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.

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 and Auld, 2004). Moreover, bread from extensively sprouted wheat show very poor characteristics, with a sticky and gummy crumb (McCleary and 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 et al., 2015) and sensory (Heiniö et al., 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 et al., 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 et al., 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 $*10^{-4}$ J; P/L = 2.08) used for blending studies.

90 Malt (M; Matlo 5, Bona s.r.l., Monza, Italy) and the enzymatic improver (EI,
91 PowerBake950, Danisco, Copenhagen, Denmark) were added to CTRL at 0.5% level,
92 which represents conventional amount used in bread-making (De Leyn, 2006). SWF
93 was used at 0.5, 1, 1.5, and 2%.
94
95 **2.2 Sprouting process**
96 Commercial wheat kernels were sprouted in an industrial sprouting plant (Bühler AG,
97 Uzwil, Switzerland). Wheat (10 tons) was soaked in water (kernels:water ratio of 1:2)
98 for 12-24h at 20°C, germinated for 72-90h at 20 °C, dried at 50 °C for 32 h.
99 Unsprouted and sprouted wheat were milled in the same industrial plant (Bühler AG,
100 Uzwil, Switzerland), and the related flours – USWF and SWF, respectively - were
101 obtained.
102
103 **2.3 Chemical composition**
104 Moisture, starch, protein, lipid and ash contents were assessed by AACC standard
105 methods (44-15.02, 76-13.01, 46-12.01, 30-10.01, and 08-01.01, respectively; AACC
106 2001). Sugars were determined by HPLC by Anion Exchange Chromatography with
107 Pulsed Amperometric Detection (HPAEC-PAD) (Zygmunt et al. 1982). Total, soluble
108 and insoluble dietary fiber content was quantified by enzymatic–gravimetric procedure
109 (AOAC Method 991.43).
110
111 **2.4 Enzymatic activities**
112 Proteolytic activity was determined in triplicate in the conditions proposed by Arnon
113 (1970) and using azocasein (Sigma Chemical Co., St Louis, MO, USA) as the
114 substrate. Alpha-amylase activity was determined in triplicate according to AACC

115 standard method n. 303, by using the Megazyme Amylase Assay Procedure
116 (Megazyme International Ireland Ltd., Wicklow, Ireland). Xylanase activity was
117 determined in triplicate using the Azo-wheat arabinoxylan kit (K-AZOWAX 09/04)
118 provided by Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland).
119
120 **2.5 Rheological properties**
121 **2.5.1 Pasting properties**
122 Pasting properties were measured in duplicate using a Micro-Visco-Amylograph device
123 (MVAG, Brabender GmbH & Co. KG, Duisburg, Germany). An aliquot of sample (12
124 g) was dispersed in 100 mL of distilled water and stirred at 250 rpm. The following
125 temperature profile was applied: heating from 30 °C to 95 °C at a rate of 3 °C/min,
126 holding at 95 °C for 20 min, cooling from 95 °C to 30 °C at a cooling rate of 3 °C/min,
127 and holding at 30 °C for 1 min.
128
129 **2.5.2 Gluten aggregation properties**
130 Gluten aggregation properties were measured at least in triplicate using the GlutoPeak
131 device (Brabender GmbH & Co. KG, Duisburg, Germany), as reported by Marti et al.
132 (2015a).
133
134 **2.5.3 Leavening properties**
135 Leavening properties of doughs were assessed in duplicate with a
136 Rheofermentometer® device (Chopin, Tripette & Renaud, Villeneuve La Garenne
137 Cedex, France). Dough samples were prepared in an automatic spiral mixer (Bomann,
138 Clatronic s.r.l., Piadena, Italy) with 1.5% NaCl and 1.5% bakers' yeast. Mixing time
139 (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 1.5g/100g 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, two hours 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

190 al. (2014). A 30 mm diameter cylindrical aluminum probe and a test speed of 2 mm/s
191 were used. Crumb hardness was measured (n = 6) after 0 (two hours after baking), 1, 2
192 and 3 storage days and expressed as the load (N) at 30% strain.
193
194 **2.8 Statistics**
195 Analysis of variance (ANOVA) was performed utilizing Statgraphics XV version
196 15.1.02 (StatPoint Inc., Warrenton, VA, USA). Different dough samples were
197 considered as factors for ANOVA. When a factor effect was found significant
198 ($p \leq 0.05$), significant differences among the respective means were determined using
199 Fisher's Least Significant Difference (LSD) test.
200
201 **3. Results and Discussion**
202 **3.1 Chemical composition and enzymatic activities before and after sprouting**
203 Wheat kernels were germinated in an industrial plant by modulating temperature and
204 humidity conditions, in order to promote a controlled sprouting (Figure S1). The
205 sprouting process did not affect the ash, protein, lipids, and fiber contents (Table S1).
206 On the other hand, after sprouting, the starch content decreased and, consequently, the
207 amount of total sugars increased, with particular regards to maltose, sucrose and
208 glucose (Table S1). These variations are due to the high enzymatic activities after
209 sprouting. Indeed, SWF had much more enzymatic activities (amylases, proteases and
210 xylanases) than USWF (Table 1). The enzymatic data confirm the synthesis and
211 accumulation of enzymes during the germination phase. This phenomenon is necessary
212 to assure the hydrolysis of proteins, polysaccharides and lipids to allow the growth of
213 the embryo (Nelson et al., 2013). Table 1 also showed the enzymatic activities of a
214 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 and 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-

240 dimensional network capable of promoting an important increase in viscosity during
241 cooling (Dobraszczyk and Dendy 2001). This trend could be of great interest, since low
242 setback values indicate low rate of starch retrogradation and syneresis. This aspect
243 would contribute to the maintenance of a soft crumb during bread storage.
244 The addition of 0.5% EI (having xylanase as the main activity, Table 1) lead to
245 no significant changes in the pasting properties of the CTRL, despite previous studies
246 showed that xylanase cleaves the arabinoxylans into oligomers resulting in the decrease
247 in peak viscosity (Hemalatha et al., 2010). Differences in xylanase activity among
248 commercial improvers might account for the differences in results.
249 As expected the addition of malt – even if at low level (0.5%) - causes a
250 considerable decrease in pasting temperature, maximum viscosity, and peak
251 temperature (Table 2), in agreement with the studies of Rao, Manohar, &
252 Muralikrishna (2007). Due to the high amount of α-amylase, this mixture did not show
253 the typical pasting profile of wheat flour; in particular, there is no real viscosity peak
254 and the curve is flat throughout the analysis period.
255
256 **3.3 Gluten Aggregation Properties**
257 The GlutoPeak indices of the commercial wheat flour (CTRL) or added to malt (0.5%
258 M), to the enzymatic improver (0.5% EI), or to the sprouted wheat flour (0.5, 1, 1.5,
259 2% SWF) are shown in Table 2.
260 GlutoPeak is a new device proposed for gluten quality evaluation, by measuring
261 protein aggregation capability (Marti et al., 2015a). Bread flours with poor
262 technological quality (e.g. resulting in a low bread volume) are usually characterized by
263 a rapid build-up in consistency and a sharply defined peak followed by a rapid

264 breakdown, while high bread quality flours have a much slower build-up in dough
265 consistency and require more time to reach peak consistency (Marti et al., 2015a,b).
266 Adding M or EI at the 0.5% no significant differences in the maximum consistency
267 value were observed. A similar result was obtained when 0.5% SWF was added;
268 whereas, increasing SWF levels (1-2%) determined a significant ($p \leq 0.05$) increase in
269 maximum torque (Table 2).
270 As regards the time at which the maximum aggregation occurred, a significant ($p \leq$
271 0.05) decrease in value has been measured when M, EI, and SWF have been added to
272 flour. The faster aggregation was measured for SWF at levels ≥1.5%. The decrease in
273 time can be related to gluten dilution, since the same phenomenon was observed adding
274 1% of starch (data not shown). Nevertheless, the action of proteases, which are
275 synthetized during germination, could be responsible for changing the aggregation
276 properties. In general, the shorter the time until the formation of gluten, the lower the
277 quality of the network (Melnyk et al., 2012). However, on the basis on previous work
278 (Marti et al., 2015a,b) the mixtures with germinated wheat flour show a gluten
279 aggregation kinetic similar to that of a flour with good bread-making quality. Indeed, it
280 seems that wheat sprouting under controlled conditions determined protein hydrolysis
281 without compromising their ability of aggregating and forming gluten network.
282 More recently the area under the peak – which takes into account both maximum
283 torque and maximum peak time - has been found the most suitable parameter for
284 predicting conventional parameters related to dough strength and extensibility (Marti et
285 al., 2015b). The energy value decreased when either M or EI were added to the CTRL.
286 Interestingly, when SWF was present at 1 or 1.5%, samples showed a similar energy
287 value as the CTRL (Table 2), suggesting that the enrichment of 1.5% SWF did not
288 compromise the gluten aggregation properties of the flour.

289

290 **3.4 Leavening properties**

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

305 Adding SWF led to increase the development of the dough (Table 2). The
306 maximum dough height was reached in the mixture with ≥1.0% SWF. Even the time
307 when this maximum height is reached, which is in closed relation to the yeast activity
308 (Huang et al., 2008), is similar for all samples. However, the mixture with 1.5 % and
309 2.0% SWF showed a better response than the other percentages.

310 Rheofermentometer analysis yields insight into CO_2 production, retention and
311 dough height throughout the dough fermentation process and therefore gives a good
312 indication of yeast fermentation performance. Either the improvers conventionally used
313 in bread-making or SWF affect the porosity time (corresponding to the loss of CO_2

314 from the dough; Table 2). On the contrary all of them, but EI, positively affected the
315 total volume of CO_2 produced and retained into the dough. Previous studies have also
316 shown that gas formation of doughs prepared with fungal α-amylase during
317 fermentation generally increased significantly (Penella et al., 2008).
318 The quantity of CO_2 lost by the dough when proofing is directly linked to the
319 porous nature of the dough, which appears more or less prematurely and is closed
320 linked with the quality of the protein network. The highest amount of retained gases is
321 observed in presence of either malt or 2% SWF. According to literature, the α-amylase
322 provoked a negative effect in the gas retention coefficient, associated with an increase
323 in dough permeability. According to Penella et al. (2008), this phenomenon was
324 induced by increased hydrolysis of starch chains.
325
326 **3.5 Bread Properties**
327 Based on the results obtained on dough rheological properties, we decided to compare
328 the bread-making performance of CTRL, with that of 0.5% EI, 0.5% M, and 1.5%
329 SWF. Crumb porosity is shown in Fig. 1, whereas bread characteristics are reported in
330 Table 3. Adding 1.5% SWF significant increased the porosity area from 44.5% (CTRL)
331 to 54.9%. This figure was similar to that of bread with 0.5% EI (53.9%) and higher
332 than sample with 0.5% M (52.4%). Looking at the cells, despite the number of cells of
333 each class was very similar among the samples (data not shown), differences in cell
334 area were observed (Fig. 1). In particular, small cells (<5 mm^2) area represented more
335 than 70% of the total pore area in the CTRL bread and about 40% in 0.5% M, 0.5% EI
336 and 1.5% SWF products. Crumb of bread with M, EI, and SWF was characterized by
337 the presence of large cells (5-50 mm^2) whose area accounted for the 60% of the total
338 porosity.

339 The effect of SWF on crumb colour was similar to that of malt. Both of them
340 significantly decreased the lightness and increased the redness compared to the control
341 bread, with no effect on yellowness. Once again, this result could be related to the
342 increased amount of amylases in the flour mixture of this two bread types.
343 As expected, adding malt or germinated wheat flour resulted in a decrease in
344 luminosity, redder and more yellow crust compared to CTRL. These changes were
345 likely caused by increase in Maillard reaction extent (Hefni and Witthöft, 2011) due to
346 the hydrolytic action of amylases and proteases (Goesaert et al., 2006). On the contrary,
347 the use of EI did not affect the bread crust colour, likely due to the low amylase content
348 and thus to low levels of released glucose.
349 The highest specific volume was observed for the bread with SWF, whereas no
350 significant differences were observed in presence of either 0.5% EI or 0.5% M (Table
351 3). Enzymes concentrations seem not to account for the observed differences in bread-
352 making performance. On the other hand, the nature of sample should be considered.
353 Indeed, adding SWF contains also proteins that might contribute to gluten formation
354 and thus maintain the structure during baking. Also Mäkinen and Arendt (2012)
355 reported no significant increased bread volume with 0.5% malt. The effectiveness of
356 xylanase present in EI (Table 1) in improving bread volume is contributing to result in
357 the redistribution of water from the pentosane phase to the gluten phase. The increase
358 in gluten volume fraction assures more extensibility to gluten and consequently a better
359 oven-spring (Goesaert et al., 2006). However, it should be considered that the improver
360 used in our study was not a pure enzyme but included various enzymatic activities,
361 with xylanase as the highest activity.
362 The presence of either malt or SWF improved the textural properties of the
363 bread by significantly decreasing the crumb firmness of fresh samples (2h after baking)

364 (Fig. 2). On the contrary, EI at 0.5% did not affect the crumb texture. During storage
365 (up to 3 days), all the samples exhibited lower firmness than CTRL (Fig. 2). The best
366 result in terms of increasing crumb softness and lowering the staling process was
367 obtained in presence of M or SWF. Differences in bread textural properties cannot be
368 related to bread crumb moisture nor to water activity, as no significant differences were
369 observed among the samples (data not shown).
370 The results of our study confirm the positive effects of amylase, proteases and
371 xylanase on crumb firmness and bread staling (Caballero, Gómez, & Rosell, 2007). The
372 antistaling effect of these enzymes have been widely reviewed (De Leynm, 2006;
373 Goesaert et al., 2006). In particular, α-amylase has been proved to be useful for
374 reducing amylopectin retrogradation and the firming rate of wheat bread crumb
375 (Champenois et al., 1999). Through studies on model systems, Rojas, Rosell, & De
376 Barber (2001) stated that maltodextrins were responsible for the antistaling effect
377 promoted by addition of α -amylase to bread formulation. Jiménez and Martínez-
378 Anaya (2001) proved that water-insoluble pentosans were positively correlated with
379 crumb elasticity and hardness during storage. Xylanases would lead to cleavage of the
380 backbone of arabinoxylans, with the consequent release of water and decrease in water-
381 insoluble pentosans (Rouau, El-Hayek, & Moreau, 1994). Both phenomena could
382 explain the positive effects of xylanases in bread freshness. Similarly, the improvement
383 of bread shelf-life through protease addition possibly would be tied with the increase of
384 the water available for starch, in conjunction with a simultaneous diminution of starch–
385 protein interactions as consequence of the hydrolysis of peptide bonds in the protein
386 molecules. In addition to enzymatic activities, during germination the lipid hydrolysis
387 promotes the production of mono- and diglycerides. This process slows the staling of
388 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 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.

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505 **List of Tables**

516

517 **List of Figures**

518 **Fig. 1.** Pictures of the bread prepared from commercial wheat flour (CTRL), with either
519 malt (0.5% M), enzymatic improver (0.5% EI), or sprouted wheat flour (1.5% SWF) (a)
520 and crumb porosity by image analysis (b). Bars associated with different letters in the
521 same class of pores are significantly different (one-way ANOVA, LSD test, $p \leq 0.05$).

522

523 **Fig. 2.** Crumb firmness of bread prepared from commercial wheat flour (CTRL), with
524 either malt (0.5% M), enzymatic improver (0.5% EI), or sprouted wheat flour (1.5%
525 SWF) during storage. Values associated with different letters are significantly different
526 (one-way ANOVA, LSD test, $p \leq 0.05$).

527

528 **Table 1.** Enzymatic activities of flour from unsprouted (USWF) and sprouted (SWF)

529 wheat, malt (M) and enzymatic improver (EI).

530

	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]

531

532 Values associated with different letters in the same row are significantly different

533 (one-way ANOVA, LSD test, $p \leq 0.05$).

534 EI, enzymatic improver; M, malt; SWF, flour from sprouted wheat; USWF, flour

535 from un-sprouted wheat

536