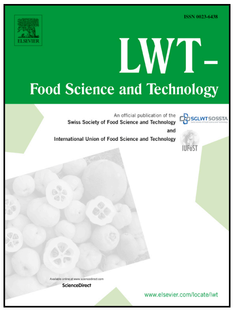


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Alessandra Marti, Gaetano Cardone, Maria Ambrogina Pagani, Maria Cristina Casiraghi

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# Flour from sprouted wheat as a new ingredient in bread-making

Alessandra Marti*, Gaetano Cardone, Maria Ambrogina Pagani & Maria Cristina Casiraghi

Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Italy

* alessandra.marti@unimi.it

Via G. Celoria 2, 20133 Milan, Italy

**Abstract**

Despite the nutritional and sensory improvements associated with sprouted grains, their use in baking has been limited until recently. Indeed, severe and uncontrolled grain sprouting induces high accumulations of enzymatic activities that negatively affect dough rheology and baking performance. In this study, wheat was sprouted under controlled conditions and the effects of enrichment (i.e. 15%, 25%, 33%, 50%, 75% and 100%) of the related refined flour (SWF) on dough rheological properties, baking performances and starch digestibility were assessed. Adding SWF to flour significantly decreased dough water absorption, development time, and stability during mixing, which suggests a weakening of the gluten network. However, no significant changes in mixing properties and gluten aggregation kinetics were measured from 25 to 75% SWF. Regardless of the amount added, SWF improved dough development and gas production during leavening. Decreases in gas retention did not compromise bread-making performances. The best result – in terms of bread volume and crumb porosity – was obtained with 50% SWF instead of using SWF alone. Interestingly, in 100 % SWF bread the slowly digestible starch fraction significantly increased.

**Keywords:** sprouting; dough rheology; bread- making; starch digestibility

## 1. Introduction

Sprouts from cereals and pulses have been used as food sources for centuries, especially in Africa and Asia, where sprouting (or germination) is mainly carried out in households to improve the sensory quality (Bellaio, Kappeler, & Zamprogna Rosenfeld, 2013). Moreover, germination is also associated with the improvement of the nutritional values of the grains, as recently reviewed by several authors (Hübner & Arendt, 2013; Omary, Fong, Rothschild, & Finney, 2012). The nutritional benefits promoted by germination include: (i) an increase in the bioavailability of several minerals and vitamins; (ii) an increase in antioxidant activity; (iii) a decrease in anti-nutrients, such as enzyme inhibitors and metal-chelating species (i.e. phytates) (Mäkinen & Arendt, 2015; Singh, Rehal, Kaur, & Jyot, 2015). Therefore, using sprouted grains in food formulations is becoming increasingly popular in the marketplace and represents an emerging trend in health foods. Downside of sprouted grains is starch digestibility, that generally increases significantly after germination, due to the increased α-amylase activity induced by the treatment (Dhital, Warren, Butterworth, Ellis, & Gidley, 2017). Unlike pulses (Hoover & Zhou, 2003), less work has been done to evaluate the effect of germination on the starch digestibility of cereals and their products (e.g. bread). Moreover, differences in types of cereal, flour refinement level, and methodology might account for contrasting results (Cornejo, Caceres, Martínez-Villaluenga, Rosell, & Frias, 2015; Świeca, Dziki, & Gawlik-Dziki, 2017).

As regards functionality, the hydrolytic enzyme activities induced by germination such as amylases and proteases – if excessive - negatively affect the technological performances of wheat, which thus becomes unsuitable for baked foods (Morris & Rose, 1996). This might occur directly in the field (i.e. pre-harvest sprouting) - when grains are exposed to prolonged wet or foggy conditions - or when the germination process is carried out under uncontrolled conditions of moisture, temperature and/or time (Nielsen, McCrate, Heyne, & Paulsen, 1984).

Germination under controlled conditions has been proposed at an industrial scale to determine the extent of the modifications occurring in germinated grains. Besides the improvement in sensory attributes of bread (Richter, Christiansen, & Guo, 2014), the native enzymes present in sprouted wheat could help decrease or substitute the use of commercially enzymes, such as flour improvers that are commonly present in the formulation of baked products (Marti, Cardone, Nicolodi, Quaglia, & Pagani, 2017). The effects of high percentages (>10%) of refined flour from germinated wheat on bread-making performances have not been investigated yet. In food formulations, balancing nutritional and/or sensory improvements while maintaining technological quality is a challenge. Therefore, the aim of this study was to investigate how gluten aggregation kinetics, dough formation, leavening performance and bread characteristics are affected by blending commercial wheat flour with refined flour from sprouted wheat. This study also aimed at determining the maximum level of sprouted wheat enrichment suitable for obtaining a product with enhanced sensory and nutritional benefits, without compromising the bread-making performance and the *in vitro* starch digestibility.

## 2. Materials and methods

### 2.1 Materials

Refined flour from sprouted wheat (SWF; starch: 79 g/100 g $_{db}$; protein: 12 g/100 g $_{db}$; lipid: 1.5 g/100 g $_{db}$; ash: 0.5 g/100 g $_{db}$) was kindly provided by Molino Quaglia (Molino Qualia S.p.A., Vighizzolo d'Este, Italy). Wheat kernels were sprouted in an industrial sprouting plant (Bühler AG, Uzwil, Switzerland) and milled as described in a previous work Marti et al. (2017a) with few modifications. Briefly, wheat was soaked in water (kernels:water ratio of 1:2) for 24h at 20 °C, germinated for 48 h at 20 °C, dried at 60 °C for 12 h.

SWF was used alone (100%) or blended with a commercial wheat flour (CTRL; Molino Quaglia S.p.A., Vighizzolo d'Este, Italy) characterized by the following alveographic indices: W (dough strength) = 280 * $10^{-4}$ J; P/L (tenacity:extensibility ratio) =1.16. In details, 15 g, 25 g, 33 g, 50 g, and 75 g of SWF were added to 85 g, 75 g, 67 g, 50 g, and 25 g of CTRL, respectively.

**2.2 Gluten aggregation properties**

Gluten aggregation properties were measured at least in triplicate with the GlutoPeak device (Brabender GmbH & Co. KG, Duisburg, Germany) as reported by Marti et al. (2017a). The following indices were automatically recorded by the software provided with the device (GlutoPeak version 2.0.1; Brabender GmbH & Co. KG, Duisburg, Germany): (i) Maximum Torque (MT, expressed in Brabender Equivalents, BE), corresponding to the peak occurring due to gluten aggregation; (ii) Peak Maximum Time (PMT, expressed in s), corresponding to the time before torque decreasing, when gluten breaks down; (iii) Energy (expressed in GlutoPeak Equivalent, GPE) corresponding to the area under the curve from the beginning of the test and 15 s after MT.

**2.3. Mixing properties**

Water absorption, development time, stability and degree of softening were measured, at least in duplicate, with the Brabender® Farinograph-E (Brabender GmbH & Co.

KG, Duisburg, Germany) equipped with a 50 g mixing bowl according to ICC 115/1 Approved Method (ICC, 1992).

### 2.4 Leavening properties

Dough development during leavening and its gas production and retention were assessed on two independent dough samples. CTRL, SWF and their blends were mixed with bakers' yeast and salt (1.5 g/100 g flour), previously dissolved in water. The required amount of water was previously determined by a farinograph until the mixing curve reached 500 BU. For each sample, the ingredients were mixed in an automatic spiral mixer (Bomann, Clatronic s.r.l., Italy) for 8 min and placed (315 g) in the Chopin Rheofermentometer F4 (Chopin, Tripette & Renaud, Villeneuve La Garenne Cedex, France) for recording changes in dough height and gas production during leavening (3 h at 30 °C).

### 2.4 Bread-making

Dough samples, which were prepared as described in the previous section, were divided into two portions of 250 g, molded into cylinder shapes, and put in tin pans (height: 8 cm; length: 15 cm; depth: 5 cm) in a proofing chamber for 60 min at 30 °C and 70% of relative humidity. Bread was baked in an oven (Self Cooking Center®, Rational International AG, Mestre, VE, Italy) for 4 min at 120 °C adding vapor until 90% relative humidity was reached. Then, the oven temperature was increased up to 230°C and bread was baked for 11 min. Samples were analyzed two hours after baking. Bread loaves were packaged in perforated oriented polypropylene film and stored at controlled conditions (20 °C, 60% relative humidity) for six days for texture analysis. Three central slices (15 mm thickness) were selected from each loaf and used for crumb color, porosity and texture analysis. For each flour mixture, two experimental baking tests were performed and six loaves were obtained from each baking test.

## 2.5 Bread properties

### 2.5.1 Colour and specific volume

Colour determination was carried out using a reflectance color meter (CR 210, Minolta Co., Osaka, Japan) to measure the lightness and saturation of the color intensity of bread crumb and crust. Results were expressed in the CIE L* a* b* colour space. Measurements of bread crust were performed in triplicate on three loaves for each bread-making process (n=18). Measurements of bread crumbs were performed on three bread slices of one loaf from each bread-making test (n=6).

The volume of three loaves from two independent baking tests (n=6) was evaluated by using the sesame displacement method after mechanically compacting the bread to exclude all empty spaces. Weight was assessed using a technical scale (Europe 1700, Gibertini, Novate, Italy). The specific volume (n=6) was determined by the volume/mass ratio and expressed in mL/g.

### 2.5.2 Crumb moisture and water activity

Crumb moisture was evaluated using a moisture analyzer (MA 210.R, Radwag Wagi Elektroniczne, Poland) drying the sample at 130 °C until the weight did not change by 1 mg for 120 s. Crumb water activity ($a_w$) was measured by an electronic hygrometer (Acqua Lab, CX-2 – Decagon Devices, Pullman, WA). Both crumb moisture and $a_w$ were measured on three central slices of one loaf from each bread-making trials (n=6).

### 2.5.3 Crumb porosity

Crumb porosity was evaluated as described in Marti et al. (2017a). Images of three central slices (15 mm thick) of one loaf from each bread-making trial were acquired with a flatbed scanner (Epson Perfection 3170 Photo, Seiko Epson Corp., Japan) at a resolution of 600 dpi (dots for inch). For each image, a single square field of view (49.5 mm x 49.5 mm) was selected. The images were calibrated, standardized and optimized by applying appropriate filters to evaluate the morphological characterization of the bubble area ($mm^2$) and porosity (%) using Image-Pro Plus 6.0 software (Media Cybernetics Inc., USA).

Moreover, bubbles, were classified into four different size classes according to their surface: class 1: bubble area between < 0.99 $mm^2$; class 2: bubble area between 1.00 and 4.99 $mm^2$; class 3: bubble area between 5.00 and 49.99 $mm^2$; class 4: bubble area greater than 50.00 $mm^2$. Porosity (i.e. the area of pores over the total area), and the area occupied by each class of pores (i.e. area of each dimensional class of pores over the total pore-area) were also calculated.

**2.5.4 Texture**

Crumb texture characteristics were analyzed by using a texture analyzer (Z005, Zwick Roell, Ulm, Germany), equipped with a 100 N load cell as described by Marti et al. (2017a). To evaluate crumb hardness, three central slices (15 mm thick) of one loaf from each bread-making trial were compressed (speed: 2 mm/s) to 30% of their height by using a 30 mm diameter cylindrical aluminum probe. Crumb hardness (n=6) was measured after 0 (two hours after baking), 1, 3 and 6 storage days and expressed as the load (N) at 30% strain.

**2.6 In vitro starch digestibility of the bread**

According to the method described by Englyst et al. (2000), in vitro starch digestibility was assessed by the estimation of rapidly (RDS) and slowly (SDS) digestible starch fractions that are likely to become available for rapid or slow absorption by the small intestine, thus modulating glycemic response. Bread was minced to simulate mastication (particle size less than 0.9 cm) and treated as reported in Marti et al. (2017b). Duplicates from two independent baking trials were averaged (n=4). Rapidly (RDS) and slowly (SDS) digestible starch fractions were calculated from the glucose-released data at 20 min and between 20 and 120 min of incubation with a mixture of hydrolytic enzymes. RDS and SDS fractions were expressed as the percentage of digested starch per 100 g of bread portion. Glucose, fructose and maltose concentrations were evaluated (in samples before digestion) by HPLC Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) (Marti et al., 2017a).

### 2.7 Statistics

The data was subjected to analysis of variance (ANOVA) to determine significant ($p \leq 0.05$) differences among the samples. ANOVA analysis was performed by utilizing Statgraphics XV version 15.1.02 (StatPoint Inc., Warrenton, VA, USA). Different dough, bread, or cells were considered as factors. When a factor effect was found to be significant ($p \leq 0.05$), significant differences among the respective averages were determined using Fisher's Least Significant Difference (LSD) test.

## 3. Results and discussion

### 3.1 Gluten aggregation properties

The GlutoPeak device has been proposed as a rapid and reliable method for evaluating gluten aggregation kinetics in wheat samples (Marti, Augst, Cox, &

Koehler, 2015; Marti, Ulrici, Foca, Quaglia, & Pagani, 2015; Melnyk, Dreisoerner, Marcone, & Seetharaman, 2012). Typical GlutoPeak curves for a wheat flour (CTRL) and a sprouted wheat flour (SWF) are shown in Fig. S1. During the test, the sample slurry is subjected to intense mechanical action promoted by the speed of the rotating element, which facilitates the formation of gluten. Thus, a rapid increase in torque is registered until the maximum value (i.e. MT) is reached. Further mixing breaks the network, with a concomitant decline in torque (Marti et al., 2015a). Generally, flours for bread-making showed higher peaks and faster gluten aggregation than flours for cakes or biscuits (Lu & Seetharaman, 2014; Marti et al., 2015b; Quayson, Atwell, Morris, & Marti, 2016).

Results suggest a weakening of the gluten network (Table 1). Indeed, germination promoted the hydrolysis of gluten forming proteins by proteases and the formation of soluble peptides (Koehler, Hartmann, Wieser, & Rychlik, 2007), compromising gluten aggregation properties. In particular, replacing wheat flour with SWF significantly decreased MT, and a linear response was observed with the enrichment level ($R^2$=0.80).

As regards the time at which maximum aggregation occurred, a no linear response was found for SWF blends. PMT did not change when up to 25% SWF was used. However, the PMT value significantly decreased when the level of SWF was increased, except for 50% level. A maximum PMT seemed to exist when SWF was blended with control bread flour in equal portions (i.e., 50:50).

A similar trend in GlutoPeak test has been shown when soft and hard wheat flours were blended in equal portions (Lu and Seetharaman, 2014). This phenomenon – which was not observed in any other rheological test - may be related to differences in interactions between gluten proteins from SWF and CTRL, similar to that observed for soft wheat and hard wheat gluten proteins (Melnyk et al., 2012; Quayson, Marti, Bonomi, Atwell, & Seetharaman, 2016). This hypothesis will need to be investigated further before any definitive conclusions can be drawn.

One of the most suitable parameters for predicting conventional parameters related to dough strength, beside PMT and MT, is found in the area under the curve which takes into account both maximum torque and PMT (Marti et al., 2015b; Quayson et al., 2016a). The presence of SWF significantly decreased this parameter, which yielded a linear response ($R^2$=0.85). The results suggest that SWF has a negative effect on gluten aggregation properties, likely due to the action of proteases, thus confirming previous findings (Marti et al., 2017a). However, SWF enrichment at 25, 50, and 70% did not significantly affect the energy value ($p \leq 0.05$).

Finally, on the basis on previous works (Marti et al., 2015a,b), the mixtures with SWF – regardless of how much was added – show a gluten aggregation kinetic similar to that of a flour with good bread-making qualities.

**3.2 Mixing properties**

The effects of incorporation of germinated wheat flour on dough mixing characteristics are shown in Table 1. Dough from CTRL was characterized by high water absorption (57.8%) and very high stability (18.8 min) (Table 1), which are typical of strong wheat flour (Fig. S2).

Replacing CTRL with SWF brought about a significant ($p \leq 0.05$) decrease in water absorption (Table 1) and resulted in a linear response ($R^2$=0.96). According to Dojczew & Sobczyk (2007), decrease in water absorption could mainly be due to proteins de-polymerization as a consequence of the intense protease activity in germinated wheat. Dough development time and dough stability sharply decreased up to 15% SWF enrichment (Table 1), indicating dough weakening. Interestingly, these two parameters did not further decrease with increasing amounts of SWF (>15%), with the exception of 25% SWF. The reduced development time and stability could be due to the disruption of the gluten matrix by enzymes (i.e. proteases).

**3.3 Leavening properties**

Rheofermentometer analysis provides information on dough leavening performance (i.e. dough height, $CO_2$ production and retention). Table 1 shows the data obtained from this test carried out on the different mixtures. Adding SWF to wheat flour increased both dough height (up to 75%) and leavening time (Table 1). The results confirmed the positive effect of α-amylase activities on dough leavening properties (Marengo et al., 2016; Marti et al., 2017a; Sanz Penella, Collar, & Haros, 2008). In fact, high levels of sugars - which result from starch hydrolysis by α-amylase – are used from the yeast during leavening, resulting in greater dough development in a shorter time, compared to CTRL. No linear response was detected for dough height, since no significant differences were observed from 15% to 75% SWF enrichment. Despite the positive effect of germination on dough development, adding SWF to common bread flour decreased height at the end of the leavening step, suggesting the collapse of the dough structure when the leavening time lasts more than 2h. This is due to the decrease in the ability of the gluten structure to withstand the physical stresses as a result of proteolytic activity.

The indices obtained from the gas release curves are summarised in Table 1. These results indicated that doughs with increasing amount of SWF had a higher volume of $CO_2$ release than CTRL. If gas is efficiently retained in the dough, an optimal final bread volume can be expected (Huang, Kim, Li, & Rayas-Duarte, 2008). The increasing availability of mono- and disaccharides as substrates promoted the carbon dioxide production during fermentation (Verheyen, Jekle, & Becker, 2014). In addition, in the presence of SWF from 33% to 100%, high amounts of retained and lost carbon dioxide resulted (Table 1) and no linear response was found for these parameters. The coefficient of retention - which is defined as the ratio expressed as percentage between the volume retained in the dough and the total volume of gas produced during the test - decreased from 94.6% (CTRL) to about 89% for 50% SWF and 100% SWF. Enzymatic activity that developed during germination might have negatively affected the gas retention capacity, which is associated with an increase of dough permeability due to dough weakening by the increased hydrolysis of starch chains (Sanz Penella et al., 2008). In addition, protease hydrolyses peptide linkages, which might have induced a partial destruction of the protein network and thus lowered the capacity of the dough to enclose air compared to CTRL sample.

**3.4 Bread properties**

Based on the dough mixing and leavening properties (Table 1), blends enriched with SWF at 50% and 75% level did not show significant differences. Only their gluten aggregation properties differed (i.e. PMT), suggesting peculiar protein interactions in 50% SWF. Thus, bread-making performance of 50% SWF was compared to that of CTRL and 100% SWF.

As shown in Figure 1, SWF did not lead to a worsening of bread-making performance. Moreover, 50% SWF enriched-bread, produced greater volume and more porosity than CTRL and 100% SWF samples (Table 2).

Adding SWF to CTRL resulted in a darker (decrease in L*) and redder crust (higher a*) (Table 2). Changes in crust might be associated with Maillard reactions (Hefni & Witthöft, 2011), which can be expected to be more intense in SWF-enriched samples. Indeed, amylases and proteases affect the Maillard reaction, the former by degrading starch to reducing sugars, whereas the latter increase the amount of free peptides and amino acids (Goesaert et al., 2005). As regards crumbs, an important difference in both redness and yellowness was observed when sprouted wheat flour was added (Table 2). These changes were also probably due to the increase in the Maillard reaction.

Specific bread volume significantly differed for the three samples (Fig. 1, Table 2), with 50% SWF having the highest specific volume. The amount of α-amylase developed during germination could have played a key role in increasing loaf volume. At the same time, sprouting under controlled conditions limited the proteases activity and its dramatic effects on the gluten network (Marti et al., 2017a) that are generally observed in pre-harvest sprouted wheat grains.

**3.4.1 Crumb porosity**

Using SWF sample significantly increased the area of porosity from 45.82% (CTRL) to 49.09% (50% SWF), which was similar to that of bread with 100% SWF (46.14%) (Table 2). This result is obviously related to the increase in volume associated with the addition of SWF and can be related to the amylase activity developed during wheat germination, whose effect on crumb porosity has been observed elsewhere (Goesaert, Slade, Levine, & Delcour, 2009). As for cells, although the number of each class was very similar for all the samples (data not shown), differences in cell area were observed (Fig. 2). A significantly larger area of small pores (<5 $mm^2$) was present in CTRL samples than in 50% and 100% samples. In fact, the small cell area represented around 60% of the total pore area in CTRL bread and only about 40% for 50% and 100% SWF. An opposite trend was observed for pore area in the medium dimensional class (5.00 - 49.99 $mm^2$), as the area occupied by this class of pores was higher in both SWF samples than CTRL samples. Moreover, larger pores (>50 $mm^2$)

were found only in bread with SWF, whose area accounted for the about 20% of the total porosity. From these results, it can be deduced that enzymes produced by germination, especially α-amylases, favor gas cell coalescence (Lagrain, Leman, Goesaert, & Delcour, 2008).

**3.4.2 Texture**

SWF addition had also a positive effect on crumb firmness (Table 2). Decrease in firmness in the presence of SWF cannot be related to differences in crumb moisture, since SWF-enriched bread showed low firmness and low crumb moisture. Unlike the Scanlon & Zghal (2001) study, crumb firmness did not increase with increasing density (Table 2).

Indeed, even during storage, bread containing either 50% or 100% SWF exhibited lower firmness than the control (Fig. 3). As observed on fresh bread (t0, 2h after baking), differences in firmness during storage were not related to either crumb moisture or water activity, (data no shown). On the other hand, several works demonstrated that production of hydrolytic enzymes during germination were responsible for improving crumb softness up to six days of storage (De leyn, 2006; Goesaert et al., 2005, 2009). In particular, α-amylase decreases amylopectin retrogradation and the firming rate of wheat bread crumb (Champenois, Della Valle, Planchot, Buleon, & Colonna, 1999). In addition, the firmness of 50% SWF bread after three days of storage was similar to that shown by CTRL bread after just one day of storage, whereas 100% SWF sample after six days exhibited firmness values similar to those of CTRL bread after just one day of storage. A similar effect was detected when SWF was included at low levels (<2%) in bread formulation (Marti et al., 2017a).

### 3.5. In vitro starch digestibility

The effects of refined flour from sprouted wheat on starch digestibility was assessed by a well-established *in vitro* assay, which allows the determination of both rapidly and slowly digestible starch fractions (RDS and SDS, respectively). By measuring the susceptibility of starch to digestive enzymes, this assay is internationally endorsed to estimate the potential glycaemic response of foods (EFSA, 2011). Significant differences in starch susceptibility to digestive enzymes were observed in bread samples (Fig 4). In particular, in 100% SWF bread the RDS and SDS fractions were significantly ($p \leq 0.05$) lower and higher, respectively, than those determined in CTRL and 50% SWF bread. These data partially agree with those reported by Świeca et al. (2017), which evidenced a decrease in starch digestibility in bread with 20% of sprouted wheat. This result was attributed to an increase in the aliquot of resistant starch and/or to a high phenolics content of sprouted wheat (Świeca et al., 2017). A comparison of our results with those of Świeca et al. (2017) is difficult, since different *in vitro* methods were used. Secondly, sprouting conditions and percentages of flour enrichment were different. The differences in starch digestibility (RDS) measured between CTRL and 50% SWF suggest that differences in chemical composition did not play a key role in starch digestibility. It is likely that the different starch digestibility (i.e. increase in SDS) assessed in 100% SWF was related to differences in bread structure, consequent to modification to wheat flour promoted by germination, that become evident only when native wheat flour was absent. This feature may be of interest from a nutritional point of view, since it could reduce the glycemic potential of this new bread formulation. Indeed, the glycaemic response appears to be directly related to the amount of RDS while insulin demand is inversely correlated to the SDS fraction (Garsetti, Vinoy, Lang, Holt, Loyer, Brand-

Miller, 2005). The effects of germination on protein structure and its impact on starch digestibility needs further investigation.

In contrast, the total number of free "glycemic" sugars significantly and non-linearly increased with SWF substitution (3.0% in CRTL vs 7.3% in 50% SWF vs 8.3% in 100% SWF), with maltose increase as the main determinant (Table 3). This trait, probably attributable to α-amylase developed during germination, could be of interest from a sensory point of view (i.e. sweet flavour note) but may promote an increased glycemic response. Further in vivo studies are needed to assess how the rate of starch digestibility and the increase in free "glycemic" sugars in 100% SWF bread impact on post-prandial glycemic response.

## 4. Conclusions

Flour from sprouted wheat has always been considered to be of poor baking quality. Indeed, the relevant amylase and protease activities accumulated into the grain during germination are responsible for intense hydrolytic phenomena at the expense of gluten and starch, the holding-structure macromolecules in the dough. The hydrolysis of these macromolecules is clearly highlighted by the rheological tests conventionally used for predicting flour baking behavior.

Although we are aware that uncontrolled wheat sprouting, in the field during wheat growing is a phenomenon associated with a sharp deterioration of dough consistency and handling and bread characteristics, our results show that controlled (i.e. in an industrial factory) sprouted wheat flour could be used as new ingredient in bread making. Gluten proteins, though weakened by proteolytic activity, do not lose their ability to aggregate and form a network suitable for leavening, as the GlutoPeak test indicated. The molecular changes associated with this behavior need to be carefully understood, evaluated and quantified, together with the actual impact of these potential functional breads on glucose metabolism. In particular, the effect of sprouting on quality-related protein fractions, starch and lipid molecules and their potential interactions should be taken into consideration as a molecular explanation for the positive effects of sprouting on bread properties.

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**Fig. 1.** Pictures of the bread prepared from commercial wheat flour (CTRL), with either 50% level of sprouted wheat flour (50% SWF), or 100% sprouted wheat flour (100% SWF).

**Fig. 2.** Area of each dimensional class of pores. Colors used: black: CTRL; light grey: 50% SWF; dark grey: 100% SWF. Different letters indicate significant differences (one-way ANOVA, LSD test, $p \leq 0.05$).

**Fig. 3.** Crumb firmness of bread prepared from commercial wheat flour (CTRL – black circle), blend with 50% of sprouted wheat flour (50% SWF – white circle), 100% sprouted wheat flour (100% SWF – black triangle) during storage. Different letters indicate correspond significant differences (one-way ANOVA, LSD test, $p \leq 0.05$).

**Fig. 4.** Rapidly (RDS, black bars) and Slowly (SDS, grey bars) digestible starch fractions of bread prepared from commercial wheat flour (CTRL), blend with 50% of sprouted wheat flour (50% SWF) and 100% sprouted wheat flour (100% SWF). Different letters (lowercase letters refer to RDS; capital letters refer to SDS) indicate significant differences (one-way ANOVA, LSD test, $p \leq 0.05$).

**Table 1.** Gluten aggregation, mixing and leavening properties of commercial wheat flour (CTRL), with increasing amount of germinated wheat flour (15%, 25%, 33%, 50%, 75%) or 100% germinated wheat flour (SWF).

| | | | CTRL | 15% SWF | 25% SWF | 33% SWF | 50%SWF | 75% SWF | 100% SWF |
|---|---|---|---|---|---|---|---|---|---|
| GLUTEN AGGREGATION PROPERTIES (GlutoPeak Test) | Peak maximum time | (s) | 186±1[c,d] | 191±1[d] | 182±3[b,c] | 172±2[a] | 197±6[e] | 177±3[a,b] | 181±2[b,c] |
| | Maximum torque | (BE) | 39±1[d] | 37±1[c] | 35.7±0.6[b,c] | 37±1[c,d] | 34±1[a,b] | 35±1[b] | 32±1[a] |
| | Energy | (GPE) | 3293±76[e] | 2993±47[d] | 2845±98[c] | 2752±142[b,c] | 2722±65[b,c] | 2682±76[b] | 2408±33[a] |
| MIXING PROPERTIES (Farinograph Test) | Water absorption | (%) | 57.8±0.1[d] | 57.4±0.3[c,d] | 56.9±0.2[c] | 57.3±0.1[c,d] | 55.3±0.1[b] | 54.8±0.3[b] | 54±1[a] |
| | Development time | (min) | 8.4±1.2[b] | 1.9±0.2[a] | 2.0±0.2[a] | 1.8±0.1[a] | 1.8±0.3[a] | 2.3±0.4[a] | 2.1±0.4[a] |
| | Stability | (min) | 18.8±0.1[d] | 5±1[b,c] | 7±2[c] | 3.4±0.5[a] | 5.1±0.2[a,b,c] | 4.6±0.2[a,b] | 3.6±0.4[a,b] |
| | ICC Degree of softening | (FU) | 9±8[a] | 67±8[b] | 67±9[b] | 83±1[b] | 92±5[b] | 114±11[b] | 75±12[b] |
| LEAVENING PROPERTIES (Rheofermentometer Test) | Maximum dough height | (mm) | 39±1[a] | 48.8±0.5[b] | 50±2[b] | 51±6[b] | 50±3[b] | 50±1[b] | 40±2[a] |
| | Final dough height | (mm) | 39±5[b] | 41.9±0.4[b] | 46±5[b] | 39.3±0.5[b] | 45.5±0.1[b] | 46±1[b] | 23±6[a] |
| | Leavening Time | (min) | 172±6[b] | 143±7[a,b] | 152±12[a,b] | 126±1[a] | 128±1[a] | 169±37[b] | 117±5[a] |
| | Total $CO_2$ | (mL) | 1200±29[a] | 1465±9[b,c] | 1402±80[b] | 1566±51[d] | 1556±1[c,d] | 1597±43[d] | 1537±17[c,d] |
| | $CO_2$ retained | (mL) | 1135±25[a] | 1329±15[b,c] | 1290±59[b] | 1388±13[c,d] | 1382±7[c,d] | 1398±1[d] | 1365±4[c,d] |
| | $CO_2$ released | (mL) | 65±5[a] | 136±5[b,c] | 111±21[a,b] | 179±37[c,d] | 174±8[c,d] | 199±42[d] | 172±13[c,d] |
| | $CO_2$ retention coefficient | (%) | 94.6±0.3[d] | 90.8±0.4[b,c] | 92±1[c,d] | 89±2[a,b] | 88.8±0.6[a,b] | 88±2[a] | 88.8±0.7[a,b] |
| | Porosity time | (h) | 1.69±0.09[a] | 1.44±0.02[a] | 1.54±0.05[a] | 1.43±0.11[a] | 1.46±0.09[a] | 1.41±0.19[a] | 1.44±0.19[a] |

Different letters in the same row indicate significant differences (one-way ANOVA, LSD test, $p \leq 0.05$).

CTRL, control wheat flour; SWF, flour from sprouted wheat

Peak maximum time: time before torque decreased due to gluten break down; Maximum torque: peak occurring as gluten aggregates; Energy: area under the curve until 15s after the maximum torque; Water absorption: amount of water needed to reach the optimal consistency (500±20 FU); Dough development time: time from first addition of water to the point of maximum consistency range; Stability: time difference between when the curve reaches (arrival time) and leaves (departure time) the 500 FU line; Degree of softening: difference between the centre of the curve at the end of the dough development time and the centre of the curve 12 minutes after this pint; Maximum dough height: maximum height achieved during the test; Final dough height: height at the end of the test; Leavening time: time required for maximum dough development; Maximum height: maximum height of gaseous production; Porosity time: time when the porosity of the dough developed; Total $CO_2$: total production of $CO_2$; $CO_2$ retained: amount of $CO_2$ retained in the dough during the test; $CO_2$ released: amount of $CO_2$ released during the test; $CO_2$ retention coefficient: ratio between $CO_2$ retained and total $CO_2$.

BE: Brabender Equivalent; FU: Farinograph Units; GPE: GlutoPeak Equivalent

**Table 2.** Properties of fresh bread from commercial wheat flour alone (CTRL) or with sprouted wheat flour (50%, 100% SWF).

| | | CTRL | 50% SWF | 100% SWF |
|---|---|---|---|---|
| Bread | Specific volume (mL/g) | 2.8±0.1[b] | 3.3±0.1[c] | 2.5±0.1[a] |
| Crust | Luminosity (L*) | 69.01±1.80[c] | 63.79±4.20[b] | 54.68±1.73[a] |
| | Redness (a*) | 5.86±1.02[a] | 9.48±1.40[b] | 12.36±1.08[c] |
| | Yellowness (b*) | 31.78±1.46[a] | 32.84±0.92[a] | 32.25±2.34[a] |
| | Browning (100-L*) | 30.99±0.80[a] | 36.21±4.20[b] | 45.32±4.79[c] |
| Crumb | Luminosity (L*) | 71.22±2.68[a] | 72.41±2.89[a] | 64.61±2.51[b] |
| | Redness (a*) | -1.04±0.08[a] | -0.67±0.08[b] | -0.41±0.06[c] |
| | Yellowness (b*) | 14.50±0.55[c] | 13.04±0.71[a] | 13.55±0.76[b] |
| | Browning (100-L*) | 28.78±2.68[a] | 27.59±2.89[a] | 35.39±2.51[b] |
| | Porosity (%) | 45.82±0.37[a] | 49.09±0.92[b] | 46.14±1.03[a] |
| | Moisture (%) | 41.3±0.5[c] | 37.4±0.9[a] | 39.4±0.4[b] |
| | Water activity | 0.939±0.005[b] | 0.932±0.013[b] | 0.917±0.006[a] |
| | Firmness (N) | 4.92±0.77[b] | 3.01±0.52[a] | 2.65±0.53[a] |

Different letters in the same row indicate significant differences (one-way ANOVA, LSD test, $p \leq 0.05$).

CTRL, control wheat flour; SWF, flour from sprouted wheat

**Table 3.** Free sugars content of fresh bread from commercial wheat flour alone (CTRL) or with sprouted wheat flour (50%, 100% SWF).

| | CTRL | 50%SWF | 100% SWF |
|---|---|---|---|
| Total free sugars (%) | 3.0±0.4[b] | 7.0± 0.4[a] | 8.3±1.3[a] |
| Glucose (%) | 0.1±0.0 | 0.2±0.1 | 0.3±0.1 |
| Fructose (%) | 0.2±0.1 | 0.4±0.2 | 0.3±0.1 |
| Maltose (%) | 2.8±0.3[c] | 6.4±0.2[b] | 7.8±1.1[a] |

Different letters in the same row indicate significant differences (one-way ANOVA, LSD test, $p \leq 0.05$).


**Fig. 1.** Pictures of the bread prepared from commercial wheat flour (CTRL), with either 50% level of sprouted wheat flour (50% SWF), or 100% sprouted wheat flour (100% SWF).

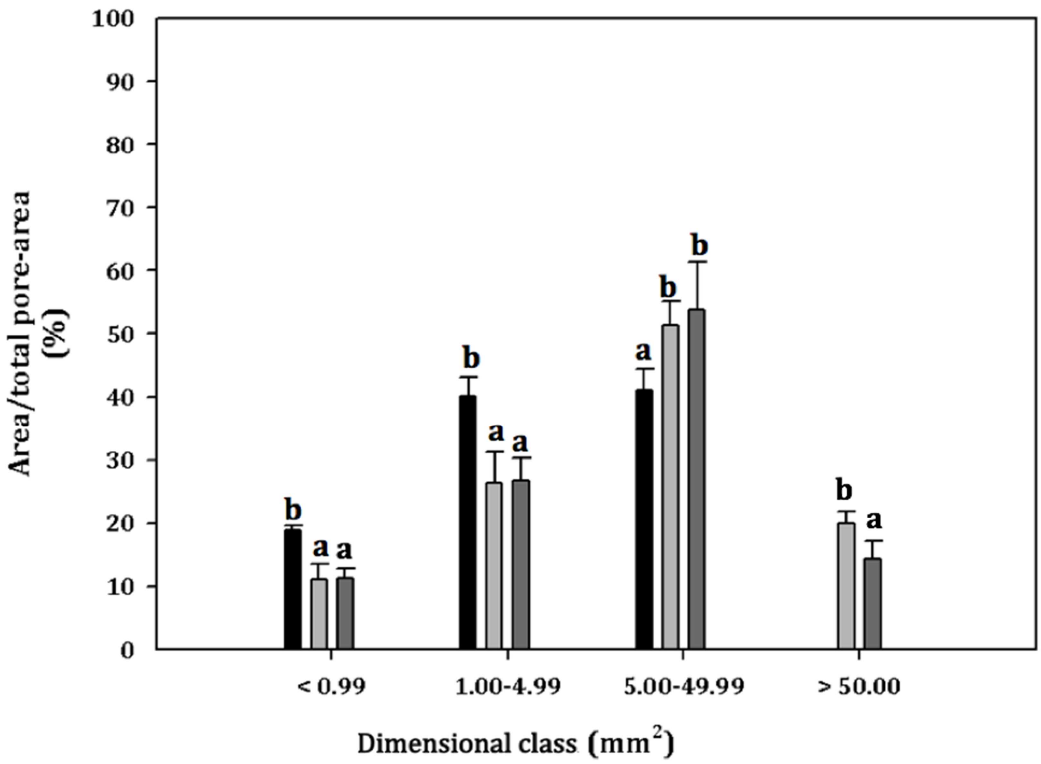
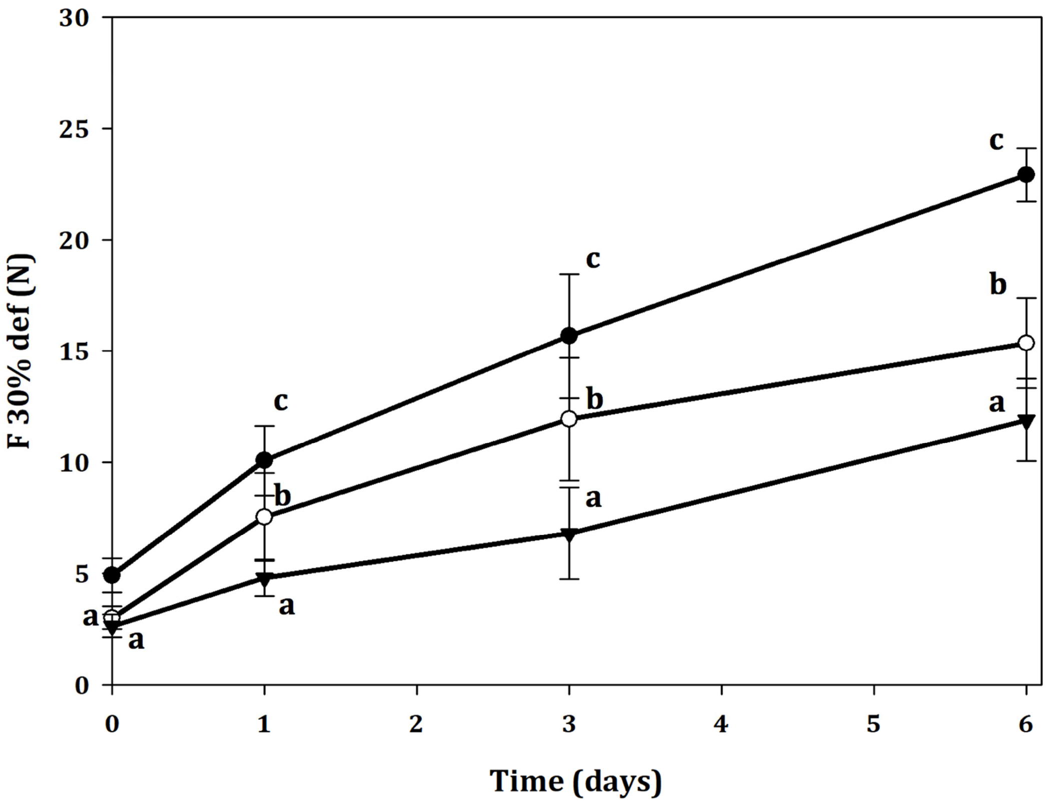



**Fig. 2.** Area of each dimensional class of pores. Colors used: black: CTRL; light grey: 50% SWF; dark grey: 100% SWF. Different letters indicate significant differences (one-way ANOVA, LSD test, p≤0.05).

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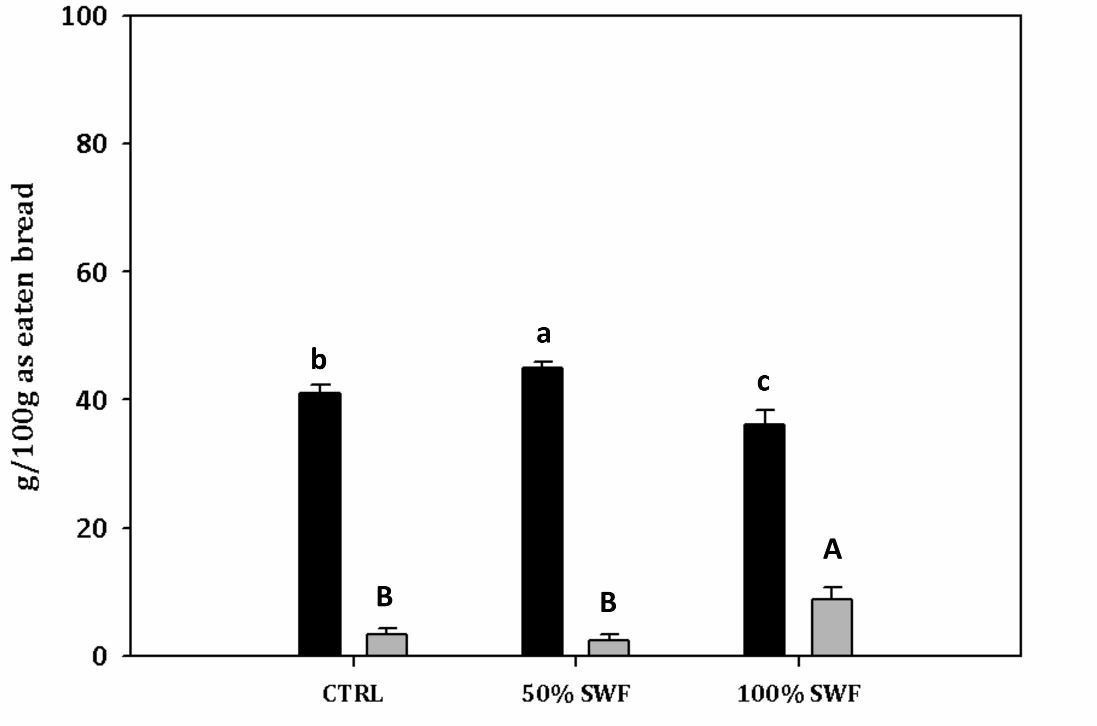


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**Highlights:**

- Sprouting was carried out in an industrial plant under controlled conditions
- High levels of wheat flour (SWF) enrichment affect dough rheology
- SWF improved the dough development and gas production during leavening
- The best bread performance was obtained with 50% SWF
- 100 % SWF increased the slowly digestible starch fraction