Effect of sprouting on nutritional quality of pulses

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ABSTRACT

The nutritional quality of chickpeas and green peas as affected by industrial-scale sprouting was investigated, analysing the ultrastructure, chemical composition, antioxidant capacity, starch digestibility, and mineral content and accessibility of pulses before and after cooking. Sprouting did not deeply affect raw seed structure, although after cooking starch granules appeared more porous and swelled. Compared to unsprouted seeds, raw sprouted ones displayed higher protein (+10%, p <0.05) and total sugar content (+90% in peas, p <0.05), except for raffinose. After sprouting and cooking phytic acid amounts (≥ -35%, p <0.01) and antioxidant capacity (≥56%) decreased in both pulses, but no changes in starch digestibility and mineral accessibility were observed in chickpeas. In conclusion, sprouting on an industrial-scale induced mild structural modifications in chickpeas and peas, sufficient to reduce the antinutritional factors, without strongly affecting their nutritional quality. These products could represent an interesting nutritional tool for different dietary patterns as well as for enriched cereal-based foods.

Keywords: sprouting; pulses; mineral accessibility; starch digestibility; antioxidant capacity; nutritional composition.

1: Introduction

Pulses have been long known for their nutritional and health-promoting properties, being a good source of fibre, proteins, antioxidant compounds - including phenolic acids, polyphenols, and flavonoids- and having a low-glycaemic index (Hall et al. 2017). Because of these properties, pulses are considered an excellent way to satisfy the needs of emerging diets such as vegetarian, vegan, or gluten-free ones, accounting for the growing interest in this food category. Despite that, the consumption of pulses is still underexploited by the Western consumers, due to the presence of antinutrients, such as phytic acid and trypsin inhibitors, and raffinose, belonging to undigested oligosaccharides family, responsible for the decrease in nutritional value and digestive discomfort, respectively (Hall et al. 2017). Last, but not least, the presence of off-flavours discourages the consumption of pulses (Roland et al. 2017). Considering these aspects and following the scientific evidence to recommend an increased consumption of pulses to improve health, several processes – including soaking, dehulling, cooking, extrusion, cooking and fermentation - have been applied to pulses (Patterson et al. 2017). Among those technological processes, sprouting (or germination) is continuing to gain traction in the marketplace and represents a re-emerging trend in healthy foods, thanks to the positive effects on the enhancement of the nutritional properties (Ghavidel and Prakesh 2007) and taste (Roland et al. 2017).

Traditionally, the germination process has been performed at the household level. The basic process consists of steeping grains in water until they reach the moisture content needed to initiate the seedling. After the steeping water is drained, the seeds are allowed to germinate. Some challenges – including the safety risk and the process reproducibility - need to be overcome in order to carry out this process at an industrial scale and deliver a safe product with consistent features. In this context, a germination plant has been developed by Buhler AG (Uzwil, Switzerland), in which grains are partially germinated under controlled conditions (i.e. temperature and relative humidity) and stabilised through drying with hot air to extend the product shelf-life. The control of the process seems the only way to balance the nutritional and sensory improvements with the maintenance of

flour performance and to ensure consistent functionality to the product (Marti et al., 2018). Recently, partial germination has been carried out on chickpeas and the resulting flour has been proposed as an interesting ingredient for the production of enriched cereal-based foods with improved rheological characteristics (Marengo et al. 2017). However, the nutritional qualities of sprouted pulses have not yet been explored.

Therefore, the present study aims at understanding the impact of sprouting – carried out at an industrial-scale level and under controlled conditions – on the nutritional profile of chickpeas and green peas. To this aim, chemical composition, microscopy features, antioxidant capacity, starch digestibility, total content (Ca, Mg, Fe, Zn and P) and accessibility of minerals (Ca and Mg) were assessed in both unsprouted and sprouted pulses, also taking into consideration the effect of cooking on the nutritional traits.

2: Experimental

2.1 Chemicals and reagents

Cellulose (powder from spruce), ABTS [2,2-azino-bis(3-ethylbenzothiazoline- 6-sulfonic acid) diammonium salt], Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid), potassium persulfate (dipotassiumperoxydisulfate), ethanol at analytical grade, enzymes, bile salts and phytic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and chemicals from Merck KGaA (Darmstadt, Germany). Milli Q water was used throughout the experiments.

2.2 Materials

Chickpea and green peas were provided by Molino Quaglia (Vighizzolo d'Este, Italy). Samples were soaked in water in 1:2 proportion (w/v) for 24h and then germinated - in darkness for 3 days, at 22 °C and 90% relative humidity- in an industrial sprouting plant (Bühler AG, Uzwil, Switzerland). After sprouting, grains were dried at 50 °C for 10 h until reaching a final moisture of about 8%, and stored at room temperature.

Experimental plan is summarized in supplemental materials (Figure S1). An aliquot of unsprouted and sprouted pulses was grinded into powder (henceforth "raw"; < 0.5 mm particle size) in a pin

86 mill (Bühler AG, Uzwil, Switzerland) and used as such for the evaluation of chemical composition,
87 phytic acid, mineral content and antioxidant capacity. Moreover, grinded pulses (40g) were used to
88 prepare a porridge by cooking in boiling water (200mL) for 3 min, to evaluate the starch
89 digestibility.
90 Intact seeds were also processed to simulate domestic preparation. Briefly, pulses were soaked in
91 excess of plain tap water, for 12 h at room temperature (henceforth "soaked"). After draining,
92 samples were cooked in plain tap boiling water for 45 min in 1:3 w/w ratio (henceforth "cooked").
93 Samples were cooked in duplicate, and final samples were combined and used for analysis. Both
94 soaked and cooked samples were used as such for light microscopy or homogenised (7011S-Waring
95 Blender Commercial, Torrington, CT, USA) for further analyses.
96 ***2.3 Light Microscopy***
97 Soaked and cooked intact seeds were sectioned with a vibratome in 20-30 μm thick sections. These
98 were stained with 0.1% water solution of toluidine blue for 1 min, or Lugol's iodine for 5 min. Then,
99 the samples were observed by an Olympus BX50 light microscope (Olympus, Tokyo, Japan)
100 equipped with Nomarski differential interference contrast.
101 ***2.4 Chemical Composition***
102 Chemical composition was performed on the unsprouted and sprouted raw pulses. Moisture,
103 starch, protein, lipid and ash content was assessed by AACC standard methods (44-15.02, 76-13.01,
104 46-12.01, 30-10.01, and 08-01.01, respectively) (AACC 2001). Sugars were evaluated by HPLC Anion
105 Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) (Englyst et al.
106 2000). Total, soluble and insoluble dietary fibre content was evaluated by the enzymatic–
107 gravimetric procedure (AOAC 1995). The analysis of phytic acid was performed by HPLC with
108 spectrophotometric detection as previously described on raw and cooked pulses (Erba et al. 2017).
109 Moisture, starch, protein, lipid, ash and phytic acid contents were determined in triplicate and
110 expressed as g kg^{-1} on the dry weight (d.w.).
111 ***2.5 Total Mineral Content and Ca and Mg Accessibilities***

112 Total contents of Ca, Mg, Fe, Zn and P were determined on raw and cooked samples (both sprouted
113 and unsprouted pulses) and, after an enzymatic digestion of cooked samples, on the supernatant
114 solutions containing the soluble mineral fraction. To investigate the uptake of minerals from tap
115 water during the domestic treatments, mineral levels were measured in tap water by atomic
116 absorption spectroscopy. For total mineral analysis, samples were dry-ashed (550 °C, overnight)
117 and dissolved with an acid solution, as previously reported (Erba et al. 2011). For Ca and Mg
118 accessibility, cooked (both sprouted and unsprouted) samples were subjected to two enzymatic
119 digestion stages, gastric and intestinal, to simulate the human gastro-intestinal system and minerals
120 in the soluble fractions were analysed (Erba et al. 2017). Mineral concentrations were determined by
121 Atomic Absorption Spectroscopy (AAnalyst800 Perkin Elmer, Waltham, MA, USA), against
122 standard curves (Ca: 0.5-4 mgL^{-1}; Mg: 0.1-0.4 mgL^{-1}; Fe: 1-4 mgL^{-1}; Zn: 0.1-0.8 mgL^{-1}), using
123 lanthanum solution to avoid interferences for Ca and Mg analyses (1 g kg^{-1} w/v). Phosphorous (P)
124 was determined by a colorimetric method (Erba et al. 2011). The accuracy of mineral analyses was
125 checked by using certified values of reference material (NCS ZC 85006). All analyses were
126 performed in triplicate and results were expressed as total mineral content (mg kg^{-1} d.w., mean ±
127 SD) and accessible Ca and Mg were expressed as percentage of soluble Ca and Mg with respect to
128 total content.

129 ***2.6 In vitro Starch Digestibility***

130 The *in vitro* starch digestibility was assessed on cooked pulses (as seeds or porridge), according to
131 Englyst's method (Englyst et al. 2000). Rapidly (RDS) and slowly (SDS) digestible as well as total
132 starch fractions were calculated from the glucose released data (at 20 and 120 min of hydrolysis,
133 respectively), determined by HPLC. Six sets of data from three independent cooking trials were
134 averaged. RDS and SDS fractions were expressed as the percentage of available starch (AVST=RDS
135 + SDS).

136 ***2.7 Determination of Total Antioxidant Capacity***

137 The total antioxidant capacity (TAC) of raw, soaked and cooked samples, was determined with a
138 direct measurement according to Açaret al. (2009) without any sample preparation. If required,
139 samples were diluted with cellulose powder, which was found to be inert toward ABTS reagent
140 (Serpen et al. 2007). The total antioxidant capacity was expressed as mmol of Trolox equivalent
141 antioxidant capacity (TEAC) per kg of dry sample, by means of an at least five points dose-response
142 curve. The analyses were repeated six times and data were presented as mean ± SD.

143 ***2.8 Statistical Analysis***

144 Analysis of variance (ANOVA) was performed utilising Statgraphics XV version 15.1.02 (StatPoint
145 Inc., Warrenton, VA, USA) to assess the effects of sprouting and processing; differences between
146 means were further evaluated by Tukey's post hoc test (p <0.05). The effect of sprouting on the
147 accessibility of Ca and Mg in cooked pulses and on the total antioxidant capacity was analysed by
148 Student's t-test (p <0.05).

149 **3 Results and Discussion**

150 ***3.1 Microscopy Features***

151 Figure 1 shows the effect of sprouting on the microstructure of the whole pulses – soaked and
152 cooked - captured under polarised light conditions. In order to highlight the cell walls, samples
153 were stained with a solution of Toluidine blue, which is a generic dye for plant tissues.
154 The arrangement of the cells in the seed tissue was clearly visible in all the samples, regardless of
155 the applied process. The sprouting conditions adopted in the present study did not dramatically
156 affect the seed structure of either pea (Figure 1b) or chickpea (Figure 1f), when compared with the
157 unsprouted pulses (Figure 1a and 1e for peas and chickpeas, respectively). The cell walls were
158 visible even after cooking in both unsprouted (Figure 1c for peas and Figure 1g for chickpeas) and
159 sprouted pulses (Figure 1d for peas and Figure 1h for chickpeas), appearing as grey lines. This
160 observation suggests that they had and maintained a high degree of ordered structure (Brummer et
161 al. 2015). Interestingly, in sprouting seeds, cells appeared slightly more stained by Toluidine,
162 indicating the presence of mobilised proteins (more accessible to the stain).

163 The majority of the interior of the cells was occupied by starch granules, which in Figure 2 appear
164 in dark as samples were stained with Lugol. The surface of the granules was generally smooth
165 without any fissures, cracks or pores. A granule size between 2.72 μm and 31.8 μm was observed
166 for pea starches (Aggarwal et al. 2004). The large oval to small spherical shaped granules were
167 reported to be present in starch from different chickpea cultivars (Brummer et al 2015).
168 Starch granules of sprouted pulses (Figure 2b, d, f and h) appeared darker compared to the related
169 unsprouted samples (Figure 2a, c, e and g) suggesting the formation of a more porous structure
170 after sprouting. Cooking of whole pulses in boiling water resulted in the swelling of starch
171 granules, but not in the disruption of the cell walls or the starch granules, consistently with other
172 studies (Brummer et al. 2015).
173 ***3.2 Chemical Composition***
174 The partial germination process did not promote significant modifications in pulses regarding
175 starch, lipid and fibre content, while protein significantly increased after sprouting (Table 1). Other
176 studies reported an increase in protein in germinated grains, that has been attributed to the
177 synthesis of enzymes (for example, proteases) during germination (Masood et al. 2014) or to the
178 compositional changes following the degradation of other constituents (Bau et al. 1997).
179 In sprouted samples, the content of fibre did not change, whereas many studies have shown that
180 the germination process has a significant impact on dietary fibre fractions in pulses (Martin-
181 Cabrejas et al. 2003; Masoodet al. 2014; Duenas et al. 2016). Differences among studies are likely
182 related to the type of legume (chickpeas, beans, etc.) and the germination conditions (e.g. sprouting
183 time, temperature and relative humidity, light/darkness conditions, drying temperature)(Martín-
184 Cabrejas et al. 2003).
185 In chickpeas, the total amount of free sugars did not change, whereas a significant increase ($p<0.05$)
186 was found in peas. As regards the qualitative distribution of sugars (Figure 3), we evidenced an
187 increase in sucrose and the disappearance of raffinose after the sprouting of both pulses. Soaking
188 and sprouting are the most efficient biological treatments for removing α-galactosides,

189 oligosaccharides derived from sucrose, containing 1–3 units of galactose linked by α-1,6 linkages.
190 These oligosaccharides, commonly present in legumes and rapidly fermented by the human colonic
191 microbiota, are responsible for flatulence in individuals that do not consume pulses on a regular
192 basis (Hall et al. 2017). The increase in the relative percentage of sucrose in the sprouted products
193 was likely the result of the compositional changes following the degradation of raffinose, probably
194 attributable to the endogenous or microbial enzymatic activities developed during sprouting (Cai et
195 al. 1997; Mäkinen and Arendt 2015). The sucrose increase in flour from germinated chickpeas
196 improved the leavening properties of dough enriched with sprouted pulses (Marengo et al. 2017)
197 and may positively contribute to the sensorial characteristics of such products.
198 Germination is reported as the more effective process for reducing phytic acid in pulses (Patterson
199 et al. 2017). This compound is an antinutritional factor with a marked chelating ability - in
200 particular for calcium – and it is linked to the inhibition of digestive enzymes, such as protease, α-
201 amylases and trypsin. In this study, myo-inositol hexaphosphate (IP6) was the only inositol
202 phosphate found. As observed in previous studies (Egli et al. 2002), the content of phytic acid in
203 chickpeas, after germination, significantly decreased by ~5% (19 ± 0.0 vs 18 ± 0.0, $p<0.05$) (Table 2).
204 Such effect was probably related to the activation of endogenous phytase (Egli et al. 2002),
205 occurring during the initial period of germination.
206 In cooked seeds, a high and significant ($p< 0.01$) reduction in IP6 levels was assessed in both
207 germinated pulses, accounting for more than 30% reduction in comparison with unsprouted ones.
208 The decrease in IP6 suggesting a potential leaching of the phytate into the soaking and/or cooking
209 water, owing to its water solubility. Compared to unsprouted pulses, the leaching effect was more
210 pronounced in the sprouted ones, likely due to their porous structure (Figures 1b, d, f and h).
211 ***3.3 Total Mineral Contents and Ca and Mg Accessibility***
212 Total mineral content was influenced by both the type of pulse (chickpeas or green peas) and the
213 treatments (sprouting and cooking)(Table 2). Cooking led to a more than two-fold increase in Ca
214 levels in both legumes, independently by the sprouting process ($p <0.01$), likely as a result of the Ca-

215 uptake from the tap water (whose Ca level was 72.5 mgL^{-1}) and a consistent increase of legume
216 weight of about 2.5-fold. Tap water was used for cooking pulses because it represents the most
217 common domestic practice, even though its influence on mineral content of cooked seed was
218 predictable. Notwithstanding, the Ca level of tap water used in this study is consistent with the
219 mean level in Italy (62.3 mgL^{-1}, Dinelli et al. 2012), thus our results do not refer to a singular
220 condition. Differently, Mg levels in the unsprouted samples - raw and cooked - were similar, even
221 though the Mg content in tap water (12 mgL^{-1}). In sprouted pulses, cooking significantly decreased
222 the Mg contents - ~27% - probably due to the mineral leaching into cooking water favoured by the
223 more porous structure of pulses. In the latter case, a significant interaction between sprouting and
224 cooking was found ($p < 0.05$).
225 Concerning trace minerals, varieties, sprouting and cooking differently affected total Zn and Fe.
226 Cooking significantly decreased the Fe content in unsprouted pulses (≥35%), but in sprouted seeds
227 cooking increased Fe in chickpeas (+20%) and decreased Fe in green peas (-28%) . As regards to Zn
228 content, it increased after cooking of both unsprouted (+112%) and sprouted (+44%) chickpeas,
229 probably due to the Zn uptake from tap water (0.3 mgL^{-1}), while in green peas Zn was only
230 minimally affected. The occurrence of different phenomena could explain the results: in chickpea,
231 the leaching of Fe into cooking water seems to be prevented by sprouting, while germination
232 negatively affects the uptake of Zn from cooking water. The observed discrepancies could be
233 attributed to the different localization of the trace mineral in seeds (Dalfollo Ribeiro et al. 2012)
234 and/or the different effects of germination on seed matrix (protein fraction) and mineral binding
235 compounds, such as tannins, phytic acid and polyphenols.
236 After cooking, P was significantly reduced in both sprouted pulses, in accordance with the decrease
237 of phytic acid (Table2), suggesting that the release of P into the cooking water is favoured by seed
238 structure modification.

239 The total mineral contents of raw unsprouted pulses were consistent with data reported in the
240 literature (Ray et al. 2014), taking into account the known variability due to different accessions of
241 legumes and the influences of the agronomic practices (Dalfollo Ribeiro et al. 2012).
242 Mineral contents in raw pulses were only partially affected by germination: Ca and Fe significantly
243 decreased in chickpeas and Mg in green peas after sprouting. Contrasting results have been
244 reported on the effect of sprouting on total mineral contents in raw pulses. Some studies showed
245 significant mineral decreases after sprouting, likely due to the leaching of solid matter in soaking
246 water (Ghavidel and Prakash, 2007; Audu and Aremu 2011). In contrast, El-Adawy (2002) did not
247 find any significant effect of germination and Pal et al. (2016) even found increases of Ca and Fe in
248 germinated horsegram.
249 Boiling was reported to influence the total mineral contents of legumes in relation to variety of
250 pulses, and therefore food matrices (i.e. chelating compounds), and kind of mineral (Alajaji and El-
251 Adawy 2006; Wang et al. 2010). Only few data are available about the total mineral contents in
252 sprouted pulses, after cooking. Bains et al. (2014) found a significant decrease in Fe and Ca after
253 chickpeas germination (by 7 % and 8 %, respectively) and a further decrease in Fe, of about 3%,
254 after pressure cooking and microwaving of sprouted pulses. Conversely, the Zn content was not
255 influenced by germination, but both methods of cooking decreased the Zn content of about 7%.
256 Unfortunately, those Authors did not consider boiling and did not compare unsprouted versus
257 sprouted pulses.
258 The content of phytic acid in our unsprouted and sprouted chickpeas was more than 3-fold higher
259 compared to levels found in other varieties (Ghavidel and Prakash 2007). This major chelating
260 properties of matrix could have hindered the improvement of mineral accessibility in sprouted and
261 cooked pulses. In fact, the *in vitro* accessibility of Mg and Ca in cooked chickpeas was similar,
262 regardless of germination, and mineral accessibility in cooked sprouted green peas was even lower
263 than in unsprouted ones (Table 3).

264 Soaking, germination and cooking are generally reported as processes able to improve mineral
265 accessibility in pulses by a reduction of antinutritional factors (Viadel et al. 2006; Ghavidel and
266 Prakash 2007), although conflicting results have been reported. For example, Hemalatha et al. (2007)
267 failed to demonstrate any increase in Zn bioavailability in chickpeas after germination.
268 To sum up, these data suggest that the accessibility of minerals in pulses is the result of interactions
269 of many factors - like the type of mineral, the composition and structure of pulses and the processes
270 - that cannot be easily predicted.

271 ***3.4 In Vitro Starch Digestibility***

272 Although several studies on starch digestibility in unsprouted and sprouted pulses have been
273 conducted, most have focused on raw/cooked flours (Ghavidel and Prakash 2007; Uppal and Bains
274 2012) or on isolated starches (Hoover et al. 2010), whereas no information is available on the effect
275 of sprouting on starch digestibility of whole pulses as eaten.
276 In this study, we evaluated the starch digestibility of unsprouted and sprouted pulses after cooking,
277 by a well-established and extensively employed *in vitro* assay, which allowed the determination of
278 nutritionally important starch fractions, rapidly and slowly digestible starch (RDS and SDS,
279 respectively). By measuring the susceptibility of starch to digestive enzymes, this assay is a
280 commonly used method to estimate the potential glycaemic response of foods (EFSA 2011). Indeed,
281 the glycaemic response appears to be directly related to the amount of RDS and the insulin demand
282 is inversely correlated to SDS fraction (Garsetti et al. 2005). As expected, different starch
283 susceptibility (Figure 4A) was observed in native pulses. In chickpeas, the RDS fraction was lower
284 and the SDS one was higher (~20%; $p<0.05$) than those determined in green peas. Differences in the
285 *in vitro* digestibility of native starches among and within species have been attributed to the
286 interplay of many factors, such as starch source, granule size, degree of crystallinity, type of
287 crystalline polymorphic (A, B, or C) form, amylose/amylopectin ratio, molecular structure of
288 amylopectin, amylose chain length etc. (Hoover et al. 2010). Moreover, differences in seed size and
289 structure, as well the thickness of seed coat, represent crucial parameters for water imbibition of

290 seeds during soaking and cooking and, consequently, on starch gelatinization (Klamczynska et al.
291 2001).
292 Sprouting under controlled conditions caused minor and not statistically significant variations of
293 the RDS and SDS percentages in both intact legumes. This suggests that the industrial treatment
294 considered in this study did not promote changes in the rate of starch digestibility in intact pulses,
295 likely maintaining a high degree of ordered structure in cell walls (Figure 1 b, f). Consequently, the
296 cooking of whole pulses in boiling water resulted in the swelling of the starch granules, but not in
297 the disruption of the cell walls or the starch granules (Figure 2 d, h). This phenomenon of
298 intracellular gelatinization without disruption of the starch granules could account for the observed
299 low rate of starch digestion in germinated pulses. Numerous studies investigated the digestibility
300 of starch in legume flours after germination. Benítez et al. (2013) reported a decrease of the total
301 starch content and a significant increase in the percentage of available starch after germination,
302 ascribing these changes to the increased α-amylase activity induced by the treatment. The Authors
303 also suggested that the reduction of antinutritional factors in the seeds after germination could
304 promote the starch digestibility. The partial removal of phytic acid and tannins, which takes place
305 during germination, probably created a large space within the matrix increasing the susceptibility
306 to enzymatic activity and, consequently, improving the starch digestibility.
307 The findings of the present study seem quite interesting in view of the effects of the consumption of
308 sprouted legumes on glycaemic metabolism. In fact, the maintenance of the characteristics of slow
309 digestibility of starch, comparable to those of unsprouted pulses, should give the germinated
310 products a reduced glycaemic impact, not unlike that typical of legumes (www.glycemicindex.com)
311 (Sievenpiper et al. 2009; Benítez et al. 2013). However, this potential should be demonstrated in
312 further *in vivo* studies.
313 Besides the consumption of pulses as whole seeds, pulses are commonly used as food ingredients,
314 thanks to their chemical composition, which improves the nutritional quality of the finished
315 products. Thus, we evaluated the starch digestibility of their flours after cooking, thereby

316 simulating a potential use in porridge or baby food. In contrast to what observed in intact pulses,
317 the changes in the structure of the seeds resulting from the grinding before cooking induced a
318 significant (p <0.01) increase in RDS fraction (Figure 4 B), thus promoting the digestibility of starch
319 in both cooked chickpea and pea flours, regardless of the process of germination. It is likely that
320 grinding opened up the cell walls and released the starch granules, favouring their dispersion in
321 water and the gelatinization during cooking (Brummer et al. 2015). Although these features can
322 greatly reduce the hypoglycaemic properties assessed in intact seeds, on the other hand, they may
323 be considered as positive in view of the formulation of products characterized by readily available
324 energy.

325 ***3.5 Total Antioxidant Capacity***

326 The effect of the sprouting on the TAC of the analysed pulses is shown in Table 4. Germination had
327 a notable effect in lowering the TAC of chickpeas and green peas. The highest decrease of TAC was
328 remarkable in both chickpea and green pea soaked samples, with an about 40% decrease, raising to
329 about 60% decrease for germinated cooked green peas with respect to raw ones.
330 These results may be explained considering that there is an increasing of reactive oxygen
331 substances at the beginning of the sprouting phase (Bailly et al. 2008) mainly produced by
332 hydrogen peroxide, which is a physiological signalling mediator, after superoxide dismutase and
333 catalase enzymes catalysis (Wojtyla et al. 2016). Phenolic compounds and other antioxidants could
334 have counteracted such reactive oxygen substances with a net decrease in the antioxidant capacity
335 of sprouted pulses. Amarowicz and Pegg (2008) evidenced a similar trend for lentil samples,
336 suggesting that only after the fourth day of germination an inverse trend of the antioxidant capacity
337 takes place. Moreover, an enhancement of the polyphenol oxidase activity, which oxidises
338 polyphenols mainly responsible for the TAC of pulses (Sharma and Sehgal 1992), cannot be ruled
339 out after the sprouting and the further soaking process.
340 On the contrary, a significant increase in TAC was observed in both pulses when raw and soaked
341 unsprouted samples were compared. Results are in contrast to those of Xu and Chang (2008), who

342 showed about a ~50% decrease of antioxidant capacity (ORAC assay) of soaked green peas with
343 respect to raw ones and a 4% to 30% decrease of soaked chickpeas with respect to raw ones. It
344 should be considered that Authors performed an extraction with organic solvents, which may have
345 not solubilised all the phenolics, such as the conjugated ones, highly present in pulses samples
346 (Wang et al. 2016). On the contrary, by using the method of Açaret al. (2009), all the phenolics
347 contained in the seed are allowed to react with ABTS ethanol reagent, resulting in a higher
348 antioxidant capacity. Our findings are also partially in accordance with those of Segev et al. (2011),
349 which evidenced that the soaking process did not significantly affect the antioxidant capacity of
350 non-coloured pulses, compared to raw ones. However, as the soaking process in our unsprouted
351 pulses minimally affected the integrity of the external layers, we hypothesized that soaking water
352 remained into the seed and may have extracted both soluble free and conjugated phenolics, by
353 increasing the antioxidant capacity.
354 Concerning the cooking process, our data showed a significant 30% TAC reduction in both the
355 pulses samples, compared to the raw ones. Data are in according to Xu and Chang (2008), which
356 reported a 57% - 77% decrease of antioxidant capacity of cooked green peas and chickpeas.
357 Interestingly, the cooking process had a similar influence on the TAC of sprouted and unsprouted
358 pulses, suggesting that, despite the almost intact cell structure (Figure 1c, d, g and h), cooked pulses
359 lost a part of the antioxidant potential probably due to the leaching of phenolic compounds in the
360 cooking water and their degradation (Xu and Chang 2008).
361 **4 Conclusions**
362 The availability of partially germinated and stabilized grains, such as pulses, at an industrial scale
363 expands the potential for the integration of these novel ingredients into the food and feed market
364 and helps to meet increasing consumer demands for natural healthy food products.
365 Although the performed sprouting process did not improve the mineral accessibility as expected
366 and promoted a decrease of antioxidant capacity, this study provides evidence that sprouted
367 chickpeas and green peas maintain their relevant nutritional traits. Moreover, flatulence-related

368 oligosaccharides greatly decreased and phytic acid level was significantly reduced. The lack of
369 replication of sprouting process of pulses might represents a weakness of the present study.
370 However, a previous study on grains sprouted in the same plant, did not show any significant
371 differences in three independent samples.
372 Overall, sprouted pulses seem to offer an excellent opportunity for developing new products aimed
373 at improving the nutrient profile of products targeting users relying on vegetarian or vegan diets.

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Xu B, Chang SK. 2008. Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chem. 110:1-13.

485 Table 1. Effect of sprouting on the chemical composition of raw chickpea and green
486 pea[a].

	chickpea		green pea	
	unsprouted	**sprouted**	**unsprouted**	**sprouted**
Protein	186 ± 3 d	202 ± 1 b	194 ± 0 c	216 ± 2 a
Lipid	73 ± 2 a	73 ± 5 a	24 ± 1 b	25 ± 2 b
Starch	518±44 a	545±78 a	590 ± 63 a	539 ± 70 a
Free sugars	56± 1 b	54 ± 3 b	46 ± 7 b	83 ± 3 a
Ash	28 ± 1 a	26 ± 0 b	27 ± 0 ab	27 ± 0 ab
Soluble Fibre	12 ± 2c	19± 3 bc	31 ± 56 a	27 ± 2 ab
Insoluble Fibre	169± 3 a	172 ± 1 a	155 ± 11 a	160 ± 8 a

487
488
489 [a]Values are presented as mean ± SD (n = 3) and expressed as g kg^{-1} dry weight. Data
490 in the same row with different letters are significantly different (p <0.05).
491

Table 2. Total mineral[a] and phytic acid[b] contents in raw and cooked chickpea and green pea (unsprouted and sprouted).

	chickpea			
	unsprouted		sprouted	
	raw	cooked	raw	cooked
Ca	791 ± 17 c	2434 ± 105 a	545 ± 72 d	2052 ± 57 b
Mg	942 ± 77 a	929 ± 15 a	978 ± 54 a	705 ± 2 b
Fe	70 ± 2 a	38 ± 2 c	63 ± 2 b	75 ± 4 a
Zn	25 ± 1 c	53 ± 1 a	27 ± 1 c	39 ± 1 b
P	4037 ± 28 a	3982 ± 29 a	4160 ± 224 a	2726 ± 37 b
Phytic Acid	19 ± 0a	18 ± 1ab	18 ± 0 b	11 ± 0c
	green pea			
	unsprouted		sprouted	
	raw	cooked	raw	cooked
Ca	769 ± 56 b	1967 ± 86 a	824 ± 127 b	1966 ± 52 a
Mg	958 ± 27 a	855 ± 29 b	870 ± 26 b	637 ± 21 c
Fe	62 ± 3 a	40 ± 5 b	58 ± 1 a	42 ± 9 b
Zn	38 ± 1 a	32 ± 1 b	38 ± 2 a	36 ± 0 a
P	4172 ± 167 a	3928 ± 159 a	4560 ± 73 a	2592 ± 77 b
Phytic Acid	17 ± 0a	16 ± 0a	17 ± 1a	11 ± 0b

[a]Values are presented as mean ± SD (n = 3) and expressed as mg kg^{-1} dry weight. [b]Values are presented as mean ± SD (n = 3) and expressed as g kg^{-1} dry weight. Data in the same row with different letters are significantly different (p <0.05).

499 Table 3. Ca and Mg accessibilities in cooked chickpea and green pea
500 (unsprouted and sprouted) [a].

501

	chickpea		**green pea**	
	unsprouted	sprouted	unsprouted	sprouted
Ca	33.5 ± 4.4	40.0 ± 5.9	30.5 ± 4.5	19.6 ± 1.6 *
Mg	45.8 ± 7.0	41.0± 2.9	43.0 ± 3.7	23.4 ± 3.7 *

502 [a]Values are presented as mean ± SD n= 5 independent measurements and
503 expressed as percentage of soluble mineral in relation to total mineral
504 content. Asterisks indicate statistical differences from the relative unsprouted
505 samples (p <0.05).

Table 4. Total antioxidant capacity in raw, soaked and cooked chickpea and pea (unsprouted and sprouted)[a].

	chickpea		**green pea**	
	unsprouted	sprouted	unsprouted	sprouted
raw	27.14 ± 1.57 b	24.89±1.29 A,*	27.13±1.62 b	24.08± 0.77 A,*
soaked	40.83±12.45 a	16.97±1.88 B,*	32.27±5.61 a	13.00±2.01 B,*
cooked	18.84±3.72 c	11.98±2.82 C,*	13.25±2.24 c	8.16±1.18 C,*

[a]Values are presented as the mean ± SD, n=6 independent measurements and expressed as mmol TEAC/kg dry weight. Lowercase letters indicate statistical differences among the unsprouted raw, soaked and cooked pulses, while capital letters indicate statistical differences among the sprouted raw, soaked and cooked pulses (p <0.05). Asterisks indicate statistical differences from the relative unsprouted samples (p <0.05).