Title: Sprouting improves the bread-making performance of whole wheat flour (*Triticum aestivum* L.)

Running title: Sprouted wheat: gluten functionality and bread-making performance

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ABSTRACT:

BACKGROUND: Pre-harvest sprouting of wheat is negatively considered because of the high enzymatic activities that lead to the worsening of bread-making performance of the related flours. On the contrary, improvements in bread properties (i.e. volume and crumb softness) are reported when sprouted wheat under controlled conditions is used in mixture with a commercial flour. However, knowledge about the effects of sprouting on gluten functionality and its relationship with bread features is still limited, especially in the case of whole wheat flour.

RESULTS: Under the conditions applied in this study (48 h, 20° C and 90% relative humidity), proteins of sprouted wheat were still able to aggregate, even if changes in gluten aggregation kinetics suggested gluten weakening. On the other hand, sprouting led to an increase in gluten stretching ability, suggesting an increase in dough extensibility. In the dough system, sprouting was responsible for the decrease in water absorption, development time, and stability during mixing. However, optimizing the bread-making conditions, sprouting improved bread height (~ 20%), specific volume (~ 15%), and crumb softness (~ 200% after 24 h of storage) even when whole wheat flour was used.

CONCLUSION: By optimizing the baking conditions, it is possible to produce bread with improved volume and crumb softness using whole wheat flour from sprouted kernels. Thus, sprouting can be exploited as a pre-treatment to improve the bread-making performance of fiber-enriched systems.

Keywords: sprouting; gluten; whole wheat flour; bread-making; ultrastructure.

Introduction

The interest in enriching cereal-based products in sprouted grains is constantly increasing,[1] because of the improved nutritional and sensory profile associated with the chemical and biochemical changes promoted by sprouting. Such changes strongly depend on the sprouting conditions adopted (i.e. temperature and time) as well as grain species, varieties and cultivars.[2] However, a prolonged and uncontrolled sprouting could represent a negative event since the high accumulation of hydrolytic enzymes developed during the process makes the flour unsuitable for bread-making. Consequently, the resulting bread will be characterized by low volume and sticky and gummy crumb.[3] Thus, controlled sprouting might be a useful process to achieve the perfect balance between nutritional advantages and technological performance.[4] In this context, Grassi *et al*. proposed the use of a portable Micro NIR device to monitor the sprouting process.[5] Although the study was carried out at lab scale (1 kg of kernels), the analysis of the spectra suggested that the greatest changes in both starch (1480-1526 nm) and protein (1500-1530 nm) fractions occurred in the first 48 h, whereas longer germination time generated no further relevant changes.[5] For what concerns the nutritional traits, Poudel *et al*. highlighted the effects of sprouting time (up to 72 h) on the increase in γ-aminobutyric acid, asparagine, and lysine, and on the decrease in thiamine and phytic acids upon sprouting time.[6]

Apart from the nutritional feature, the relation between changes induced by sprouting on starch and protein functionality and the quality of the product have been poorly studied so far. The absence of such information, makes difficult to elucidate if sprouting may improve the technological performance of wheat. This aspect is worthy of interest, especially in the case of whole wheat flour, whose use in bread formulations is growing due to its nutritional and health benefits. To the best of our knowledge, most of the available studies focused on the use of refined flour[7] or whole grain[6] flour from sprouted wheat in mixture (<10%) with commercial flours. Furthermore, in most of the studies, sprouting was carried out at lab or pilot scale, neglecting the scale-up of the process, that as

well-known might represent a critical point at industrial level and deserve to be investigated to help companies in formulating cereal based-products with constant characteristics.

In this context, this work aimed at (1) assessing starch and gluten functionality before and after controlled sprouting of common wheat at industrial scale; (2) relate such changes to bread-making performance of both 100% whole wheat and refined flours. The effects of sprouting on wheat were explored by using ultrastructure techniques in combination with empiric rheology to elucidate the relationship between macromolecular features and bread-making performance.

Materials and methods

Sample preparation

Kernels of common wheat (*Triticum aestivum* L.) were divided into three aliquots. An aliquot was grinded into a M20 Universal Mill (IKA, Werke Staufen, Germany) to obtain a whole wheat flour (80% particle size < 500 μm). Another aliquot was milled using a Bona laboratory mill (Labormill, Monza, Italy) obtaining a refined flour (95% particle size < 250 μm). The third aliquot was sprouted in an industrial plant (Bühler Pargem, Bühler AG, Uzwil, Switzerland) using the following conditions: soaking for 24 h at 20° C, 90% relative humidity; sprouting for 48 h at 20° C, 90% relative humidity; drying for 9 h at 60° C. Sprouted grains were milled into whole wheat and refined flours as described for the unsprouted kernels.

Chemical composition and enzymatic activities

Protein, total starch, and damaged starch content were evaluated according to AACCI methods 46-12.01, 76-13.01, and 76-31.01 (AACCI 2001), respectively.[8] Sugars were quantified by means of the Megazyme Maltose/Sucrose/D-Glucose Assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). α-amylase activity was determined according to AACCI method 22-02.01 (AACCI 2001),[8] whereas β-amylases as reported by Betamyl-3 Assay (Megazyme, Bray, Ireland). All the analyses were carried out in triplicate.

Visco-elasticity and aggregation properties of gluten

A creep-recovery test was carried out using the Glutograph-E® (Brabender GmbH & Co. KG, Duisburg, Germany). The wet gluten obtained from 10 g of each sample was used to evaluate its stretching and elastic properties, following the procedure reported in the manufacturer's manual. Shear and relaxation angles were calculated from the curve.

Gluten aggregation kinetics were assessed on flours by using the GlutoPeak® (Brabender GmbH&Co. KG, Duisburg, Germany) device as reported by Marti *et al*.[9]

Both analyses were carried out in triplicate.

Mixing properties

Mixing properties were studied in duplicate following the ICC method 115/1 (1992),[10] by means of the Farinograph® (Brabender GmbH & Co. KG, Duisburg, Germany) equipped with a 50 g bowl.

Micro-baking test

The bread was obtained by kneading flour (70 g) with compressed yeast (1.05 g) and salt (0.7 g). The amount of water added to the bread formulation was in accordance with the water absorption index previously determined by the farinographic analysis. Specifically, 44.0 mL and 39.4 mL of water for whole wheat flours from unsprouted and sprouted wheat, respectively, and 38.7 mL and 36.9 mL of water for refined flours before and after sprouting, respectively. The dough was prepared in a spiral mixer (KitchenAid® Artisan, St. Joseph, Michigan) for a time corresponding to the development time obtained from the farinographic test. After kneading, a portion of 80 g of dough was obtained, shaped in cylindrical forms, placed in baking pan (length: 9 cm; height: 6; width: 4 cm) and left to rise at 29±1° C (70% relative humidity) for 90 min. Successively, the bread was baked at 220° C for 20 min (Self Cooking Center®, Rational International AG). For each type of sample two baking tests were performed and each loaf was characterized two hours after baking.

Bread properties

Specific volume was determined by the ratio between apparent volume - assessed by the sesame replacement method - and weight. Loaf height was determined by image analysis (Image ProPlus, v6; Media Cybernetics, Inc; Maryland) measuring the highest point of the two central slices of each loaf (n=4). Crumb firmness was assessed on two slices from two loaves (n=4) and measured 2 h (t0) and 24 h (t1) after baking as described by Marti *et al*.[7]

Ultrastructure

Ultrastructure of kernels was investigated by scanning electron microscopy (SEM) at the end soaking and after 48 h of sprouting. Kernels were air dried overnight on filter paper and cut/cracked with a razor blade to obtain a transversal section. Samples were mounted on circular specimen holders (Agar Scientific, Plain stubs 10 × 10 mm) with double carbon tape (Agar Scientific, Carbon Tabs 9 mm). Samples gold coated with a sputter coater (SEMPREP2; Nanotech) were observed with a Zeiss LEO 1430 SEM at 3 kV.

The microstructure of the samples collected from GlutoPeak® at the maximum torque was analyzed by using an inverted confocal laser scanning microscope (CLSM, Nikon A1+, Minato, Japan). A concave microscope slide was filled with sample that was directly stained by adding 15 µL of Fast Green FCF (0.1 mg/mL in water) (Sigma, MO, USA) for protein labelling. The excitation/emission wavelengths were set at 638 nm/660–740 nm for Fast Green FCF and at 405 nm/440-530 nm to visualize auto-fluorescent bran particles in whole wheat samples. Images are presented as maximum projection of 150 layers of 512*512 pixel images that are stacked together with separation between layers set at 0.30 µm (ImageJ software Research Services Branch, National Institute of Health and Medicine, Maryland).

125 Statistical analysis

126 Statistical differences (t-Test; two-tailed distribution) were evaluated using the Statgraphics Plus
127 5.1 (Statpoint Inc., Warrenton, Virginia). Differences at $P < 0.05$ (*); $P < 0.01$ (**) and $P < 0.001$
128 (***) were considered significant.

129 ## Results

130 Chemical composition and enzymatic activities

131 The protein content in whole wheat did not changed after 48 h of sprouting (Table S1). In the case
132 of refined flour, proteins significantly decreased after sprouting (from 12.65 to 11.69 g/100 g db).
133 Total starch significantly decreased in whole wheat only (from 64.8 to 63.4 g/100 g db), likely
134 because enzymatic hydrolysis proceeds from the outside to the inner part of the kernel leaving intact
135 the starch granules in the kernel core, as shown by SEM images (Fig. 1). Indeed, the characteristic
136 pitting of granules was found only on those located at the periphery of the endosperm, close to
137 aleurone cells (Fig. 1(b) red head-arrows). The increase in simple sugars in sprouted samples can be
138 also attributed to the α-amylase activity (> 600 folds). Compared to α-amylase, β-amylase activity
139 increased at lower extent (~ 1%).

140 Gluten functionality

141 Washed gluten showed a typical profile of strong gluten when examined by GlutoGraph® (Fig. 2(a)
142 and (b)), namely low stretch and relaxation angle (Table 1). The sprouting process led to a
143 significant increase in stretch angle, whereas no differences in relaxation angle were found.
144 The GlutoPeak® test showed that the sprouting process caused a decrease in all the parameters
145 taken into consideration (Fig. 2(c) and (d); Table 1). Specifically, the sprouted samples showed a
146 lower peak maximum time and maximum torque, resulting in lower aggregation energy.
147 In the refined flour from unsprouted wheat, the protein matrix (Fig. 3(a), in green) was organized in
148 thick strands giving rise to a network suitable to surround and contain the starch granules.

Differently, few signs of fibrous protein organization were found in whole wheat flour from unsprouted wheat (Fig. 3(b)). As regards the effect of sprouting, apparently, a more compact protein structure was observed in the refined sample (Fig. 3(c)), while the ability to organize a network and form aggregates was almost absent in the whole wheat (Fig. 3(d)). Indeed, in sprouted wheat flour, the protein matrix was mainly arranged into clumps which are homogeneously distributed and often connected to each other by short protein fibers (Fig. 3(e)). In addition to proteins, CLSM images highlighted the presence of bran fragments as well as aleurone layer cells (Fig. 3, in blue). Specifically, bran particles ~ 500 μm in size were detected in whole wheat from unsprouted kernels, while bran particles up to ~ 250 μm were observed after sprouting, suggesting weakening of bran layers in sprouted kernels during milling.

Bread-making properties

Regardless the refinement level (whole wheat vs refined flour), sprouted samples required less water to form a dough with optimal consistency (500 UF) (Table 1). Furthermore, the sprouting process caused a significant decrease in both dough development time and stability (Table 1). Moreover, after sprouting, flours gave weaker dough with increased degree of softening compared to reference samples (Figs. 2(e) and (f)). Similar results were shown in either pre-harvest and controlled sprouted wheat.[11]

Images of bread samples together with specific volume and height are shown in Fig. 4(a). Although the bread made from sprouted samples showed a significant increase in loaf height compared to the unsprouted ones, only the whole wheat bread from sprouted sample showed a significant increase in specific volume (Fig. 4(a)). In addition, the specific volume of this sample was similar to that of bread from refined flour from unsprouted wheat. Crumb bread from sprouted wheat exhibited a lower firmness than control samples, even after one day of storage (Fig. 4(b)). In particular, the positive effect of sprouting on delaying crumb firmness during storage was more effective when whole wheat flour was used (-42% vs -36%, for whole wheat and refined samples).

Discussion

The biochemical changes occurring during sprouting are the driving force for the well-documented enhancement in nutritional and sensory properties of sprouted grains.[12] Besides that, high synthesis and accumulation of hydrolytic enzymes might lead to relevant changes in dough properties, responsible for an overall decrease in bread-making performance.[3] This behavior is typical of wheat subjected to pre-harvest sprouting directly in field. In such conditions, the amount of α-amylase increases as much as several thousand folds,[13] due to the exposure of plant to the alternation of hot and humid weather conditions after maturity and before harvesting. On the contrary, the sprouting conditions applied in this study (48 h at 20 °C) allowed the increase in α-amylase activity by about 600 times, while β-amylases increased less than one time (Table S1), since they are inactivated during drying at 60° C (data not shown). Among the various enzymatic activities developed during sprouting, α-amylase activity is considered the most important in defining wheat quality,[13] and the easiest one to be monitored during the process. Indeed, the available methods are based on the direct or indirect (i.e. by measuring changes in viscosity due to starch hydrolysis) quantification of amylases present in the flour.

By assessing the kinetics of the sprouting process using both conventional and spectroscopic data, Grassi *et al*. monitored the starch pasting properties upon sprouting time.[5] A quick loss of gelatinization and retrogradation capacity was detected after 38 h as effect of starch degradation by amylases. However, when the test was carried out in the presence of $AgNO_3$, to prevent the activation of α-amylases, data suggested that the capability of granules to gelatinize may have been masked by the presence of high levels of α-amylase during the test running rather than during the sprouting process.[5] However, amylolytic enzymes might hydrolyze starch during kneading, leavening and the first stages of baking.[3] Starch degradation - also assessed as damaged starch (i.e. the fraction of starch readily hydrolyzed by α-amylases) - was more intense in whole wheat compared to the refined flour (Table S1), because the most hydrolyzed endosperm regions at 48 h

of sprouting are very close to the bran layers, and thus maintained in the whole wheat flour as shown by the SEM images in Fig. 1.

As regards proteins, protease activity developed during 48 h of sprouting seems to only partially degrade gluten proteins. Indeed, Marti *et al*. found that the proteolytic activity in refined flour from 72 h-sprouted wheat increased only by 1 fold.[7] In the present study, sprouted wheat was still able to form a network (see GlutoPeak® pattern; Figs. 2(c) and (d)) and maintain viscoelastic properties (see GlutoGraph® indices; Figs.2 (a) and (b)). On the other hand, sprouting longer than 48 h caused gluten degradation due to an excessively high proteases accumulation.[14] Specifically, glutenins are mainly hydrolyzed during the first 48 h of sprouting, while longer times are needed for gliadin hydrolysis (about 102 h).[14] Thus, the unexpected good baking performance observed in our study might be due to limited changes in gliadin fraction after 48 h of sprouting. However, this aspect need to be further investigated. According to Marti *et al*., the maximum torque in the GlutoPeak® profile is mainly related to the amount of gliadins, whereas both the time and energy aggregation are influenced by glutenins.[9] In accordance to empiric rheology (i.e. gluten aggregation properties), CLSM showed gluten weakening upon sprouting, since clumped proteins kept together by tiny fibrils are evident, especially in whole wheat flour (Fig. 3). However, this peculiar and unusual protein organization was still able to assure a volume development during baking (Fig. 4(a)). Sprouting also affected dough mixing properties (i.e. decrease in water absorption, development time, and stability; Table 1) as the macroscopic effects of partial hydrolysis of starch and proteins. Specifically, the decrease in dough water absorption and mixing time could be due to the low molecular weight of the hydrolyzed gluten proteins.[15] Another negative effect is the increase in dough weakening (Table 1), usually related to dough stickiness.[16] Even if, in this study dough did not stick to hands or bowl during mixing and processing, evaluating the effects of sprouting on dough stickiness by an objective approach is worthy of interest.

By optimizing processing conditions - including the amount of water and the mixing time, as suggested by the farinograph test (Table 1) - it was also possible to prepare bread from 100%

sprouted wheat showing high volume without collapsing after baking (Fig. 4(a)). At the same time, rheological findings showed sprouted wheat to be more extensible than the unsprouted one, without losing the ability to recover its initial structure following deformation (Fig. 2(a) and (b); Table 1).

The increase in specific volume in sprouted samples (Fig. 4(a)) could be related to the increase in simple sugars (Table S1) that are an available substrate the growth of yeast and the production of CO_2. In addition to the increased volume, sprouting was effective in decreasing the crumb staling (Fig. 4(b)). A similar effect has been shown even at low percentage of sprouted flour addition (< 2%).[7] This result might be due to the lower retrogradation properties of sprouted flours,[5] confirming that α-amylases are useful to decrease amylopectin retrogradation and the firmness rate of crumb. Interestingly, the lowest staling rate was observed in the whole wheat bread due to its high α-amylase activity after sprouting (Table S1).

In conclusion, this study provides information about the relationship between the kernel biochemical modifications induced by sprouting and gluten functionality in both dough and bread systems, as well as baking performance of both whole wheat and refined flours. Starting from wheat that has been sprouted at industrial level, it is possible to obtain bread with improved volume and crumb softness by optimizing the baking conditions. Thus, sprouting might represent a biotechnological process able to improve the bread-making performance of fiber-rich flours. Although after sprouting gluten proteins were still able to aggregate and maintain peculiar visco-elasticity properties, potential changes in quality-related gluten fractions (i.e. gliadin and glutenin) need to be further investigated and related to gluten functionality.

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Conflicts of interest: The author declare no conflict of interest.

References

1. Pagand J, Heirbaut P, Pierre A and Pareyt B, The magic and challenges of sprouted grains. Cereal Foods World. **62**:221–6 (2017).
2. Ding J and Feng H, Controlled germination for enhancing the nutritional value of sprouted grains, in: *Sprouted Grains*, ed. by Feng H, Nemzer B and Devries J. Elsevier Inc. in cooperation with AACC International press, St. Paul, pp. 91–112 (2019).
3. Olaerts H and Courtin CM, Impact of preharvest sprouting on endogenous hydrolases and technological quality of wheat and bread: a review. Compr Rev Food Sci Food Saf. **17**:698–713 (2018).
4. Marengo M, Carpen A, Bonomi F, Casiraghi MC, Meroni E, Quaglia L, *et al*., Macromolecular and Micronutrient Profiles of Sprouted Chickpeas to Be Used for Integrating Cereal-Based Food. Cereal Chem J. **94**:82–8 (2017).
5. Grassi S, Cardone G, Bigagnoli D and Marti A, Monitoring the sprouting process of wheat by non-conventional approaches. J Cereal Sci. **83**:180–7 (2018).
6. Poudel R, Finnie S and Rose DJ, Effects of wheat kernel germination time and drying temperature on compositional and end-use properties of the resulting whole wheat flour. J Cereal Sci. **86**:33–40 (2019).
7. Marti A, Cardone G, Nicolodi A, Quaglia L and Pagani MA, Sprouted wheat as an alternative to conventional flour improvers in bread-making. LWT - Food Sci Technol. **80**:230–6 (2017).
8. AACC International. Approved methods of analysis, 11th Ed. AACC International. AACC International Inc, St. Paul.
9. Marti A, Augst E, Cox S and Koehler P, Correlations between gluten aggregation properties defined by the GlutoPeak test and content of quality-related protein fractions of winter wheat flour. J Cereal Sci. **66**:89–95 (2015).
10. International Association for Cereal Science and Technology. Standard No. 115/1, Method for using the Brabender Farinograph (1992).

11. Dojczew D and Sobczyk M, The effect of proteolytic activity on the technological value of wheat flour from pre-harvest sprouted grain. Acta Sci Pol Technol Aliment. **6**:45–53 (2007).
12. Lemmens E, Moroni AV, Pagand J, Heirbaut P, Ritala A, Karlen Y, *et al*., Impact of Cereal Seed Sprouting on Its Nutritional and Technological Properties: A Critical Review. Compr Rev Food Sci Food Saf. **18**:305–28 (2019).
13. Kruger JE, Enzymes of sprouted wheat and their possible technological significance, in *Wheat: Chemistry and Technology*, ed. by Khan K and Shewry PR. Springer, Boston, pp 143–53 (1994).
14. Koehler P, Hartmann G, Wieser H and Rychlik M, Changes of folates, dietary fiber, and proteins in wheat as affected by germination. J Agric Food Chem. **55**:4678–83 (2007).
15. Delcour JA and Hoseney RC (eds). *Principles of Cereal Science and Technology*, 2nd edn. AACC International Inc, St. Paul (2010).
16. Caramanico R, Marti A, Vaccino P, Bottega G, Cappa C and Lucisano M, Rheological properties and baking performance of new waxy lines: Strengths and weaknesses. LWT - Food Sci Technol. **88**:159–64 (2018).

Figure captions

Fig. 1. SEM images of the kernel after 48 h of sprouting showing starch granules in native conditions in the core of the endosperm (a), or partially hydrolyzed (red head-arrows) in the outermost portion of the endosperm in contact with bran layers (white arrow) (b). Scale bar is 10 μm.

Fig. 2. Profiles of wholegrain (black) and refined (grey) flours from unsprouted (solid lines) and sprouted (dash lines) wheat profiles obtained by GlutoGraph® (a, b); GlutoPeak® (c, d) and Farinograph® (e, f) test.

Fig. 3. Microstructure of slurries from GlutoPeak® test. Refined (a) and wholegrain (b) flours from unsprouted wheat; refined (c) and wholegrain (d) flours from sprouted wheat. Panel "e" is an enlarged frame of panel "d" where filamentous protein (FP, arrows) connecting clumped protein (CP) is evident. Protein is green and auto-fluorescent bran is blue. Scale bar is 50 μm.

Fig. 4. Bread crumb images, specific volume (SV), height (H) (a) and firmness after 2 h (black) and 24 h of storage (white) (b).
WU: wholegrain flour from unsprouted wheat; WS: wholegrain flour from sprouted wheat; FU: refined flour from unsprouted wheat; FS: refined flour from sprouted wheat.
The asterisks indicate significant differences between the means of the unsprouted and sprouted samples of each class (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; t-Test). ns: not significant differences. Mean (n=2 for SV and H; n=4 for firmness).

Figure 1

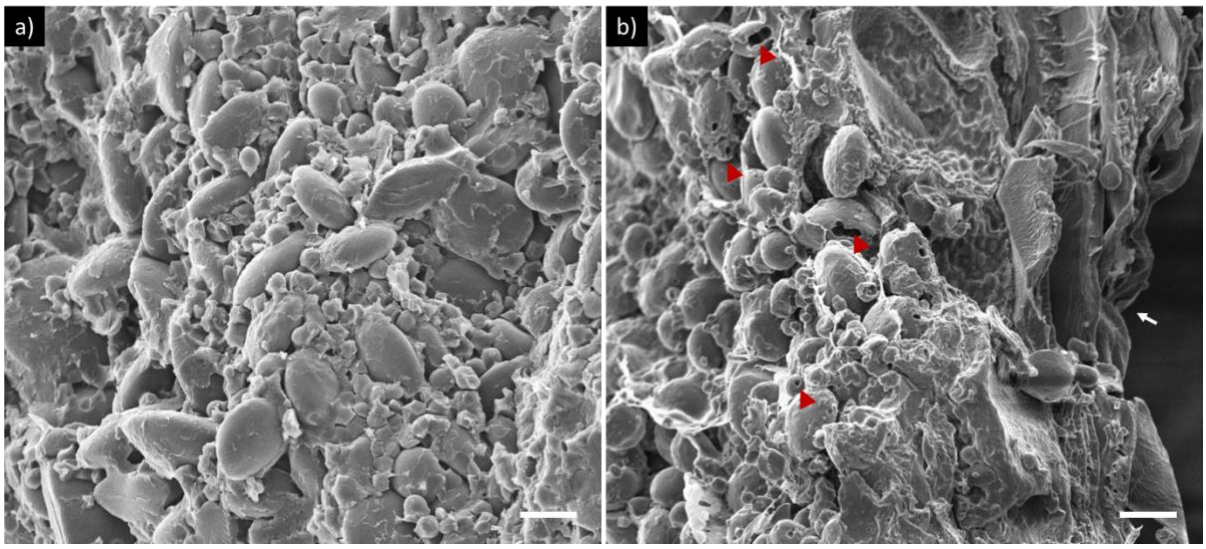


Figure 2

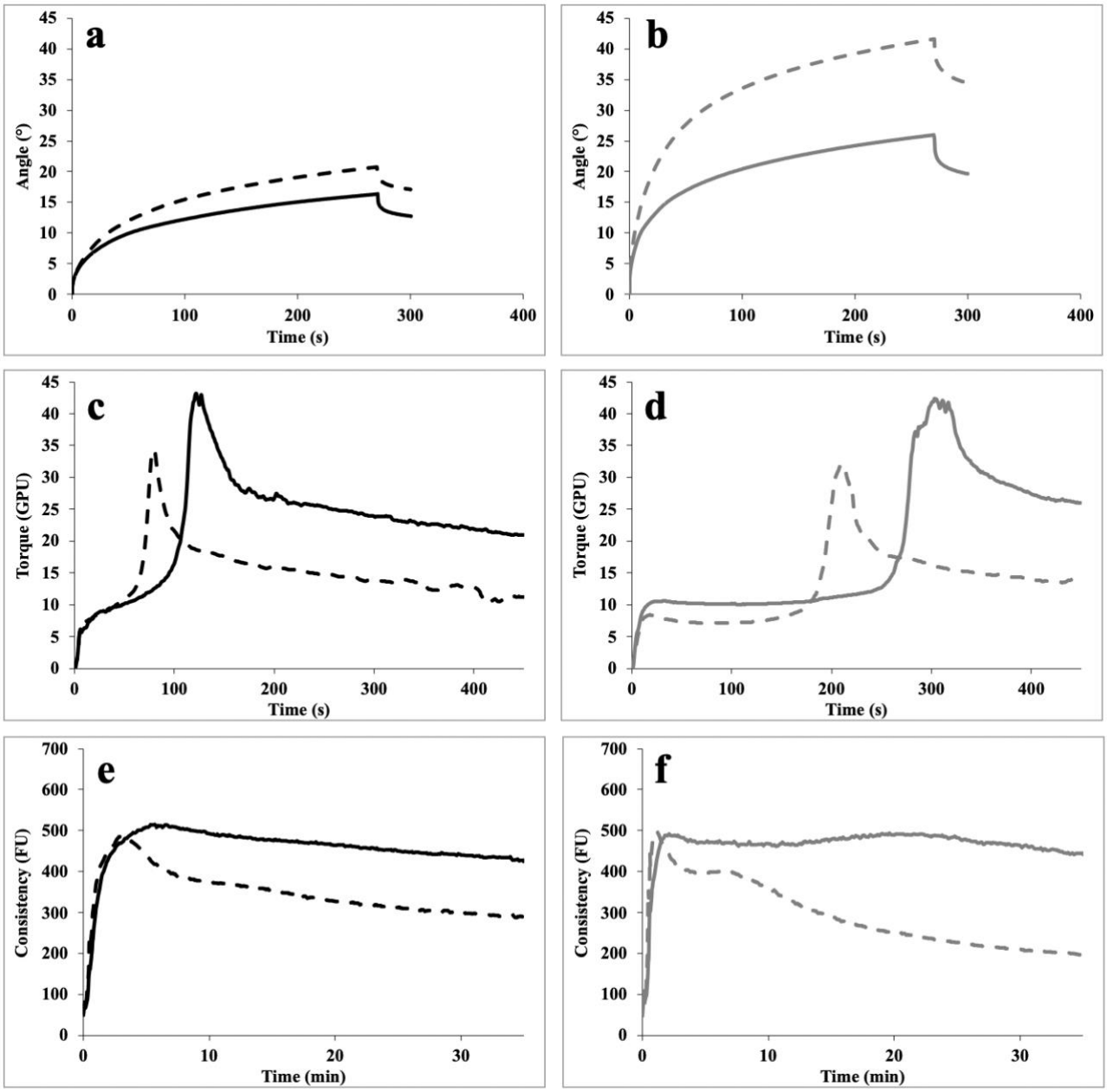

a
Angle (°)
Time (s)
b
Angle (°)
Time (s)
c
Torque (GPU)
Time (s)
d
Torque (GPU)
Time (s)
e
Consistency (FU)
Time (min)
f
Consistency (FU)
Time (min)

Figure 3

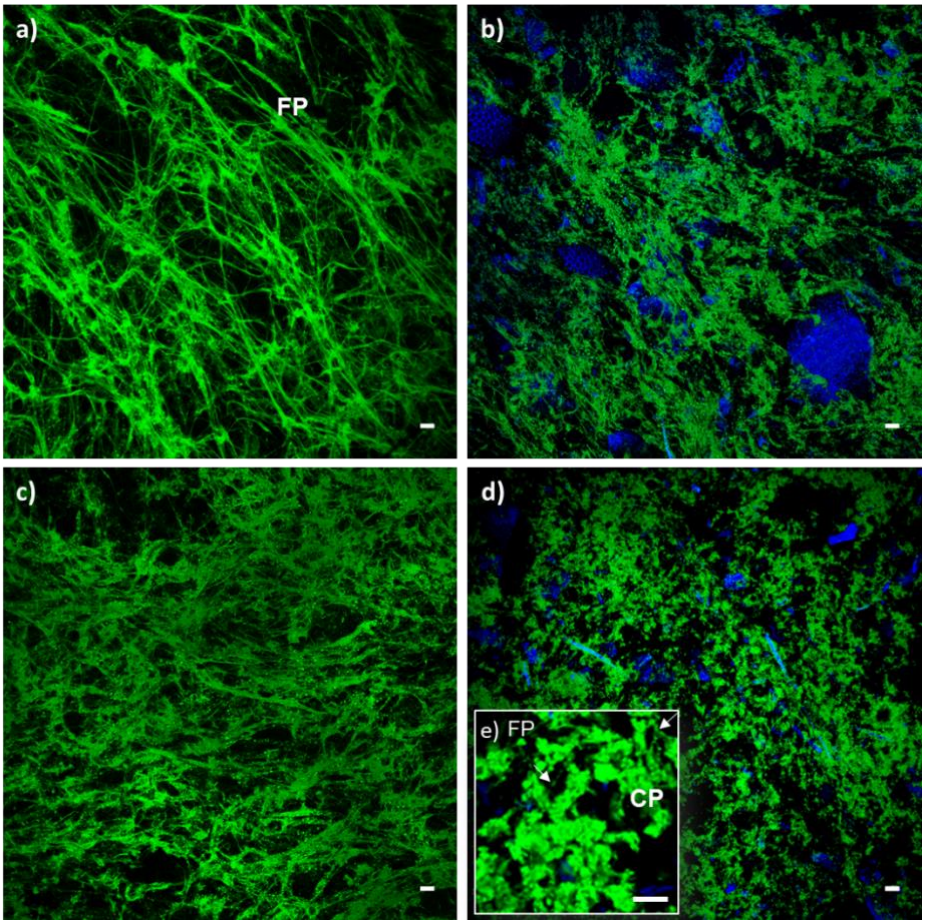
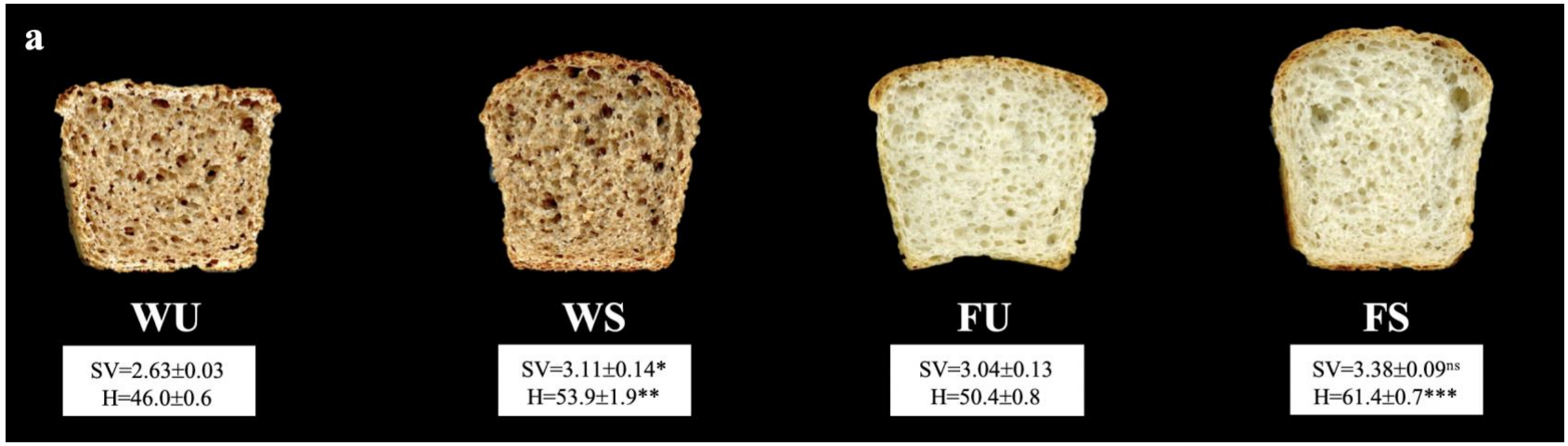


Figure 4


a
WU
SV=2.63±0.03
H=46.0±0.6
WS
SV=3.11±0.14*
H=53.9±1.9**
FU
SV=3.04±0.13
H=50.4±0.8
FS
SV=3.38±0.09ns
H=61.4±0.7***

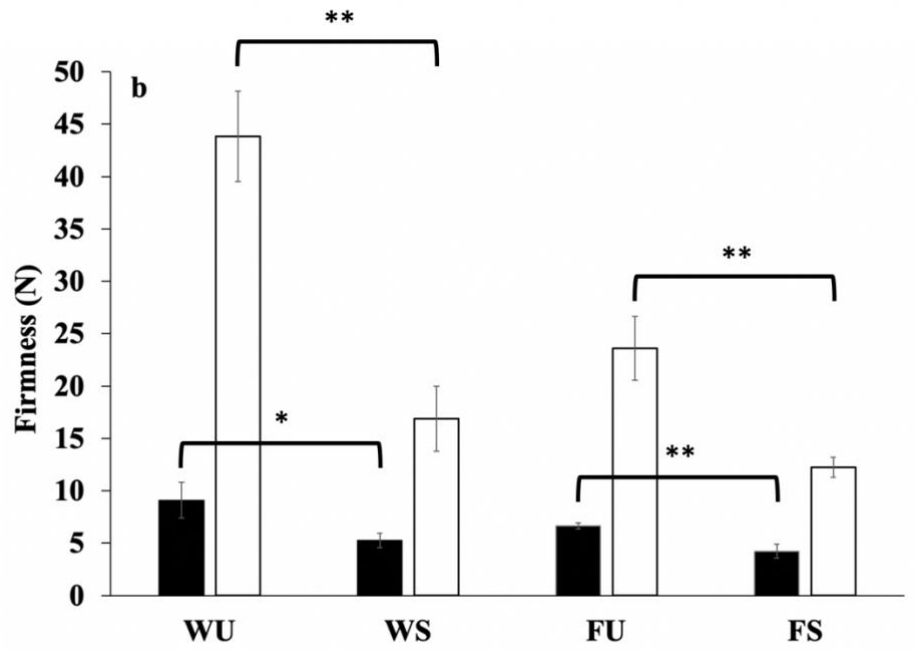

b
Firmness (N)
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Table 1. Effect of sprouting on pasting, gluten and mixing properties of wholegrain and refined flours.

		WHOLEGRAIN FLOUR		REFINED FLOUR	
		UNSPROUTED	SPROUTED	UNSPROUTED	SPROUTED
GlutoGraph test	Stretch Angle (°)	16.3±0.1	20.0±1.1*	23.1±4.0	40.8±1.1*
	Relaxation Angle (°)	3.7±0.2	3.5±0.2$_{ns}$	5.8±0.8	7.5±0.4$_{ns}$
GlutoPeak test	Maximum Torque (GPU)	43.6±0.6	34.1±0.4**	42.8±0.6	31.7±0.4**
	Peak Maximum Time (s)	123±1	80±1***	297±8	210±1**
	Aggregation Energy (GPE)	1130±11	797±10**	1247±21	858±10**
Farinograph test	Water absorption (%)	62.9±0.1	56.3±0.1***	55.3±0.1	52.7±0.1**
	Dough Development Time (min)	5.9±0.2	3.1±0.1**	20.3±0.1	1.1±0.1***
	Stability (min)	11±1	2.5±0.1**	28.6±1.1	1.0±0.1***
	Degree of softening (%)	8	39	8	28

Mean (n=2 for Farinograph® test; n=3 for GlutoGraph® and GlutoPeak® test). The asterisks indicate significant differences between the means of the unsprouted and sprouted samples of each class (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; t-Test). ns: not significant differences.

Table S1. Effect of sprouting on chemical composition and enzymatic activities of wholegrain and refined flours.

	WHOLEGRAIN FLOUR		REFINED FLOUR	
	UNSPROUTED	SPROUTED	UNSPROUTED	SPROUTED
Protein (g/100 g db)	13.29±0.08	13.40±0.05$_{ns}$	12.65±0.03	11.69±0.21*
Total starch (g/100 g db)	64.8±0.8	63.4±0.6*	77.4±1.0	76.0±0.7$_{ns}$
Damaged starch (g/100 g starch)	6.2±0.3	9.6±0.4***	7.2±0.2	9.9±0.4***
Maltose (g/100 g db)	0.51±0.05	3.41±0.01***	0.58±0.05	7.53±0.01***
Sucrose (g/100 g db)	0.93±0.07	2.63±0.05***	0.29±0.08	0.98±0.13***
D-glucose (g/100 g db)	0.19±0.07	0.85±0.04***	0.06±0.02	0.63±0.09***
α-amylase activity (UC/g db)	0.110±0.001	69.3±0.6***	0.082±0.003	48.6±1.8***
β-amylase activity (UC/g db)	28.6±0.4	29.5±0.3*	27.6±0.5	30.2±0.7*

Mean (n=3). The asterisks indicate significant differences between the means of the unsprouted and sprouted samples of each class (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; t-Test). ns: not significant differences.