



## 11 **Abstract**

12 The impact of 48 h sprouted quinoa (SQ) was assessed in bread-making. Wheat flour (WF) was  
13 replaced with SQ at different levels (i.e., 10:90, 20:80 and 30:70, SQ:WF ratio). Once the optimal  
14 replacement level of SQ was identified, the bread-making performance of this ingredient was  
15 compared with those of pearled quinoa (PQ), commonly used in bread-making.

16 Starch pasting properties and gluten aggregation behavior were not strongly affected at 20:80 level,  
17 even if statistically significant ( $p \leq 0.05$ ). Regardless the replacement level, SQ caused an increase in  
18 dough water absorption and in softening degree, and a decrease in stability, suggesting weakening of  
19 the gluten network. During leavening, SQ improved dough development and gas production, due to  
20 increased sugar content. The best bread-making performance (highest bread specific volume and  
21 lowest crumb firmness) was obtained at 20:80 replacement level. Compared to PQ, SQ exhibited the  
22 best leavening capacity (high dough development, gas production and gas retention) and bread  
23 properties (high specific volume and low crumb firmness), likely due to its higher sugar content.  
24 Moreover, 20SQ bread was characterized by a decreased bitterness assessed by electronic tongue. In  
25 conclusion, sprouting might be considered a valid alternative to pearling to improve the  
26 characteristics of quinoa enriched bread.

27 **Keywords:** Chenopodium quinoa; germination; dough rheology; electronic sensing

28 **Abbreviations:** 10SQ, blend composed by sprouted quinoa and wheat flour at 10:90 ratio; 20PQ,  
29 blend composed by pearled quinoa and wheat flour at 20:80 ratio; 20SQ, blend composed by sprouted  
30 quinoa and wheat flour at 20:80 ratio; 30SQ, blend composed by sprouted quinoa and wheat flour at  
31 30:70 ratio; BU, Brabender Unit; FU, Farinograph Unit; GPE, GlutoPeak Equivalent; GPU,  
32 GlutoPeak Unit; PQ, pearled quinoa; SP, sprouted quinoa; SV, specific volume; WF, wheat flour.

## 33 **1. Introduction**

34 Quinoa is a gluten-free grain from both agronomic and nutritional standpoint. Specifically,  
35 quinoa is particularly high in lysine, which is the limiting amino acid in cereals, it is a good source

36 of minerals, phenolic compounds, dietary fiber and polyunsaturated fatty acids (Tang and Tsao,  
37 2017). All these compositional traits account for the potential health benefits of quinoa seeds in  
38 contributing to the prevention of various diseases such as cancer, diabetes, cardiovascular diseases,  
39 and aging (Tang and Tsao, 2017). Thus, these characteristics are the driving force for enhancing the  
40 consumption of quinoa not only as seeds but also as an ingredient in various food applications,  
41 including both enriched wheat-based goods and gluten free products.

42 Despite the well-known nutritional features of quinoa, its consumption is limited by the bitter  
43 and astringent taste, due to saponin compounds (Suárez-Estrella et al., 2018). Nowadays, pearling is  
44 one of the main processes applied to quinoa to improve its acceptability in food formulation; it  
45 consists in the removal of the seed external layers, which are rich in saponins (Suárez-Estrella et al.,  
46 2018). On the other hand, a significant loss of bioactive compounds occurs during the pearling  
47 process (Suárez-Estrella et al., 2018). Nowadays, quinoa is proposed in bread-making only as flour  
48 from pearled grains. Specifically, in wheat-based bread, 250 g/kg of pearled quinoa seems to be the  
49 threshold level in terms of dough rheological properties and sensory acceptability (Rosell et al.,  
50 2009); conversely, bitter aftertaste was detected at higher quinoa enrichment levels (Lorenz and  
51 Coulter, 1991).

52 Recently, several authors reported the possibility to exploit sprouted grains to enhance the  
53 bread-making attitude of wholewheat (Cardone et al., 2020b), brown-rice (Watanabe et al., 2004),  
54 and pulses (Hallén et al., 2004; Marengo et al., 2017b). The improved bread characteristics (i.e. high  
55 specific volume and crumb softness) are mainly attributable to the activity of the hydrolytic enzymes  
56 (e.g.  $\alpha$ -amylase) developed during sprouting (Goesaert et al., 2009). Moreover, sprouting process is  
57 associated with several grain nutritional and sensory improvements, in terms of increasing mineral  
58 and vitamin bio-availability and of decreasing antinutritional factors (e.g. phytic acid, trypsin  
59 inhibitors) (Lemmens et al., 2019). Thus, the sprouting of quinoa might be considered a useful  
60 approach to improve both its nutritional value and its bread-making attitude.

61 Up to now, the effects of sprouting on technological and sensory properties of quinoa dough  
62 and bread have not been extensively reported. In this context, Park & Morita (2005) studied the effects  
63 of the enrichment level of wheat flour with sprouted quinoa (up to 72 h), at 100g/kg replacement level  
64 only. Starting from the consideration above, the aim of this research was to assess the maximum  
65 enrichment level of sprouted quinoa suitable for achieving good bread-making performance. Once  
66 the optimal replacement level of sprouted quinoa was identified, the bread-making performance of  
67 this ingredient was compared with those of pearled quinoa, in order to assess the potential use of  
68 sprouted quinoa in bread-making.

## 69 **2. Materials and methods**

### 70 *2.1 Materials*

71 Quinoa seeds (*Chenopodium quinoa* Willd. var. *Titicaca*) were provided by Quinoa Marche  
72 s.r.l. (Ancona, Italy), who also carried out the pearling process on the seeds. The untreated seeds (5  
73 kg) were sprouted at lab scale (Memmert GmbH Co. KG, Schwabach, Germany) at 22 °C for 48 h  
74 and dried (Self Cooking Center®, Rational International AG, Mestre, Italy) at 55 °C for 6 h, as  
75 previously reported by Suárez-Estrella (2019). Sprouting time was selected based on preliminary  
76 results: the maximum intensity of the macromolecular modifications can be seen at 48 h of sprouting,  
77 without compromising functionality, in terms of starch gelatinization and foaming capacity and  
78 stability (Suárez-Estrella, 2019).

79 Pearled (PQ) and sprouted (SQ) quinoa seeds were grinded by means of a Cyclotec 1093 (Foss  
80 Sample Mill, Höganäs, Sweden) lab-scale mill, in order to obtain flours with particle size < 250 µm.  
81 Commercial wheat flour (WF; protein: 123 mg/g db; W: 290\*10<sup>-4</sup> J) was provided by Molino Quaglia  
82 S.p.A. (Vighizzolo D'Este, Italy) and it was used alone or in mixture with either PQ or SQ flours. In  
83 particular, three sprouted quinoa:wheat blend ratios were investigated: 10:90 (10SQ), 20:80 (20SQ),  
84 and 30:70 (30SQ). In the second part of this study, the pearled quinoa:WF blend (20:80; 20PQ) was  
85 considered.

### 86 *2.2 Methods*

87 *2.2.1. Pasting properties*

88 Starch pasting properties were investigated by means of the Micro Visco-Amylograph  
89 (Brabender GmbH & Co. KG, Duisburg, Germany) as reported by Elkhailifa et al. (2017) with a  
90 modification (i.e. 3 min of pre-treatment at 30 °C).

91 *2.2.2. Gluten aggregation properties*

92 The aggregation kinetics of gluten protein were studied by using the GlutoPeak device  
93 (Brabender GmbH & Co. KG, Duisburg, Germany). Flour (9 g) was dispersed in distilled water (9  
94 mL), scaling both of them on a 14% sample moisture basis. The test was performed by setting the  
95 paddle speed at 2750 rpm and the circulating water bath at 35 °C.

96 *2.2.3. Mixing properties*

97 Mixing properties were performed by means of the Farinograph-E (Brabender GmbH & Co.  
98 KG, Duisburg, Germany) with a 50 g kneading bowl following the ICC 115/1 Approved Method  
99 (ICC, 1992).

100 *2.2.4. Leavening properties*

101 Dough samples were prepared using commercial baker's yeast (25 g/kg flour; Carrefour®)  
102 and salt (15 g/kg flour; Candor®). The bread-making conditions (i.e. amount of water and mixing  
103 time) were previously determined by means of the farinographic test. Dough samples were prepared  
104 with a lab-scale kneading (Artisan 5KSM150PS KitchenAid, St. Joseph, USA) equipped with a hook.  
105 At the end of mixing, an aliquot of 315 g of the dough was placed in the Rheofermentometer F4  
106 device (Chopin, Tripette & Renaud, Villeneuve La Garenne, France) for 3 h at 30 °C, to measure  
107 dough development and gas production and retention during leavening.

108 *2.2.5. Bread-making*

109 The dough – prepared in the conditions reported in the Section 2.2.4 – was left to rest for 10  
110 min at room temperature (20±1 °C), divided in three portions of 250 g each, molded into cylindrical

111 shapes, placed into baking pans (length: 12.5 cm, width: 7.5 cm; height: 5 cm), and leavened at 30  
112 °C (70% relative humidity) in a climate chamber (Self Cooking Centre®). The time necessary for  
113 leavening varied from 75 to 85 min, until the dough exceeded the top of the pans by 2.5 cm. Samples  
114 were baked at 220 °C for 25 min (Self Cooking Center®), with steam injection for 5 s.

#### 115 2.2.6. *Bread characterization*

116 Bread loaves were analysed 2 h after baking. Specific volume was obtained by the ratio  
117 between the loaf apparent volume, by sesame replacement method, and loaf weight. Crumb softness  
118 was measured according to the Approved Method AACC 74-09.01 (AACC 2001) by using a Texture  
119 Analyzer TA.XT plus C (Stable Micro Systems, Surrey, UK), equipped with a 100 kg\*m/s<sup>2</sup> load cell.  
120 Specifically, an aluminum probe (36 mm Radiused Cylinder Probe) and a test speed of 100 mm/min  
121 were used. Samples were analysed after 2, 24 and 72 h from baking, keeping the loaves in a plastic  
122 bag at room temperature until test.

#### 123 2.2.7. *Electronic tongue assessment*

124 Electronic-tongue (e-tongue) assessment was performed (n=3) on whole breads enriched in  
125 either sprouted or pearled quinoa at 20:80 replacement level, as well as on crusts and crumbs  
126 separately. The breads were freeze-dried (-80 °C for 72 h; Alpha 1-2 LD plus; Deltek s.r.l., Naples,  
127 Italy) and milled in a lab scale mill (IKA M20, Staufen, Germany). Analyses were performed with  
128 the Taste-Sensing System SA 402B (Intelligent Sensor Technology Co. Ltd, Atsugi, Japan) according  
129 to (Marengo et al., 2017a), with some modifications. Briefly, 10 g of samples were suspended in 150  
130 mL of distilled water and centrifuged at 5,000 x g for 10 min at 20 °C. After centrifugation, the  
131 supernatants were tested.

#### 132 2.2.8. *Statistics*

133 All the rheological analyses were carried out in triplicate. As regards bread-making, three  
134 baking tests were performed for each sample and three loaves were obtained from each baking test.

135 Thus, specific volume was replicated nine times while crumb firmness was measured on the three  
136 central slices of each bread obtained from each baking trial.

137 Analysis of variance (one-way ANOVA;  $\alpha=0.05$ ) was assessed by Statgraphics Plus 5.1  
138 (StatPoint Inc., Warrenton, USA) using the samples as factors. The significant differences ( $p\leq 0.05$ )  
139 were determined by using Tukey HSD test. A t-test was applied for comparing sprouted with pearled  
140 samples. Data from e-tongue measurements were elaborated by Principal Component Analysis (PCA)  
141 using MINITAB 14 (v.12.0; Minitab Inc, State College, USA) software package.

## 142 **3. Results**

### 143 *3.1. Effects of enrichment in sprouted quinoa*

#### 144 *3.1.1 Pasting properties*

145 As the level of SQ increased, no significant differences ( $p>0.05$ ) were measured in terms of  
146 pasting temperature ( $62.2\pm 1.2$  °C for WF to  $64.0\pm 0.1$ ,  $64.7\pm 1.5$  and  $63.6\pm 1.2$  BU for 10SQ, 20SQ  
147 and 30SQ, respectively), instead, viscosity during both heating and cooling steps decreased ( $p\leq 0.05$ )  
148 (Figure 1a). Also, breakdown values decreased ( $p\leq 0.05$ ) from  $128\pm 8$  BU for WF to  $93\pm 5$ ,  $71\pm 6$  and  
149  $56\pm 4$  BU for 10SQ, 20SQ and 30SQ, respectively, suggesting increase in heating stability in presence  
150 of quinoa. This behavior is due to the quinoa starch granules, that did not show a sharp peak but a  
151 plateau (Suárez-Estrella, 2019). Moreover, quinoa starch granules might be modified by sprouting,  
152 inducing a lower intensity of gelatinization (Suárez-Estrella, 2019). The decrease in viscosity during  
153 cooling resulted in a decrease ( $p\leq 0.05$ ) in setback values (from  $505\pm 11$  BU for WF to  $393\pm 14$ ,  $326\pm 5$   
154 and  $288\pm 7$  BU for 10SQ, 20SQ and 30SQ, respectively), which seems to be related to starch  
155 retrogradation tendency.

#### 156 *3.1.2 Gluten aggregation properties*

157 The gluten aggregation kinetics of WF was typical of a strong flour with good bread-making  
158 performance that is usually characterized by long aggregation time ( $104\pm 3$  s), high maximum torque  
159 ( $61\pm 0.3$  GPU) and energy (i.e., the area under the curve till 15 s after the maximum torque) values

160 (1480±4 GPE) (Figure 1b). Replacing WF with SQ promoted a significant decrease ( $p\leq 0.05$ ) in  
161 maximum torque (60±0.6, 53±2.3 and 40±1.3 GPU for 10SQ, 20SQ and 30SQ, respectively) and  
162 energy values (1239±25, 1105±34 and 916±16 GPE for 10SQ, 20SQ and 30SQ, respectively). This  
163 trend suggested gluten weakening as the amount of quinoa increased. As regards the time required  
164 for gluten aggregation, the value did not follow a consistent trend. Specifically, the peak maximum  
165 time decreased ( $p\leq 0.05$ ) in 10SQ (96±2 s), did not change ( $p>0.05$ ) in 20SQ blend (102±1 s) and  
166 increased ( $p\leq 0.05$ ) in 30SQ (123±2 s).

### 167 *3.1.3 Dough mixing properties*

168 WF showed a long dough development time and high stability (Figure 1c), which is a common  
169 characteristic for strong flours. Replacing WF up to 200 g/kg significantly increased ( $p\leq 0.05$ ) the  
170 amount of water (from 555±4 for WF to 572±1 and 580±1 g/kg for 10SQ and 20SQ, respectively) to  
171 achieve the optimal dough consistency (i.e., 500 FU). The further increase in SQ did not result in a  
172 significant increase ( $p>0.05$ ) in water absorption (582±2 g/kg for 30SQ). Up to 200 g/kg of the  
173 enrichment level, adding SQ to WF decreased ( $p\leq 0.05$ ) the development time (from 6.8±0.3 for WF  
174 to 6.1±0.1 and 5.6±0.1 min for 10SQ and 20SQ, respectively) needed to reach optimal consistency,  
175 with no further decreasing at 300 g/kg of replacement level (5.5±0.3 min for 30SQ). The same trend  
176 was registered for stability time (23.8±1.2 min for WF to 5.6±0.2, 3.5±0.1 and 3.3±0.2 min for 10SQ,  
177 20SQ and 30SQ, respectively), whose decrease was in agreement with the increase ( $p\leq 0.05$ ) in the  
178 degree of softening, that varied from 17±2 FU for WF to 93±4, 132±5 and 152±3 min for 10SQ,  
179 20SQ and 30SQ, respectively.

### 180 *3.1.4 Dough leavening properties*

181 At the beginning of the leavening phase (up to 1 h), the sprouted quinoa-enriched dough  
182 exhibited a rapid dough development, regardless of the quinoa enrichment (Figure 1d). Replacing  
183 WF up to 200 g/kg level led to an increase ( $p\leq 0.05$ ) in dough development from 51±1 to 60±1 mm,  
184 respectively. Instead, higher replacement level (i.e., 30:70) increased ( $p\leq 0.05$ ) this index up to 56±1



185 mm. Moreover, both WF and 10SQ dough samples required longer ( $p>0.05$ ) leavening time (~ 2 h  
186 and 20 min) to reach the maximum dough height, compared to 20SQ and 30SQ samples (~ 1 h and  
187 50 min). After 2 h of leavening, either dough with 20SQ or 30SQ were not able to hold gas inside the  
188 dough, resulting in a decrease ( $p\leq 0.05$ ) in dough height, as a consequence of the weakening of the  
189 gluten network. Dough weakening was less dramatic in 10SQ and therefore no loss in maximum  
190 height was detected up to 2.5 h of leavening. Finally, gas production increased ( $p\leq 0.05$ ) from  
191  $1250\pm 54$  mL for WF to  $1426\pm 26$ ,  $1469\pm 10$  and  $1464\pm 9$  mL for 10SQ, 20SQ and 30SQ, respectively,  
192 whereas the dough retention capacity slightly decreased ( $p\leq 0.05$ ) in presence of SQ, with no  
193 significant differences ( $p>0.05$ ) according to the enrichment level ( $93\pm 1\%$  for WF to  $90\pm 1$ ,  $90\pm 1$  and  
194  $88\pm 1\%$  for 10SQ, 20SQ and 30SQ, respectively).

### 195 *3.1.5 Bread characteristics*

196 Replacing WF with SQ did not cause negative effects on bread-making properties, except for  
197 10SQ sample. Indeed, at this replacement level, the resulting bread was characterized by the lowest  
198 specific volume and the highest crumb firmness ( $p\leq 0.05$ ) (Figure 2). 20SQ showed the best baking  
199 performance in terms of specific volume, whose value were even higher ( $p\leq 0.05$ ) compared with WF  
200 and 30SQ loaves (Figure 2).

201 Unlike 10SQ bread, high replacement levels (20SQ and 30SQ) significantly decreased  
202 ( $p\leq 0.05$ ) crumb firmness, contributing to high crumb softness, not only in fresh bread (Figure 2) but  
203 also during storage (up to 72 h; data not shown).

### 204 *3.2. Comparison between sprouted and pearled quinoa*

205 Compared to using PQ, the blend enriched in SQ was characterized by a higher water  
206 absorption (~3%), shorter development time (-20%), lower stability (-49%), and higher degree of  
207 softening (76%) ( $p\leq 0.05$ ) (Table 1).

208 As regards dough performance during leavening, 20SQ dough showed a higher ( $p\leq 0.05$ )  
209 maximum dough height (~22%) and retention capacity coefficient (~15%) than 20PQ dough (Table

210 1). In addition, the best leavening performance accounted for the highest specific volume of SQ-  
211 enriched bread ( $3.61\pm 0.11$  vs  $2.60\pm 0.10$  mL/g for SQ and PQ, respectively). Moreover, the presence  
212 of SQ improved ( $p\leq 0.05$ ) also crumb softness not only of fresh bread (2 h after baking) but also during  
213 storage (up to 72 h), compared to PQ-enriched bread (Figure 3).

214 The sensory traits of quinoa-enriched bread obtained from e-tongue measurement and  
215 elaborated through the Principal Component Analysis (PCA) are shown in Figure 4. The two main  
216 components accounted for 81.5% of the total variance. As shown in the score plot (Figure 4a),  
217 samples were clearly discriminated on PC1 (48.9% of the total variance) based on the treatment  
218 applied to seeds before milling (pearling or sprouting). In fact, the sprouted samples (S) were located  
219 on the right side (positive) of PC1. On the contrary, samples with pearled quinoa (P) were located on  
220 the left side (negative) of PC1. PC2 discriminated the samples (32.6% of the total of the variance)  
221 according to the assessed bread sections (whole bread, crumb, or crust). In particular, whole bread  
222 (W) as well as crumb (C) were located on the upper (positive), without great differences between  
223 them. Indeed, crumb represents more than 90% of the whole bread (data not shown). Whereas, bread  
224 crust (O) was located on the lower (negative) of PC2.

#### 225 **4. Discussion**

226 The effects of replacing wheat flour with quinoa on dough and bread properties have been  
227 shown in previous studies (Chauhan, Zillman, and Eskin, 1992; Lorenz and Coulter, 1991). Briefly,  
228 when quinoa is blended with wheat, the dough water absorption and mixing tolerance index (or degree  
229 of softening) increased, whereas dough development time and loaf volume decreased (Chauhan et  
230 al., 1992; Lorenz and Coulter, 1991). At the same time, a worsening in crumb softness and overall  
231 acceptability have been reported (Lorenz and Coulter, 1991). To the best of our knowledge, most of  
232 the studies have been carried out on pearled quinoa, since pearling has been shown to improve product  
233 acceptability by decreasing the amount of saponins (Gómez-Caravaca et al., 2014). Beside pearling,  
234 sprouting has been shown to enhance the sensory profile of grains mainly due to the production of

235 simple sugars (Heiniö et al., 2001). However, till now, the effects of sprouting on quinoa acceptability  
236 have not been yet addressed. On the other hand, from a technological standpoint, sprouted quinoa  
237 showed enhanced functional properties (i.e., increased foam stability, decreased retrogradation  
238 degree) encouraging its use as an ingredient in bread-making (Suárez-Estrella, 2019).

239 The impact of sprouted quinoa was assessed in bread-making in light of results previously  
240 reported in this study. Specifically, sprouted quinoa was added to wheat at different enrichment levels  
241 (i.e., 10:90, 20:80, and 30:70, sprouted quinoa:wheat ratio).

242 The first part of the study focused on starch and protein functionality of sprouted quinoa  
243 blends. Understanding the effect on starch is important because this component is responsible for  
244 bread staling. Instead, gluten properties are important because gluten plays a key role in leavened  
245 products by retaining the gas produced during fermentation. The pasting profile of quinoa blends  
246 suggested a gradual loss of the ability to gelatinize and retrograde up to 20:80 substitution level  
247 (Figure 1a). Changes in starch properties could be due to various factors: (1) the dilution effect, since  
248 the starch content in sprouted quinoa is lower than in wheat (Suárez-Estrella, 2019); (2) presence of  
249 fiber that restricts starch swelling during the initial stages of gelatinization (Collar et al., 2009); (3)  
250 starch hydrolysis by the amylases developed during sprouting, and formation of small glucose  
251 polymers that are less prompted to absorb water and gelatinize (Suárez-Estrella, 2019). The lower  
252 retrogradation tendency of 20SQ and 30SQ blends might account for the decrease in bread staling  
253 and the preservation of crumb softness even during storage (Figure 2b). A similar effect has been  
254 shown in wheat bread (Cardone et al., 2020a,b; Marti et al., 2018, 2017).

255 Regarding proteins, gluten protein aggregation in different conditions of hydration and shear  
256 stress, namely in slurry (i.e. GlutoPeak test) and in dough (i.e. Farinograph test) systems, was  
257 addressed. The former measures the gluten aggregation kinetic which is solely affected by gluten  
258 quality (Goldstein et al., 2010); the latter measures the dough formation which is affected by other  
259 components, including starch and fiber (Ahmed et al., 2013; Soh et al., 2006). Replacing WF, up to  
260 20:80 replacing level, seems to have only a partial effect on gluten aggregation behavior, mainly

261 affecting maximum torque rather than peak maximum time (Figure 1b). Since the maximum torque  
262 is correlated to gluten content (Marti et al., 2015a), its decrease upon quinoa enrichment might be  
263 related to gluten dilution. Similar trends have already observed in previous studies where flours high  
264 in fiber and low in gluten-forming proteins were added to wheat (Marti et al., 2015b). Increasing the  
265 amount up to 30:70 substitution level, the maximum torque decreased while the peak maximum time  
266 increased (Figure 1b), resulting in a decrease in the aggregation energy, and suggesting an extensive  
267 gluten weakening, unsuitable for bread-making. Indeed, usually flours for bread-making exhibit faster  
268 gluten formations and higher peaks compared to those for cookies or cakes (Lu and Seetharaman,  
269 2014). Regardless the enrichment level, the GlutoPeak profile of quinoa-enriched flours showed a  
270 sharper peak compared to WF profile (Figure 1b), suggesting low resistance to intense shear stresses.

271 Findings on gluten weakening were confirmed on the dough system by using the farinograph  
272 test. Specifically, the worsening of dough mixing properties were evaluated by the decrease in  
273 stability and the increase in softening degree (Figure 1c). Gluten dilution, together with fiber  
274 enrichment, might account for such modification at high levels of quinoa enrichment (20:80 and  
275 30:70). Moreover, the increasing replacement level caused an increase in water absorption, likely due  
276 to the higher fiber content present in the quinoa flour. It is well known the great ability of fibers to  
277 bound a high amount of water leading to a higher water absorption index, thanks to the presence of  
278 its large number of hydroxyl groups able to establish interactions with water through hydrogen bonds  
279 (Sudha et al., 2007). However, the water absorption of 30SQ dough was not different from the value  
280 of 20SQ. Our results partially confirmed previous study of Park et al. (2005) who reported that  
281 replacing 100 g/kg of wheat with 48 h sprouted quinoa did not result in any modification of the dough  
282 development time, while it caused an increase in the water absorption and a decrease in the stability  
283 indices. Differences in sprouting conditions (i.e., temperature, relative humidity) and grain variety  
284 might account for different results. The gluten dilution in SQ samples affects also the dough capacity  
285 to maintain its shape during proofing (Figure 2a). However, by carefully following the results  
286 provided by the farinographic test (i.e., water absorption, mixing time) (Figure 1c,d), the production

287 of wheat bread enriched in sprouted quinoa was possible even at the highest replacement level  
288 (30:70). The best result in terms of specific volume was obtained by using 20SQ (Figure 2), in  
289 agreement with the results on dough properties during both mixing and leavening (Figure 1c,d).  
290 Dough development increased, as well as the leavening rate, likely due to the higher presence of  
291 simple sugars in sprouted quinoa (Suárez-Estrella, 2019), usable by the yeasts for CO<sub>2</sub> production.  
292 Indeed, the presence of sprouted quinoa also led to high gas production during leavening, in  
293 agreement with bread volume (Figure 2). The high bread volume might account for the crumb  
294 softness of sprouted quinoa-enriched bread (Figure 2b). The positive effect of sprouted quinoa on  
295 bread features was evident only at high enrichment levels (20SQ and 30SQ).

296 Taking into consideration both the dough and bread features, results showed that the 20SQ  
297 blend is the most suitable for bread-making. For this reason, the second part of the study focused on  
298 the comparison between sprouting and pearling as pre-processing for producing quinoa-enriched  
299 bread.

300 Despite the dilution of gluten proteins, the enrichment in sprouting quinoa was associated with  
301 the best leavening properties, in terms of dough development and gas production and retention, in  
302 comparison with pearled quinoa (Table 1). As stated above, the best dough leavening performance in  
303 sprouted quinoa was due to the higher sugar content (Suárez-Estrella, 2019). Specifically, using  
304 sprouted quinoa improved bread volume and crumb softness in both fresh (2h after baking) and stored  
305 (upon 72h) bread (Figure 3), thanks to the increased  $\alpha$ -amylase activity during sprouting. The positive  
306 effects of  $\alpha$ -amylase activity in bread-making have already been reported (Goesaert et al., 2009; De  
307 Leyn, 2006).

308 Sprouting should be preferred to pearling also in relation to the sensory properties, as assessed  
309 by electronic-tongue (Figure 4). The loading plot (Figure 4a) evidenced the tendency of bread made  
310 with sprouted quinoa to umami, richness, sourness and astringency and bitterness aftertastes; while,  
311 pearled quinoa samples were located on the left side of PC1, in correspondence of saltiness, bitterness

312 and astringency. The location of sprouted samples at the opposite side of bitterness is an indicative  
313 of the suitability of sprouting process to decreasing the bitter perception of quinoa enriched bread.

## 314 **5. Conclusions**

315 Using sprouted quinoa at 20:80 replacement level in wheat formulation, it was possible to  
316 produce enriched bread with high specific volume, keeping low the crumb firmness even during  
317 storage (up to 72 h). Therefore, sprouting could be a suitable strategy for producing quinoa-enriched  
318 bread in order to increase the production and consumption of fiber-rich products, together with  
319 proteins characterized by high biological value.

320 In addition, comparing sprouting to pearling, which is the process actually used for enhancing  
321 the sensory acceptability of quinoa seeds and flours, results on both dough and bread clearly showed  
322 that sprouting was more effective in improving bread properties (i.e. specific volume and crumb  
323 softness) as well as decreasing bread bitterness. Thus, although pearling is - nowadays - the main pre-  
324 treatment of quinoa to decrease its bitter taste, sprouting might represent a valid alternative to this  
325 process to increase the use of quinoa in bread and other baked products. Moreover, sprouting is a  
326 quite simple process, requiring non technologically-advanced plants and easily transferable in low-  
327 income countries, as the world main producers are. Finally, the effect of sprouting on the actual  
328 saponins content – the main cause of quinoa bitterness – is worthy of interest. The effects related to  
329 the instrumental sensory properties of samples bode well.

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336 The authors declare that there is no conflict of interest.

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## Figure captions

**Figure 1.** Pasting (a), gluten aggregation (b), mixing (c) and leavening (d) properties of wheat (WF; solid line), and with increasing replacement level of sprouted quinoa (10SQ: dotted line; 20SQ: dash line; 30SQ: dash-dotted line). 10SQ, blend composed by sprouted quinoa and wheat flour at 10:90 ratio; 20SQ, blend composed by sprouted quinoa and wheat flour at 20:80 ratio; 30SQ, blend composed by sprouted quinoa and wheat flour at 30:70 ratio.

**Figure 2.** Specific volume and crumb firmness (2 h after baking) of bread from wheat flour (WF) and with increasing replacement level of sprouted quinoa. 10SQ, blend composed by sprouted quinoa and wheat flour at 10:90 ratio; 20SQ, blend composed by sprouted quinoa and wheat flour at 20:80 ratio; 30SQ, blend composed by sprouted quinoa and wheat flour at 30:70 ratio.

Different letters in the same row indicate a statistically significant difference among samples (Tukey test HSD;  $p \leq 0.05$ ).

**Figure 3.** Crumb firmness of wheat bread enriched in sprouted (triangle) or pearled (circle) quinoa. The asterisks indicate a statistically significant difference between the mean values (t-Test;  $***p \leq 0.001$ ).

**Figure 4.** Score plot (a) and loading plot (b) from e-tongue PCA of bread with pearled (circle) or sprouted (diamond) quinoa. P: Pearled; S: Sprouted; W: whole bread; C: crumb; O: crust. Aftertaste-A: aftertaste-astringency; Aftertaste-B: aftertaste-bitterness

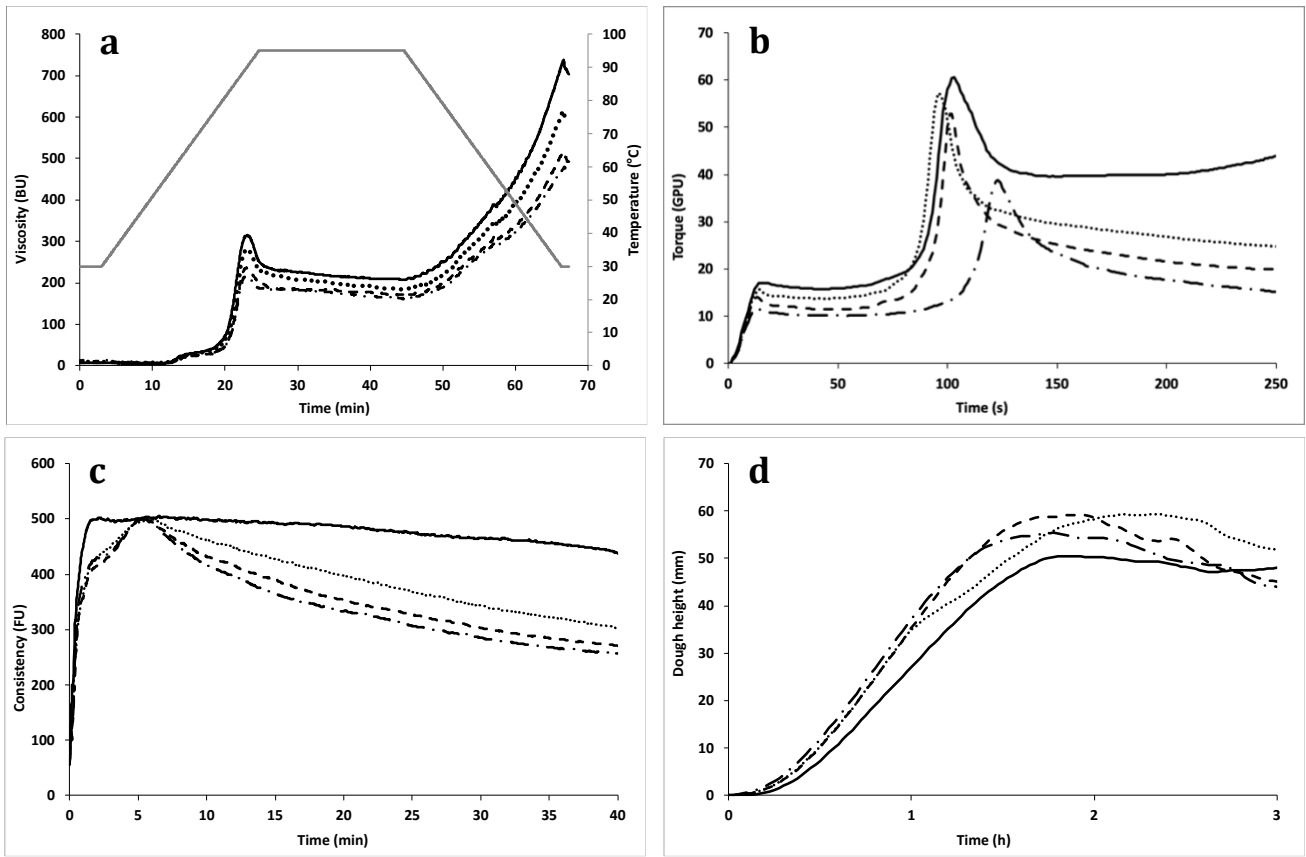



Figure 1.

	WF	10SQ	20SQ	30SQ
				
Specific volume (mL/g)	3.37±0.11 <sup>b</sup>	3.18±0.04 <sup>a</sup>	3.61±0.11 <sup>c</sup>	3.38±0.10 <sup>b</sup>
Crumb firmness (kg*m/s <sup>2</sup> )	9.4±1.0 <sup>c</sup>	8.8±0.9 <sup>c</sup>	5.6±0.7 <sup>a</sup>	6.8±0.9 <sup>b</sup>

**Figure 2.**

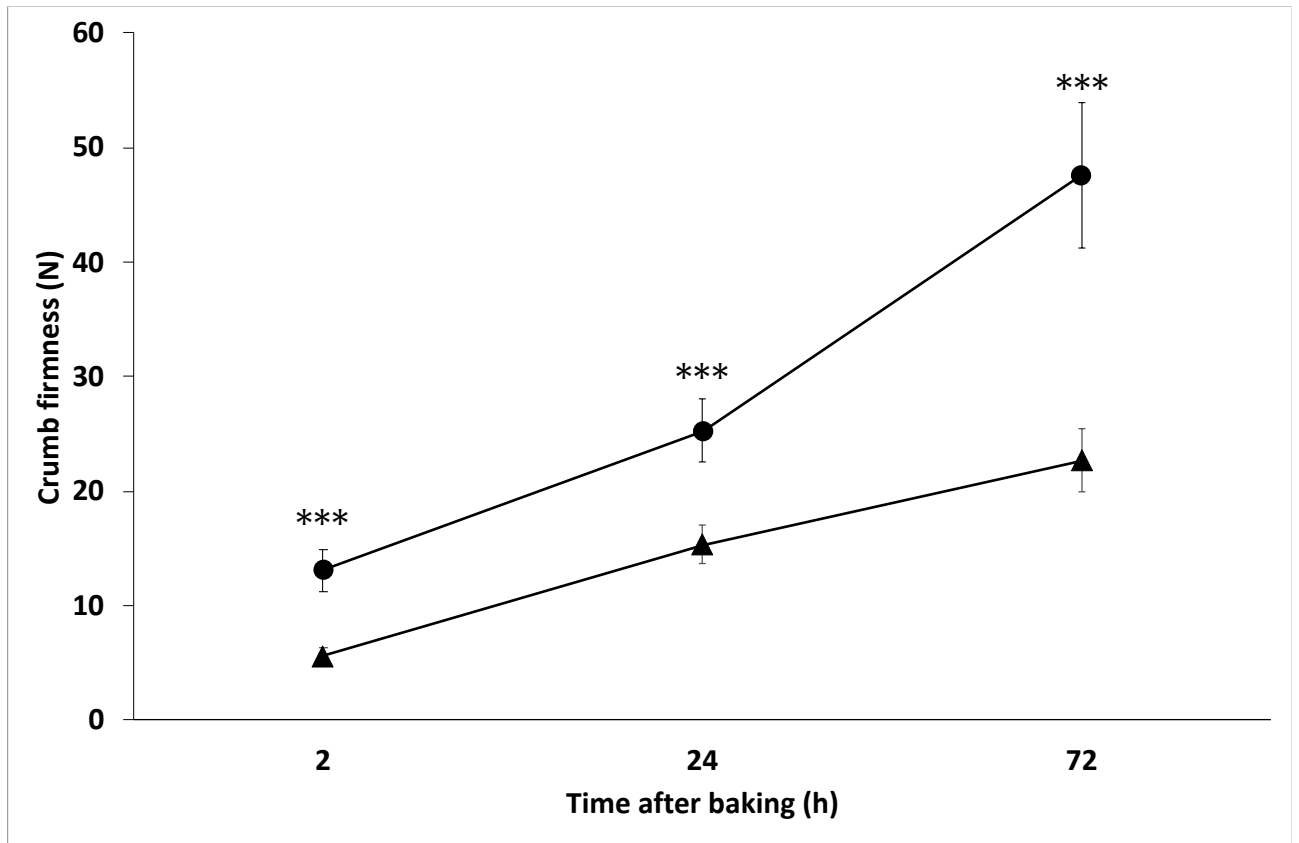


Figure 3.

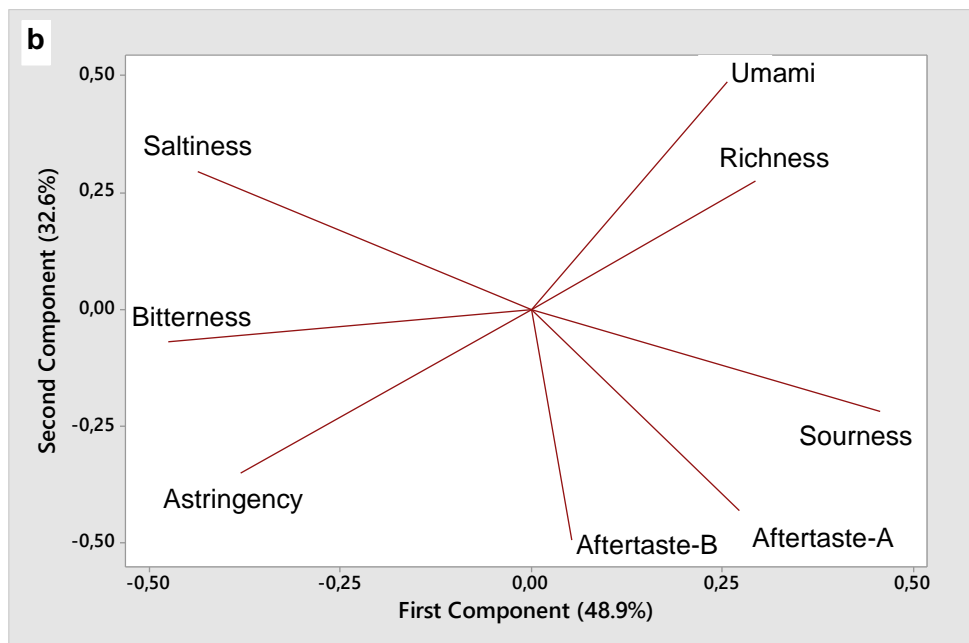
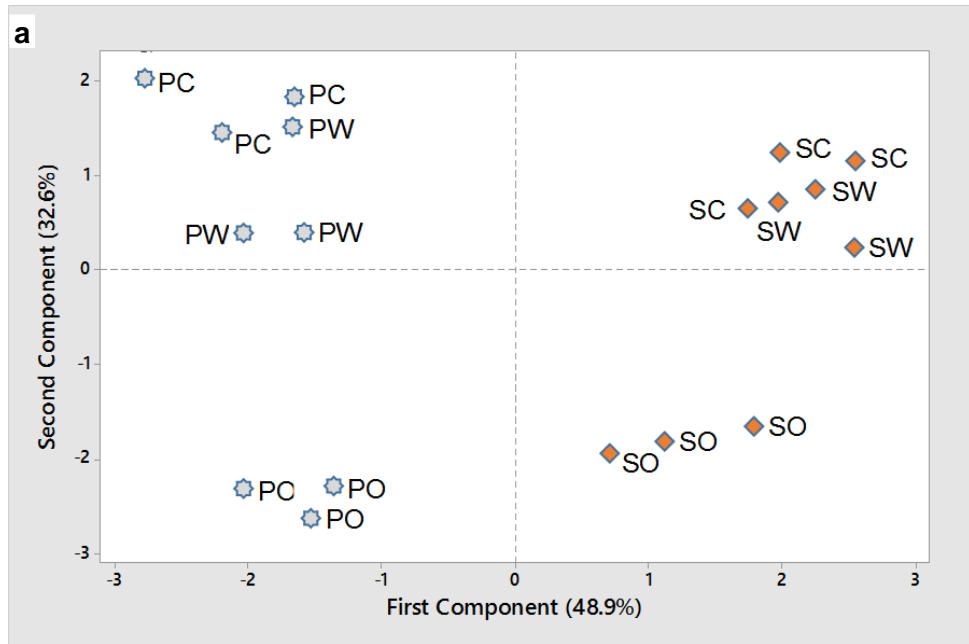


Figure 4.

**Table 1.** Mixing and leavening properties of enriched dough enriched in sprouted or pearled quinoa.

		20SQ	20PQ
Mixing properties	Water absorption (g/kg)	580±1	563±1***
	Development Time (min)	5.6±0.1	7.0±0.1***
	Stability (min)	3.5±0.1	6.8±0.7**
	Degree of Softening (FU)	132±5	75±5***
Leavening properties	Maximum dough height (mm)	60±1	49±2***
	Maximum height time (h)	1.9±0.1	2.0±0.2 n.s.
	Porosity time (h)	1.4±0.1	1.0±0.1***
	Total CO <sub>2</sub> (mL)	1469±10	1900±20***
	CO <sub>2</sub> retained (mL)	1315±9	1475±15***
	CO <sub>2</sub> released (mL)	153±1	424±13***
	CO <sub>2</sub> retention coefficient (%)	90±1	78±1***

20SQ, blend composed by sprouted quinoa and wheat flour at 20:80 ratio; 20PQ, blend composed by pearled quinoa and wheat flour at 20:80 ratio

The asterisks indicate significant differences between the mean values of the sprouted and pearled quinoa samples (\*\*\* $p \leq 0.001$ ; t-Test). n.s. indicates no statistical difference.

## Sprouting as a pre-processing for producing quinoa-enriched bread

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### Highlights:

- Enrichment of wheat bread with sprouted quinoa
- Using sprouted quinoa improved dough leavening properties and bread features
- Using 20% sprouted quinoa led to the highest bread volume
- Sprouting has to be preferred to pearling in quinoa bread-making



