Sprouted wheat as an alternative to conventional flour improvers in bread-making

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ABSTRACT

Sprouting is a natural process that enhances the nutritional and sensory profile of cereal-based foods. The present work addressed the possibility of using refined flour from sprouted wheat (SWF) to improve the bread-making performance of some flours in place of conventional improvers - i.e. enzymatic improver (EI) and malt (M). Either 0.5% EI or M was added to the control flour (CTRL), as conventionally used in bakeries, whereas SWF was used up to 2%. Unlikely EI and M, 1.5% SWF showed a gluten aggregation strength similar to that of the CTRL, suggesting no worsening of the protein network characteristics. As for the leavening properties, dough development increased, thanks to the enrichment with 1.5% SWF. In addition, presence of SWF improved the amount of gas production during leavening-resulting in bread with high specific volume - and the crumb softness during storage. Addition of SWF may represent a valid alternative to enzymatic improvers or malt for improving the technological performance of wheat flours.

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1. Introduction

During germination (or sprouting), high levels of hydrolytic enzymes - such as amylases and proteases — are accumulated in the cereal seed, so that the insoluble endosperm starch and protein reserves are hydrolyzed into soluble forms that can be transported to the embryo to meet the needs of the growing plant. Significant correlations between xylanase activity levels and sprouting-related parameters, such as α-amylase activity, and viscous properties of flour-water suspensions, have been reported (Dornez et al., 2008).

Under ideal growth conditions, ripe grains contain only small amount of enzymes and the resulted flour can be used to produce a wide range of cereal-based products. On the other hand, under non ideal conditions - e.g. when the grains are exposed to prolonged wet or foggy conditions — amylases, proteases, and xylanases may be retained or synthesized prior to harvest and as a consequence, the flour is unsuitable for baked products (Prasada and Hemalata, 2014).

Indeed, pre-harvest sprouted wheat is usually associated with dough weakening and stickiness, and with worsening of dough handling (Paulsen & Auld, 2004). Moreover, bread from extensively sprouted wheat show very poor characteristics, with a sticky and gummy crumb (McCleary & Sturgeon, 2002). Finally, the crumb color of the breads is darker and the grain and texture inferior compared to bread baked from non-germinated wheat (Finney et al., 1980).

On the other hand, since the nutritional (Hubner and Arendt, 2013; Singh, Rehal, Kaur, & Jyot, 2015) and sensory (Heiniö, Oksman-Caldentey, Latva-Kala, Lehtinen, & Poutanen, 2001) benefits of germination have been extensively documented, using of sprouted grains in food formulations is continuing to gain traction in the marketplace and represents a re-emerging trend in healthy foods.

Recent studies reported that the use of flour from whole wheat germinated in controlled conditions improved loaf volume and crumb texture (Bellaio, Kappeler, Rosenfeld, & Jacobs, 2014; Richter, Christiansen, & Guo, 2014). These positive effects were ascribed to the natural enzymes expressed during the germination process that might decrease or completely replace the quantity of commercial enzymes added to bread formulation. Nonetheless, the use of

sprouted wheat as alternative to conventional flour improvers (e.g. enzymes, malt) has not been thoroughly investigated up to now.

Using enzymes as flours improvers is a frequent practice for flour standardization and also as baking aids. Enzymes — such as amylases, proteases and xylanases - are usually added to modify dough rheology, gas retention and crumb softness in bread-making (Goesaert, Gebruers, Courtin, Brijs, & Delcour, 2006). Those enzymes can be added individually or in complex mixtures, which may act in a synergistic way in the production of baked goods.

The present work addressed the possibility of using refined flour from controlled-sprouted wheat, as source of enzymes, to improve the bread-making performance of flours. The effects of the enrichment with low level (0.5—2%) of sprouted wheat on dough rheology and bread-making performance were assessed and compared to those of the improvers (e.g. malt and enzymatic improver) conventionally used in bread making.

2. Materials and methods

2.1. Materials

Flours from unsprouted wheat (USWF) and sprouted wheat (SWF) were kindly provided by Molino Quaglia (Molino Qualia S.p.A., Vighizzolo d'Este, Italy), as the commercial wheat flour (CTRL; $W = 260 \times 10^{-4}$ J; P/L = 2.08) used for blending studies. Malt (M; Malto 5, Bona s.r.l., Monza, Italy) and the enzymatic improver (EI, PowerBake950, Danisco, Copenhagen, Denmark) were added to CTRL at 0.5% level, which represents conventional amount used in bread-making (De Leyn, 2006). SWF was used at 0.5, 1, 1.5, and 2%.

2.2. Sprouting process

Commercial wheat kernels were sprouted in an industrial sprouting plant (Bühler AG, Uzwil, Switzerland). Wheat (10 tons) was soaked in water (kernels:water ratio of 1:2) for 12—24 h at 20 °C, germinated for 72—90 h at 20 °C, dried at 50 °C for 32 h. Unsprouted and sprouted wheat were milled in the same industrial plant (Bühler AG, Uzwil, Switzerland), and the related flours — USWF and SWF, respectively - were obtained.

2.3. Chemical composition

Moisture, starch, protein, lipid and ash contents were assessed by AACC standard methods (44—15.02, 76—13.01, 46—12.01, 30—10.01, and 08—01.01, respectively; AACC 2001). Sugars were determined by HPLC by Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) (Zygmunt et al., 1982). Total, soluble and insoluble dietary fiber content was quantified by enzymatic—gravimetric procedure (AOAC Method 991.43).

2.4. Enzymatic activities

Proteolytic activity was determined in triplicate in the conditions proposed by Arnon (1970) and using azocasein (Sigma Chemical Co., St Louis, MO, USA) as the substrate. Alpha-amylase activity was determined in triplicate according to AACC standard method n. 303, by using the Megazyme Amylase Assay Procedure (Megazyme International Ireland Ltd., Wicklow, Ireland). Xylanase activity was determined in triplicate using the Azo-wheat arabinoxylan kit (K-AZOWAX 09/04) provided by Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland).

2.5. Rheological properties

2.5.1. Pasting properties

Pasting properties were measured in duplicate using a Micro-Visco-Amylograph device (MVAG, Brabender GmbH & Co. KG, Duisburg, Germany). An aliquot of sample (12 g) was dispersed in 100 mL of distilled water and stirred at 250 rpm. The following temperature profile was applied: heating from 30 °C to 95 °C at a rate of 3 °C/min, holding at 95 °C for 20 min, cooling from 95 °C to 30 °C at a cooling rate of 3 °C/min, and holding at 30 °C for 1 min.

2.5.2. Gluten aggregation properties

Gluten aggregation properties were measured at least in triplicate using the GlutoPeak device (Brabender GmbH & Co. KG, Duisburg, Germany), as reported by Marti, Augst, Cox, and Koehler (2015).

2.5.3. Leavening properties

Leavening properties of doughs were assessed in duplicate with a Rheofermentometer[®] device (Chopin, Tripette & Renaud, Villeneuve La Garenne Cedex, France). Dough samples were prepared in an automatic spiral mixer (Bomann, Clatronic s.r.l., Piadena, Italy) with 1.5% NaCl and 1.5% bakers' yeast. Mixing time (1.6—1.8 min) and amount of water (54.5—55%) were those determined by the Farinograph test, according to the ICC Standard Method 115/1 (ICC 1992). The rheofermentographic test was performed on 315 g portion of the dough and carried out at 30 °C for 3 h.

2.6. Bread-making

Either wheat flour or blends were mixed with compressed yeast and salt, each comprising 1.5 g/100 g of the total mixture, and previously dissolved in water. The amount of water added to each formulation varied according to the farinographic water absorption index, previously determined. For each formulation, the ingredients were mixed in an automatic spiral mixer (Bomann, Clatronic s.r.l., Italy), for 8 min. Immediately after mixing, the dough was left to rest for 10 min at room temperature. After that, the dough was divided into portions of 250 g, molded into cylinder shapes, put in baking pans (8 × 15 × 5 cm) and left to rest for 60 min in a proofing chamber at 30 °C and 70% RH. Samples were baked in an oven (Self Cooking Center[®], Rational International AG) for 4 min at 120 °C with vapor injection for 7 s. Then, the oven temperature was increased to 230 °C for 11 min. Two hours after removing loaves from the oven, they were packaged in perforated orientated polypropylene film and stored at controlled conditions (20 °C, 60% RH) for three days. For each sample, two baking experimental tests were performed and three loaves were obtained from each baking test.

2.7. Bread properties

A reflectance color meter (CR 210, Minolta Co., Osaka, Japan) was used to measure the lightness and saturation of the color intensity of bread crumb and crust. Each measurement was replicated five times and the average value was used.

The apparent volume (n = 6) was determined by the rapeseed displacement method, 2 h after baking. The weight of the bread (n = 6) was recorded and the specific volume was determined through the volume/mass ratio and expressed in mL/g.

Three central slices (15 mm thickness) were selected from each bread and used for crumb moisture, water activity, porosity and texture analysis.

Moisture content of the crumb was measured in triplicate by drying the sample at 130 °C until the weight will not change of 1 mg

for 60 s, by an infrared balance (MA 210.R, Radwag Wagi Elektroniczne, Poland). The crumb core water activity (aw) was measured in triplicate by an electronic hygrometer (Aqua Lab, CX-2 – Decagon Devices, Pullman, WA).

Crumb porosity was evaluated by image analysis. The images were acquired at a resolution of 600 dpi (dots for inch) using a flatbed scanner (Epson Perfection 3170 Photo, Seiko Epson Corp., Japan). The images were converted to 8 bit grey scale and subjected to spatial calibration before the analysis. The images were calibrated, standardized and optimized applying appropriate filters to evaluate the morphological characterization of the bubbles area (mm^2) and porosity (%) using an Image-Pro Plus 6.0 (Media Cybernetics Inc., USA) software. The bubbles, moreover, have been classified into four different size classes according to their surface: class 1: bubbles area between 0.01 and 0.99 mm^2; class 2: bubbles area between 1.00 and 4.99 mm^2; class 3: bubbles area between 5.00 and 49.99 mm^2; class 4: bubbles area greater than 50.00 mm^2. The number of pores and the area occupied by each class (expressed as percentage of the total number of pores and total pore-area, respectively) were also evaluated.

Crumb texture characteristics were assessed using a testing machine (Z005, Zwick Roell, Ulm, Germany), equipped with a 100 N load cell as described by Marti et al. (2014). A 30 mm diameter cylindrical aluminum probe and a test speed of 2 mm/s were used. Crumb hardness was measured (n = 6) after 0 (2 h after baking), 1, 2 and 3 storage days and expressed as the load (N) at 30% strain.

2.8. Statistics

Analysis of variance (ANOVA) was performed utilizing Statgraphics XV version 15.1.02 (StatPoint Inc., Warrenton, VA, USA). Different dough samples were considered as factors for ANOVA. When a factor effect was found significant ($p \leq 0.05$), significant differences among the respective means were determined using Fisher's Least Significant Difference (LSD) test.

3. Results and discussion

3.1. Chemical composition and enzymatic activities before and after sprouting

Wheat kernels were germinated in an industrial plant by modulating temperature and humidity conditions, in order to promote a controlled sprouting (Fig. S1). The sprouting process did not affect the ash, protein, lipids, and fiber contents (Table S1).

On the other hand, after sprouting, the starch content decreased and, consequently, the amount of total sugars increased, with particular regards to maltose, sucrose and glucose (Table S1). These variations are due to the high enzymatic activities after sprouting. Indeed, SWF had much more enzymatic activities (amylases, proteases and xylanases) than USWF (Table 1). The enzymatic data confirm the synthesis and accumulation of enzymes during the germination phase. This phenomenon is necessary to assure the hydrolysis of proteins, polysaccharides and lipids to allow the growth of the embryo (Nelson, Stojanovska, Vasiljevic, & Mathai,

2013). Table 1 also showed the enzymatic activities of a commercial malt (M) and an enzymatic improver (EI) that are conventionally used in bread-making to improve the baking performance and shelf-life of the product. In the following sections, the effects of small amounts of SWF (0.5–2%) on dough rheology and bread quality will be compared with those promoted by conventional flour improvers at similar dosage (De Leyn, 2006).

3.2. Pasting properties

The MVAG indices of commercial wheat flour alone (CTRL) or after addition of malt (0.5% M), enzymatic improver (0.5% EI), or sprouted wheat flour (0.5, 1, 1.5, 2% SWF) are reported in Table 2. The progressive addition of SWF (from 0.5 to 2%) resulted in a significantly ($p \leq 0.05$) decrease in viscosity during heating and cooling phase as a consequence of the high amylase activity in germinated wheat (Table 1). The effect of amylase activity on paste viscosity has been already documented (Dobraszczyk & Dendy, 2001).

Although a decrease in peak viscosity has been measured in presence of SWF, the starch in the mixture has still the ability to form a gel at temperature lower than 95 °C. This result is of great interest in view of incorporating SWF in food formulation, without dramatically compromising the starch behavior during baking. In presence of SWF, peak temperature significantly ($p \leq 0.05$) decreased, indicating the starch granules reached maximum viscosity earlier compared to CTRL.

During the cooling step the gelatinized starch is reorganized, giving the structure of a gel. The setback value - which reflects the retrogradation tendency of amylose in a starch paste - decreased with increasing percentage of SWF (Table 2), suggesting a decrease in starch retrogradation compared to the CTRL. The outer branches of the amylopectin are hydrolyzed by the alpha-amylase and thus made unavailable for forming large amylopectin crystals. These small crystallites do not form a three-dimensional network capable of promoting an important increase in viscosity during cooling (Dobraszczyk & Dendy, 2001). This trend could be of great interest, since low setback values indicate low rate of starch retrogradation and syneresis. This aspect would contribute to the maintenance of a soft crumb during bread storage.

The addition of 0.5% EI (having xylanase as the main activity, Table 1) lead to no significant changes in the pasting properties of the CTRL, despite previous studies showed that xylanase cleaves the arabinoxylans into oligomers resulting in the decrease in peak viscosity (Hemalatha, Rao, Leelavathi, & Salimath, 2010). Differences in xylanase activity among commercial improvers might account for the differences in results.

As expected the addition of malt – even if at low level (0.5%) - causes a considerable decrease in pasting temperature, maximum viscosity, and peak temperature (Table 2), in agreement with the studies of Rao, Manohar, and Muralikrishna (2007). Due to the high amount of α-amylase, this mixture did not show the typical pasting profile of wheat flour; in particular, there is no real viscosity peak and the curve is flat throughout the analysis period.

Table 1
Enzymatic activities of flour from unsprouted (USWF) and sprouted (SWF) wheat, malt (M) and enzymatic improver (EI).

	USWF	SWF	M	EI
α-amilase (ceralpha unit * g^{-1})	0.094 ± 0.001[a]	12.904 ± 0.040[b]	247.744 ± 0.298[c]	0.118 ± 0.006[a]
Xylanase (unit * g^{-1})	0.701 ± 0.003[a]	2.316 ± 0.032[b]	80.47 ± 0.08[c]	256.27 ± 0.17[d]
Protease (unit * g^{-1})	0.66 ± 0.90[a]	1.43 ± 0.29[b]	8.280 ± 0.057[d]	4.290 ± 0.124[c]

Values associated with different letters in the same row are significantly different (one-way ANOVA, LSD test, $p \leq 0.05$).
EI, enzymatic improver; M, malt; SWF, flour from sprouted wheat; USWF, flour from un-sprouted wheat.

Table 2
Rheological properties of commercial wheat flour (CTRL), with either malt (0.5% M), enzymatic improver (0.5% EI), or sprouted wheat flour (0.5, 1, 1.5, 2% SWF).

			CTRL	0.5% SWF	1% SWF	1.5% SWF	2% SWF	0.5% M	0.5% EI
Pasting properties (MVAG Test)	Pasting temperature	(°C)	63.7 ± 0.7[b]	63.4 ± 0.7[b]	61.9 ± 1.0[ab]	63.7 ± 1.6[b]	63.7 ± 1.2[b]	60.7 ± 1.7[a]	63.3 ± 1.7[b]
	Peak viscosity	(BU)	308 ± 11[f]	215 ± 12[d]	192 ± 7[d]	147 ± 4[c]	131 ± 7[b]	58 ± 2[a]	320 ± 6[f]
	Peak temperature	(°C)	90.0 ± 0.3[e]	88.2 ± 0.2[d]	87.6 ± 0.2[cd]	86.9 ± 0.3[bc]	86.5 ± 0.2[b]	73.0 ± 1.5[a]	90.3 ± 0.1[e]
	Final viscosity	(BU)	816 ± 5[f]	548 ± 29[e]	477 ± 14[d]	404 ± 16[c]	369 ± 8[b]	106 ± 13[a]	841 ± 32[f]
	Breakdown	(BU)	81 ± 8[d]	71 ± 3[c]	74 ± 3[c]	60 ± 2[b]	59 ± 1[b]	33 ± 2[a]	85 ± 5[d]
	Setback	(BU)	585 ± 17[e]	403 ± 19[d]	348 ± 13[c]	298 ± 16[b]	276 ± 8[b]	72 ± 12[a]	602 ± 19[e]
Gluten aggregation properties (GlutoPeak test)	Peak maximum time	(s)	296 ± 6[d]	263 ± 3[bc]	265 ± 3[bc]	252 ± 4[a]	250 ± 1[a]	260 ± 7[b]	267 ± 6[c]
	Maximum torque	(BE)	31.5 ± 0.6[a]	32.3 ± 1.0[a]	35.0 ± 0.1[b]	35.0 ± 0.1[b]	34.8 ± 0.5[b]	31.5 ± 1.0[a]	32.3 ± 1.0[a]
	Energy	(GPE)	3326 ± 80[de]	3153 ± 81[ab]	3385 ± 60[e]	3262 ± 52[cd]	3199 ± 85[bc]	3058 ± 16[a]	3208 ± 53[bc]
Leavening properties (Rheofermentometer Test)	Maximum dough height	(mm)	57.3 ± 6.4[a]	65.1 ± 1.2[ab]	69.3 ± 0.1[b]	69.8 ± 0.9[b]	69.8 ± 5.5[b]	69.6 ± 4.0[b]	59.9 ± 1.9[a]
	Final dough height	(mm)	55.9 ± 5.4[a]	64.6 ± 0.5[bc]	69.3 ± 0.1[c]	69.7 ± 1.0[c]	69.0 ± 6.2[c]	69.2 ± 4.5[c]	57.2 ± 0.4[ab]
	Maximum height	(mm)	62.7 ± 1.1[a]	62.0 ± 0.9[a]	62.2 ± 1.0[a]	62.9 ± 0.7[a]	64.0 ± 0.4[a]	64.3 ± 0.4[a]	62.9 ± 2.3[a]
	Total CO$_2$	(mL)	1272 ± 20[a]	1403 ± 14[b]	1444 ± 15[bc]	1458 ± 4[cd]	1487 ± 12[d]	1494 ± 18[d]	1286 ± 31[a]
	CO$_2$ retained	(mL)	1213 ± 5[a]	1306 ± 8[b]	1348 ± 3[c]	1365 ± 6[cd]	1384 ± 8[de]	1395 ± 2[e]	1229 ± 24[a]
	CO$_2$ released	(mL)	60 ± 15[ab]	98 ± 6[c]	96 ± 12[c]	93 ± 9[c]	103 ± 21[c]	100 ± 21[c]	57 ± 7[a]
	CO$_2$ retention coefficient	(%)	95.3 ± 1.1[bc]	93.1 ± 0.4[a]	93.4 ± 0.8[ab]	93.7 ± 0.6[abc]	93.1 ± 1.3[ab]	93.4 ± 1.3[abc]	95.6 ± 0.5[c]
	Porosity time	(h)	1.55 ± 0.04[a]	1.55 ± 0.11[a]	1.64 ± 0.05[a]	1.60 ± 0.04[a]	1.60 ± 0.04[a]	1.51 ± 0.05[a]	1.58 ± 0.11[a]

Values associated with different letters in the same row are significantly different (one-way ANOVA, LSD test, p ≤ 0.05).
CTRL, control wheat flour; EI, enzymatic improver; M, malt; SWF, flour from sprouted wheat; USWF, flour from un-sprouted wheat.
Pasting temperature: temperature at which an initial increase in viscosity occurs; Peak viscosity: maximum viscosity achieved during the heating cycle; Peak temperature: temperature at the maximum viscosity; Final viscosity: viscosity at the end of the test; Breakdown: viscosity difference between peak and after holding at 95 °C; Setback: difference between the final viscosity at 30 °C and the viscosity reached after the holding period at 95 °C; 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; Maximum dough height: maximum height achieved during the test; Final dough height: height at the end of the test; 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; BU: Brabender Units; GPE: GlutoPeak Equivalent.

3.3. Gluten aggregation properties

The GlutoPeak indices of the commercial wheat flour (CTRL) or added to malt (0.5% M), to the enzymatic improver (0.5% EI), or to the sprouted wheat flour (0.5, 1, 1.5, 2% SWF) are shown in Table 2.

GlutoPeak is a new device proposed for gluten quality evaluation, by measuring protein aggregation capability (Marti, Augst et al., 2015). Bread flours with poor technological quality (e.g. resulting in a low bread volume) are usually characterized by a rapid build-up in consistency and a sharply defined peak followed by a rapid breakdown, while high bread quality flours have a much slower build-up in dough consistency and require more time to reach peak consistency (Marti, Augst et al., 2015; Marti, Ulrici, Foca, Quaglia, & Pagani, 2015).

Adding M or EI at the 0.5% no significant differences in the maximum consistency value were observed. A similar result was obtained when 0.5% SWF was added; whereas, increasing SWF levels (1–2%) determined a significant (p ≤ 0.05) increase in maximum torque (Table 2).

As regards the time at which the maximum aggregation occurred, a significant (p ≤ 0.05) decrease in value has been measured when M, EI, and SWF have been added to flour. The faster aggregation was measured for SWF at levels ≥1.5%. The decrease in time can be related to gluten dilution, since the same phenomenon was observed adding 1% of starch (data not shown). Nevertheless, the action of proteases, which are synthetized during germination, could be responsible for changing the aggregation properties. In general, the shorter the time until the formation of gluten, the lower the quality of the network (Melnyk, Dreisoerner, Marcone, & Seetharaman, 2012). However, on the basis on previous work (Marti, Augst et al., 2015; Marti, Ulrici et al., 2015) the mixtures with germinated wheat flour show a gluten aggregation kinetic similar to that of a flour with good bread-making quality. Indeed, it seems that wheat sprouting under controlled conditions determined protein hydrolysis without compromising their ability of aggregating and forming gluten network.

More recently the area under the peak — which takes into account both maximum torque and maximum peak time - has been found the most suitable parameter for predicting conventional parameters related to dough strength and extensibility (Marti, Ulrici et al., 2015). The energy value decreased when either M or EI were added to the CTRL. Interestingly, when SWF was present at 1 or 1.5%, samples showed a similar energy value as the CTRL (Table 2), suggesting that the enrichment of 1.5% SWF did not compromise the gluten aggregation properties of the flour.

3.4. Leavening properties

The Rheofermentometer allows evaluating the proofing behavior of doughs by measuring dough development and gas release during the fermentation process. The main indices obtained from the curves during dough development and gas production are summarized in Table 2. Adding 0.5% EI to control flour did not affect either the dough height or the gas production and retention. Both samples showed a slight dip in height after 1 h and 30 min of proofing (data not shown). When 0.5% M was added to the flour, dough developed without showing any decrease in height within the first 2 h of proofing. Moreover, the use of malt increased the dough final height from 57 to 70 mm (Table 1), likely due to the more intense yeast activity in presence of free sugars formed from the starch hydrolysis from α-amylase. The positive effect of α-amylase on dough leavening properties have been already demonstrated (Penella, Collar, & Haros, 2008). The height reached by dough during fermentation is related to loaf specific volume; therefore, maximum height is an important parameter when evaluating baking performance.

Adding SWF led to increase the development of the dough (Table 2). The maximum dough height was reached in the mixture with ≥1.0% SWF. Even the time when this maximum height is reached, which is in closed relation to the yeast activity (Huang, Kim, Li, & Rayas-Duarte, 2008), is similar for all samples. However, the mixture with 1.5% and 2.0% SWF showed a better response than the other percentages.

Rheofermentometer analysis yields insight into CO$_2$ production,

retention and dough height throughout the dough fermentation process and therefore gives a good indication of yeast fermentation performance. Either the improvers conventionally used in bread-making or SWF affect the porosity time (corresponding to the loss of CO_2 from the dough; Table 2). On the contrary all of them, but EI, positively affected the total volume of CO_2 produced and retained into the dough. Previous studies have also shown that gas formation of doughs prepared with fungal α-amylase during fermentation generally increased significantly (Penella et al., 2008).

The quantity of CO_2 lost by the dough when proofing is directly linked to the porous nature of the dough, which appears more or less prematurely and is closed linked with the quality of the protein network. The highest amount of retained gases is observed in presence of either malt or 2% SWF. According to literature, the α-amylase provoked a negative effect in the gas retention coefficient, associated with an increase in dough permeability. According to Penella et al. (2008), this phenomenon was induced by increased hydrolysis of starch chains.

3.5. Bread properties

Based on the results obtained on dough rheological properties,

we decided to compare the bread-making performance of CTRL, with that of 0.5% EI, 0.5% M, and 1.5% SWF. Crumb porosity is shown in Fig. 1, whereas bread characteristics are reported in Table 3. Adding 1.5% SWF significant increased the porosity area from 44.5% (CTRL) to 54.9%. This figure was similar to that of bread with 0.5% EI (53.9%) and higher than sample with 0.5% M (52.4%). Looking at the cells, despite the number of cells of each class was very similar among the samples (data not shown), differences in cell area were observed (Fig. 1). In particular, small cells (<5 mm^2) area represented more than 70% of the total pore area in the CTRL bread and about 40% in 0.5% M, 0.5% EI and 1.5% SWF products. Crumb of bread with M, EI, and SWF was characterized by the presence of large cells ($5-50$ mm^2) whose area accounted for the 60% of the total porosity.

The effect of SWF on crumb color was similar to that of malt. Both of them significantly decreased the lightness and increased the redness compared to the control bread, with no effect on yellowness. Once again, this result could be related to the increased amount of amylases in the flour mixture of this two bread types.

As expected, adding malt or germinated wheat flour resulted in a decrease in luminosity, redder and more yellow crust compared to CTRL. These changes were likely caused by increase in Maillard
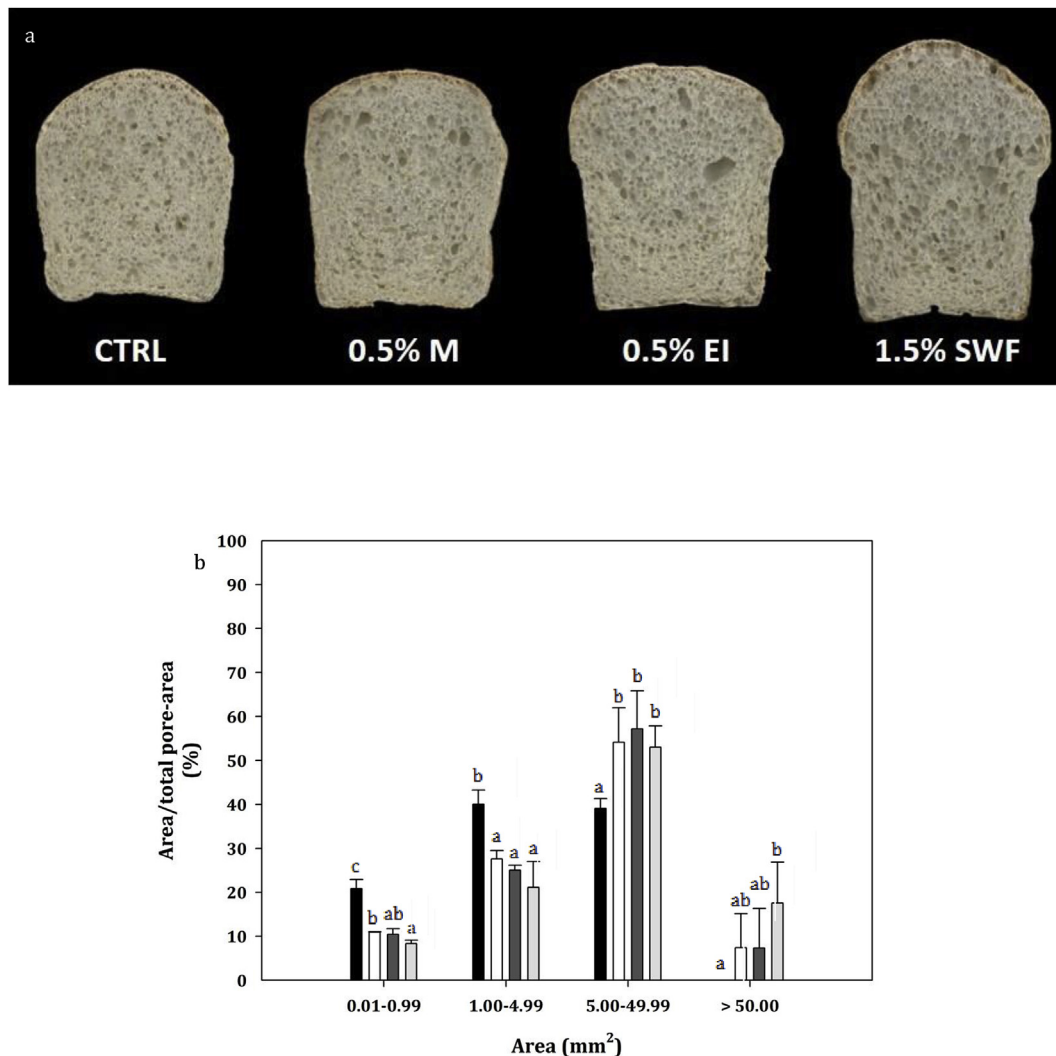

Fig. 1. Pictures of the bread prepared from commercial wheat flour (CTRL), with either malt (0.5% M), enzymatic improver (0.5% EI), or sprouted wheat flour (1.5% SWF) (a) and crumb porosity by image analysis (b). Colors used: black: CTRL; white: 0.5% M; dark grey: 0.5% EI; light grey: 1.5% SWF. Values associated with different letters in the same area's group are significantly different (one-way ANOVA, LSD test, p ≤ 0.05).

Table 3
Specific volume, moisture, water activity, color and firmness of bread from commercial wheat flour (CTRL), with either malt (0.5% M), enzymatic improver (0.5% EI), or sprouted wheat flour (1.5% SWF).

		CTRL	0.5% M	0.5% EI	1.5% SWF
Bread	Specific volume (mL/g)	3.0 ± 0.5^a	3.2 ± 0.1^a	3.0 ± 0.4^a	3.6 ± 0.1^b
Crust	Luminosity (L*)	76.47 ± 2.86^c	71.77 ± 1.15^b	77.58 ± 1.02^c	69.69 ± 1.08^a
	Redness (a*)	3.62 ± 0.51^a	6.13 ± 0.55^b	3.29 ± 0.35^a	7.64 ± 0.67^c
	Yellowness (b*)	29.57 ± 1.21^a	31.89 ± 1.55^b	28.94 ± 0.90^a	34.24 ± 0.72^c
	Browning (100-L*)	23.53 ± 2.86^a	28.23 ± 1.15^b	22.42 ± 1.02^a	30.31 ± 1.08^c
Crumb	Luminosity (L*)	69.14 ± 2.76^b	66.43 ± 4.32^a	70.97 ± 3.19^b	66.08 ± 2.50^a
	Redness (a*)	-1.70 ± 0.09^b	-1.63 ± 0.09^c	-1.83 ± 0.14^a	-1.63 ± 0.11^c
	Yellowness (b*)	14.66 ± 0.73^a	14.86 ± 1.13^a	15.33 ± 1.60^a	14.94 ± 1.10^a
	Browning (100-L*)	30.86 ± 2.76^a	33.57 ± 4.32^b	29.03 ± 3.19^a	33.92 ± 2.50^b
	Moisture (%)	42.4 ± 1.0^a	42.0 ± 0.8^a	41.7 ± 1.5^a	42.0 ± 1.7^a
	Water activity	0.927 ± 0.006^{ab}	0.921 ± 0.008^a	0.926 ± 0.007^{ab}	0.932 ± 0.012^b
	Firmness (N)	3.54 ± 1.60^b	2.27 ± 0.26^a	3.65 ± 1.20^b	2.36 ± 0.31^a

Values associated with different letters in the same row are significantly different (one-way ANOVA, LSD test, $p \leq 0.05$).
CTRL, control wheat flour; EI, enzymatic improver; M, malt; SWF, flour from sprouted wheat.

reaction extent (Hefni & Witthöft, 2011) due to the hydrolytic action of amylases and proteases (Goesaert et al., 2006). On the contrary, the use of EI did not affect the bread crust color, likely due to the low amylase content and thus to low levels of released glucose.

The highest specific volume was observed for the bread with SWF, whereas no significant differences were observed in presence of either 0.5% EI or 0.5% M (Table 3). Enzymes concentrations seem not to account for the observed differences in bread-making performance. On the other hand, the nature of sample should be considered. Indeed, adding SWF contains also proteins that might contribute to gluten formation and thus maintain the structure during baking. Also Mäkinen and Arendt (2012) reported no significant increased bread volume with 0.5% malt. The effectiveness of xylanase present in EI (Table 1) in improving bread volume is contributing to result in the redistribution of water from the pentosane phase to the gluten phase. The increase in gluten volume fraction assures more extensibility to gluten and consequently a better oven-spring (Goesaert et al., 2006). However, it should be considered that the improver used in our study was not a pure enzyme but included various enzymatic activities, with xylanase as the highest activity.

The presence of either malt or SWF improved the textural properties of the bread by significantly decreasing the crumb firmness of fresh samples (2 h after baking) (Fig. 2). On the contrary, EI at 0.5% did not affect the crumb texture. During storage (up to 3 days), all the samples exhibited lower firmness than CTRL (Fig. 2). The best result in terms of increasing crumb softness and lowering the staling process was obtained in presence of M or SWF. Differences in bread textural properties cannot be related to bread crumb moisture nor to water activity, as no significant differences were observed among the samples (data not shown).

The results of our study confirm the positive effects of amylase, proteases and xylanase on crumb firmness and bread staling (Caballero, Gómez, & Rosell, 2007). The antistaling effect of these enzymes have been widely reviewed (De Leyn, 2006; Goesaert et al., 2006). In particular, α-amylase has been proved to be useful for reducing amylopectin retrogradation and the firming rate of wheat bread crumb (Champenois, Della Valle, Planchot, Buleon, & Colonna, 1999). Through studies on model systems, Rojas, Rosell, and De Barber (2001) stated that maltodextrins were responsible for the antistaling effect promoted by addition of α-amylase to bread formulation. Jiménez and Martínez-Anaya (2001) proved that water-insoluble pentosans were positively correlated with crumb elasticity and hardness during storage. Xylanases would lead to cleavage of the backbone of arabinoxylans, with the consequent release of water and decrease in water-insoluble pentosans (Rouau,

El-Hayek, & Moreau, 1994). Both phenomena could explain the positive effects of xylanases in bread freshness. Similarly, the improvement of bread shelf-life through protease addition possibly would be tied with the increase of the water available for starch, in conjunction with a simultaneous diminution of starch–protein interactions as consequence of the hydrolysis of peptide bonds in the protein molecules. In addition to enzymatic activities, during germination the lipid hydrolysis promotes the production of mono- and diglycerides. This process slows the staling of bread, which corresponds to a longer shelf life of the product.

4. Conclusions

This study provides evidence that refined flour from sprouted wheat can be considered as an ingredient for improving the technological performance of commercial flours. Refined flour from industrial-scale germinated wheat shows increased enzymatic activities without compromising the aggregation properties of gluten proteins. Wheat sprouting under controlled conditions increases sugar production with a concomitant improvement of dough leavening properties. The bread-making performance evaluated in terms of loaf volume and crumb softness, confirms that flour from
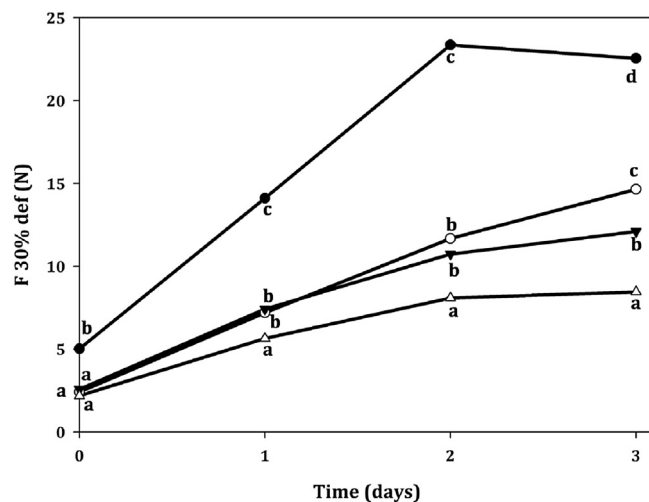
Fig. 2. Crumb firmness of bread prepared from commercial wheat flour (CTRL — black circle), with either malt (0.5% M — white circle), enzymatic improver (0.5% EI — black triangle), or sprouted wheat flour (1.5% SWF — white triangle) during storage. Values associated with different letters in the same day are significantly different (one-way ANOVA, LSD test, $p \leq 0.05$).

sprouted wheat is a promising and interesting ingredient for formulating baked products, avoiding the use of enzymatic improvers or malt with a positive impact on consumers' acceptance and facilitating the adoption of clean label.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2017.02.028.

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