al., 2022). Geometric features such as two-dimensional (2D) areas were evaluated by digital image analysis using FIJI-ImageJ 2.9.0_172 (National Institutes of Health (NIH), Rockville, MD, USA).

The chemical composition of flour such as moisture, ash, protein, and fat was estimated according to the AACCI (2010), approved methods 44-15.02, 08-01.01, 46-11.01, and 30-25.01, respectively.

2.4. Alpha-amylase activity assessment

The alpha-amylase activity was assessed using both indirect and direct methods. In the first case, the official standard method 56–81.03 (AACC International, 2010) using the Perten FN system (FN 1310, Perten Instruments AB, Stockholm, Sweden), and the Rapid Visco Analyzer (RVA-4, Newport Scientific, Warriewood, Australia) following ICC standard method 162 (ICC, 1996) were used. Simultaneously, all the RVA assays were also run in the presence of silver nitrate (1 mM) instead of solely water, to inhibit the alpha-amylase as described by Santamaria et al. (2023). Silver nitrate 10 mM and 100 mM were also tested when 1 mM was insufficient to recover the original starch viscosity.

For the direct method, the alpha-amylase activity was quantified using the alpha-amylase assay kit (Neogen-Megazyme International Ireland Ltd, Bray, Ireland) and following the official ICC standard method number 108 (ICC, 1998).

2.5. Total and damaged starch contents

Total starch content was quantified by using a Total Starch Assay Kit (Neogen-Megazyme International Ireland Ltd, Bray, Ireland) following the method 76-13.01 (AACCI, 2010). The damaged starch content quantification was carried out using the Megazyme Starch Damage Kit K-SDAM 06/18) (Megazyme International Ireland Ltd, Bray, Ireland) and following the method 76-30.02 (AACCI, 2010).

2.6. Evaluation of flour thermal properties

Differential scanning calorimetry (DSC) was used to calculate the Onset temperature (T_o), peak temperature (Tp), conclusion temperature (Tc), and the enthalpy of starch gelatinization (ΔHg). Analysis was done in a DSC Q series 200 (TA Instruments, New Castle, DE, USA) using alminium pans (PE 0319-0218). Wheat flour and distilled water (1:3 w: w) were added to pans, hermetically sealed, and allowed to stand overnight at room temperature. The heating conditions were set as follows: 30 °C for 2 min, and heated from 30 to 130 °C at 10 °C/min.

2.7. Assessment of gluten quantity and quality

Wet gluten and gluten index were determined according to the AACCI official method 31-12 (AACCI, 2010). Besides to standard method mentioned, analyses also included a modified method for gluten index determination. In brief, washed gluten was kept in a water bath at 37 °C for different time intervals (0, 30, and 120 min) and subsequently, the gluten index was determined (Aja et al., 2004).

2.8. Solvent retention capacity

Solvent retention capacity (SRC), which is defined as the weight of solvent retained by wheat flour after centrifugation, was evaluated following the procedure outlined in method 56-11.01 (AACCI, 2010). The outcomes are presented as a percentage of the flour weight, based on a 14% moisture content. Four different solvents were employed to generate four distinct SRC values: water SRC, 50% sucrose SRC, 5% sodium carbonate SRC, and 5% lactic acid SRC. Furthermore, the Gluten Performance Index was calculated using the ratio of SRC values of 5% lactic acid to the combined SRC values of 5% sodium carbonate and 50% sucrose (Kweon et al., 2011).

2.9. Mixing properties of flours

Flour was mixed with water at a constant temperature (30 °C) and speed (63 rpm) using a Micro-dough LAB device (Perten Instruments, Hägersten, Sweden) for 20 min. The dough behavior was recorded and the mixing parameters determined by the equipment were: dough development time (min), stability (min), peak torque (mNm), bandwidth, and mixing tolerance index (mNm) as described in method 54–70.01 (AACCI, 2010).

2.10. Breadmaking process

A basic recipe was utilized, comprising 1% dry yeast, 2% salt, and water as determined by micro-doughLAB (Perten Instruments, Hägersten, Sweden). The ingredients were mixed in a lab mixer at 25 °C for 10 min. Micro-breads were made by using the scale-down method proposed by (Garzon et al., 2017). The dough (4 g) was placed in greased mini pans and proofed at 85% RH and 30 °C and baked at 130 °C for 7 min. These breadmaking conditions ensure obtaining both crumb structure and texture comparable to a 500 g loaf, but preventing the crust formation (Garzon et al., 2017). The optimum proofing time at 30 °C and 85% RH was previously determined as the time needed to reach 75% of the maximum dough volume as previously reported (Beltrao Martins et al., 2022). The volume increase (ΔV) was recorded every 10 min up to 120 min and the optimum time was identified.

2.11. Bread characterization

The 2D area and crumb structure of the slices were assessed using the procedure reported by Espinosa-Ramirez et al. (2018) with slight modifications. Briefly, pictures of the bread slices were captured by a Perfection V39 flatbed scanner (Seiko Epson Corp., Nagano, Japan) at 1200 dpi, and then analyzed using FIJI-ImageJ 2.9.0_172 (National Institutes of Health (NIH), Rockville, MD, USA). The images were then processed by splitting channels and contrast enhancement was applied, as well as a predefined algorithm ("Otsu") was used for segmentation. Two slices from three different breads of each batch were analyzed.

The color of the breadcrumb was analyzed by using a Croma Meter CR-400 (Konica-Minolta Sensing Americas, New Jersey, USA). The L*, a*, and b* were considered and stand for lightness, green (−) to red (+), and blue (−) to yellow (+), respectively (Garzon et al., 2017).

The texture profile analysis of a 10 mm thickness piece of crumb was determined through a Texture Analyzer TA.XT2i (Stable Micro Systems, Surrey, UK). Sample taken from the center of each slice was compressed up to 50% of strain at a speed of 1 mm s^{-1} using a 36 mm aluminum probe (Garzon et al., 2017). From the compression curve, the following parameters were calculated: hardness (g), springiness, cohesiveness, chewiness (g), and resilience.

2.12. Statistical analysis

The results were expressed as mean ± standard deviation (n ≥ 3). Statistical analyses were conducted using Statgraphics Centurion 19-X64 software (2019) (Statistical Graphics Corporation, Rockville, MD, USA). One-way analysis of variance (ANOVA) was employed to assess significant differences among samples at a 95% confidence interval, using Fisher's least significant differences (LSD) test. Pearson correlation coefficient (r) and associated *p*-values were utilized to identify correlations, whereas Principal Component Analysis (PCA), was employed to differentiate among the samples.

3. Results and discussion

3.1. Kernel morphology and proximate composition

Kernel area increased significantly ($p < 0.05$) during germination varying from 14.43 mm^2 (control) to 20.42 mm^2 at 36 h of germination (Table 1), due to kernel wetting and inner changes associated with germination. According to morphology, wheat kernels sprouted for 12–24 h would be classified as sprouted kernels, whereas grains germinated for 36 h would fall in the category of severely sprouted kernels because the broken sprout protrudes beyond the contour of the germ.

Germination did not significantly ($p < 0.05$) affect protein content, determined as nitrogen content of flour (Table 1). No trend was observed with the ash content. Still, the fat content increased significantly ($p < 0.05$) compared to the control, particularly after 36 h of germination (from 2.97 to 3.89 g/100 g db), which could be associated with the generation of lipids related to the seed growth, as has been previously reported after 48 and 72 h controlled wheat germination (Baranzelli et al., 2018). As expected, the most affect constituent during germination was the starch content, but the decrease was only observed after 36 h. Conversely, Grassi et al. (2018) reported a decrease in starch content after 72 h of germination only in whole grain flour and not in refined flour.

3.2. Alpha-amylase activity of germinated flours

The alpha-amylase activity was determined by direct and indirect methods (Table 2), the latter usually employed for wheat quality assessment. The total alpha-amylase activity values of flour significantly ($p < 0.05$) increased with germination time from 0.14 to 6.96 CU/g after 36 h. Cardone et al. (2020a,b) also reported the increase in α-amylase activity by about 600 times in whole wheat flour sprouted for 48 h at 20 °C. Simultaneously, FN significantly ($p < 0.05$) decreased (Table 2). The same trend has been reported in other studies, where the FN values decreased over the germination time (Olaerts et al., 2016; Ding et al., 2018). There was a significant negative correlation ($p < 0.0005$, $r = -0.84$) between FN and alpha-amylase activity, consistent with previous findings by Hareland (2003), Cauduro et al. (2023) and Kiszonas et al. (2018). This was expected as prolonged germination times increase alpha-amylase activity that breaks down starch structure, reducing its ability to form a viscous gel, leading to lower FN values.

Pasting properties were assessed to indirectly estimate the alpha-amylase activity in wheat flour. Plots of the pasting behavior of the germinated flours are displayed in Fig. 1. Apparent peak viscosity, trough, final viscosity, and setback significantly ($p < 0.05$) decreased at germination for 12 h (Table 2). After 24 h germination, the apparent peak viscosity was barely detectable (Table 2). This reduction in peak viscosity is associated to the degradation of starch into smaller molecules caused by enzyme activity over time (Grassi et al., 2018; Ding et al., 2018). A similar trend was found by Grassi et al. (2018), Baranzelli et al. (2018), and Cardone et al. (2020a,b). The average breakdown values decreased from 444 to 80 mPa s as germination duration

Table 1
Proximate composition of germinated and non-germinated wheat flour, expressed in g/100 g (DM).

Composition	Control	G0	G6	G12	G18	G24	G36
Kernel 2D area (mm^2)	14.43 ± 0.37^{a}	17.01 ± 0.26^{b}	18.49 ± 0.26^{c}	17.37 ± 0.20^{b}	18.37 ± 0.35^{c}	19.50 ± 0.09^{d}	20.42 ± 0.54^{e}
Moisture	13.20 ± 0.31^{ab}	11.57 ± 0.7^{a}	12.54 ± 1.76^{ab}	12.83 ± 1.46^{ab}	12.62 ± 1.92^{ab}	13.71 ± 0.46^{b}	11.87 ± 0.13^{ab}
Ash	1.77 ± 0.11^{a}	2.05 ± 0.11^{c}	1.89 ± 0.13^{abc}	1.92 ± 0.12^{abc}	2.01 ± 0.14^{bc}	1.96 ± 0.04^{bc}	1.86 ± 0.07^{ab}
Protein	16.98 ± 0.24^{a}	16.79 ± 0.00^{a}	17.05 ± 0.50^{a}	16.66 ± 0.16^{a}	16.64 ± 0.83^{a}	16.96 ± 0.44^{a}	17.06 ± 0.58^{a}
Fat	2.97 ± 0.03^{a}	3.41 ± 0.05^{b}	3.71 ± 0.13^{cd}	3.56 ± 0.07^{bc}	3.64 ± 0.30^{bcd}	3.64 ± 0.13^{bcd}	3.89 ± 0.11^{d}
Total starch	62.89 ± 2.82^{b}	61.09 ± 1.37^{b}	61.64 ± 4.60^{b}	62.40 ± 3.88^{b}	64.61 ± 3.72^{b}	63.54 ± 0.54^{b}	52.88 ± 1.94^{a}

* Differences in each parameter are highlighted by different superscript letters at $p < 0.05$.
* Flours obtained from the following wheats: Sound Control, G0: grain subjected to soaking, G6: sprouted for 6 h, G12: sprouted for 12 h, G18: sprouted for 18 h, G24: sprouted for 24 h G36: sprouted for 36 h.

Table 2
Effect of sprouting on physico-chemical properties of flour.

Parameter	Control	G0	G6	G12	G18	G24	G36
Alpha-amylase activity (CU/g)[a]	0.14 ± 0.04^{a}	0.11 ± 0.02^{a}	0.12 ± 0.03^{a}	0.18 ± 0.09^{a}	0.42 ± 0.08^{b}	1.56 ± 0.58^{c}	6.96 ± 1.30^{d}
Falling number (s)	385 ± 1^{a}	389 ± 9^{a}	389 ± 1^{a}	359 ± 8^{b}	259 ± 10^{c}	108 ± 2^{d}	63 ± 2^{e}
Total damaged starch (g/100 g)	6.86 ± 0.76^{a}	6.96 ± 0.90^{ab}	7.65 ± 0.71^{ab}	8.19 ± 0.32^{bc}	7.95 ± 0.44^{abc}	8.95 ± 1.07^{c}	9.15 ± 0.33^{c}
Pasting properties							
Apparent Peak viscosity (mPa·s)	1192 ± 51^{e}	1176 ± 99^{e}	1191 ± 67^{e}	1043 ± 68^{d}	649 ± 39^{c}	139 ± 4^{b}	85 ± 18^{a}
Trough (mPa·s)	677 ± 67^{e}	689 ± 49^{e}	695 ± 51^{e}	571 ± 18^{d}	242 ± 25^{c}	36 ± 5^{b}	16 ± 0^{a}
Breakdown (mPa·s)	444 ± 48^{bc}	490 ± 55^{d}	496 ± 22^{d}	472 ± 51^{cd}	407 ± 14^{b}	103 ± 0^{a}	80 ± 12^{a}
Final viscosity (mPa·s)	1275 ± 94^{d}	1303 ± 66^{d}	1292 ± 67^{d}	1099 ± 32^{c}	529 ± 34^{b}	44 ± 1^{a}	24 ± 0^{a}
Thermal properties							
T_o (°C)	60.08 ± 0.27^{a}	59.54 ± 0.76^{a}	60.02 ± 0.08^{a}	59.80 ± 0.72^{a}	60.40 ± 0.08^{ab}	61.02 ± 0.54^{b}	62.37 ± 0.15^{c}
T_p (°C)	65.34 ± 0.29^{a}	65.17 ± 0.17^{a}	65.20 ± 0.07^{a}	65.34 ± 0.04^{a}	65.33 ± 0.15^{a}	65.89 ± 0.10^{b}	66.43 ± 0.16^{c}
T_C (°C)	74.84 ± 1.31^{d}	73.70 ± 0.34^{cd}	73.09 ± 0.31^{bc}	72.32 ± 0.27^{ab}	72.07 ± 1.17^{ab}	71.91 ± 0.81^{ab}	71.18 ± 0.27^{a}
ΔH (J/g)	4.86 ± 0.81^{d}	4.09 ± 0.29^{c}	3.89 ± 0.31^{c}	3.54 ± 0.11^{bc}	3.51 ± 0.22^{bc}	2.96 ± 0.22^{ab}	2.65 ± 0.06^{a}
Gluten performance							
Gluten index at 0 min (%)	92 ± 0^{c}	93 ± 4^{c}	92 ± 8^{c}	90 ± 9^{c}	90 ± 9^{c}	84 ± 9^{b}	63 ± 4^{a}
Gluten index at 30 min (%)	91 ± 0^{b}	93 ± 0^{b}	92 ± 2^{b}	93 ± 2^{b}	92 ± 1^{b}	93 ± 1^{b}	84 ± 5^{a}
Gluten index at 120 min (%)	90 ± 0^{a}	91 ± 2^{ab}	93 ± 0^{b}	92 ± 2^{ab}	92 ± 2^{ab}	92 ± 1^{ab}	91 ± 3^{a}
Solvent retention capacity							
W-SRC (%)	78.06 ± 2.32^{ab}	75.89 ± 1.17^{a}	81.59 ± 3.37^{bc}	87.20 ± 1.48^{de}	85.46 ± 1.26^{cd}	87.92 ± 2.96^{de}	91.18 ± 1.13^{e}
LA-SRC (%)	92.79 ± 0.73^{c}	92.35 ± 0.80^{c}	91.29 ± 3.92^{c}	89.83 ± 7.14^{bc}	89.86 ± 1.23^{bc}	82.26 ± 2.08^{ab}	82.21 ± 1.45^{a}
Suc-SRC (%)	108.16 ± 5.19^{a}	109.30 ± 3.58^{a}	108.52 ± 3.82^{a}	107.90 ± 3.65^{a}	107.95 ± 3.50^{a}	108.65 ± 2.76^{a}	107.17 ± 1.44^{a}
SC-SRC (%)	95.35 ± 0.71^{a}	93.97 ± 0.41^{a}	94.79 ± 2.99^{a}	95.75 ± 0.01^{a}	94.06 ± 1.48^{a}	97.06 ± 1.63^{b}	105.97 ± 6.99^{c}
GPI	0.46 ± 0.01^{b}	0.45 ± 0.01^{b}	0.45 ± 0.01^{b}	0.44 ± 0.03^{b}	0.44 ± 0.01^{b}	0.40 ± 0.01^{a}	0.39 ± 0.02^{a}

* Differences in each parameter among different germination times are highlighted by different letters at $p < 0.05$.
* Sound Control, G0: grain subjected to soaking, G6: sprouted for 6 h, G12: sprouted for 12 h, G18: sprouted for 18 h, G24: sprouted for 24 h G36: sprouted for 36 h.
[a] CA: Ceralpha Units, MTI: Mixing tolerance index, SRC: Solvent retention capacity, W: Water, LA: Lactic acid, Suc: Sucrose, SC: Sodium carbonate, GPI: Gluten performance index, T_o (°C); onset temperature, T_p (°C); peak temperature, T_C (°C); conclusion temperature, ΔH (J/g); enthalpy of starch gelatinization.

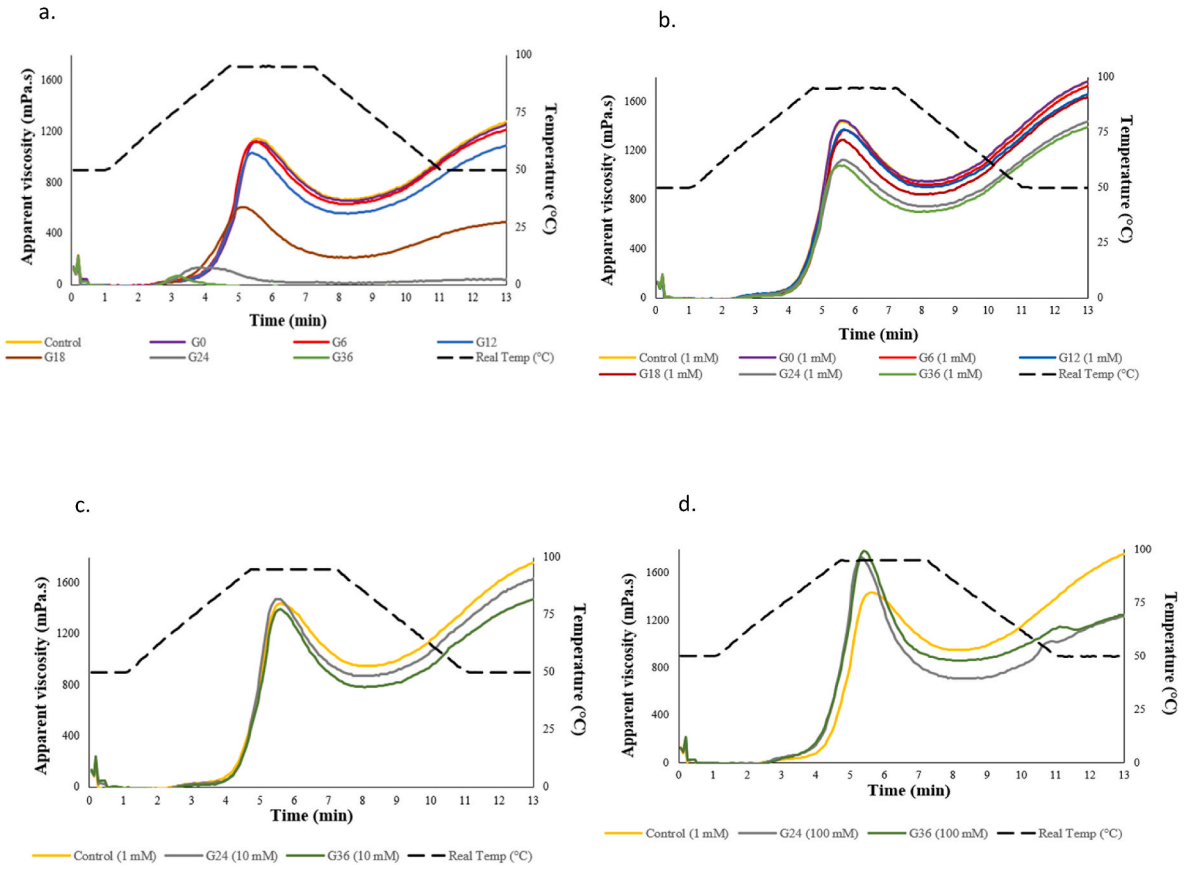

Fig. 1. RVA profiles a. non-germinated and germinated wheat flours, b. Flours in the presence of 1 mM silver nitrate, c. G24 and G36 in the presence of 10 mM silver nitrate, d. G24 and G36 in the presence of 100 mM silver nitrate compared to control.

progressed, indicating the reduction of the stability of the starch paste under heat and shear. Significant negative correlations ($p < 0.005$) were found between alpha-amylase activity with every parameter of RVA, comprising apparent peak viscosity (r = −0.79), trough (r = −0.70), breakdown (r = −0.83), final viscosity (r = −0.73) and setback (r = −0.74). Additionally, FN was also significantly ($p < 0.0005$) positively

correlated with apparent peak viscosity (r = 0.99), trough (r = 0.96), breakdown (r = 0.95), final viscosity (r = 0.98), and setback (r = 0.99). Olaerts et al. (2018) highlighted the significant correlation between FN and RVA parameters (peak viscosity, final viscosity, and setback viscosity) and with the alpha-amylase activity of preharvest sprouted wheat flour. Hence, these correlations indicate that RVA measurements can effectively predict enzymatic activity in flours with alpha-amylase content. To determine if starch properties were modified due to germination, the pasting properties were evaluated in the presence of silver nitrate (1, 10, or 100 mM) to inhibit the alpha-amylase activity. Even the lowest concentration of silver nitrate (1 mM) was able to inhibit alpha-amylase activity and restore the peak and final viscosity in the samples germinated for up to 18 h (Fig. 1). Higher concentration of silver nitrate (10 mM and 100 mM) was required to inhibit the levels of alpha-amylase existing in germinated wheat flour at 24 and 36 h (Fig. 1). At 10 mM silver nitrate (Fig. 1 c), the pasting profile during heating of G24 and G36 significantly ($p < 0.05$) increased, reaching the same values recorded in the control samples. However, apparent viscosity during cooling was kept lower than the original one, suggesting that amylose chains might be affected by amylases, reducing its ability to recrystallize. With the addition of a more concentrated silver nitrate solution (100 mM), the apparent peak viscosity of G24 and G36 further increased going beyond the control reducing even more the final viscosity (Fig. 1 d). This increase in peak viscosity might be due to interactions between starch chains and the silver nitrate or induced by this salt, which also decreased the amylose retrogradation.

An increase in damaged starch content as well as decreased total starch content was noticed after 18 h of germination, compared to the control (Tables 1 and 2). Grassi et al. (2018) reported an increase in the peak viscosity in the laboratory-sprouted wheats for 24 h or 38 h, due to minor alterations in starch structure, as well as the increase in damaged starch. Nevertheless, in the present study, higher damaged starch was obtained with G24 and G36, which showed lower apparent viscosity.

To gain further understanding about the impact of germination on the starch properties, the thermal properties of wheat flour were assessed (Table 2). Onset temperature (T_o) increased after 18 h of germination. G24 and G36, germinated for 24 h and 36 h, respectively, showed significantly ($p < 0.05$) higher T_o, and peak temperature (T_p). Conversely, conclusion temperature (T_c) decreased with longer germination times, indicating reduced thermal stability likely due to starch enzymatic degradation. The impact of germination on the thermal properties was significantly reflected on the enthalpy (ΔH) which decreased significantly ($p < 0.05$) with sprouting, being reduced by 39% and 46%, in G24 and G36, respectively. Enzyme starch hydrolysis during sprouting decreases the energy required for starch gelatinization. Statistically significant correlations among changes in the thermal properties and the rest of the starch properties reflected the impact of germination on starch. ΔH was significantly ($p < 0.005$) positively correlated with peak viscosity (r = 0.81), trough viscosity (r = 0.73), breakdown (r = 0.74), final viscosity (r = 0.73), and setback value (r = 0.73) while a negative correlation was found with total alpha-amylase (r = −0.66) and damaged starch content (r = −0.77). Increased alpha-amylase levels decrease enthalpy, reflecting energy required for gelatinization is reduced with higher damaged starch content, as supported by these correlations. In fact, Benavent-Gil and Rosell (2017) reported that alpha-amylase damages the starch granule surface creating pores that result in lower gelatinization enthalpy.

3.3. Gluten properties of flour

Germination's effect on gluten properties was assessed using gluten index (GI) measurements at 0, 30, and 120 min, providing an indirect record of the changes induced by protease activity on gluten (Table 2). A comparison of GI at 30 and 120 min could serve as a measure of protein degradation in damaged wheat (Aja et al., 2004). Wheat germinated at 24 and 36 h showed significantly ($p < 0.05$) lower GI at 0 min, indicating reduced gluten strength and flour quality due to protease breakdown. Nevertheless, when extending the incubation time to 30 min, differences in GI values were negligible across samples, except for G36. By 120 min, all the GI showed no significant ($p < 0.05$) difference, likely due to limited protease accumulation during controlled germination. Results indicate that despite proteases degradation of gluten into shorter protein chains, those were able to aggregate and form a network. Marti et al. (2017) also observed that gluten proteins still retain their ability to aggregate and form a network suitable for leavening when analysing wheat blends made with sprouted wheat samples by using Glutopeak device.

The gluten performance index (GPI), assessed through the Solvent Retention Capacity (SRC), also varied during germination (Table 2). Germination significantly increased Water-SRC (W-SRC) from 78.06% to 91.18%, likely due to higher damaged starch content absorbing more water (Liu et al., 2017), since Carbonate-SRC (SC-SRC), indicative of damaged starch levels, rose from 95.35% to 105.97% in G24 and G36, respectively. In fact, W-SRC was found to be significantly positively ($p < 0.05$) correlated with damaged starch (r = 0.87), and negatively correlated with RVA parameters such as peak viscosity (r = −0.79), trough viscosity (r = −0.76), breakdown (r = −0.66), final viscosity (r = −0.77), and gluten index (r = −0.63). Therefore, W-SRC had significant correlations with parameters related to starch and gluten performance, likely due to the interaction of water molecules with all the major components of flour (Hammed et al., 2015). LA-SRC and GPI values were notably lower in G24 and G36 (Table 2), indicating weaker gluten strength influenced by endoprotease activity during germination. Similarly, Boukid et al. (2018) observed decreased LA-SRC, and GPI values in durum wheat flour after longer (6 days) germinations. Therefore, GI at 0 min and GPI confirmed the protease activation during germination, although it requires longer time than the amylase. Those measurements allowed identifying the activity of protease in germinated wheat, which has been slightly mentioned or neglected in many previous manuscripts. Besides to that, the modified gluten index method allowed to hypothesize that proteases are breaking down the structure of the gluten into fragments long enough that with time they can be rearranged and keep the gluten strength. Again, it must be highlighted the correlations found between starch and gluten dependent parameters of the germinated flours. Significant ($p < 0.05$) positive correlations were found between GPI and alpha-amylase activity (r = 0.80) and GI at 0 min (r = 0.60), supported by trends in GI values for longer germination periods (Table 2).

3.4. Mixing properties of flour

The parameters that identify the mixing performance are shown in Table 3. Significantly ($p < 0.05$) shorter DDT and stability were obtained in G24 and G36, suggesting protease activity weakened the gluten matrix. Marti et al. (2018) also noted a decrease in DDT and dough stability when adding 15% sprouted wheat flour. Germinated flour showed higher MTI than for control (Table 3), highlighting reduced resistance to overmixing due to greater breakdown susceptibility. This aligns with Baranzelli et al. (2018) results on Mixolab parameters (lower DDT, stability, and MTI) in laboratory-induced and pre-harvest sprouted wheat owing to the gliadins and glutenin hydrolysis. The increase in dough softening also confirms the gluten weakening. In fact, significant ($p < 0.05$) positive correlation was found between GI at 0 min with dough development time (r = 0.80) and negatively with softening (r = −0.68) and MTI (r = −0.60).

3.5. Bread characteristics

Breadmaking was carried out with the germinated flours adapting the hydration, as in Table 3, and the fermentation time (Table 4). Dough made from germinated flour G36 had a significantly ($p < 0.05$) higher fermentation rate in comparison to other doughs, which might be

Table 3
Effect of germination on flour and dough properties.

Parameter	Control	G0	G6	G12	G18	G24	G36
Mixing parameters							
Water absorption (g/100 g)	60.97 ± 0.40^{a}	62.60 ± 0.69^{a}	61.70 ± 1.85^{a}	61.33 ± 1.67^{a}	61.37 ± 1.80^{a}	60.65 ± 0.21^{a}	62.40 ± 0.17^{a}
Development time (min)	3.1 ± 0.0^{c}	3.1 ± 0.1^{c}	3.2 ± 0.1^{c}	3.6 ± 0.1^{d}	3.2 ± 0.0^{c}	2.9 ± 0.0^{b}	2.6 ± 0.0^{a}
Softening (mNm)	90 ± 0^{a}	103 ± 11^{a}	118 ± 5^{b}	129 ± 3^{bc}	134 ± 6^{c}	135 ± 16^{c}	150 ± 3^{d}
Stability (min)	1.8 ± 0.0^{bcd}	1.9 ± 0.0^{cd}	2.0 ± 0.0^{d}	1.80 ± 0.0^{bcd}	1.75 ± 0.0^{bc}	1.50 ± 0.1^{a}	1.6 ± 0.1^{ab}
Bandwidth (mNm)	65 ± 12^{b}	70 ± 4^{b}	70 ± 6^{b}	64 ± 5^{b}	65 ± 1^{b}	55 ± 8^{a}	50 ± 5^{a}
MTI	107 ± 17^{a}	105 ± 17^{a}	118 ± 13^{b}	129 ± 10^{bc}	131 ± 8^{c}	133 ± 8^{bc}	134 ± 8^{c}

* Differences in each parameter between different sprouted times are highlighted by different letters at $p < 0.05$.
* Flours obtained from the following wheat: Sound Control, G0: grain subjected to soaking, G6: sprouted for 6 h, G12: sprouted for 12 h, G18: sprouted for 18 h, G24: sprouted for 24 h G36: sprouted for 36 h.

Table 4
Impact of germination time on fermentation rate and bread parameters.

Parameter	Control	G0	G6	G12	G18	G24	G36
Fermentation rate (mL/min)	0.22 ± 0.01^{a}	0.22 ± 0.02^{a}	0.23 ± 0.02^{a}	0.24 ± 0.01^{a}	0.24 ± 0.01^{a}	0.24 ± 0.00^{a}	0.28 ± 0.03^{b}
Area of bread (mm^2)	332.41 ± 10.14^{a}	364.62 ± 9.76^{ab}	351.59 ± 32.69^{ab}	379.34 ± 16.71^{bcd}	377.49 ± 13.68^{bc}	415 ± 23.88^{d}	408.97 ± 25.74^{cd}
Hardness (g)	2678 ± 101^{e}	2564 ± 119^{e}	2080 ± 166^{d}	1715 ± 84^{c}	1641 ± 173^{bc}	1403 ± 188^{ab}	1345 ± 191^{a}
Chewiness (g)	1860 ± 122^{d}	1881 ± 37.60^{d}	1547 ± 148^{c}	1260 ± 164^{b}	1236 ± 131^{b}	1096 ± 97^{b}	875 ± 136^{a}
Cohesiveness	0.75 ± 0.02^{bc}	0.75 ± 0.01^{abc}	0.77 ± 0.02^{abc}	0.78 ± 0.01^{bc}	0.77 ± 0.01^{c}	0.74 ± 0.02^{b}	0.71 ± 0.02^{a}
Springiness	0.95 ± 0.01^{b}	0.96 ± 0.02^{bcd}	0.98 ± 0.01^{d}	0.98 ± 0.02^{cd}	0.98 ± 0.01^{bcd}	0.96 ± 0.02^{bc}	0.92 ± 0.01^{a}
Resilience	0.36 ± 0.00^{c}	0.37 ± 0.01^{cd}	0.39 ± 0.01^{ef}	0.40 ± 0.01^{f}	0.38 ± 0.00^{de}	0.34 ± 0.01^{b}	0.31 ± 0.01^{a}
L^*	54.87 ± 0.55^{c}	54.44 ± 0.81^{c}	54.50 ± 0.49^{c}	53.45 ± 0.74^{b}	52.90 ± 0.14^{b}	53.28 ± 0.46^{b}	50.92 ± 0.23^{a}
a^*	8.36 ± 0.10^{a}	8.55 ± 0.25^{ab}	8.70 ± 0.13^{bc}	8.94 ± 0.08^{cde}	9.02 ± 0.07^{de}	8.85 ± 0.11^{cd}	9.18 ± 0.11^{e}
b^*	22.26 ± 0.17^{a}	22.70 ± 0.24^{b}	22.90 ± 0.23^{bc}	23.36 ± 0.17^{d}	23.46 ± 0.27^{d}	23.17 ± 0.26^{cd}	23.52 ± 0.18^{d}

*Differences within each parameter between different sprouted times are highlighted by different letters at $p < 0.05$.
* Sound Control: flour as is, G0: sound control subject to soaking, G6: Whole wheat flour sprouted for 6 h, G12: Whole wheat flour sprouted for 12 h, G18: Whole wheat flour sprouted for 18 h, G24: Whole wheat flour sprouted for 24 h G36: Whole wheat flour sprouted for 36 h.

related to higher amount of damaged starch and/or free sugars available for yeast metabolism. Changes in the functional characteristics of the flours during germination had a significant impact on bread features. The bread area varied significantly across different germination times. Control and G6 had the smallest area (332.41 ± 10.14 mm^2), while G24 (415 ± 23.88 mm^2), and G36 (408.97 ± 25.74 mm^2) showed larger areas. G24 had a slightly higher area than G36, though not statistically significant (Fig. S1). In explaining the larger area in G24 compared to G36, despite its lower fermentation rate, is important to consider that amylase is an endohydrolase, releasing dextrins or shorter amylose or amylopectin chains. Those fragments are not readily fermentable by the yeast and some additional time is required for the beta-amylase to produce the sugars that could be metabolized by the yeast. In this picture is also important to consider that the fermentation rate was only considering the proofing at 30 °C, but no the oven rise occurring during the early baking stage. In that stage, the joint action of amylase and protease must be considered. The observed increase in loaf's volume (up to 25%) can be attributed to the structural properties of gluten, particularly its ability to retain carbon dioxide during fermentation and baking, which is influenced by gluten's elasticity and extensibility. Weaker gluten is typically more extensible till certain level, allowing it to stretch and accommodate the gas produced during fermentation. This extensibility aids in trapping CO_2 bubbles, resulting in a better rise of the dough. As germination progresses, proteolytic activity increases, leading to weaken gluten in G36, as indicated the GI and gluten quality parameters such as GPI and LA-SRC, affecting gas retention in the dough, and contributing to a lower area than G24. However, Cardone et al. (2020a,b) found a significantly increased loaf height of whole wheat bread made from flour germinated for 48 h due to higher gas production linked to an increased amount of fermentable sugars released by amylase activity, but no comparison could be made based on the amylase activity of those flours. Further, the area of bread was found to be significantly ($p < 0.05$) positively correlated with alpha-amylase activity (r = 0.51) and negatively with FN (r = −0.74). Nevertheless, excessive germination might result in a reduced loaf volume attributed to compromised gluten properties caused by increased peptidase activities, which reduce dough's ability to retain gas (Olaerts et al., 2018).

Texture profile parameters for the mini-breads are shown in Table 4. Significant variation was found among samples in hardness, chewiness, springiness, and resilience ($p < 0.05$). Increased germination time provided softer micro-breads than control (sound and soaked) samples, as evidenced by lower crumb hardness, which are directly related to their major expansion. Significant reductions in chewiness and cohesiveness were also observed as germination time increased. Control bread has the highest chewiness and cohesiveness, while G36 has significantly ($p < 0.05$) the lowest (Table 4). Springiness was maintained until G24 but significantly reduced in G36, indicating poor shape recovery after compression. The reduced resilience of mini bread was linked to the excessive starch breakdown, which hinders the recrystallization of amylose and amylopectin fragments upon cooling, resulting in a softer and stickier crumb texture (Olaerts et al., 2018). Bread parameters such as crumb hardness ($p < 0.05$, r = −0.54), chewiness ($p < 0.05$, r = −0.64), cohesiveness ($p < 0.0005$, r = −0.73), springiness ($p < 0.0005$, r = −0.72), and resilience ($p < 0.0005$, r = −0.86) showed strong negative significant correlations with the alpha-amylase activity of the flour used. Therefore, controlled germination of wheat or sprouted wheat to a certain level can improve crumb texture properties.

The L* value indicating brightness decreased in micro-breads with longer germination times, while a^* (redness) and b^* (yellowness) values increased (Table 4). These changes resulted in darker and more intensely colored bread crusts, consistent with findings by Baranzelli et al. (2018) regarding L^* and a^* values. Yellowness (b^*) showed no significant alteration.

4. Principal component analysis (PCA)

Principal Component Analysis (PCA) identified five principal components; those with eigenvalues above average were retained. PC1 and PC2 effectively summarized 80.55% of the variance (PC1 = 62.55%; PC2 = 18.00%). The biplot (Fig. 2) highlights differences among the

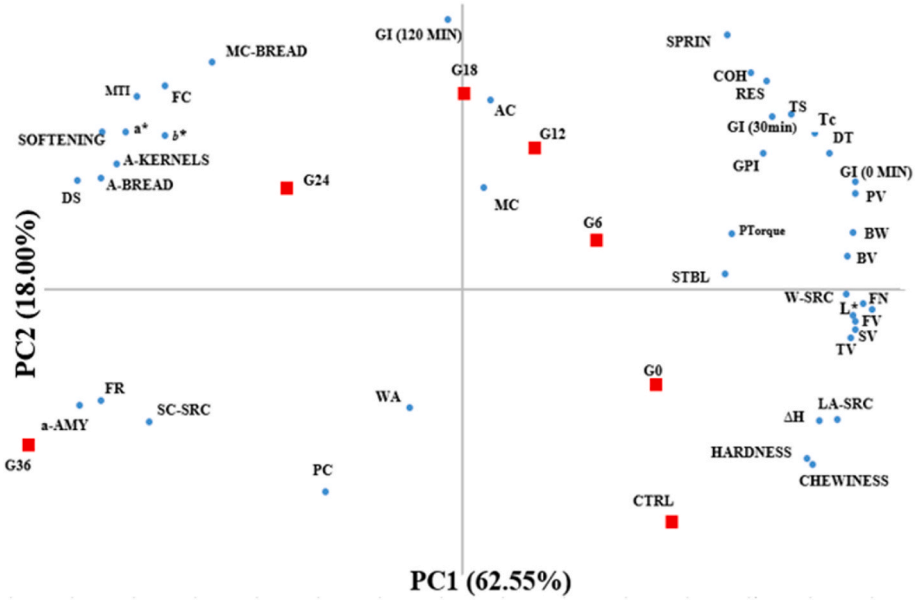

Fig. 2. Principal component analysis (biplot) on data collected from kernels, flour, dough, and bread. GI: Gluten index, AC: ash content, MC: Moisture content, PC: Protein content, FC: Fat content, SPRIN: Springiness, T_c=Conclusion temperature, COH: Cohesiveness, TS: Total starch, DS: Damaged starch, DT: Dough development time, PV: Apparent peak viscosity, BW: Bandwidth, BV: Breakdown, SRC: Solvent retention capacity, W: Water, LA: Lactic acid, SC: Sodium carbonate, FN: Falling number, FV: final viscosity, SV: Setback value, TV: Trough viscosity, ΔH: Enthalpy, CTRL: Control, A-Area, MTI: Mixing tolerance index, *L**: Lightness, FR: Fermentation rate, a-AMY: alpha-amylase, WA: Water absorption. The number following letters corresponds to germination time.

samples, particularly along PC1, which differentiates based on germination times. Control and G0 showed positive PC1 values, whereas samples G24 and G36, exhibited negative PC1 values. Positive PC1 values were associated with pasting and thermal properties, gluten quality, and crumb texture properties. Alpha-amylase (a-AMY), fermentation rate (FR), SC-SRC, water absorption (WA) along with damaged starch (DS), softening, MTI, moisture content of bread (MC-Bread), area of kernels and bread (A-kernels, A-Bread) were responsible for negative PC1 values in samples sprouted for 24 h and 36 h (Fig. 2). The PC2 effectively explains the distinction between the control, G0, and germinated samples up to 18 h (G6, G12, G18). Parameters such as Falling number (FN), pasting and thermal properties, W-SRC, LA-SRC, and bread parameters such as hardness and chewiness were the predominant factors that distinguished the control and G0 from the sprouted samples. Therefore, sound and G0 were separated from the germinated samples, forming one cluster together, the second group is of G6, G12, and G18 clustered together and G24 and G36 showed significant differences between them. The second group with G6, G12, and G18 were characterized by better gluten quality and strength (gluten performance index and gluten index), mixing properties (stability (STBL), dough development time), pasting properties (apparent peak viscosity, breakdown), bread-characteristics (cohesiveness, resilience, springiness). G24 with the higher area of bread among all samples and G36 was characterized by the highest enzyme activity and SC-SRC, thus the latter could be a potential predictor of wheat sprouting.

5. Conclusions

Kernels, flours and breads obtained from controlled germination under different times were characterized to identify their potential used in breadmaking as solely flours, instead of blending with sound flours. Germination of the kernels affects their geometry, showing an increased size due to water imbibition. Moderate germination, like that observed in G6, G12, and G18, provides adequate gluten amount and quality (gluten performance index and gluten index), mixing properties (stability, maximum torque, dough development time), pasting properties (apparent peak viscosity and breakdown), bread-characteristics (cohesiveness, resilience, and springiness). All those properties indicative that the starch and gluten functionality required for the breadmaking were preserved or improved. More extensive germination as occurred in G24 led to the higher 2D area of bread, among all samples, whereas G36 was characterized by the highest enzyme activity that has a noticeable negative impact on gluten, starch, and dough mixing properties. These data indicate that germinated flours even with FN between 200 s and 100 s could be suitable for breadmaking, using adapted hydration and fermentation time. These findings provide valuable insights for the wheat industry to minimize losses due to sprout damage and improve bread quality.

CRediT authorship contribution statement

Nancydeep Kaur: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Nicola Gasparre:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **Cristina M. Rosell:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: On behalf of all the authors I declare that none have conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2024.104059.

Data availability

Data will be made available on request.

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