Effect of sprouting on nutritional quality of pulses

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Running title: Nutritional quality of sprouted pulses
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.
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- tetramethylichroman-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
mill (Bühler AG, Uzwil, Switzerland) and used as such for the evaluation of chemical composition, phytic acid, mineral content and antioxidant capacity. Moreover, grinded pulses (40g) were used to prepare a porridge by cooking in boiling water (200mL) for 3 min, to evaluate the starch digestibility.

Intact seeds were also processed to simulate domestic preparation. Briefly, pulses were soaked in excess of plain tap water, for 12 h at room temperature (henceforth “soaked”). After draining, samples were cooked in plain tap boiling water for 45 min in 1:3 w/w ratio (henceforth “cooked”). Samples were cooked in duplicate, and final samples were combined and used for analysis. Both soaked and cooked samples were used as such for light microscopy or homogenised (7011S-Waring Blender Commercial, Torrington, CT, USA) for further analyses.

2.3 Light Microscopy

Soaked and cooked intact seeds were sectioned with a vibratome in 20-30 µm thick sections. These were stained with 0.1% water solution of toluidine blue for 1 min, or Lugol’s iodine for 5 min. Then, the samples were observed by an Olympus BX50 light microscope (Olympus, Tokyo, Japan) equipped with Nomarski differential interference contrast.

2.4 Chemical Composition

Chemical composition was performed on the unsprouted and sprouted raw pulses. Moisture, starch, protein, lipid and ash content was assessed by AACC standard methods (44-15.02, 76-13.01, 46-12.01, 30-10.01, and 08-01.01, respectively) (AACC 2001). Sugars were evaluated by HPLC Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) (Englyst et al. 2000). Total, soluble and insoluble dietary fibre content was evaluated by the enzymatic–gravimetric procedure (AOAC 1995). The analysis of phytic acid was performed by HPLC with spectrophotometric detection as previously described on raw and cooked pulses (Erba et al. 2017). Moisture, starch, protein, lipid, ash and phytic acid contents were determined in triplicate and expressed as g kg⁻¹ on the dry weight (d.w.).

2.5 Total Mineral Content and Ca and Mg Accessibilities
Total contents of Ca, Mg, Fe, Zn and P were determined on raw and cooked samples (both sprouted and unsprouted pulses) and, after an enzymatic digestion of cooked samples, on the supernatant solutions containing the soluble mineral fraction. To investigate the uptake of minerals from tap water during the domestic treatments, mineral levels were measured in tap water by atomic absorption spectroscopy. For total mineral analysis, samples were dry-ashed (550 °C, overnight) and dissolved with an acid solution, as previously reported (Erba et al. 2011). For Ca and Mg accessibility, cooked (both sprouted and unsprouted) samples were subjected to two enzymatic digestion stages, gastric and intestinal, to simulate the human gastro-intestinal system and minerals in the soluble fractions were analysed (Erba et al. 2017). Mineral concentrations were determined by Atomic Absorption Spectroscopy (AAAnalyst800 Perkin Elmer, Waltham, MA, USA), against 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 lanthanum solution to avoid interferences for Ca and Mg analyses (1 g kg⁻¹ w/v). Phosphorous (P) was determined by a colorimetric method (Erba et al. 2011). The accuracy of mineral analyses was checked by using certified values of reference material (NCS ZC 85006). All analyses were performed in triplicate and results were expressed as total mineral content (mg kg⁻¹ d.w., mean ± SD) and accessible Ca and Mg were expressed as percentage of soluble Ca and Mg with respect to total content.

2.6 In vitro Starch Digestibility

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

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

### 2.8 Statistical Analysis

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

### 3 Results and Discussion

#### 3.1 Microscopy Features

Figure 1 shows the effect of sprouting on the microstructure of the whole pulses – soaked and cooked - captured under polarised light conditions. In order to highlight the cell walls, samples were stained with a solution of Toluidine blue, which is a generic dye for plant tissues. The arrangement of the cells in the seed tissue was clearly visible in all the samples, regardless of the applied process. The sprouting conditions adopted in the present study did not dramatically affect the seed structure of either pea (Figure 1b) or chickpea (Figure 1f), when compared with the unsprouted pulses (Figure 1a and 1e for peas and chickpeas, respectively). The cell walls were visible even after cooking in both unsprouted (Figure 1c for peas and Figure 1g for chickpeas) and sprouted pulses (Figure 1d for peas and Figure 1h for chickpeas), appearing as grey lines. This observation suggests that they had and maintained a high degree of ordered structure (Brummer et al. 2015). Interestingly, in sprouting seeds, cells appeared slightly more stained by Toluidine, indicating the presence of mobilised proteins (more accessible to the stain).
The majority of the interior of the cells was occupied by starch granules, which in Figure 2 appear in dark as samples were stained with Lugol. The surface of the granules was generally smooth without any fissures, cracks or pores. A granule size between 2.72 µm and 31.8 µm was observed for pea starches (Aggarwal et al. 2004). The large oval to small spherical shaped granules were reported to be present in starch from different chickpea cultivars (Brummer et al. 2015). Starch granules of sprouted pulses (Figure 2b, d, f and h) appeared darker compared to the related unsprouted samples (Figure 2a, c, e and g) suggesting the formation of a more porous structure after sprouting. Cooking of whole pulses in boiling water resulted in the swelling of starch granules, but not in the disruption of the cell walls or the starch granules, consistently with other studies (Brummer et al. 2015).

3.2 Chemical Composition

The partial germination process did not promote significant modifications in pulses regarding starch, lipid and fibre content, while protein significantly increased after sprouting (Table 1). Other studies reported an increase in protein in germinated grains, that has been attributed to the synthesis of enzymes (for example, proteases) during germination (Masood et al. 2014) or to the compositional changes following the degradation of other constituents (Bau et al. 1997). In sprouted samples, the content of fibre did not change, whereas many studies have shown that the germination process has a significant impact on dietary fibre fractions in pulses (Martin-Cabrejas et al. 2003; Masood et al. 2014; Duenas et al. 2016). Differences among studies are likely related to the type of legume (chickpeas, beans, etc.) and the germination conditions (e.g. sprouting time, temperature and relative humidity, light/darkness conditions, drying temperature)(Martín-Cabrejas et al. 2003).

In chickpeas, the total amount of free sugars did not change, whereas a significant increase (p<0.05) was found in peas. As regards the qualitative distribution of sugars (Figure 3), we evidenced an increase in sucrose and the disappearance of raffinose after the sprouting of both pulses. Soaking and sprouting are the most efficient biological treatments for removing α-galactosides,
oligosaccharides derived from sucrose, containing 1–3 units of galactose linked by α-1,6 linkages. These oligosaccharides, commonly present in legumes and rapidly fermented by the human colonic microbiota, are responsible for flatulence in individuals that do not consume pulses on a regular basis (Hall et al. 2017). The increase in the relative percentage of sucrose in the sprouted products was likely the result of the compositional changes following the degradation of raffinose, probably attributable to the endogenous or microbial enzymatic activities developed during sprouting (Cai et al. 1997; Mäkinen and Arendt 2015). The sucrose increase in flour from germinated chickpeas improved the leavening properties of dough enriched with sprouted pulses (Marengo et al. 2017) and may positively contribute to the sensorial characteristics of such products.

Germination is reported as the more effective process for reducing phytic acid in pulses (Patterson et al. 2017). This compound is an antinutritional factor with a marked chelating ability - in particular for calcium – and it is linked to the inhibition of digestive enzymes, such as protease, α-amylases and trypsin. In this study, myo-inositol hexaphosphate (IP6) was the only inositol phosphate found. As observed in previous studies (Egli et al. 2002), the content of phytic acid in chickpeas, after germination, significantly decreased by ~5% (19 ± 0.0 vs 18 ± 0.0, p<0.05) (Table 2). Such effect was probably related to the activation of endogenous phytase (Egli et al. 2002), occurring during the initial period of germination. In cooked seeds, a high and significant (p< 0.01) reduction in IP6 levels was assessed in both germinated pulses, accounting for more than 30% reduction in comparison with unsprouted ones. The decrease in IP6 suggesting a potential leaching of the phytate into the soaking and/or cooking water, owing to its water solubility. Compared to unsprouted pulses, the leaching effect was more pronounced in the sprouted ones, likely due to their porous structure (Figures 1b, d, f and h).

3.3 Total Mineral Contents and Ca and Mg Accessibility

Total mineral content was influenced by both the type of pulse (chickpeas or green peas) and the treatments (sprouting and cooking)(Table 2). Cooking led to a more than two-fold increase in Ca levels in both legumes, independently by the sprouting process (p <0.01), likely as a result of the Ca-
uptake from the tap water (whose Ca level was 72.5 mg\textperLD\textsuperscript{-1}) and a consistent increase of legume weight of about 2.5-fold. Tap water was used for cooking pulses because it represents the most common domestic practice, even though its influence on mineral content of cooked seed was predictable. Notwithstanding, the Ca level of tap water used in this study is consistent with the mean level in Italy (62.3 mg\textperLD\textsuperscript{-1}, Dinelli et al. 2012), thus our results do not refer to a singular condition. Differently, Mg levels in the unsprouted samples - raw and cooked - were similar, even though the Mg content in tap water (12 mg\textperLD\textsuperscript{-1}). In sprouted pulses, cooking significantly decreased the Mg contents - ~27% - probably due to the mineral leaching into cooking water favoured by the more porous structure of pulses. In the latter case, a significant interaction between sprouting and cooking was found (p <0.05).

Concerning trace minerals, varieties, sprouting and cooking differently affected total Zn and Fe. Cooking significantly decreased the Fe content in unsprouted pulses (≥35%), but in sprouted seeds cooking increased Fe in chickpeas (+20%) and decreased Fe in green peas (-28%). As regards to Zn content, it increased after cooking of both unsprouted (+112%) and sprouted (+44%) chickpeas, probably due to the Zn uptake from tap water (0.3 mg\textperLD\textsuperscript{-1}), while in green peas Zn was only minimally affected. The occurrence of different phenomena could explain the results: in chickpea, the leaching of Fe into cooking water seems to be prevented by sprouting, while germination negatively affects the uptake of Zn from cooking water. The observed discrepancies could be attributed to the different localization of the trace mineral in seeds (Dalfollo Ribeiro et al. 2012) and/or the different effects of germination on seed matrix (protein fraction) and mineral binding compounds, such as tannins, phytic acid and polyphenols.

After cooking, P was significantly reduced in both sprouted pulses, in accordance with the decrease of phytic acid (Table2), suggesting that the release of P into the cooking water is favoured by seed structure modification.
The total mineral contents of raw unsprouted pulses were consistent with data reported in the literature (Ray et al. 2014), taking into account the known variability due to different accessions of legumes and the influences of the agronomic practices (Dalfollo Ribeiro et al. 2012). Mineral contents in raw pulses were only partially affected by germination: Ca and Fe significantly decreased in chickpeas and Mg in green peas after sprouting. Contrasting results have been reported on the effect of sprouting on total mineral contents in raw pulses. Some studies showed significant mineral decreases after sprouting, likely due to the leaching of solid matter in soaking water (Ghavidel and Prakash, 2007; Audu and Aremu 2011). In contrast, El-Adawy (2002) did not find any significant effect of germination and Pal et al. (2016) even found increases of Ca and Fe in germinated horsegram.

Boiling was reported to influence the total mineral contents of legumes in relation to variety of pulses, and therefore food matrices (i.e. chelating compounds), and kind of mineral (Alajaji and El-Adawy 2006; Wang et al. 2010). Only few data are available about the total mineral contents in sprouted pulses, after cooking. Bains et al. (2014) found a significant decrease in Fe and Ca after chickpeas germination (by 7 % and 8 %, respectively) and a further decrease in Fe, of about 3%, after pressure cooking and microwaving of sprouted pulses. Conversely, the Zn content was not influenced by germination, but both methods of cooking decreased the Zn content of about 7%.

Unfortunately, those Authors did not consider boiling and did not compare unsprouted versus sprouted pulses.

The content of phytic acid in our unsprouted and sprouted chickpeas was more than 3-fold higher compared to levels found in other varieties (Ghavidel and Prakash 2007). This major chelating properties of matrix could have hindered the improvement of mineral accessibility in sprouted and cooked pulses. In fact, the in vitro accessibility of Mg and Ca in cooked chickpeas was similar, regardless of germination, and mineral accessibility in cooked sprouted green peas was even lower than in unsprouted ones (Table 3).
Soaking, germination and cooking are generally reported as processes able to improve mineral accessibility in pulses by a reduction of antinutritional factors (Viadel et al. 2006; Ghavidel and Prakash 2007), although conflicting results have been reported. For example, Hemalatha et al. (2007) failed to demonstrate any increase in Zn bioavailability in chickpeas after germination. To sum up, these data suggest that the accessibility of minerals in pulses is the result of interactions of many factors - like the type of mineral, the composition and structure of pulses and the processes - that cannot be easily predicted.

### 3.4 In Vitro Starch Digestibility

Although several studies on starch digestibility in unsprouted and sprouted pulses have been conducted, most have focused on raw/cooked flours (Ghavidel and Prakash 2007; Uppal and Bains 2012) or on isolated starches (Hoover et al. 2010), whereas no information is available on the effect of sprouting on starch digestibility of whole pulses as eaten.

In this study, we evaluated the starch digestibility of unsprouted and sprouted pulses after cooking, by a well-established and extensively employed in vitro assay, which allowed the determination of nutritionally important starch fractions, rapidly and slowly digestible starch (RDS and SDS, respectively). By measuring the susceptibility of starch to digestive enzymes, this assay is a commonly used method to estimate the potential glycaemic response of foods (EFSA 2011). Indeed, the glycaemic response appears to be directly related to the amount of RDS and the insulin demand is inversely correlated to SDS fraction (Garsetti et al. 2005). As expected, different starch susceptibility (Figure 4A) was observed in native pulses. In chickpeas, the RDS fraction was lower and the SDS one was higher (~20%; p<0.05) than those determined in green peas. Differences in the in vitro digestibility of native starches among and within species have been attributed to the interplay of many factors, such as starch source, granule size, degree of crystallinity, type of crystalline polymorphic (A, B, or C) form, amylose/amylopectin ratio, molecular structure of amyllopectin, amylose chain length etc. (Hoover et al. 2010). Moreover, differences in seed size and structure, as well the thickness of seed coat, represent crucial parameters for water imbibition of
seeds during soaking and cooking and, consequently, on starch gelatinization (Klamczynska et al. 2001).

Sprouting under controlled conditions caused minor and not statistically significant variations of the RDS and SDS percentages in both intact legumes. This suggests that the industrial treatment considered in this study did not promote changes in the rate of starch digestibility in intact pulses, likely maintaining a high degree of ordered structure in cell walls (Figure 1 b, f). Consequently, the cooking of whole pulses in boiling water resulted in the swelling of the starch granules, but not in the disruption of the cell walls or the starch granules (Figure 2 d, h). This phenomenon of intracellular gelatinization without disruption of the starch granules could account for the observed low rate of starch digestion in germinated pulses. Numerous studies investigated the digestibility of starch in legume flours after germination. Benítez et al. (2013) reported a decrease of the total starch content and a significant increase in the percentage of available starch after germination, ascribing these changes to the increased α-amylase activity induced by the treatment. The Authors also suggested that the reduction of antinutritional factors in the seeds after germination could promote the starch digestibility. The partial removal of phytic acid and tannins, which takes place during germination, probably created a large space within the matrix increasing the susceptibility to enzymatic activity and, consequently, improving the starch digestibility.

The findings of the present study seem quite interesting in view of the effects of the consumption of sprouted legumes on glycaemic metabolism. In fact, the maintenance of the characteristics of slow digestibility of starch, comparable to those of unsprouted pulses, should give the germinated products a reduced glycaemic impact, not unlike that typical of legumes (www.glycemicindex.com) (Sievenpiper et al. 2009; Benítez et al. 2013). However, this potential should be demonstrated in further in vivo studies.

Besides the consumption of pulses as whole seeds, pulses are commonly used as food ingredients, thanks to their chemical composition, which improves the nutritional quality of the finished products. Thus, we evaluated the starch digestibility of their flours after cooking, thereby
simulating a potential use in porridge or baby food. In contrast to what observed in intact pulses, the changes in the structure of the seeds resulting from the grinding before cooking induced a significant ($p < 0.01$) increase in RDS fraction (Figure 4 B), thus promoting the digestibility of starch in both cooked chickpea and pea flours, regardless of the process of germination. It is likely that grinding opened up the cell walls and released the starch granules, favouring their dispersion in water and the gelatinization during cooking (Brummer et al. 2015). Although these features can greatly reduce the hypoglycaemic properties assessed in intact seeds, on the other hand, they may be considered as positive in view of the formulation of products characterized by readily available energy.

3.5 Total Antioxidant Capacity

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

On the contrary, a significant increase in TAC was observed in both pulses when raw and soaked unsprouted samples were compared. Results are in contrast to those of Xu and Chang (2008), who
showed about a ~50% decrease of antioxidant capacity (ORAC assay) of soaked green peas with respect to raw ones and a 4% to 30% decrease of soaked chickpeas with respect to raw ones. It should be considered that Authors performed an extraction with organic solvents, which may have not solubilised all the phenolics, such as the conjugated ones, highly present in pulses samples (Wang et al. 2016). On the contrary, by using the method of Açar et al. (2009), all the phenolics contained in the seed are allowed to react with ABTS ethanol reagent, resulting in a higher antioxidant capacity. Our findings are also partially in accordance with those of Segev et al. (2011), which evidenced that the soaking process did not significantly affect the antioxidant capacity of non-coloured pulses, compared to raw ones. However, as the soaking process in our unsprouted pulses minimally affected the integrity of the external layers, we hypothesized that soaking water remained into the seed and may have extracted both soluble free and conjugated phenolics, by increasing the antioxidant capacity.

Concerning the cooking process, our data showed a significant 30% TAC reduction in both the pulses samples, compared to the raw ones. Data are in according to Xu and Chang (2008), which reported a 57% - 77% decrease of antioxidant capacity of cooked green peas and chickpeas. Interestingly, the cooking process had a similar influence on the TAC of sprouted and unsprouted pulses, suggesting that, despite the almost intact cell structure (Figure 1c, d, g and h), cooked pulses lost a part of the antioxidant potential probably due to the leaching of phenolic compounds in the cooking water and their degradation (Xu and Chang 2008).

4 Conclusions

The availability of partially germinated and stabilized grains, such as pulses, at an industrial scale expands the potential for the integration of these novel ingredients into the food and feed market and helps to meet increasing consumer demands for natural healthy food products.

Although the performed sprouting process did not improve the mineral accessibility as expected and promoted a decrease of antioxidant capacity, this study provides evidence that sprouted chickpeas and green peas maintain their relevant nutritional traits. Moreover, flatulence-related
oligosaccharides greatly decreased and phytic acid level was significantly reduced. The lack of replication of sprouting process of pulses might represents a weakness of the present study. However, a previous study on grains sprouted in the same plant, did not show any significant differences in three independent samples. Overall, sprouted pulses seem to offer an excellent opportunity for developing new products aimed at improving the nutrient profile of products targeting users relying on vegetarian or vegan diets.

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REFERENCES


AOAC International.


Table 1. Effect of sprouting on the chemical composition of raw chickpea and green pea*.

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<tr>
<th></th>
<th>chickpea</th>
<th>green pea</th>
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<tbody>
<tr>
<td></td>
<td>unsprouted</td>
<td>sprouted</td>
</tr>
<tr>
<td>Protein</td>
<td>186 ± 3 d</td>
<td>202 ± 1 b</td>
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<tr>
<td>Lipid</td>
<td>73 ± 2 a</td>
<td>73 ± 5 a</td>
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<tr>
<td>Starch</td>
<td>518±44 a</td>
<td>545±78 a</td>
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<tr>
<td>Free sugars</td>
<td>56± 1 b</td>
<td>54 ± 3 b</td>
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<td>Ash</td>
<td>28 ± 1 a</td>
<td>26 ± 0 b</td>
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<tr>
<td>Soluble Fibre</td>
<td>12 ± 2c</td>
<td>19± 3 bc</td>
</tr>
<tr>
<td>Insoluble Fibre</td>
<td>169± 3 a</td>
<td>172 ± 1 a</td>
</tr>
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*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 ($p <0.05$).
Table 2. Total mineral\(^a\) and phytic acid\(^b\) contents in raw and cooked chickpea and green pea (unsprouted and sprouted).

<table>
<thead>
<tr>
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<th>green pea</th>
<th></th>
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<tbody>
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<td></td>
<td>sprouted</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>cooked</td>
<td>raw</td>
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<tr>
<td>Ca</td>
<td>791 ± 17 c</td>
<td>2434 ± 105 a</td>
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<td>Mg</td>
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<td>Fe</td>
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<td>53 ± 1 a</td>
<td>27 ± 1 c</td>
<td>39 ± 1 b</td>
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<td>P</td>
<td>4037 ± 28 a</td>
<td>3982 ± 29 a</td>
<td>4160 ± 224 a</td>
<td>2726 ± 37 b</td>
</tr>
<tr>
<td>Phytic Acid</td>
<td>19 ± 0a</td>
<td>18 ± 1ab</td>
<td>18 ± 0 b</td>
<td>11 ± 0c</td>
</tr>
</tbody>
</table>

|        | 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 (\(p < 0.05\)).
Table 3. Ca and Mg accessibilities in cooked chickpea and green pea (unsprouted and sprouted)\(^a\).

<table>
<thead>
<tr>
<th></th>
<th>chickpea</th>
<th>green pea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>unsprouted</td>
<td>sprouted</td>
</tr>
<tr>
<td>Ca</td>
<td>33.5 ± 4.4</td>
<td>40.0 ± 5.9</td>
</tr>
<tr>
<td>Mg</td>
<td>45.8 ± 7.0</td>
<td>41.0 ± 2.9</td>
</tr>
</tbody>
</table>

\(^a\)Values are presented as mean ± SD n= 5 independent measurements and expressed as percentage of soluble mineral in relation to total mineral content. Asterisks indicate statistical differences from the relative unsprouted samples (\(p <0.05\)).
Table 4. Total antioxidant capacity in raw, soaked and cooked chickpea and pea (unsprouted and sprouted)\(^a\).

<table>
<thead>
<tr>
<th></th>
<th>chickpea</th>
<th>green pea</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>unsprouted</td>
<td>sprouted</td>
<td>unsprouted</td>
</tr>
<tr>
<td>raw</td>
<td>27.14 ± 1.57 b</td>
<td>24.89±1.29 A,*</td>
<td>27.13±1.62 b</td>
</tr>
<tr>
<td>soaked</td>
<td>40.83±12.45 a</td>
<td>16.97±1.88 B,*</td>
<td>32.27±5.61 a</td>
</tr>
<tr>
<td>cooked</td>
<td>18.84±3.72 c</td>
<td>11.98±2.82 C,*</td>
<td>13.25±2.24 c</td>
</tr>
</tbody>
</table>

\(^a\)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 (\(p < 0.05\)).