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Bread-making performance of durum wheat as affected by sprouting

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ABSTRACT

The effects of sprouting duration (24 h, 38 h, 48 h, and 62 h) were assessed on durum wheat kernel characteristics (hardness, test weight), semolina chemical composition, pasting and gluten aggregation properties, and leavening and bread-making performance (bread volume and crumb porosity). Sprouting decreased both kernel hardness (~29%) and test weight (~19%). Starch gelatinization and retrogradation capability, as well as the gluten aggregation properties, decreased as sprouting duration increased. The 62 h sample showed the worst aggregation properties leading to a bread with the lowest specific volume (2.69 mL/g). The best results in terms of bread specific volume (3.08 mL/g) and crumb porosity distribution were obtained using semolina from sprouted wheat up to 38 h. A multivariate approach by Principal Component Analysis and clustering confirmed the relationships between all the considered variables and allowed to assess three sprouting levels: 24–38 h with improved bread-making performance; 48 h with decreased overall quality; 62 h with the worst quality. In conclusion, the sprouting of durum wheat up to 38 h could improve its bread-making attitude.

1. Introduction

Durum wheat (*Triticum turgidum* subsp. *durum*) is characterized by a peculiar hard and vitreous endosperm which influences its milling behavior, e.g., milling energy, yield and the starch damage (Turnbull & Rahman, 2002). The strength and poor extensibility of its gluten network makes durum wheat the ideal raw material for pasta-making but unsuitable for baked-goods (Ammar, Kronstad, & Morris, 2000). Despite the enhanced nutritional traits thanks to the carotenoids (Pasqualone, Caponio, & Simeone, 2004), using durum wheat in bread-making results in low loaf volume and dense crumb structure (Sissons, 2008). However, dough extensibility and bread volume improved using sourdough fermentation, since the combination of acidity and hydrolytic activity of both lactic acid bacteria and yeasts positively affect durum wheat gluten functionality (Barber, Ortolá, Barber, & Fernández, 1992). Considering the above, this study investigated the exploitation of the enzymatic pattern developed throughout sprouting to improve the bread-making performance of durum wheat. Although, an excessive accumulation of enzymes in wheat has always represented a negative event from a technological standpoint, recently it has been reported that sprouting improved the bread-making performance of common wheat (Cardone, D'Incecco, Pagani, & Marti, 2020a; Marti, Cardone, Nicolodi, Quaglia, & Pagani, 2017; Marti, Cardone, Pagani, & Casiraghi, 2018). In the case of durum wheat, the sprouting process have been recently investigated in relation to bioactive compounds (Jribi, Sahagùn, Debbabi, & Gomez, 2019a) and functional properties (Jribi, Sahagùn, Debbabi, & Gomez, 2019b) of wholemeal semolina. To the best of our knowledge, no study has focused yet on the relationship between sprouting and bread-making performance of durum wheat. Since the understanding of flour functionality is a key element in the production of cereal-based products, the aim of this study was to evaluate the effects of sprouting duration on durum wheat kernel characteristics, starch and gluten behavior, and their relationship with the bread characteristics also from a multivariate point of view, thus applying Principal Component Analysis and clustering.

Abbreviations: A_0, radial area of the dough at the beginning of the leavening; A-am, α-amylase activity; AgEn, Aggregation Energy; A_t, radial area of the dough at time t; BD, Breakdown index; CTRL, unsprouted durum wheat; DS, Damaged Starch; FV, Final Viscosity; Glu, D-glucose; GPE, GlutoPeak Equivalent; GPU, GlutoPeak Unit; Mal, Maltose; MT, Maximum Torque; PCA, Principal Component Analysis; PMT, Peak Maximum Time; Prot, Protein; PV, Peak Viscosity; SpV, Specific Volume; Suc, Sucrose; TS, Total Starch; V, bread volume.

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2. Materials and methods

2.1. Sample preparation

Five aliquots (1 kg each) of durum wheat (*Triticum durum* Desf.), supplied by Molino Quaglia S.p.A. (Vighizzolo d'Este, Italy) were sprouted at 20 °C for 24 h, 38 h, 48 h and 62 h and dried at 50 °C for 9 h, as previously reported by Grassi, Cardone, Bigagnoli, and Marti (2018). Unsprouted durum wheat was used as control (CTRL). Unsprouted and sprouted samples were conditioned until they reached 165 g/kg of water content and milled into refined semolina using a laboratory mill (RM1300, Erkaya, Ankara, Turkey), equipped with a 250 μm sieve.

2.2. Kernel hardness and test weight

Kernel hardness was assessed by NIR (6500, Foss, Hilleroed, Denmark) following the AACC method 39–70.02 (AACC International, 2001). Test weight was determined with a Grain Analysis Computer (2100b, DICKEY-john, Auburn, USA). These tests were carried out in single.

2.3. Chemical composition and α-amylase activity

Total and damaged starch content were evaluated according toAACC methods (76–13.01 and 76–31.01, respectively; AACC International, 2001). Simple sugars were quantified by means of the Maltose/Sucrose/D-Glucose Assay kit commercialized by Megazyme (Wicklow, Ireland). Protein content was quantified by following the ISO method 20483:2006 (ISO, 2006). α-amylase activity was determined according to the AACC method 22–02.01 (AACC International, 2001). All the measurements were carried out in triplicate.

2.4. Pasting properties

Starch pasting properties were evaluated in duplicate by using the Rapid Viscoanalyzer® (4500, Perten Instrument, Stockholm, Sweden) according to the AACC International, 2001 method 76–21.01 (AACCI, 2001) in presence of either water or silver nitrate ($AgNO_3$; 0.001 mol/L) as enzymatic inhibitor.

2.5. Gluten aggregation properties

Gluten aggregation kinetic was assessed in triplicate by using the GlutoPeak® (Brabender GmbH&Co, Duisburg, Germany) device, according to (Marti, Cecchini, D'Egidio, Dreisoerner, and Pagani, 2014), by using 9 g of distilled water instead of 10 g..

2.6. Dough preparation and leavening properties

Semolina was kneaded with fresh yeast (30 g/kg semolina; Carrefour, Milan, Italy) and salt (15 g/kg semolina; Candor®, Com-Sal s.r.l, Pesaro, Italy) in an automatic mixer equipped with a spiral hook (KitchenAid 5KSM125EER, Whirlpool, St. Paul, USA) for 6 min, until a smooth and non-sticky dough was obtained. The amount of water used in the formulations has been added on the basis on preliminary farinographic tests. Specifically, 645 g/kg of water was added to CTRL and 24 h sample, 605 g/kg of water for 38 h and 48 h samples and, finally, 585 g/kg of water for 62 h sample. Three portions (5 g each) of the resulted doughs were molded in a spherical shape and then placed in three Petri dishes, and subjected to leavening at 30 °C. The Petri dishes were scanned at 300 dpi with a flatbed scanner (Epson Perfection 550 Photo, Seiko-Epson, Suwa, Japan) at the beginning of the test, and after 15 min, 30 min, 45 min, 60 min, 90 min, 120 min and 180 min. The radial increase of the dough area (mm^2) was determined by image analysis using the Image Pro Plus software v. 6.0 (Media Cybernetics, Inc, Rockville, USA) and it was used to determine the relative increase of dough surface (A_t/A_t0), through the ratio between the area at time t (A_t) and the area of the dough at the beginning of the test (A_t0), according to (Caramanico et al., 2018).

2.7. Micro-baking test

Dough samples were obtained as reported in the previous paragraph. Samples were shaped, left to rise (90 min at 30 °C) and baked (20 min at 200 °C) as reported by Cardone, D'Incecco, Pagani, and Marti (2020). The obtained loaves were characterized 2 h after baking. One baking test was performed for each sample and two loaves were obtained.

2.8. Bread properties

Each loaf was characterized for specific volume (SpV) through the ratio between the bread volume, evaluated by seed replacement method (AACC International, 2001 10–05.01; AACC International, 2001) and the bread weight. Crumb porosity was assessed on three slices from each loaf as described by Marti et al. (2017) with some modifications about pore dimensional classes (i.e. < 0.09 mm^2; 0.10–0.99 mm^2; 1.00–2.99 mm^2; 3.00–9.99 mm^2; >10.00 mm^2). Crumb yellowness was evaluated on three points of three central slices from each loaf by means of digital colorimeter (Digital Color Meter, Apple Inc, Cupertino, USA).

2.9. Statistical analysis

Data were elaborated by a paired *t*-Test ($\alpha = 0.05$) through the software StatPlus:mac (v.7.3.31, (Analystsoft, Inc, Walnut, USA), to compare differences between mean for unsprouted (CTRL) and each sprouted sample for different duration for each parameter. Moreover, a type of homoscedastic or heteroscedastic *t*-Test was selected according to whether the variance of the pair of the tested samples was equal or different, respectively. In order to provide the precision of the measurements, for the parameters in which the variance of the samples was comparable, the pooled SD (i.e. the square-root of a pooled variance estimator) was calculated. Data were also explored by Principal Component Analysis (PCA) after data mean centering by means of Matlab software (v. 2016a, Mathworks, Inc, Natick, USA). Samples grouping was confirmed by K-Nearest Neighbor cluster analysis (PLS toolbox, v. 8.5, Eigenvector Research, Inc, Manson, USA).

3. Results

3.1. Kernel characteristics

The sprouting process caused a significant decrease in both kernel hardness (from 112 to 78 after 24 h of sprouting) and test weight (from 80 kg/hL to 69 kg/hL after 24 h of sprouting).

3.2. Chemical composition and α-amylase activity

Sprouting did not affect the starch content, instead the damaged starch fraction statistically ($p = 9.01 \times 10^{-5}$) increased after 38 h of sprouting (Table 1). As the damaged starch increased also simple sugars significantly dincreased; in particular, maltose increased ($p = 4.47 \times 10^{-4}$) after 24 h, instead sucrose ($p = 3.44 \times 10^{-2}$) after 38 h, and glucose ($p = 4.61 \times 10^{-2}$) after 48 h of sprouting (Table 1). α-amylase activity significantly ($p = 1.60 \times 10^{-4}$) increased by about 260 folds, already after 24 h of sprouting (Table 1).

Sprouting duration did not strongly affect the protein content of semolina, which decreased by about 6% (Table 1).

3.3. Pasting properties

Regardless the sprouting duration, in presence of water, sprouted samples showed low viscosity values (<0.1 Pa × s), in both heating and

Table 1
Chemical composition (starch, simple sugar and protein contents) and α-amylase activity of semolina from unsprouted (CTRL) and sprouted durum wheat at different sprouting duration (24 h, 38 h, 48 h and 62 h).

	CTRL	24 h	38 h	48 h	62 h	Pooled SD
Total starch	710	710^ns	720^ns	710^ns	690^ns	10
Damaged starch	103	97*	132*	134*	159*	3
Maltose	3	21*	47*	54*	66*	4
Sucrose	15	19^ns	20*	20*	21*	2
D-glucose	20	16^ns	3^ns	41*	42*	2
Protein	141	141^ns	138^ns	138*	133*	0.3
α-amylase activity	0.089 ± 0.004	3.8 ± 0.3*	9.9 ± 0.5*	21.6 ± 0.9*	24.3 ± 0.2*	–

Chemical data are expressed as g/kg sample (dry basis). Damaged starch is expressed as g/ kg of total starch (dry basis). α-amylase activity is expressed as Ceralpha Units/g flour (dry basis). Asterisk indicates a significant difference between CTRL and each sprouted sample (paired *t*-Test; $\alpha = 0.05$; $n = 3$). CTRL: unsprouted durum wheat; 24 h, 38 h, 48 h, 62 h: sprouting duration; ns: not significant difference.

cooling stages (data not shown). Inhibiting the amylase activity with a solution of silver nitrate ($AgNO_3$; 0.001 mol/L), all samples showed a higher viscosity, indicating that the pasting and gelation properties of sprouted samples were not drastically affected by sprouting (Fig. 1a). Specifically, the peak viscosity (1.866 ± 0.008 Pa × s for CTRL, 1.58 ± 0.02, 1.34 ± 0.04, 1.1755 ± 0.0007 and 1.156 ± 0.008 Pa × s for 24 h, 38 h, 48 h and 62 h, respectively) and the breakdown index (i.e. resistance of the gel to mechanical stress) (0.44 ± 0.01 Pa × s for CTRL, 0.39 ± 0.03, 0.33 ± 0.04, 0.275 ± 0.006 and 0.36 ± 0.04 Pa × s for 24 h, 38 h, 48 h and 62 h, respectively) significantly decreased after 24 h ($p = 3.46 \times 10^{-2}$) and 48 h ($p = 3.38 \times 10^{-2}$) of sprouting, respectively. Moreover, the final viscosity and the setback index (i.e. the tendency of starch to retrograde) statistically ($p = 4.34 \times 10^{-2}$) decreased as sprouting duration increased, starting from 24 h of sprouting (2.92 ± 0.04 Pa × s for CTRL, 2.471 ± 0.008, 2.163 ± 0.002, 1.95 ± 0.01 and 1.71 ± 0.08 Pa × s for 24 h, 38 h, 48 h and 62 h, respectively).

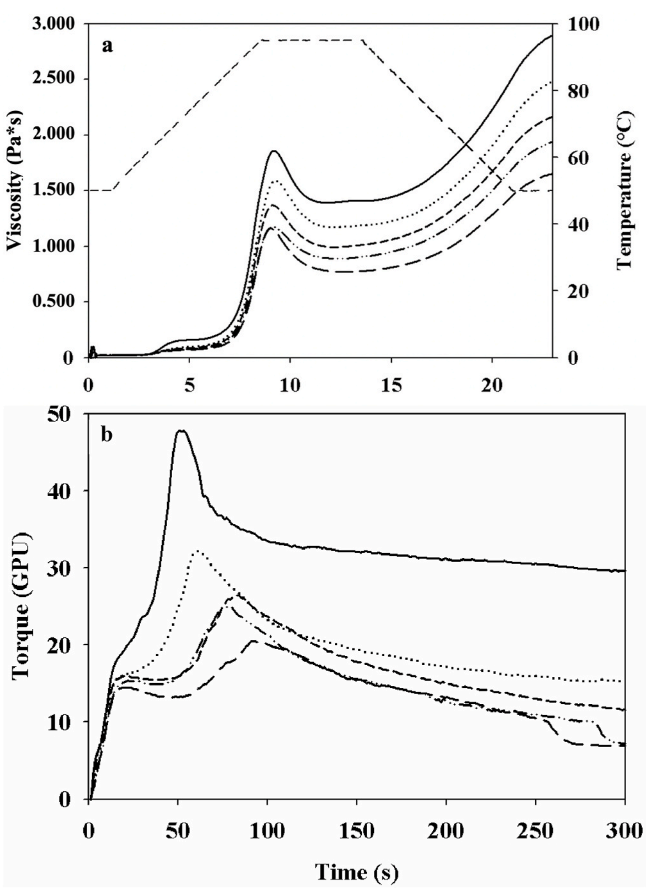

Figure 1. Rapid Viscoanalyzer (in presence of silver nitrate - AgNO3; 0.001 mol/L) (a) and GlutoPeak (b) profiles of semolina from unsprouted (CTRL) and sprouted durum wheat. CTRL: solid line; 24 h: dotted line; 38 h: short dash line; 48 h: dash-dot-dot line; 62 h: long dash line. 24 h, 38 h, 48 h, 62 h: sprouting duration; CTRL: unsprouted durum wheat; GPU: GlutoPeak Units.

3.4. Gluten aggregation properties

As regards changes in gluten aggregation kinetics (Fig. 1b), sprouting led to a significant ($p = 1.21 \times 10^{-3}$) increase in the peak maximum time starting from 38 h of sprouting (60 ± 2 s for CTRL, 62 ± 3, 83 ± 2, 77 ± 2 and 98 ± 6 s for 24 h, 38 h, 48 h and 62 h, respectively), and a significant decrease in both maximum torque ($p = 3.34 \times 10^{-4}$) (47.0 ± 0.8 GPU for CTRL, 31.8 ± 0.9, 26.4 ± 0.1, 24.2 ± 0.9 and 20.5 ± 0.7 GPU for 24 h, 38 h, 48 h and 62 h, respectively) and aggregation energy ($p = 4.48 \times 10^{-2}$) (i.e. energy required for gluten aggregation; 1239 ± 47 GPE for CTRL, 887 ± 15, 758 ± 9, 694 ± 21 and 592 ± 15 GPE for 24 h, 38 h, 48 h and 62 h, respectively), already after 24 h and 38 h of sprouting, respectively.

3.5. Dough leavening properties

Dough leavening properties were evaluated by monitoring changes in radial area. CTRL reached the maximum development in 45 min ($A\_t45/A\_t0 = 2.3$) and no longer increased up to 120 min of leavening; after that, it decreased ($A\_t180/A\_t0 = 2.0$) (Fig. 2). In contrast, the radial area of sprouted wheat dough constantly increased until the end of the test period ($A\_t180/A\_t0 = 2.7$) (Fig. 2). The fastest area expansion was observed after 24 h and 36 h of sprouting, subsequent to leavening for 15 min.

3.6. Bread-making properties

Using sprouted wheat did not lead to a drastic worsening of bread properties, in terms of volume, not even after 62 h of sprouting (178 ± 4, 173 ± 4, 180 ± 1, 180 ± 1 and 178 ± 4 mL for CTRL, 24 h, 38 h, 48 h and 62 h, respectively). Samples from 38 h sprouted wheat showed the best

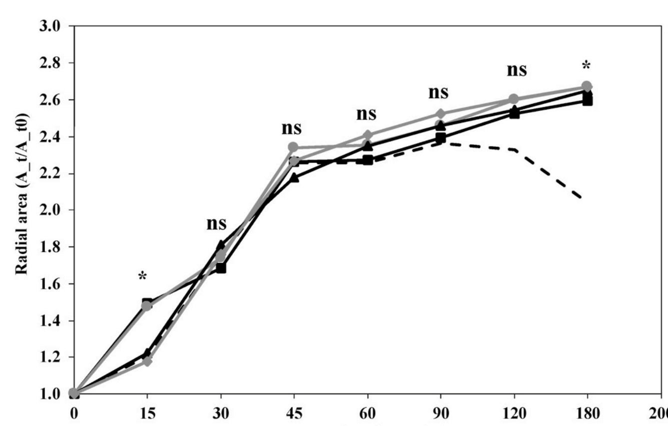

Figure 2. Increasing the radial area (A_t/A_t0) of the dough during leavening. CTRL: dash-line; 24 h: black square; 38 h: grey circle; 48 h: black triangle; 62 h: grey diamond. Asterisk indicates a significant difference between CTRL and each sample from sprouted wheat (paired *t*-Test; $\alpha = 0.05$; $n = 3$). n.s.: not significant differences. 24 h, 38 h, 48 h, 62 h: sprouting duration; A_t0, radial area of the dough at the beginning of the leavening; A_t, radial area of the dough at time t; CTRL: unsprouted durum wheat.

bread-making performances, in terms of specific volume (Fig. 3). Instead, 62 h sprouted sample showed the worst crumb structure, that appeared sticky and irregular. As regards crumb yellowness, loaves from sprouted wheat showed a more intense yellowness.

No significant differences ($p > 0.05$) were observed among the samples in terms of number of cells (data not shown). Unlike that, differences were observed in cell area (Table 2). Specifically, CTRL bread showed a crumb characterized by about 70% of small cells (<1 mm^2), instead this pore class represented about 50% of the total in loaves from sprouted wheat. Moreover, large pores (>10 mm^2) were found only in bread from sprouted wheat whose area accounted for the 10% of the total for 24 h bread, instead about 5% for 38 h and 48 h loaves.

3.7. PCA and cluster analysis

PCA results showed sample distribution according to chemical composition, α-amylase activity, dough leavening properties and bread-making properties. The scores plot defined by the first PC described almost the 83% of the data variability (PC1 = 55.87%; PC2 = 27.11%) and showed a clear separation of CTRL samples from sprouted samples (Fig. 4a). Indeed, CTRL samples assumed highly positive PC1 and PC2 values, being in the I quadrant of the plot. 24 h sprouted sample is located in the IV quarter, assuming the lowest PC2 value; 38 h sprouted samples was well separated in the III quarter; finally, 48 h and 62 h samples were grouped in the II quarter. Scores *vs* time representation (Fig. 4b) enabled to highlight that PC1 described an unique process as the scores values decreased with time progress, whereas PC2 trajectory was characterized by a sudden decrease in the first 24 h followed by an increment of the scores after 38 h and a consecutive decrement in the last sampling time. In order to uncover the variables responsible for sample grouping the loadings plot was presented (Fig. 4c). Most of the chemical indices and α-amylase drove the separation of CTRL sample from sprouted samples along PC1, together with gluten aggregation properties; whereas leavening properties and bread characteristics resulted relevant in the discrimination among samples subjected to different sprouting duration (24 h, 38 h, 48 h and 62 h).

The explorative data analysis showed a sample distribution according to the sprouting duration (Fig. 4c), envisioning the possibility of defining sprouting classes according to the considered parameters. However, the confirmation of sample grouping according to sprouting duration needed more solid bases, thus a cluster analysis was performed. The cluster analysis based on K-Nearest Neighbor algorithm identified four clusters based on the whole results collected. From the dendrogram (Fig. 4d), the first cluster, i.e. the group that differed the most from the others, was the one formed by CTRL which resulted highly different (distance = 7) from the sprouted samples, no matter the sprouting duration. By reducing the distance to 5, the analysis individuated three sprouting levels: a cluster consisting of 24 h and 38 h sprouted samples and other two separated clusters for 48 h and 62 h sprouted samples.

Table 2
Area occupied by each pore dimensional class of the bread crumb (%).

Dimensional classes (mm^2)	CTRL	24 h	38 h	48 h	Pooled SD
<0.09	8.9	8.7^{ns}	9.3^{ns}	7.8^{ns}	0.7
0.10–0.99	59	42*	47*	43*	2
1.00–2.99	26	25^{ns}	23^{ns}	28^{ns}	4
3.00–9.99	8	14^{ns}	14^{ns}	17^{ns}	3
>10.00	–	7	10	4	2

Asterisk indicates a significant difference between CTRL and each sprouted sample (paired *t*-Test; α = 0.05; n = 3). CTRL: unsprouted durum wheat; 24 h, 38 h, 48 h: sprouting duration; ns: not significant difference.

4. Discussion

Compared to common wheat, durum wheat is characterized by high kernel hardness, high gluten tenacity and intensive yellowness – due to its high carotenoid content. All these characteristics are used to evaluate the grain quality on the market. As regards the kernel characteristics, sprouting process led to a significant decrease in hardness, with the greatest changes occurring at 48 h sprouting duration (Fig. S1). The decrease in kernel hardness might positively affect the milling behavior. Indeed, hard kernels, such as durum wheat, require more energy to be milled than both soft and hard kernels (Różyło, Laskowski, & Grundas, 2003). Specifically, the decrease in kernel hardness might be attributed to the decrease in starch-protein matrix density in the endosperm. This hypothesis has been confirmed by the decrease in test weight (i.e. index related to the kernel density; Fig. S1) due to the high α-amylase activity associated with sprouting (Table 1). The effect of enzymatic activity on decreasing the endosperm density as a consequence of sprouting has been recently shown in sprouted common wheat (Cardone, D'Incecco, Casiraghi, & Marti, 2020b). Moreover, the decrease in kernel hardness and test weight were in line with previous study carried out on sprouted common wheat (Miś & Grundas, 2002; Różyło; Laskowski, & Grundas, 2003). However, both the indices seemed not to be affected by the sprouting duration (Fig. S1).

In addition to milling energy, hardness also affects the milling yield and the damaged starch content of flours (Turnbull & Rahman, 2002). In this study, the milling yield did not appear to be affected by the sprouting duration within 48 h, ranging from 490 g/kg for CTRL, to 480, 460, 470 and 380 g/kg for 24 h, 38 h, 48 h and 62 h, respectively. The low yield ratio obtained could be due to the use of a laboratory mill that allowed to extract mainly the innermost regions of the endosperm, at the expenses of the yield. The decrease in milling yield might be related to the decrease in test weight (Fig. S1), with evidence at prolonged

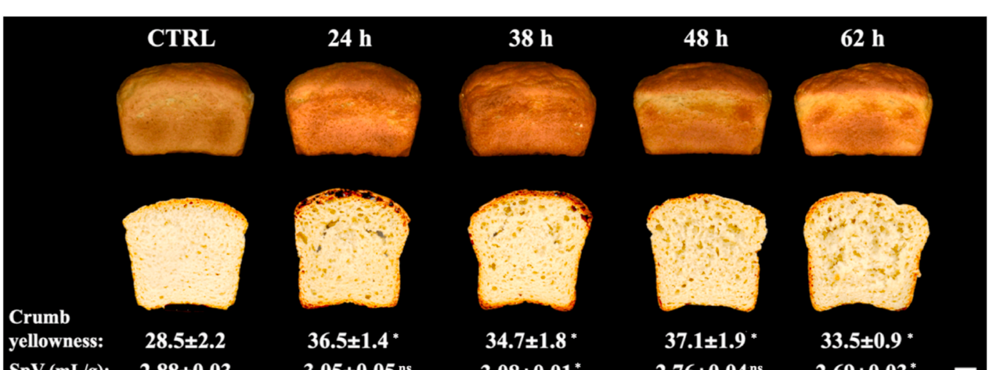

Figure 3. Pictures of the bread loaves and data related to crumb yellowness and specific volume (SpV) of bread prepared from semolina from unsprouted (CTRL) and sprouted durum wheat. Asterisk indicates a significant difference between CTRL and each bread sample from sprouted wheat (paired *t*-Test; α = 0.05; n = 5 for crumb yellowness; n = 2 for specific volume). Scale bar is 1 cm. 24 h, 38 h, 48 h, 62 h: sprouting duration; CTRL: unsprouted durum wheat.

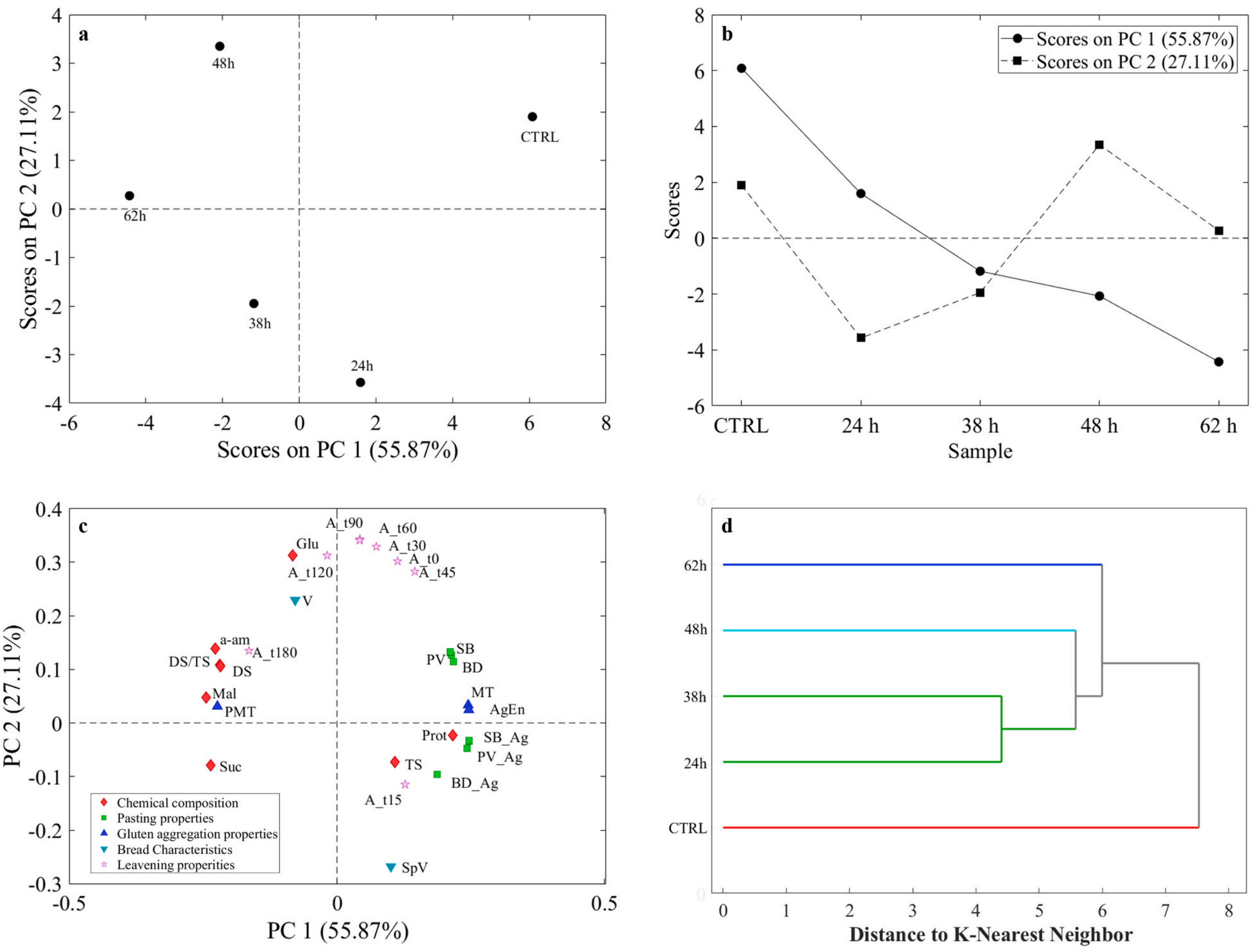

Figure 4. Multivariate data analysis on data collected for chemical composition, α-amylase activity, dough leavening properties and bread-making properties: scores plot for Principal Component Analysis (a), scores vs sprouting duration plot (b), loadings plot (c), dendrogram for cluster analysis by K-Nearest Neighbor (d). A-am, α-amylase activity; TS, Total Starch; DS, Damaged Starch; Mal, Maltose; Suc, Sucrose; Glu, D-glucose; Prot, Protein. Pasting properties: PV, Peak Viscosity; BD, Breakdown index; FV, Final Viscosity. Gluten aggregation properties: PMT, Peak Maximum Time; MT, Maximum Torque; AgEn, Aggregation Energy. Leavening properties: relative increase of dough surface at 15 min (A_t15), 30 min (A_t30), 45 min (A_t45), 60 min (A_t60), 90 min (A_t90), 120 min (A_t120) and 180 min (A_t180). Bread characteristics: SpV, Specific Volume; V, Bread Volume.

sprouting duration. Indeed, after 62 h the rootlet was quite evident (Fig. S1), suggesting an intense hydrolysis of the storage macromolecules, as confirmed by the increased α-amylase activity. It is generally recognized that the sprouting process is considered concluded when the rootlet reached the kernel length, in order to avoid strongly negative effects on the kernel properties and flour functionality (Marti, Cardone, & Pagani, 2020). During sprouting, high levels of hydrolytic enzymes – specifically α-amylases – are released and create some holes on the surface of the starch granules (Cardone, D'Incecco, Pagani, & Marti, 2020; Faltermaier, Zarnkow, Becker, Gastl, & Arendt, 2015), making them more accessible to a further enzymatic action. Thus, the level of damaged starch (which is defined as the amount of starch which is readily accessible to α-amylase) might provide information about the intensity of the sprouting process. In general, high damaged starch content adversely affects the dough handling (because of the greater water absorption and dough stickiness) and the bread characteristics (leading to a lower development in volume and darker crust color) (Sapirstein, David, Preston, & Dexter, 2007). Under the condition applied in this study, the damaged starch content increased as the sprouting duration increased too, as an effect of the increased α-amylase activity (Table 1), rather than exclusively as mechanical damage of the starch granules during milling. These findings were confirmed by the multivariate exploration by PCA, indeed damaged starch and α-amylase activity were close to each other and located in the II quarter of the loadings plot (Fig. 4c) affecting the separation of samples sprouted 48 h and 62 h from lower germination exposure (24 h and 38 h) and CTRL (Fig. 4a), thus driving the separation of these samples along PC1 according to sprouting duration (Fig. 4b).

Sprouting resulted in a lower pasting and gelation properties (Fig. 1a), because of the lower gelatinization and retrogradation ability of the smaller starch polymers accumulated in sprouted samples than CTRL. These changes were in line with other studies on sprouted durum (Jribi, Molnar, et al., 2019) and common (Cardone, D'Incecco, Pagani, & Marti, 2020; Grassi et al., 2018) wheat and also remarked by the PCA loadings plot (Fig. 4c), in which the pasting and gelation indices calculated from the analysis performed in presence of water or silver nitrate assumed positive PC1 scores, thus separating the CTRL from the sprouted samples (Fig. 4a). Furthermore, the lower retrogradation ability of the sprouted samples might have led to obtain a fresh bread with a softer crumb, compared to the CTRL one, as shown in common wheat (Cardone, D'Incecco, Pagani, & Marti, 2020,b).

As regards the proteins, the decrease (Table 1) might be attributable to their hydrolysis into soluble peptides due to the proteolytic activity (Mbithi-Mwikya, Ooghe, Van Camp, Ngundi, & Huyghebaert, 2000). On the other hand, it is reported that changes in protein content less than 10% indicates that the sprouting process did not significantly affect the protein content of grains (Lemmens et al., 2019). Similar changes are reported in previous studies on sprouted durum (Jribi, Sahagùn, et al.,

2019) and common (Cardone, D'Incecco, Pagani, & Marti, 2020; Grassi et al., 2018; Koehler, Hartmann, Wieser, & Rychlik, 2007; Marti et al., 2017) wheat.

Moving to gluten properties, the sprouting duration negatively affected the aggregation properties of the gluten-forming proteins (Fig. 1b), in terms of peak maximum time (increased by ~63% after 62 h of sprouting), maximum torque (decreased by ~56% after 62 h of sprouting) and aggregation energy (decreased by ~52% after 62 h of sprouting), suggesting a weakening of the gluten network (Grassi et al., 2018; Marti, Augst, Cox, & Koehler, 2015), as a consequence of the proteolytic activity. In general, flour with good bread-making performance are characterized by a faster aggregation (i.e., low peak maximum time) and higher maximum torque compared to those with poor bread-making attitude (Quayson, Atwell, Morris, & Marti, 2016). Actually, the aggregation properties of the gluten-forming proteins resulted the ones most affecting the separation between CTRL and the highly sprouted samples along the PC1 of the PCA scores plot (Fig. 4a), being the peak maximum time highly negative and maximum torque and aggregation energy highly positive. A possible explanation of the maximum torque and the peak maximum time shifts is that sprouting induced changes in the profile of gluten proteins (i.e. gliadin and glutenin fractions) (Koehler et al., 2007). Indeed, Marti, A., Augst, E., Cox, S., & Koehler, P. (2015) found a positive correlation between maximum torque and gliadin content and between energy and glutenin with high molecular weight. In particular, it is already reported that sprouting caused a significant degradation of glutenins, already after 48 h of sprouting, instead longer duration was required for degrading gliadins, about 102 h (Koehler et al., 2007). Although the sprouted samples showed a different gluten aggregation profiles that would suggest gluten weakening, they were still able to aggregate and form a gluten network with good performance in bread-making (Fig. 1b), confirming previous studies on common wheat (Cardone, D'Incecco, Pagani, & Marti, 2020; Marti et al., 2018). The only exception was the 62 h sample that lost its ability to form gluten (Fig. 1b), likely due to the stronger intensity of the sprouting process (Fig. S1; Table 1).

In comparison with common wheat, durum wheat is characterized by a very stiff and not very extensible gluten, making it suitable for the pasta-making but unsuitable for leavened baked-goods (Ammar et al., 2000). Indeed, the resulting bread will be characterized by a high density and a hard texture (Sissons, 2008). The interest in durum wheat bread lies in the fact that this raw material is richer in carotenoids (i.e. antioxidant compounds) compared to common wheat. Generally, to overcome the negative technological properties (i.e., low volume and high crumb density) of durum wheat bread, sourdough fermentation is used as leavening agent. Indeed, the low pH and the enzymatic activities of lactic bacteria and yeasts enhance bread-making performance (Barber et al., 1992; Pagani; Lucisano; Mariotti, 2014). In this context, the increased enzymatic activity developed during sprouting process might represent a good strategy to improve the bread-making attitude of durum wheat.

Thanks to the correlations between dough tenacity and strength and maximum torque and aggregation energy (Marti, Urlici, Foca, Quaglia, Pagani, 2015; Rakita, Dokić, Dapčević Hadnađev, Hadnađev, & Torbica, 2018), it is possible to hypothesize that sprouting could represent a good way to decrease dough tenacity and consequently improve its bread-making performance. Despite the gluten weakening (Fig. 1b), the dough from sprouted durum wheat was able to withstand the leavening stresses, expanding itself without collapsing (Fig. 2). The increased CO_2 production during leavening - thanks to the increased amount of fermentable sugars by yeasts, resulting from the α-amylase activity (Table 1) – increased loaf specific volume, mainly for the 38 h sample (Figs. 3 and 4a). Similar results are reported for common wheat (Cardone, D'Incecco, Pagani, & Marti, 2020; Marti et al., 2018). The worsening of crumb structure in bread from 62 h sprouted wheat (Fig. 3) agreed with the excessive gluten weakening (Fig. 1b). Indeed, the poor gluten aggregation properties and its gas retention capacity resulted in the lowest specific volume (Fig. 3). As regards the pore distribution, large pores (>10 mm^2) were found only in bread from sprouted wheat, probably due to the coalescence of the gas cells, favored by α-amylase activity (Lagrain, Leman, Goesaert, & Delcour, 2008). In addition, bread from sprouted wheat resulted in a higher crumb yellowness (Fig. 3), following a similar trend of the yellow index of semolina (from 19 ± 1 for CTRL to 25.4 ± 0.8 after 62h). Yang, Basu, and Ooraikul (2001) report that the β-carotene content increased upon sprouting and the color intensity of the carotenoid extract increased as the sprouting duration increased too. Although this aspect needs to be further investigated, finding suggests that sprouting process might have a positive effect on the carotenoid content in bread from sprouted durum wheat.

All the considered chemical composition, α-amylase activity, dough leavening properties and bread-making properties do not act separately but are interconnected and correlated. Thus, the multivariate approach led us to confirm the relationships between all the considered variables and to define which of them contributed most in the sample distribution, i.e. in assessing the sprouting influence in the final product, as Grassi et al. (2018) pointed out. Indeed, the dendrogram obtained by the cluster analysis (Fig. 4d) confirmed that samples sprouted up to 38 h had similar and improved bread-making performance. The two distinct clusters for 48 h and 62 h sprouted samples (Fig. 4d) indicated a progressive and significant decrease of the overall quality.

5. Conclusions

Changes induced by sprouting strongly depended on the process duration. Specifically, sprouting up to 48 h did not strongly compromise the functional properties of starch (i.e., gelatinization and retrogradation phenomena). As regards proteins, despite the sprouting process weakened the gluten network, gluten proteins were still able to aggregate and retain gas during leavening, resulting in bread with improved volume. Specifically, the best bread-making performance were achieved using durum wheat that was sprouted for 38 h.

Overall results suggest that sprouting carried out under controlled conditions could improve the bread-making attitude of durum wheat and produce a more attractive product (i.e. improved bread volume and crumb porosity) for the consumer and with high carotenoid content compared to common bread. However, the effects of sprouting process on gliadin and glutenin fractions need to be studied in depth, as well as the potential application of the process on various durum wheat varieties.

CRediT authorship contribution statement

Gaetano Cardone: Formal analysis, Investigation, Writing - original draft, Validation, Data curation. **Silvia Grassi:** Validation, Investigation, Formal analysis, Writing - original draft, Methodology, Data curation. **Anna Scipioni:** Investigation, Formal analysis, Visualization. **Alessandra Marti:** Conceptualization, Methodology, Supervision, Funding acquisition, Project administration, Resources, Validation, Writing - review & editing.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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