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Macromolecular and micronutrient profiles of sprouted chickpeas to be used for integrating cereal-based food

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ABSTRACT

Pulse flour may be used to improve nutritional traits of gluten and gluten-free formulations in traditional food such as bread or pasta. However due some intrinsic nutritional, textural, and sensory properties, the use of pulses as ingredients for production of enriched food remains limited. In this study, we investigated the modification in macromolecules and micronutrients in industrial-scale flour from partially sprouted chickpeas to define its possible use as an ingredient in cereal-based foods. Controlled sprouting resulted in significant decrease of antinutritional compounds and in an increase of free minerals and vitamins. Sprouting also affected the overall structural organization of proteins and their thiol/disulfide balance, and promoted release of peptides. All of these had a positive effect on dough mixing properties, in particular for dough development. Formulations with enrichment in sprouted chickpeas flour (wheat:chickpea ratio = 100:20) were tested also as for their dough leavening properties that improved with respect to flour from non-sprouted chickpeas. All together, taking into account the modifications induced by partial sprouting at industrial scale, we can conclude that sprouted chickpea flour represents an interesting ingredient for production of enriched cereal based food with better nutritional and rheological characteristics.

Keywords: chickpeas, sprouting, thiol accessibility, pasting properties, dough rheology

Pulses are a particularly rich source of vegetable proteins, as well as of dietary fibers, vitamins and minerals (such as folate and iron), but their nutritional value is limited by the presence of antinutrients that decrease digestibility and micronutrient bioavalaibility (Oomah et al 2011). Thus, despite Western consumers are increasingly interested in natural and healthy food products – without turning down the hedonistic aspect of food – the use of pulses are underexploited at an industrial scale, and they are seldom used as ingredients in processed foods such as bakery products, pasta and snacks (Abu-Ghannam and Gowen, 2011). Indeed, in addition to nutritional issues, consumers often identify off-flavors in products containing high levels (>10%) of pulse flours.

Sprouting (or germination) is a natural process that decreases the antinutrient content in pulses while substantially increases the micronutrient bioavailability and improving the sensory properties. The nutritional benefits of germination have been documented extensively in the scientific literature (Savelkoul et al 1992; Chitra et al 1996; Vidal-Valverde et al 2002; Ghavidel and Prakash 2007; Martín-Cabrejas et al 2008; Khandelwal et al 2010; Gujral et al 2011). During germination, natural enzymes that degrade antinutrients such as phytic acid and trypsin inhibitors are expressed, increasing the vitamin content and the bioavailability of some minerals in the products. Enzymes are also able to degrade ROF (raffinose family of oligosaccharides) into shorter carbohydrates, decreasing the digestive discomfort caused by the presence of ROF and developing a sweet flavor note in germinated pulses.

In many countries, the germination process traditionally has been performed at a household level. The basic germination process consists of steeping pulses in water until they reach the moisture content needed to initiate the seedling, after which the steeping water is drained and the pulses are allowed to germinate under cool, humid conditions. After the germination phase, the grains need to be dried since they usually have a moisture content of 30-50%. Processing conditions must be constantly and precisely monitored and controlled to standardize the properties of the final product and assure good quality is maintained (Bellaio et al 2014).

Some studies had shown that partial germination of several types of pulses decreased the antinutrient content and improved the amount of micronutrients (Ghavidel et al 2007; Bellaio et al 2012). Moreover, the content of fructose greatly increased, thus improving the ingredient taste with a desirable

sweet note (Bellaio et al 2012). In addition to nutritional aspects, germination positively affected the dehulling process for brown chickpeas, mung peas, and pigeon peas (Bellaio et al 2011). Moreover, the process can influence the cooking properties of pulses, including cooking time and dispersed solids (Zamprogna et al 2011). Finally, developing processed foods such as pasta, white layer cakes, and extruded snacks with partially germinated yellow pea reportedly resulted in end products with acceptable characteristics (Han and Buchko 2014; Frohlinch et al 2014).

The impact of sprouting – carried out at an industrial scale – on macromolecular and micronutrient profiles of chickpeas was assessed in the present study. Modification of protein overall association as well variation of specific proteins contents and starch pasting properties were investigated before and after sprouting. In addition, rheological properties of chickpea flour enriched dough were studied in view of providing a nutritious and high protein staple food for low-income end users, such as those in developing areas, and for users wishing to improve the nutritional quality of their diet.

MATERIALS AND METHODS

Materials. Raw and sprouted chickpea (Fig. S1) were provided by Molino Quaglia (Vighizzolo d'Este, Italy). Chickpeas were sprouted in an industrial sprouting plant (Bühler AG, Uzwil, Switzerland) for 3 days at 18-24 °C. Sprouted peas were dried at 50 °C, and stored at room temperature. When required, chickpeas samples (sprouted or non-sprouted) were ground into flour (0.5 mm particle size) in a pin mill (Bühler AG, Uzwil, Switzerland). A commercial common wheat flour (proteins: 13%; alveographic W: 355 $*10^{-4}$J; alveographic P/L: 0.76) was used to prepare the chickpea-enriched dough for rheology studies (wheat:chickpea ratio = 100:20).

Chemical Composition. 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 assessed by HPLC by Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) (Zygmunt et al 1982). Total, soluble and insoluble dietary fiber content was by the enzymatic–gravimetric

procedure (AOAC Method 991.43). The analysis of phytic acid was performed by HPLC with method that uses a spectrophotometric detection as described by Oberleas & Harland (2007).

Protein solubility, soluble peptides, and thiol accessibility. The solubility of proteins in chickpea flour samples (10 g) was determined by suspending the flour samples (10 g in 10 L^{-1}) in 0.05 mol L^{-1}mM sodium phosphate, 0.1 mol L^{-1} NaCl buffer, pH 7.0, containing 8 mol L^{-1} urea or 8 mol L^{-1} urea and 0.01 mol L^{-1}dithiothreitol (DTT) where indicated (Bonomi et al 2012). After 1 h stirring at 25°C, the suspensions were centrifuged (~2,500 *x g*, 30 min, 25°C), and the protein concentration in the supernatant was determined by a dye-binding method (Bradford 1976).

The amount of soluble peptides was monitored by measuring the absorbance at 280 nm in the supernatant obtained from treatment with 10% trichloroacetic acid (TCA). Typically, flour (10 g) was suspended in 10 mL of 0.05 mol L^{-1}sodium phosphate, 0.1 mol L^{-1}NaCl buffer, pH 7.0. After stirring for 30 min at room temperature, the supernatant was collected by centrifugation at 10000 *x g* for 30 min, and an appropriate aliquot was treated with an equal volume of 20% TCA. The supernatant of TCA precipitation was collected by centrifugation at 10000 *x g* for 30 min, and read at 280 nm. Results are given as arbitrary units (absorbance at 280 nm of the TCA supernatant, corrected for dilution when necessary).

Accessible thiols (expressed as µmol thiols (g flour)$^{-1}$)were determined by suspending chickpea flour in 0.05 mol L^{-1}mM sodium phosphate, 0.1 mol L^{-1}NaCl buffer, pH 7.0, containing 0.2 mmol L^{-1} 5,5'-dithiobis-(2-nitrobenzoate) (DTNB), in the presence/absence of 8 mol L^{-1} urea as described by Bonomi et al. 2012. After 1 h stirring at 25°C and centrifugation (~2,500 *x g*, 30 min, 25°C), absorbance of the supernatant was read at 412 nm.

SDS-PAGE. Proteins solubilized from chickpea flour as described above were diluted to 1/10 of their volume with SDS-PAGE denaturing buffer (0.125 mol L^{-1}Tris-HCl, pH 6.8, 500 mL glycerol L^{-1}, 17 g L^{-1} SDS, 0.1 g L^{-1}Bromophenol Blue), containing 10 mL L^{-1} of 2-mercaptoethanol when indicated, and the mixtures were heated at 100°C for 10 min. Sample volumes were adjusted to load 0.01 mg of protein per lane. SDS-PAGE

was carried out in a MiniProtein apparatus (BioRad, Richmond, VA, USA), and gels were stained with Coomassie Blue.

Trypsin inhibitor levels. Trypsin inhibitor content was assessed by using the official method IS/ISO 14902 (2001) as described in Scarafoni et al 2008, using 0.2 mmol L^{-1}solutions of the chromogenic substrate N-benzoyl-p-nitroanilide (BAPA)in 150 mM Tris/HCl, pH 8.0, and trypsin (E.C.3.4.21.4 TPCK-treated, T1426, SIGMA Aldrich, Milan, Italy). Trypsin activity was measured in the presence/absence of the buffer-soluble protein fraction prepared from chickpea flour as described above. One trypsin inhibitor unit (TIU) was defined as the amount of inhibitor that decreases trypsin activity by 50%.

Enzyme Activity Assay. Proteolytic activity was determined by the method of Arnon (1970) using azocasein (Sigma Chemical Co., St Louis, MO, USA) as the substrate. Alpha-amylase activity was determined at least in duplicate according to AACC standard method n. 303, by using the Megazyme Amylase Assay Procedure (Megazyme International Ireland Ltd., Wicklow, Ireland).

Micronutrients determination. For metal quantitative analysis, samples were analyzed by ICP-MS (Bruker AURORA M90 ICP-MS,Milan, Italy. Total metal content was measured on aliquots of a flour suspension (10 g of flour suspended in 10 mL of 100 mmol L^{-1}Tris-HCl buffer, pH 7.5) after removing solids by centrifugation at 10000 *x g* for 30 min. Unbound metals were assessed on the ultrafiltrate obtained by passing the clarified extract through a Centricon ultrafiltration device (5kDa nominal cutoff, Merck Millipore, Vimodrone, Italy).

A RP-HPLC method was used for the determination of water-soluble vitamins (B1, B2, B6, PP) according to (Aslam et al 2008), using a Symmetry® C18 3.5 column (5 µm, 4.6 x 250 mm (Waters, Vimodrone, Italy) and isocratic elution (water/methanol, 50/50 v/v, containing 0.2 % v/v PICB8) at a flow rate of 0.700 mL/min. The eluate was monitored at 440 and 256 nm, and vitamin content was calibrated with reference to appropriate standard curves. Vitamin determination was carried out on the supernatant obtained after centrifugation (10000 *x g*for 30 min) of a suspension of 10 g flour in 10 mL of0.5 molL^{-1}sodium acetate pH

5.0. Unbound vitamins were assessed on the ultrafiltrate obtained by passing the clarified extract through a Centricon ultrafiltration device (5kDa nominal cutoff, Merck Millipore, Vimodrone, Italy).

Pasting Properties. Pasting properties were measured in duplicate using a Micro-Visco-Amylograph (MVAG, Brabender GmbH & Co. KG, Duisburg, Germany), as reported by Marti et al 2013, with little modification. An aliquot of sample (15 g) was dispersed in 100 mL of distilled water (scaling both flour and water weight on a 14% flour moisture basis) and stirred at 250 rpm. The following temperature profile was applied: heating from 30 °C to 95 °C at a rate of 3 °C/min, holding at 95°C for 15 min, cooling from 95°C to 50°C at a cooling rate of 3°C/min, and holding at 50°C for 1 min.

Mixing Properties. Mixing properties of chickpea-enriched doughs (wheat:chickpea ratio = 100:20) were measured in duplicate using a Farinograph-E (Brabender GmbH & Co. KG, Duisburg, Germany)equipped with a 50g mixing bowl. AACCI Approved Method 54-21.01 (AACC 2001) was used for the identification of optimal water absorption for flours.

Leavening Properties. Leavening properties of chickpea-enriched doughs (wheat:chickpea ratio = 100:20) were assessed in duplicate with a Rheofermentometer ®(Chopin, Tripette & Renaud, Villeneuve La Garenne Cedex, France). Dough samples were prepared in an automatic spiral mixer (Bomann, Clatronic s.r.l., Piadena, Italy) with 1% NaCl and 1.5% bakers' yeast. Mixing time and amount of water were those determined by the Farinograph test. The rheofermentographic test was performed on 315 g portion of the dough and carried out at 30 °C for 3 h.

RESULTS AND DISCUSSION

Sprouting influences the chemical composition. The processing conditions used did not promote important modification in terms of starch, protein (as detected as total nitrogen), lipid and fiber content (Table I). In these chickpea samples the content of fiber did not change despite of many studies have shown

that the germination process has a significant impact on dietary fiber fractions (Chang et al 2006; Mahadevamma and Tharanathan 2004; Martin-Cabrejas et al 2003, 2008; Duenas et al 2016), but those changes are dependent on the investigated legume and germination conditions. Although the total amount of sugars did not change, sprouting promoted an important increase in sucrose and a decrease in both raffinose and glucose.

The amount of some free micronutrient as well some antinutritional compounds are positively modified by sprouting. The amount of some free micronutrient as well of some antinutritional compounds are positively modified by sprouting. Unbound metal concentrations are reported in Table II. Sprouting has an effect on the availability of some mineral micronutrients that are released from macro and micromolecules (such as phosphates and or phytate) during the process. This improvement is particularly noticeable for relevant micronutrients such as iron, copper and calcium. In this frame it is important to note that the total content of these metals did not significantly change upon sprouting (not shown), suggesting that sprouting promotes a change in speciation of these chemical species. The content of acid phytic decreases ≈10 % upon sprouting, as observed in previous studies (Chitra et al 1996). Phytates have a marked chelating ability - in particular for calcium - and interact with proteins decreasing their solubility and availability. Phytic acid has also been linked to the inhibition of digestive enzymes such as protease, alpha amylases and trypsin.

The content of unbound soluble vitamins such as B_2, B_6 and PP is not modified, while the amount of vitamin B_1 increases twice with respect to the non-sprouted sample. Sprouting lowers the levels of Serin-Protease Inhibitors (SERPINs) that are antinutrient factors broadly present in legumes. The data presented in Fig. 1 show that sprouted flour had a TIU of 42.6±3 (that is, 43% less than the non-sprouted flour (24.1±6 TIU)), confirming previous findings reviewed by Jain et al (2009).

Sprouting results in a modification of protein overall association as well in the content of specific proteins. Protein solubility studies in different media represents an useful approach for gathering information on the nature of the inter-protein interactions in legumes (Manful et al 2014) and in cereal-based materials (Iametti et al 2006; Bonomi et al 2012; Cabrera-Chávez et al 2012; Barbiroli et al 2013).

Sprouting resulted in a significant decrease of all the classes of proteins solubilized in the various media. Proteins soluble in buffered saline were the most degraded (33.3% decrease, from 141±5.4 to 94±3.8 mg (g flour)$^{-1}$), along with proteins that could only be solubilized upon treatment with dissociating agents (27.5% decrease, from 218±8.2 to 159±7.4 mg (g flour)$^{-1}$), whereas changes affecting proteins in aggregates stabilized only by hydrophobic bonds were much lower (14.9%, from 152± 7.1 to 129± 5.4 mg (g flour)$^{-1}$).

The electrophoretic profile of proteins and large peptides extracted in media of various dissociating ability and analyzed in the absence and in the presence of disulfide-reducing agents are shown in the two panels of Fig. 2, and indicate that the most evident germination-related changes involved proteins of high molecular mass, that are present in the original flour as hydrophobic aggregates stabilized by disulfide bonds. Recently, Ghumman et al (2016) shoed that pulses germination resulted in the breakdown of high molecular weight proteins to low molecular weight proteins. A comparison between the solubility data presented in the previous paragraph and the tracings in Fig. 2 indicates that small peptides (escaping detection by the dye-binding method used here, and not evident in the SDS-PAGE gels) were possibly the most abundant products of sprouting-induced protein breakdown.

This interpretation is supported by measurements of the TCA-soluble material in extracts obtained from chickpea flours with buffered saline. Small peptides are expected to be soluble in this medium, and to remain soluble even in the presence of 10% TCA. We found a ≈ 60% increase in the amount of TCA-soluble peptides in the flour obtained from sprouted chickpeas. The release of peptides during sprouting represents a positive effect, as it may improve protein digestibility, facilitate further degradation of proteins and production of bioactive species, and provide substrates for flavor formation upon further processing and baking.

The formation of a proper protein network in dough has been shown to depend on the presence and/or formation of intra- and intermolecular S-S bonds among involved proteins. Thus, sulfhydryls (-SH) and disulfides (-S-S-) have a fundamental role in defining the technological properties of flours. Quantification of the amount and accessibility of protein –SH groups has been applied to understand the nature and to evaluate the extent of process-related modifications in many cereal-based foods (Bonomi et al 2012; Cabrera-Chávez et al 2012; Barbiroli et al 2013). The arrangement of thiols in the different chickpea

samples was studied by assessing the amount of –SH groups accessible to the thiol reagent, DTNB, in the presence/absence of denaturants. Sprouting had no effect on the content of readily-accessible thiols (i.e., reacting with DTNB in the absence of denaturants) that remained constant at 120±8 nmol (g flour)$^{-1}$) in both samples. Treatment with urea increased the content in accessible thiols to 150±11 nmol (g flour)$^{-1}$) in the flour from non–sprouted chickpeas, whereas the content in urea-accessible thiols in flour from sprouted chickpeas was in excess of 350 nmol (g flour)$^{-1}$. Thus, the sprouting treatment may imply a loosening of the compact structure of legume storage proteins, making a higher number of protein thiols accessible upon protein unfolding. This new structural organization could facilitate the incorporation of these proteins into the protein network relevant in baked foods, and also improve the network-forming ability in dough containing mixtures of flour from different sources.

Sprouting also affected the endogenous levels of hydrolytic activities. The most notable increase involved alpha-amylase, that in the sprouted material was 0.125±0.02 U (g flour)$^{-1}$, that is more than twice the activity in raw chickpea flour. The endogenous proteolytic activity also reached 7.1±0.04 U/(g flour)$^{-1}$ in the flour from sprouted chickpea, 170% higher than in the non-sprouted sample. The presence of these endogenous enzymes may represent a form of added value, since sprouted materials can be used as partial or even total replacement for commonly used exogenous enzymes. This might allow to produce "clean label" products.

Sprouting does not compromise starch pasting properties. Sprouting affects pasting properties of chickpea flour (Fig. 3), decreasing the maximum viscosity reached during the heating step (from 416.5 to 339.5 BU for raw and sprouted chickpea flour, respectively). This is likely due to the alpha-amylase activity, which increases in sprouted chickpea. The action of α–amylase results in a rapid decrease in the size of the starchy molecules and, consequently, in the viscosity of the suspension (Collado and Corke 1999). A quite similar behavior was also observed on sprouted grains (Mohan et al 2010; Gamel et al 2005).

All samples show retrogradation of starch in the cooling phase (from 95 to 50°C). Retrogradation was much lower in sprouted chickpea flour, because the increased dextrinization by alpha amylase- limits starch reorganization as the suspension is cooled. The setback values were 479 and 314 BU for raw and

sprouted chickpea flour, respectively. This is an encouraging result in view of preparing chickpea enriched bread, since low setback values are related to low starch retrogradation and thus, to improved shelf life.

Dough enrichment with sprouted chickpea flour improves dough formation and leavening properties compared to raw chickpea flour. Wheat dough enrichment with sprouted chickpea determined important changes in its dough-mixing behavior as measured by the Farinograph (Fig. 4A). Incorporation of raw chickpea flours did not affect the optimal water absorption, that increased slightly when including sprouted chickpea flour. A similar effect was observed by Fernandez and Berry (1989). Differences between raw and sprouted chickpea enriched dough might be attributed to the formation of peptides and to the overall proteins structural changes observed upon sprouting.

Dough development time is the time from the first addition of water to reaching the point of greatest torque. During this phase of mixing, water hydrates the flour components and dough is developed. High level of fiber usually results in longer development time (Peressini and Sensidoni 2009). Addition of sprouted chickpea flour lowers development time much more that the non-sprouted one (5.4 vs 8.7 min). These differences could relate to the effects of sprouting on thiol group accessibility. Increase in mixing time of wheat dough with incorporation of either raw or sprouted chickpea (from 2 min of wheat flour to 8.7 min and 5.4 min for dough with raw and sprouted chickpea flours respectively) was in agreement with previous studies (Baik and Han 2012; Mohammed et al 2012; Mohammed et al 2014; Sathe et al 1981).

The dough sample containing flour from sprouted chickpea exhibits a similar stability similar than non-sprouted chickpea flour (7.5 vs 8.3 min). Despite the initial loss of consistency, the dough with sprouted chickpea flour was not subjected to further sagging, maintaining torque values very similar to unsprouted flour. The changes in dough characteristics upon addition of chickpea flour may be attributed to dilution of gluten-forming proteins causing weakening of dough. Variation in hydration behavior of two proteins may be another reason for these differences (Mohammed et al 2012; Mohammed et al 2014).

Dough leavening properties are reported in Fig. 4B and Fig. 4C, presenting dough height development and gas production and retention during proofing, respectively. Interestingly, chickpea

enrichment increases dough development during proofing (from 39.2 mm in wheat-only dough to about 47.7 mm in and chickpea-enriched dough). Enrichment with sprouted chickpea gives a similar height as non-sprouted (47.7 vs 46.5 mm), but in a shorter time (103 vs 166 min), likely due to the sucrose formation promoted by sprouting (Table I).

The enrichment in sprouted chickpea flour allows the dough to reach the maximum development faster than in the case of non-sprouted flour (Fig. 4A). Addition of chickpea flour increases gas production from 1200 ml for wheat-only to 1538 ml (non-sprouted) and 1574 ml (sprouted). The amount of retained gas is higher in enriched dough compared to the control (1135 ml for wheat-only, 1380 ml (non-sprouted) and 1397 ml (sprouted)). The relation between gas production and retention, given as a percentage of gas retained in the dough, was slightly decreased by enrichment going from ~ 95 for wheat-only to 90 % (non-sprouted) and 89% (sprouted). This parameter is related to the dough ability to be stretched in thin membranes and - again - is associated to quality of the protein network. All together, these results suggest that partial germination of chickpea promotes a more rapid dough development during proofing, without worsening the gas production and retention of the dough.

CONCLUSIONS

This study proves that sprouted chickpeas can be used as an ingredient for production of enriched food. Enrichment with industrial-scale germinated chickpea flour appears to exert beneficial effects from a nutritional standpoint, whereas the structural modifications in macromolecules make sprouted chickpea flour a promising and interesting ingredient in formulating enriched products. Thus, sprouted chickpea flour offers a good opportunity for new products aiming at improving the nutritional significance of products targeted to low-income consumers and to users relying on vegetarian or vegan diets.

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Figure legends

Figure S1. Sprouted chickpeas.

Figure 1. Effects of sprouting on trypsin inhibitory activity.

Figure 2. SDS-PAGE separation under non-reducing (top) and reducing (bottom) conditions of proteins solubilized from sprouted and non-sprouted chickpeas in different media.

Figure 3. Effect of sprouting on pasting properties of chickpea flour.

Figure 4. Effect of sprouting on mixing properties (A), dough development (B) and gas production (C) of chickpea-enriched dough.

Table I

Effect of sprouting on the chemical composition of chickpea flour

Data are expressed as g (100 g flour d.b.)$^{-1}$

	non-sprouted	sprouted
Starch	45.6 ± 1.3	42.1 ± 3.1
Protein	18.6 ± 0.3	20.2 ± 0.1
Lipid	7.3 ± 0.3	7.2 ± 0.6
Ash	3.2 ± 0.1	2.8 ± 0.0
Sugars	5.6 ± 0.1	5.6 ± 0.2
Glucose	0.1± 0.0	n.d
Fructose	0.1± 0.0	0.1± 0.0
Sucrose	3.2± 0.2	5.5± 0.2
Raffinose	2.2± 0.2	n.d.
Total Fiber	17.9 ± 1.7	18.8 ± 2.0
SolubleFiber	1.0 ± 0.1	1.6 ± 0.7
InsolubleFiber	16.9 ± 0.3	17.2 ± 0.1

TABLE II

Unbound micronutrient and phytic acid content in sprouted and non-sprouted chickpea flour.

Data are expressed as micrograms (g flour d.b.)$^{-1}$

analyte	non-sprouted	sprouted
Na	444.6 ± 10.0	442.8 ± 12.0
Mg	546.0± 16.0	455.0 ± 18.0
Ca	230.0 ± 10.0	263.0 ± 7.0
Cr	0.2±0.0	0.2 ± 0.0
Mn	1.6± 0.1	0.9± 0.0
Fe	3.9 ± 0.2	4.6 ± 0.1
Co	0.05 ± 0.01	0.08 ± 0.00
Ni	0.8 ± 0.1	1.0 ±0.0
Cu	0.4 ±0.0	1.4 ± 0.1
Zn	10.1 ± 0.3	9.9 ± 0.2
Se	0.1 ±0.0	0.2 ± 0.0
Mo	3.9 ± 0.2	4.2 ± 0.1
vit B_1	0.4 ±0.1	0.8 ± 0.1
vit B_2	0.4 ± 0.1	0.4 ± 0.0
vit B_6	4.9 ± 0.2	5.3 ± 0.4
vit PP	0.7 ± 0.0	0.8± 0.0
phytic acid	18810 ± 107	17010 ± 279

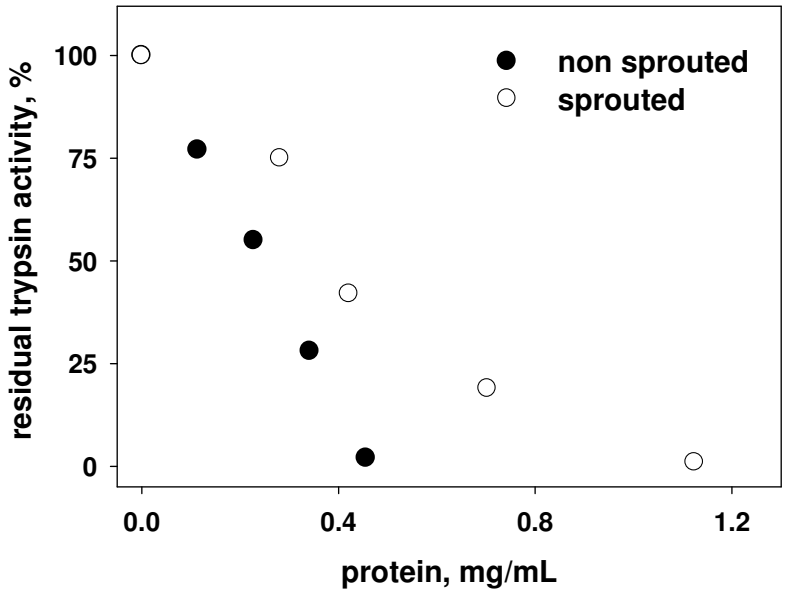


Figure 1. Effects of sprouting on trypsin inhibitory activity.

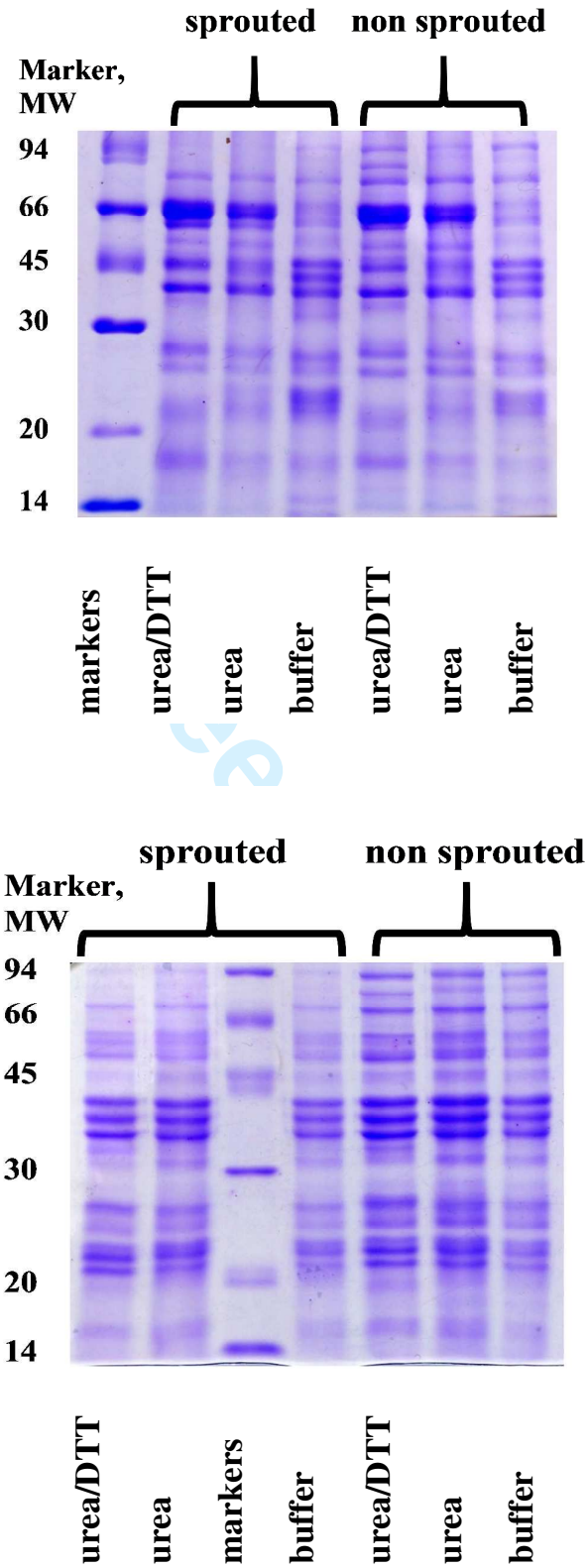


Figure 2. SDS-PAGE separation under non-reducing (top) and reducing (bottom) conditions of proteins solubilized from sprouted and non-sprouted chickpeas in different media.

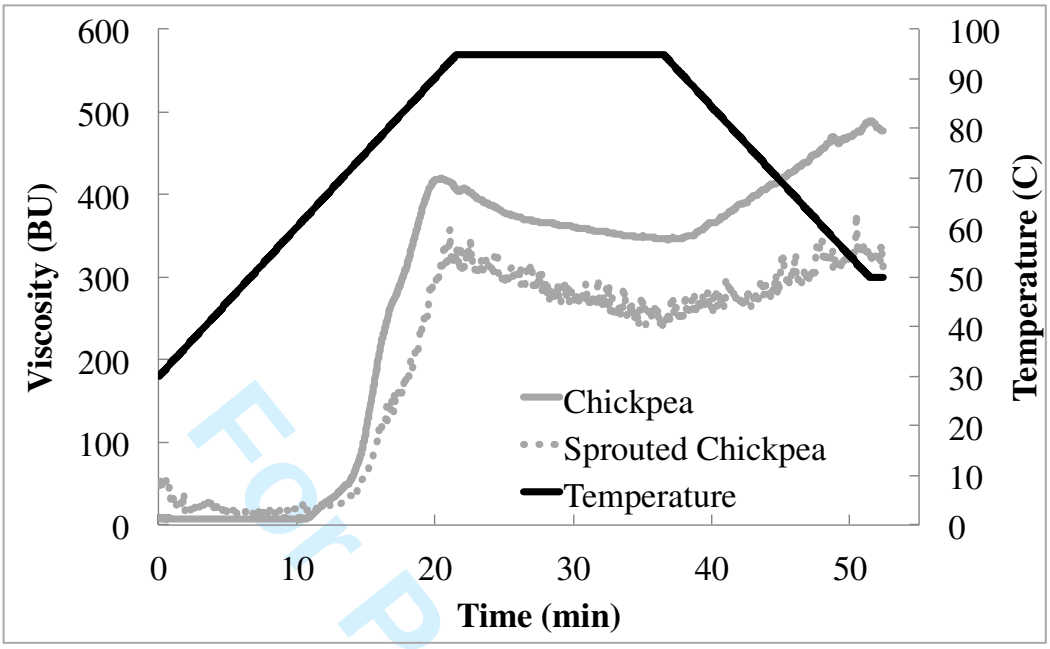
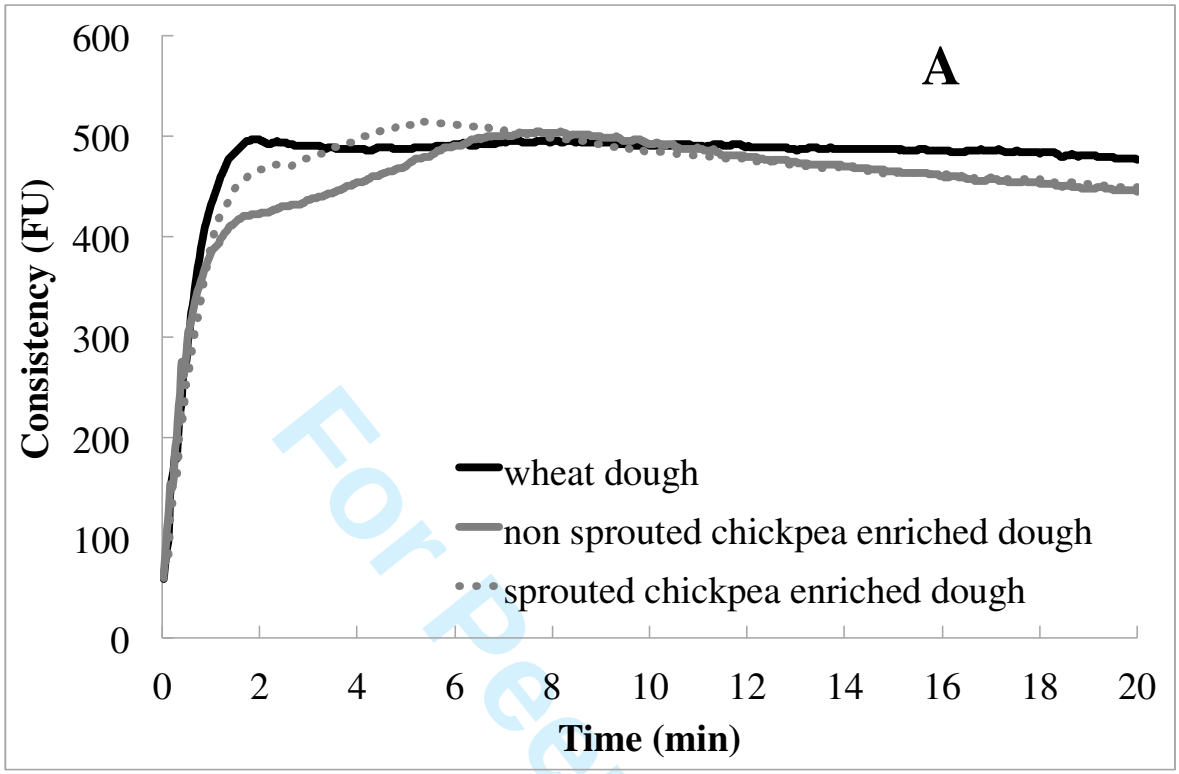
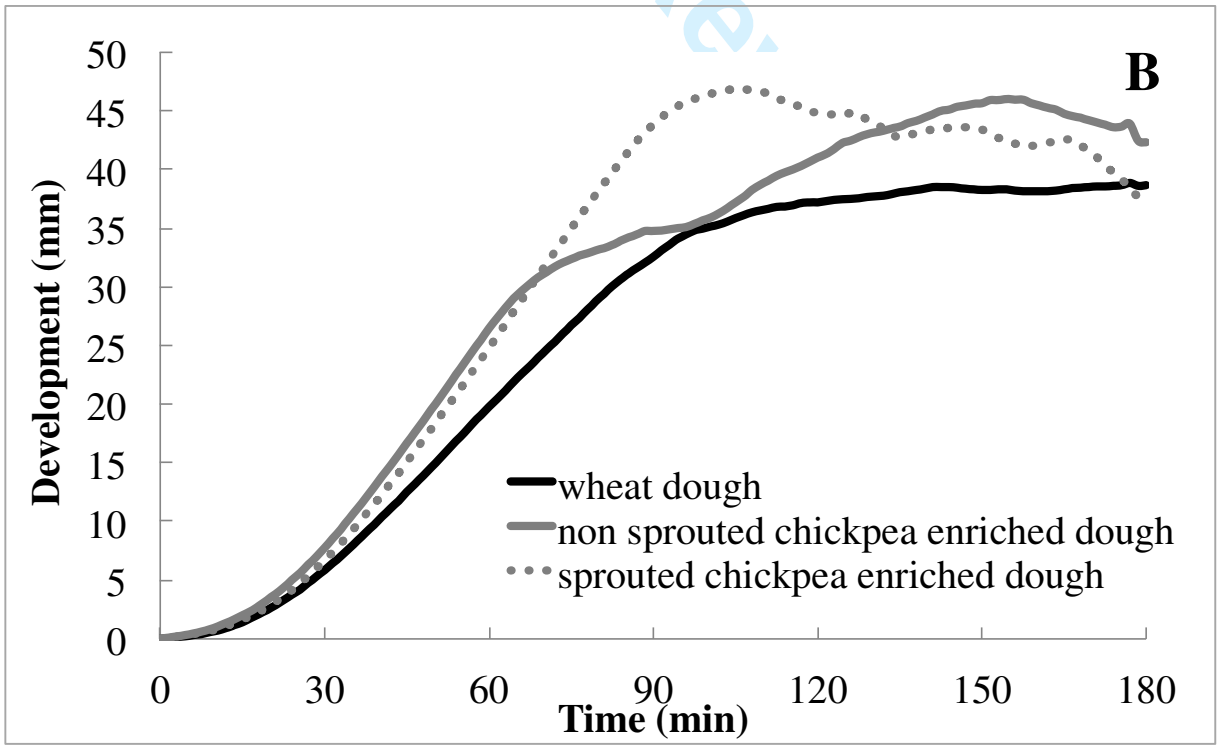


Figure 3. Effect of sprouting on pasting properties of chickpea flour.


A
Consistency (FU)
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
16
18
20
Time (min)
wheat dough
non sprouted chickpea enriched dough
sprouted chickpea enriched dough


B
Development (mm)
50
45
40
35
30
25
20
15
10
5
0
0
30
60
90
120
150
180
Time (min)
wheat dough
non sprouted chickpea enriched dough
sprouted chickpea enriched dough

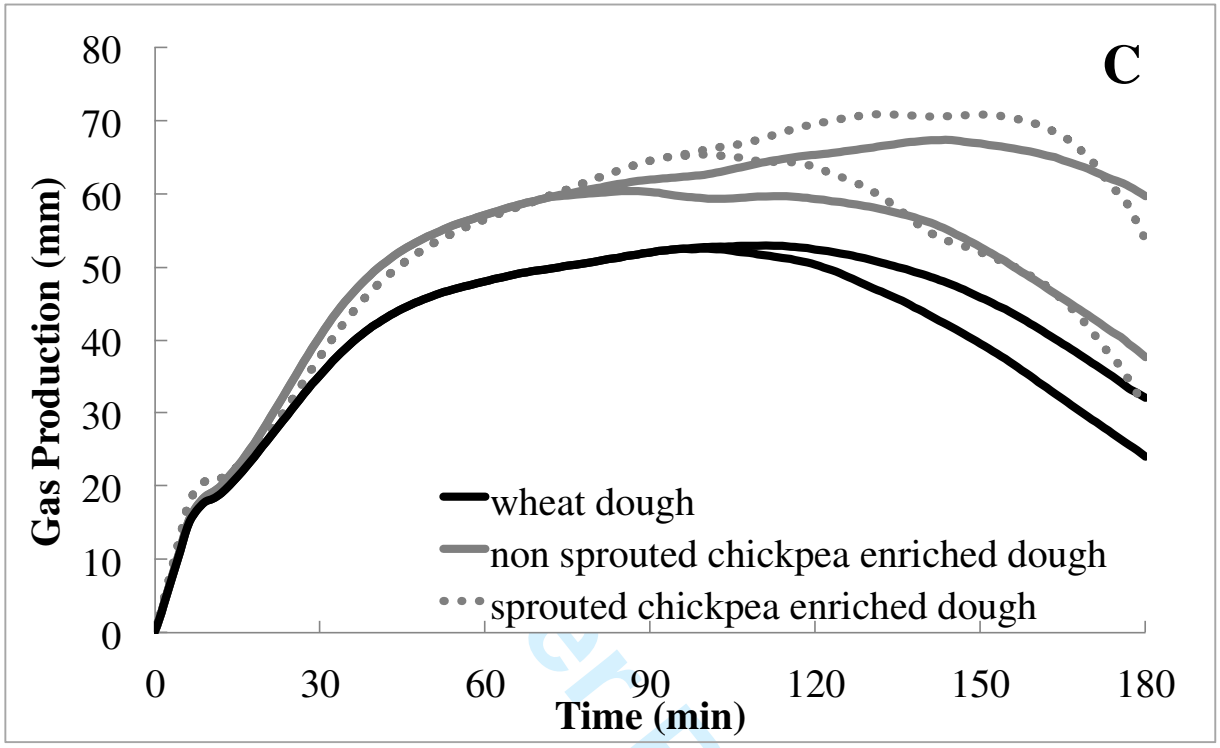


Figure 4. Effect of sprouting on mixing properties (A), dough development (B) and gas production (C) of chickpea-enriched dough.

Figure S1. Sprouted chickpeas.