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Research article

Effects of phytase-supplemented fermentation and household processing on the nutritional quality of *Lathyrus sativus* L. seeds

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ABSTRACT

Grass pea (*Lathyrus sativus* L.) is commonly consumed in cooked, fermented, and roasted forms in Ethiopia. However, the impacts of household processing practices on its nutrients, antinutrients, and toxic compounds have not been adequately studied. Therefore, the effects of household processing and fermentation in the presence and absence of a phytase on the contents of β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP), *myo*-inositol phosphates, crude protein, minerals and the *in vitro* bioaccessibility were investigated. Fermentation exhibited a significant decline in β-ODAP (13.0–62.0%) and phytate (7.3–90.5%) irrespective of the presence of phytase. Pressure and pan cooking after discarding the soaking water resulted in a 27.0 and 16.2% reduction in β-ODAP. A 30% reduction in phytate was observed during germination followed by roasting. In addition, germination resulted in a significant ($p < 0.05$) increase in crude protein. Germination and germination followed by roasting resulted in the highest Fe bioaccessibilities (more than 25 fold higher compared to untreated samples) followed by pressure cooking and soaking. Processing also improved Zn bioaccessibilities by 50.0% (soaked seed without soaking water), 22.5% (soaked seed with soaking water), and 4.3% (germination). Thus, the processing technologies applied were capable of reducing the content of phytate ($InsP_6$) and β-ODAP with a concomitant increase in mineral bioaccessibilities. Processing of grass peas could therefore contribute to their more widespread utilization.

1. Introduction

Grass pea (*Lathyrus sativus* L.) is a pulse rich in protein (26–34%), lysine and minerals such as zinc, iron, calcium, phosphorus, magnesium, and copper (Urga et al., 1995, 2005; Hanbury et al., 2000). It also has considerable antioxidants, vitamins, and health improving properties. Moreover, it is a drought-tolerant and nitrogen-fixing pulse producible under adverse conditions. In Ethiopia, grass pea seeds are consumed in different household processed forms such as sprout/soaked-boiled-'nifro', soaked-roasted-'kollo', and roasted flour-'shiro'. Grass pea flour is also commonly utilized together with flours of other legumes such as dry pea or chickpea for making unleavened bread as well as a traditional Ethiopian sauce 'shiro wott' (Hailu et al., 2015; Fikre et al., 2011). Grass pea seeds, however, lack sulfur-rich amino acids (methionine and cysteine) as well as tryptophan. They also contain antinutrients such as phytate, tannin, and trypsin inhibitors that have adverse effects on protein digestibility and mineral bioavailability in foods derived from grass peas (Urga et al., 2005; Arslan 2017). The major limitation for its consumption, however, is the presence of the non-proteinogenic amino acid, β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP), a toxic compound resulting in neurolathyrism (Tamburino et al., 2012; Mondal and Puteh, 2014). β-ODAP contents below 300 mg/100 g were reported to exhibit a low toxicity (Kumari, 2001). A daily intake of 500 mg β-ODAP per day per person for a period of 2–3 months was considered as the maximum safe limit for human consumption (Rao, 2001). Nevertheless, during processing, its concentration might be reduced to levels that pose a

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minimum threat to health (Walker and Kochhar, 1982). Low cost and conventional preparation methods such as soaking are reported to be effective in degrading antinutrients and improving nutritional quality in legumes (Wang et al., 1997; Kebede et al., 1995).

Fermentation has vital advantages of retaining nutrients, sensory attributes, and reducing the microbial load of food products (Stoica et al., 2013). During soaking the seeds absorb water, endogenous enzymes are activated, and the content of antinutritional factors is declining. Long time soaking, however, has been found to reduce the nutritional quality of legumes by leaching of nutrients into the soaking water (Hailu et al., 2015). Germination is another simple and inexpensive treatment to enhance the nutritional value of seeds by affecting respiration, subcellular structures, synthesis of macromolecules, proteolysis, conversion of seed nitrates into ammonium compounds or plant proteins and degradation of antinutrients (Hooda and Jood, 2002; Jiang et al., 2013). Furthermore, cooking was shown to reduce the content of phytate, trypsin inhibitors, and heat-labile antinutrients (Wang et al., 1997). Soaking followed by cooking was reported to be capable of converting 40.0% of β-ODAP to the non-toxic α-ODAP (Padmajaprasad et al., 1997). Igzaw et al. (2004) observed an 80.0% and 97.0% reduction in the β-ODAP present in a broad and low toxin grass pea variety, respectively, by fermentation.

However, in literature, only a few number of studies exist on grass pea seeds that primarily deal with the effects of different household processing practices on their limited nutritional quality, and there is no study dealing with the effect of fermentation in the presence of phytase. Therefore, the primary objectives of this study were to elucidate the effects of fermentation, household processing practices, and their combinations on the content of β-ODAP, *myo*-inositol phosphates, and minerals in high β-ODAP containing grass pea seeds. Furthermore, the effect of processing on the protein quality and *in vitro* mineral bioaccessibility was investigated.

2. Materials and methods

2.1. Raw materials

Grass pea (*Lathyrus sativus* L.) seed samples were provided by the Debre Zeit Agricultural Research Centre one of the federal centers of the Ethiopian Institute of Research Centre. The samples were transported to the Max Rubner-Institute, Karlsruhe, Germany, where fermentation, all household processing, and the analysis were carried out. Cleaned and graded raw grass pea seeds were kept at 4 °C until processing and further analysis. *Aspergillus niger* phytase (Natuphos®) was obtained from BASF (Ludwigshafen, Germany). Maize flour, fresh yeast, and mixed sourdough were purchased from a local supermarket, Karlsruhe, Germany. After quarter sampling, portions of raw seed samples were milled (Rommelsbacher Gewürz und Kaffeemühle, Germany) and passed through a 0.5 mm aperture sieve (Retsch testing sieve, Germany). After that, the ground material was stored at 4 °C until further processing.

2.2. Phytase activity assay

The activity phytase was determined as previously reported by Menezes-Blackburn et al. (2015) with little modification. Briefly, a 1.0 g fresh sample of grass pea/maize was extracted with 20 mM Na-acetate buffer, pH 5.0 by agitating for 2 h. The supernatant obtained after centrifugation at 10,000 rpm for 15 min was used for phytase activity determination. 350 μL 100 mM Na-acetate buffer, pH 5.5 containing 1.79 mM Na-phytate were incubated in a water bath at 37 °C for 10 min. Then, 10 μL of the phytase-containing supernatant were added and incubated at 37 °C for 30 min. Thereafter, 1.5 mL a freshly prepared solution of 10 mM ammonium molybdate:2.5 M sulfuric acid:acetone (1:1:2 v/v) and 100 μL, 1 M citric acid were added to stop the reaction. Blanks were prepared by adding the phytase-containg supernantant after the ammonium molybdate: sulfuric acid: acetone solution. Before measuring absorbance at 355 nm (Specord 200, Analytik Jena, Jena, Germany), all incubation mixtures were centrifuged in a table centrifuge at 8,000 rpm for 5 min.

2.3. Household treatments

2.3.1. Fermentation

20 g of pure grass pea flour and a blend consisting of 13.3 g grass pea flour and 6.7 g of maize flour were yeast or sourdough fermented in the presence or absence of a phytase from *Aspergillus niger* (Table 1). Either 500 or 1000 U *Aspergillus niger* phytase were added per kilogram ground grass pea or grass pea blend. All ingredients were mixed vigorously, kneaded, covered with aluminum foil, and incubated at 37 °C for 2.5 h. The progress of fermentation was followed by measuring the pH of the fermentation mixture every 30 min. Preliminary fermentation studies revealed no further decrease in the pH-value of the fermentation mixture after 2.5 h fermentation time. The samples were kept at room temperature (21 °C) for 15 min after incubation in order to prepare the samples for more effective freeze drying, besides, keeping the samples at 21 °C may give chance for further fermentation if available. The samples were kept at -20 °C for 72 h before freeze-drying. The freeze dried samples were kept in desiccators overnight, then, milled using a mortar and pistil and kept at 4 °C until further analysis.

2.3.2. Soaking

100 g grass pea seeds were soaked at 21 °C for 9 h using 400 mL of tap water. The soaked samples were either freeze-dried without discarding the soaking water or freeze-dried after discarding the soaking water. To prevent sprouting of the seeds, the soaked samples were kept 72 h at -20 °C before freeze-drying.

2.3.3. Pressure and pan cooking

Pressure and pan cooking were carried out with grass peas soaked as described in 2.3.2. Both household processes were performed with or without discarding the soaking water. A 2.5 L pressure cooker and a 2 *L pan* cooker were used. Pressure-cooking was performed for 7 min at 121 °C and pan cooking for 25 min at 100 °C. When discarding the soaking water, the same amount of tap water was added before cooking. The same cooking methods were also carried out without grass pea samples to quantify the concentrations of the minerals in the water before and after the cooking processes. No water was added during pan cooking to replace evaporated water. The cooking time was defined as the time needed to obtain the desired softness. All samples were kept at -20 °C for 72 h, freeze-dried and then kept under 4 °C until further analysis.

2.3.4. Germination

200 g of grass pea seeds were rinsed three times with distilled water and soaked for 9 h in the presence of 800 mL tap water. The seeds were placed on germination cotton-cloth previously wetted with distilled water and supported by a perforated plate. The seeds were distributed to give all seeds sufficient space for sprouting. Watering of the seeds was performed manually every hour during daytime, and germination was carried out under controlled temperature of, 25 °C for 24 h, 48 h, and 72 h. Before freeze-drying, germinated seeds were kept overnight at -20 °C to stop germination.

2.3.5. Roasting

100 g of grass pea seeds were roasted on a plate heater adjusted to a temperature of 200–250 °C for 10 min using a manual roaster made of stainless steel. Previously soaked or germinated grass peas were thawed and roasted for 15 min and 20 min, respectively. Roasting was stopped after the appearance of a light brown color. The roasted samples were cooled down to 21 °C and freeze-dried.

Table 1. Experiment design for grass pea fermentation.

Grass pea samples	*Aspergillus niger* phytase (U/kg)	further ingredients			
		yeast (g)	sour dough (g)	salt (g)	water (mL)
Grass pea	500	0.3	-	0.6	30
Grass pea	1000	0.3	-	0.6	30
Grass pea	-	0.3	-	0.6	30
Grass pea	-	-	0.3	0.6	30
Grass pea blend	500	0.3	-	0.6	30
Grass pea blend	1000	0.3	-	0.6	30
Grass pea blend	-	0.3	-	0.6	30
Grass pea blend	-	-	0.3	0.6	30

All freeze-dried samples treated by household processing practices were milled (Rommelsbacher Gewürz- und Kaffeemühle, Germany), passed through 0.5 mm aperture sieve (Retsch testing sieve, Germany) and stored at 4 °C until further analysis.

2.4. β-ODAP analysis

β-ODAP quantification is described in detail in Bekele et al. (2019). Briefly, 0.5 g of the flour sample and 10 mL 60 % ethanol were mixed and agitated (Edmund Bühler E1, Germany) thoroughly for 2 h at 21 °C. After that, the suspension was centrifuged (Thermo Scientific™ Sorvall™ LYNX 6000, Germany) for 30 min at 4000 rpm. A combination of the extracts was performed after repeating another extraction with 5 mL of ethanol. Hydrolyzing for 1.5 h in a boiling water bath was carried out combining 2 mL of the extracts and 4 mL 3 M potassium hydroxide. After cooling down to 21 °C, 0.25 mL of hydrolysates and non-hydrolyzed extracts were combined with 2 mL of *ortho*-phthalaldehyde (OPT) reagent and 0.75 mL bi-distilled water accordingly. The OPT reagent preparation can be referred from our previous publication Bekele et al. (2019). After incubation of the solution at 21 °C for 2 h, absorbance was determined at 426 nm (Specord 200, Analytik Jena, Jena, Germany). Using diaminopropionic acid (DAP) as a standard, calibration was performed in a range from 1.0 to 8.0 g x 10^{-6} per mL. The concentrations of DAP were converted to β-ODAP concentrations by a 1.69 conversion factor (Aletor et al., 1994).

2.5. Crude protein analysis

Crude protein content was analyzed according to ISO, 1978 (International Organization for Standardization (ISO), 1978). 1.0 g ground grass pea and 2 Kjeltabs CX (C. Gerhardt GmbH & Co. KG, Germany) containing anhydrous potassium sulfate and copper (II) sulfate were transferred into a Kjeldahl flask, mixed with 25 mL 95–98% sulfuric acid (Merck Chemicals GmbH, Germany) and subjected to digestion by boiling vigorously for 90 min (Turbotherm, C. Gerhardt GmbH & Co. KG, Germany). The resulting mixture was allowed to cool down to 22 °C. Then, the solution was transferred into the distillation apparatus (Vapodest 50 SC C. Gerhardt GmbH & Co. KG, Germany) and mixed with 74 mL deionized water as well as 101 mL 32% sodium hydroxide (C. Roth GmbH & Co. KG, Germany) solution. The receiver vessel of the distillation apparatus was provided with 61 mL 4% boric acid (Merck Chemicals GmbH, Germany) solution. Steam distillation was performed until at least 150 mL distillate was collected. Subsequently, the content of the receiving vessel was titrated with 0.1 N hydrochloric acid (Merck Chemicals GmbH, Germany) detecting the endpoint of the titration using a pH-combination electrode (C. Gerhardt GmbH & Co. KG, Germany). A blank test in dublicate was performed when fresh batches of reagents or freshly prepared solutions were used. The volume of hydrochloric acid required was used to calculate the nitrogen content in the sample. Glycine-a well-known nitrogen content was used as a standard to determined the recovery rate of the process. A recovery rate of 100.05% was obtained. The measurements of all samples and the respective reference material were carried out for four consecutive days. All determinations were done in duplicate. The repeatability pooled standard deviations of the reference sample and different household processed samples were calculated and obtained to be 0.14% and 0.17%. The factor used to calculate the protein content in the samples was 6.25.

2.6. Myo-inositol phosphates analysis

Myo-Inositol phosphates-InsP_6 analysis was done using High-Performance Liquid Chromatography (Dionex Ultimate 3000, Chromeleon software) (Bekele et al., 2019). 0.5 g of the samples were vigorously agitated (Edmund Bühler E1, Germany) for 2 h with 20 mL 0.5 M hydrochloric acid at 21 °C. The extracts were after that centrifuged 15,000 *g* for 30 min (Thermo Scientific™ Sorvall™ LYNX 6000, Germany), and overnight freezing at -20 °C of the supernatants was undertaken. The samples were de-frozen at 21 °C, and diluted with three times the volume of bi-distilled water. The diluted samples were applied to 0.5 g AG 1-X4 resin, (Bio-Rad Lab, United States) in a column equilibrated with 50 mL of bi-distilled water. 25 mL of 0.025 M hydrochloric acid followed by 25 mL of distilled water were used to clean the column before eluting the *myo*-inositol phosphates with 25 mL of 2 M hydrochloric acid. Using a vacuum evaporator, the eluent was evaporated, after that the dried remnant left in the rotary flask allowed to dissolve in 1 mL bi-distilled water. The sonicated liquid samples passed via a membrane filter -0.45 μm Millipore (Sartorius Stedim Biotech GmbH, Göttingen, Germany). Finally, 20 μL eluate was chromatographed to Ultrasep ES 100 RP18 (2 × 250 mm) to quantify InsP_6. The running rate of the column was 0.2 mL per min of the eluant containing (56:44:1.5:5 v/v) of methanol, formic acid, tetrabutylammonium hydroxide, and, water at 45 °C and 4.25 pH. Mixtures of inositol trisphosphate (InsP_3) to inositol hexakisphosphate (InsP_6) were prepared as standards. InsP_6 standard was used to draw a calibration curve.

2.7. In vitro mineral bioaccessibility

A simplified gastrointestinal digestion assay was adapted from Feitosa et al. (2018) to quantify bioaccessibility in raw and household processed grass pea samples. Briefly, 10 g of grass pea flours was dispersed in 60 mL of a 20 mM glycine-hydrochloric acid buffer, pH 2.0. 1.3 mL of a solution containing 1.6 g of pepsin (Sigma-Aldrich Produktions GmbH, Riedstraße, Steinheim, Germany) in 10 mL 20 mM glycine-hydrochlorid acid buffer, pH 2.0 were added. The incubation of the suspension was done in a water bath under agitation (400 rpm) at 37 °C for 2 h. Then, by subsequent addition of 1 M $NaHCO_3$, the suspension pH was regulated to be 7.2 to simulate intestinal digestion. Thirteen milliliters of a pancreatin solution prepared by dissolving 0.4 g pancreatin powder (Sigma-Aldrich Produktions GmbH, Riedstraße, Steinheim, Germany) in 100 mL of ultra-purified water was added. 2 ml ultra-purified water was added in a dialysis bag (Carl Roth GmbH + Co. KG, Schoemperlenstraße, Karlsruhe, Germany) and then put in the digested fluid followed by incubation for 2

h at 37 °C, under agitation at 255 rpm. Subsequently, after removing the dialysis bag, the analysis of iron, zinc, calcium, and phosphorus in the dialysate was carried out by Inductively Coupled Plasma Mass Spectrometry-ICP-MS. Percentage bioaccessibility (%) equal to Y/Z*100, where Y symbolizes quantified dialyzable mineral\100 g dry matter (DM) of the digested fluids whereas Z is the total amount of the mineral\100 g DM of the flours of raw and processed grass pea seeds.

2.8. Mineral analysis

The concentrations of iron, zinc, calcium and phosphorus in raw and processed grass pea flours were measured according to Feitosa et al. (2018) with modifications by ICP-MS in particular (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States, iCAP Q). A mixture of 200 mg of ground grass pea flours and 7 mL concentrated nitric acid was decomposed in a microwave (Berghof Products + Instruments GmbH, Harretstraße, Eningen, Germany). The heat was successively raised linearly till a temperature of 70 °C within 10 min at 80 W; 70 °C at 70 W for 10 min; till 150 °C within 10 min at 80 W; 150 °C at 70 W for 10 min; till 180 °C within 10 min at 80 W; 180 °C at 80 W for 20 min. After preparing sets of digestion blanks for each batch of the sample, the samples were analyzed in triplicate. Depending on the mineral content, the samples were diluted and measured in 2 % (v/v) HNO_3. The data are expressed on a dry matter basis. Table 2 showed the measurement parameters and operating conditions of ICP-MS. For calibration, the standard was added directly, whereas the LOQ (limit of quantification) was calculated according to the blanks (N = 34–40), LOQ represents the average (μ) + 10 * standard deviation (σ), N represents the number of blanks determined for calibration. Accuracy and precision of the method were determined (Table 3) using fresh kidney beans (NCS ZC73019 (GSB–12)) Sigma-Aldrich (St. Louis, MO, USA) as the reference material (n = 14). Less than 3% relative standard deviations were obtained for all analyzed elements with a confidence interval of 95%.

2.9. Statistical analysis

Mineral content and bioaccessibility analysis were conducted in triplicate, whereas crude protein, β-ODAP, and *myo*-inositol phosphate determinations were conducted in duplicate. All data were given in mean values ± standard deviation. One-way analysis of variance (ANOVA) was performed using JMP Pro 13 for windows (version 13). Statistically significant differences among raw and treated samples means ($p < 0.05$) were considered using the Student's *t* comparison test at a 95% confidence level. Excel Windows 2010 software was used for graphically compared results.

Table 2. ICP-MS operating conditions and measurement parameters.

Parameter	Value
RF power	1550 W
Argon flow rates	
Cooling	14 L min^{-1}
Auxiliary	0.75 L min^{-1}
Nebulizer	1.05 L min^{-1}
Sample cone	Ni
Skimmer cone	Ni
Analyte	56Fe, 66Zn, 43Ca, 31P
Internal standard	45Sc (Fe), 89Y (Zn, Ca, P)
Acquisition/scanning mode	STD (Ca), KED-H_2 (Zn, P), 0V–H_2 (Fe)
Sweeps per reading	100
Dwell time	10 ms (Zn, Ca, P), 40 ms (Fe)
No. of runs	5
Replicate time	21 s
Sample uptake rate	0.2 mL min^{-1}
Wash time between samples (2% HNO_3)	40 s
Uptake time	50 s

3. Results and discussion

3.1. Phytase activity of grass pea seeds and grass pea blends

Flours of grass pea seeds and flours of a grass pea blend with maize were used for the fermentation studies. The blend was used to obtain a product with a higher content in sulfur-containing amino acids (cysteine and methionine). Those amino acids are essential for humans, and in grass peas, their content is very low (Sarkar et al., 2019). The intrinsic phytase activities determined at pH 5.5 of grass pea seeds and maize were determined to be 257.7 ± 0.3 and 27.4 ± 0.6 U/kg, respectively. The obtained activity for grass pea seeds was within the range reported by Greiner & Konietzny (2006), whereas the activity obtained for maize was lower than reported by the same authors. It is, however, well established that the phytase activity of individual plant seeds differs among varieties, harvest years, or environmental conditions where the plants are grown (Steiner et al., 2007).

3.2. Impact of fermentation on β-ODAP and $InsP_6$ contents

The content of β-ODAP and $InsP_6$ after fermentation in the presence (1000 U/kg, 500 U/kg) or absence of phytase is shown in (Figure 1A, B). The β-ODAP contents of raw grass peas and the grass pea blends were found to be 825 ± 9 and 549 ± 3 mg/100 g DM, respectively. The β-ODAP content of the grass pea blend is found to be 33.5% less compared to raw grass peas, a result expected due to the dilution effect when using a β-ODAP free material such as maize for blending. The change in β-ODAP content during fermentation followed a similar trend irrespective of the use of grass pea flour or grass pea blend flour (Figure 1A).

During all the different fermentations applied, a decline in β-ODAP content was observed ranging from 12.4% to 62.0%. This behavior is in agreement to already published results (Starzyńska-Janiszewska and Stodolak, 2011; Kuo et al., 1995; Igzaw et al., 2004; Akalu et al., 1998). A 10.0% decline in β-ODAP content was reported by fermentation with *Lactobacillus plantarum* fermentation of grass peas (Starzyńska-Janiszewska and Stodolak, 2011) or fermentation with *Aspergillus oryzae* followed by *Rhizopus oligosporous* (Kuo et al., 1995). Igzaw et al. (2004) observed an 80.0% and 97.0% reduction in β-ODAP from a high and low toxin grass pea variety by fermentation with *Rhizopus oligosporous* followed by *Aspergillus oryzae*. On the other hand, neither the back slopping nor the spontaneous fermentation had a significant effect on the β-ODAP content of grass peas (Akalu et al., 1998). The mechanisms for β-ODAP reduction during fermentation is not known, but enzymes present in the yeast or the sourdough microflora might be responsible for the observed effect. Yeast was shown to be more productive with respect to the reduction of the β-ODAP content compared to sourdough (Figure 1A). The addition of phytase during yeast fermentation was found to result in a further decline of the β-ODAP contents of the grass pea flours as well as the grass pea blend flours (Figure 1A). The effect was more prominent while adding 1000 U phytase activity per kilogram of flour compared to adding only 500 U. Since phytase does not act on β-ODAP, the observed effects need to be indirect. One explanation might be that the phytase-induced dephosphorylation of phytate (see next paragraph) resulted in a release of phytate-chelated cations, and those act as a co-factor for the yeast enzyme responsible for β-ODAP degradation. This hypothesis, however, needs to be proven by further studies.

The initial $InsP_6$ contents of raw grass peas and the grass pea blend were found to be 974 ± 4 mg/100 g DM and 878 ± 3 mg/100 g DM, respectively. In general, fermentation resulted in a reduction in $InsP_6$ concentration ranging from 7.3% to 90.5% irrespective grass pea flour or grass pea blend flour was used (Figure 1B). Yeast fermentation was observed to result in slightly lower $InsP_6$ levels compared to sourdough

Table 3. ICP-MS precision and accuracy of the method.

Elements	LOQ (μg kg^{-1})	reference material measured value (mg kg^{-1})	reference material certified value (mg kg^{-1})
Fe	0.64	311 ± 17	330 ± 20
Zn	23.5	30 ± 3	32 ± 2
Ca	12.5	6088 ± 338	6700 ± 400

fermentation. This observation is in contrast to the result reported, for example, by Lopez et al. (2001), who reported that sourdough fermentation was more efficient in phytate reduction than yeast fermentation. Since the intrinsic plant phytase activity was shown to be responsible for phytate dephosphorylation during fermentation (Reale et al., 2007), the pH value during yeast fermentation seems to be more favorable for the intrinsic grass pea phytase than the pH value during sourdough fermentation. This behavior is in good agreement with the observation that legumes exhibit a considerable phytase activity around pH 7.0 (Greiner and Konietzny, 2006). Without the addition of exogenous phytase, fermentation of grass pea flour exhibited higher $InsP_6$ reductions than fermentation of grass pea blend flour. This result might be explained by the 10-fold higher intrinsic phytase activity of grass peas compared to maize. As expected from studies on the baking processes (Greiner and Konietzny, 2006; Haros et al., 2001; Požrl et al., 2009), the addition of exogenous phytase to the fermentation processes resulted in significant higher reduction compared to the fermentation process without phytase addition (Figure 1B). Irrespective of the addition of 500 or 1000 U phytase activity per kilogram of flour, at least 85% of the initial present $InsP_6$ was dephosphorylated. Yeast fermentation without phytase addition resulted in a 25% (grass pea seed flour) and a 10% (grass pea blend flour) $InsP_6$ reduction.

3.3. Impact of household processing on grass pea composition

3.3.1. Effect of household processing on the β-ODAP content of grass pea seeds

The β-ODAP levels of grass pea seeds before and after household processing are given in (Figure 2A). Soaking and discarding the soaking water followed by pressure cooking (Pcwo) resulted in a 27.0% reduction of the β-ODAP contents compared to raw grass peas seeds. Soaking and discarding the soaking water followed by pan cooking (Pacwo) resulted in 16.3% lower β-ODAP contents compared to raw grass pea seeds. Soaking while discarding the soaking water itself reduced the β-ODAP content by 12.3% (Swo). The loss of β-ODAP during soaking might be due to leaching because β-ODAP is water-soluble (Yan et al., 2006) and cooking was assumed to result in a heat-induced isomerization of β-ODAP to α-ODAP (Bell and O'Donovan 1966; Padmajaprasad et al., 1997). Heat treatment of the raw seeds by roasting (Ro) and processing and keeping the soaking water (Sr, Sw, Pacw, and Pcw) had only a minor effect on the β-ODAP contents (0.8–4.3%). The obtained results are in principle in good agreement with those previously reported. Akalu et al. (1998) obtained a 56 and 26% reduction in β-ODAP by cooking grass pea seeds in the presence of tap water with and without discarding the soaking water. Furthermore, a significant reduction in β-ODAP by roasting and soaking was observed. Tadelle et al. (2003) also observed a significant reduction in β-ODAP by soaking, roasting, and cooking, and Tarade et al. (2007) reported that pressure and pan cooking are effective in β-ODAP reduction. Thereby, a marginally higher extent of β-ODAP reduction was observed by pressure-cooking. Germination followed by roasting (Gr) did not have any effect on the β-ODAP content. This behavior could be explained by the observed significant ($p < 0.05$) increase in β-ODAP content by germination (G) (19.2%). Due to the higher β-ODAP concentration in germinated grass pea seeds, roasting resulted in loss as expected. An increase in β-ODAP by germination was already reported by Lambein et al. (1992). They obtained a two to three fold increase in β-ODAP content of grass peas by germination. Stodolak et al. (2004) found also an increase in β-ODAP by germination using a grass pea variety low in β-ODAP.

3.3.2. Effect of household processing on the phytate ($InsP_6$) content of grass pea seeds

The contents of phytate ($InsP_6$) of grass pea seeds before and after processing are given in (Figure 2B). A significant reduction (22.5%, $p < 0.05$) of the $InsP_6$ content of grass peas was only observed by germination (G). Phytate dephosphorylation during germination is due to the action of the endogenous phytases present in grass pea, and germination was already shown to increase the endogenous phytase activity (Greiner and Konietzny, 2006). Ramachandran et al. (2008) already reported a reduction of 4.4% in $InsP_6$ content in an Indian grass pea variety upon germination. Germination followed by roasting (Gr) resulted in a 30.0% reduction in the $InsP_6$ content. Roasting the raw grass pea seeds, however, did not result in a significant $InsP_6$ reduction. This difference could be explained by the significantly higher phytase activity of grass peas after germination. A decline in the $InsP_6$ content of 5.3% was observed by soaking grass peas and discarding the soaking water afterward (Swo). This behavior might be explained by leaching. All other household procedures did not have a significant effect on the $InsP_6$ content of grass peas.

3.3.3. Crude protein

The crude protein content of raw grass pea seed was determined to be 26.02 ± 0.04% DM (Table 4). This result is in good agreement with those obtained by Urga et al. (1995) and Khandare et al. (2018). According to these authors, grass peas contain between 22 and 28% DM of crude protein. Pastor-Cavada et al. (2011), however, found only 17% DM crude protein in a grass pea variety from Spain. All household procedures applied resulted in higher measurable protein contents. However, besides germination, none of the household procedures was expected to result in an increase in the protein contents compared to the raw grass peas. Nevertheless, significant increaments of crude protein contents in flours of cooked pea (25.90 ± 05% to 27.6 ± 0.2%) from Milwa variety; flours of cooked lentil (28.7 ± 0.3% to 29.2 ± 0.2%) and (28.6 ± 0.5% to 30.0 ± 0.2%) from Anita and Tina varieties compared to uncooked pea and lentil were reported by Piecyk et al. (2012). Better accessibility/release of the nitrogen during the process of protein quantification in processed compared to unprocessed samples might explain the observation.

3.3.4. In vitro mineral digestibility

Iron, zinc, calcium, and phosphorus contents of raw and different household processed grass pea seeds are shown in (Table 5). In the raw grass pea seed, 60.70 ± 03 mg/kg DM iron, 43.85 ± 0.01 mg/kg DM zinc, and 1283 ± 8 mg/kg DM calcium were determined. After the application of household processing, higher contents of Fe, Zn, and Ca were found compared to the raw grass pea seeds with some insignificantly different values. Fe increment ranged from 2.3% to 10.4 % with an exceptional higher value obtained by pressure-cooking soaked grass pea seeds while keeping the soaking water (Pcw). The mean concentration of Fe in tap water before and after the soaking and cooking processes measured 0.12 ± 0.34 mg/100 mL. The theoretical Fe added from the tap water was 0.48 mg. Thus, the maximum Fe concentration from tap water before and after

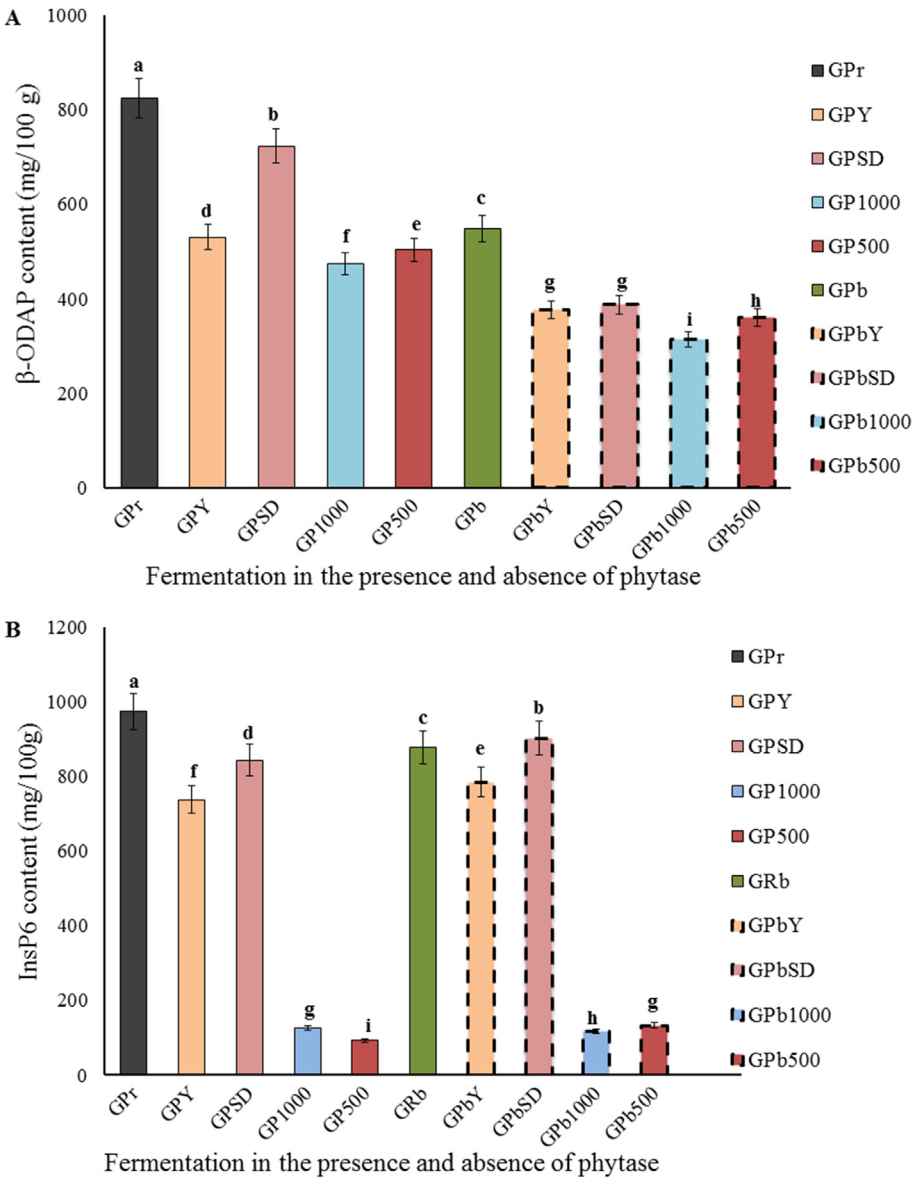
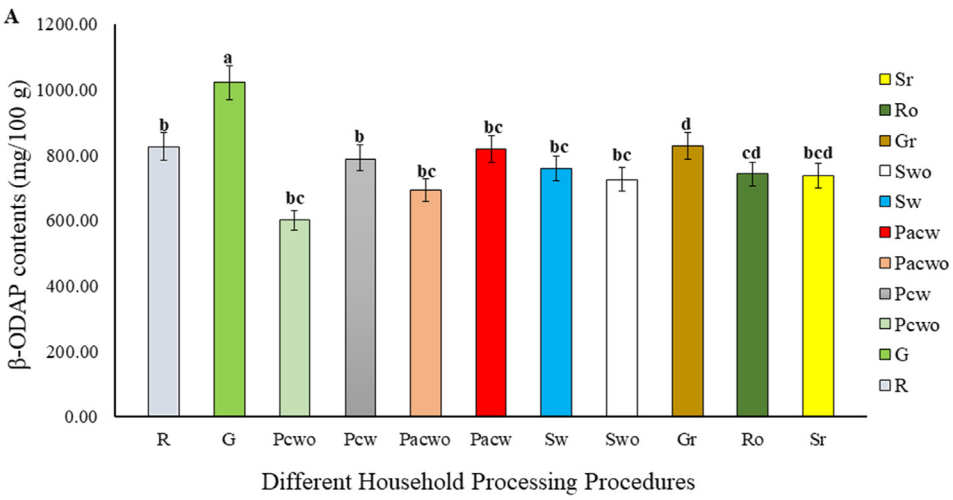


Figure 1. (A). β-ODAP content (mg/100 g DM), (B). $InsP_6$ content (mg/100 g DM) of grass pea or grass pea blend flour before and after fermentation in the presence and absence of Natuphos®. **GPr**-raw (unfermented grass pea), **GPY**-yeast fermented grass pea, **GPSD**-sourdough fermented grass pea, **GP1000**-yeast fermented grass pea in the presence of 1000 U/kg Natuphos®, **GP500**-yeast fermented grass pea in the presence of 500 U/kg Natuphos®, **GPb**-grass pea blend (unfermented), **GPbY**-yeast fermented grass pea blend, **GPbSD**-sourdough fermented grass pea blend, **GPb1000**-yeast fermented grass pea blend in the presence of 1000 U/kg Natuphos®, **GPb500**-yeast fermented grass pea blend in the presence of 500 U/kg Natuphos®.

household processing was obtained to be 7.9%. The potential Fe contamination in tap water could be in ferrous form (Fe^{2+}) which is non-visible and dissolved; and in ferric form (Fe^{3+}) that is insoluble found in water exposed to oxygen. Iron contamination could occur from rusty pipelines, appliances, even all the way through the treatment process and in lesser extent during sample preparation and measurement process. Additional Zn and Ca concentration with maximum values of 80.3% and 76.9% respectively in processed samples compared to the raw grass pea seeds were obtained during household processing. Zn mean concentrations in tap water measured 0.51 ± 0.23 mg/100 g; and the average concentrations of Zn in the boiled water after pressure and pan cooking were 0.69 ± 0.47 mg/100 g and 1.6 ± 0.8 mg/100 g respectively. Therefore, the increase in the Zn contents was found to be due to slightly tap water and more from leaching of zinc ions from the pan as well as pressure cooker surfaces. The average Ca concentration in the tap water was measure to be 15.44 ± 0.84 mg/100 mL. Thus, the mean amount of theoretical Ca added for individual household processing was 61.8 mg. The increases in Ca were found to be in a range from 28.3 ± 2.4 mg/100 g to 77 ± 1 mg/100 g. The increase in Ca concentration was observed to be due to the addition of tap water during cooking and other household processing. The additional Fe, Zn and Ca can be derived from the tap water used for soaking and cooking or being leached from the surfaces used for cooking and roasting as hypothesized by (Bolle et al., 2011; Feitosa et al., 2018; Jain, 2018). Feitosa et al. (2018) reported more than 50% increment of Zn and Ca contents irrespective of household procedures. Jain (2018) reported 20% higher Fe contents in black gram and beetroot halwa after cooking with the iron utensil, and Bolle et al. (2011) found 10–95% more Zn in tea prepared in traditional metallic teapots.

In general, a significant loss of minerals was expected while soaking, followed by discarding the soaking water because minerals leach from the food matrix into the soaking water (Lagardo et al., 2016). For Fe and Ca, the expected effect was observed (Table 5). Zn, however, was not lost during soaking. The location of Zn might explain this behavior within the food matrix and the interaction of different food constituents with Zn (Raes et al., 2014). Soaking followed by cooking is not expected to result in additional loss of minerals (Lagardo et al., 2016). Thus, pressure and pan cooking the soaked grass peas should also result in higher Fe and Ca losses when discarding the soaking water. Higher Fe loss was however observed during pan cooking than pressure cooking, which can be hypothesized as: additional Fe could be lost along with evaporated water. The missing loss of Ca might be explained by the relatively high Ca content of the tap water used to replace the discarded soaking water.



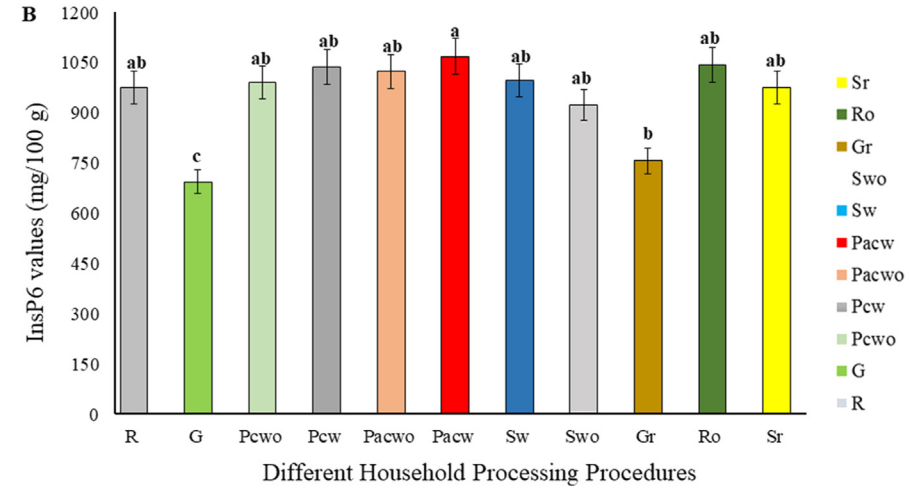


Figure 2. (A). β-ODAP contents (mg/100 g DM), (B). Phytate ($InsP_6$) contents (mg/100 g DM) of raw and processed grass pea. Values marked by different letters are significantly different to each other ($p < 0.05$). **R**-raw grass pea seeds, **G**-germination, **Pcwo**-soaking followed by pressure-cooking while discarding the soaking water, **Pcw**-soaking followed by pressure-cooking while keeping the soaking water, **Pacwo**-soaking followed by pan-cooking while discarding the soaking water, **Pacw**-soaking followed by pan-cooking while keeping the soaking water, **Sw**-soaking and keeping the soaking water, **Swo**-soaking and discarding the soaking water, **Gr**-germination followed by roasting, **Ro**-roasting, **Sr**-soaking followed by roasting.

Table 4. Total protein concentration of household processed grass pea samples.

Samples	Protein (N*6.25), %
R	$26.02^{g} \pm 0.04$
Ro	$26.60^{f} \pm 0.06$
Swo	$27.60^{de} \pm 0.08$
Sr	$27.87^{cd} \pm 0.08$
Sw	$27.93^{cd} \pm 0.08$
G	$28.70^{a} \pm 0.03$
Gr	$27.3^{e} \pm 0.1$
Pacw	$28.0^{c} \pm 0.2$
Pacwo	$28.2^{bc} \pm 0.1$
Pcw	$28.2^{bc} \pm 0.2$
Pcwo	$28.40^{ab} \pm 0.03$

R-raw grass pea seeds, **Ro**-roasting, **Swo**-soaking and discarding the soaking water, **Sr**-soaking followed by roasting, **Sw**-soaking and keeping the soaking water, **G**-germination, **Gr**-germination followed by roasting, **Pacw**-soaking followed by pan-cooking while keeping the soaking water, **Pacwo**-soaking followed by pan-cooking while discarding the soaking water, **Pcw**-soaking followed by pressure-cooking while keeping the soaking water, **Pcwo**-soaking followed by pressure-cooking while discarding the soaking water.Values marked by different letters in a column are significantly different ($p < 0.05$).

Furthermore, the transfer of minerals such as iron from the kitchen utensils used might overcome the loss of the same mineral during soaking. The observed increase in the content of Fe-2.2%, Zn-9.1% and Ca-67.3% while germinating grass peas seeds could be due an uptake of these minerals by the grass pea seeds from the water used for germination.

Bioaccessibilities of Fe, Zn, Ca, and P from raw and household processed grass pea seeds are given in (Table 6). In general, processing resulted in an improvement of the bioaccessibility Fe, Zn and P with some exceptions. The improvement was well exhibited during germination, germination followed by roasting and soaking attributing to the observed dephosphorylation of phytate. Phytate is a well-known chelator for multivalent cations such as Fe, Zn, and Ca, reducing their bioavailability. Dephosphorylation of phytate was shown to improve mineral uptake from the small intestine (Reale et al., 2007). Mineral absorption from phytate rich foods, however, cannot be determined by only considering the phytate content. All components in the diet and their interactions need to be considered (Lopez et al., 2002; Raes et al., 2014). Besides, the non-bioaccessible phytate-bound phosphate was released into an easily bioaccessible form (inorganic P) during phytate dephosphorylation (Greiner et al., 2006; Hussain et al., 1989). During applying different household processing technologies, the changes in mineral concentrations of treated samples need to be also considered while investigating the bioaccessibilities of minerals. As already mentioned leaching into the

Table 5. Mineral content of household cooked grass pea samples.

Samples	Fe (mg/kg)	Zn (mg/kg)	Ca (mg/kg)
R	$60.7^{g} \pm 0.3$	$43.85^{f} \pm 0.01$	$1283^{i} \pm 8$
Ro	$61.4^{fg} \pm 0.2$	$48.2^{d} \pm 0.3$	$1318^{h} \pm 2$
Swo	$62.2^{e} \pm 0.3$	$43.6^{f} \pm 0.2$	$1568^{f} \pm 7$
Sr	$63.0^{d} \pm 0.1$	$45.3^{e} \pm 0.1$	$1537^{g} \pm 5$
Sw	$63.1^{d} \pm 0.4$	$43.5^{f} \pm 0.1$	$1630^{e} \pm 2$
G	$62.0^{ef} \pm 0.4$	$47.8^{d} \pm 0.4$	$2146^{b} \pm 5$
Gr	$66.6^{b} \pm 0.7$	$80.4^{a} \pm 0.9$	$2270^{a} \pm 10$
Pacw	$62.5^{de} \pm 0.4$	$43.9^{f} \pm 0.3$	$1709^{d} \pm 7$
Pacwo	$63.06^{d} \pm 0.04$	$48.88^{c} \pm 0.04$	$2035^{c} \pm 9$
Pcw	$79.8^{a} \pm 0.1$	$45.04^{e} \pm 0.03$	$1724^{d} \pm 6$
Pcwo	$64.0^{c} \pm 0.3$	$53.70^{b} \pm 0.06$	$2050^{c} \pm 9$

R-raw grass pea seeds, **Ro**-roasting, **Swo**-soaking and discarding the soaking water, **Sr**-soaking followed by roasting, **Sw**-soaking and keeping the soaking water, **G**-germination, **Gr**-germination followed by roasting, **Pacw**-soaking followed by pan-cooking while keeping the soaking water, **Pacwo**-soaking followed by pan-cooking while discarding the soaking water, **Pcw**-soaking followed by pressure-cooking while keeping the soaking water, **Pcwo**-soaking followed by pressure-cooking while discarding the soaking water. Values marked by different letters in a column are significantly different ($p < 0.05$).

Table 6. Bioaccessibilities (%) of iron, zinc, calcium and phosphorus in raw and processed grass pea seeds with traditional household processes.

Samples	Fe (%)	Zn (%)	Ca (%)	P (%)
R	$0.15^{c} \pm 0.02$	$17.60^{cd} \pm 0.06$	$5.08^{a} \pm 0.09$	$12.54^{abc} \pm 0.07$
Ro	$0.16^{c} \pm 0.04$	$16.82^{cd} \pm 0.07$	$5.0^{a} \pm 0.3$	$12.5^{abc} \pm 0.2$
Swo	$0.19^{c} \pm 0.03$	$26^{a} \pm 2$	$4.6^{ab} \pm 0.3$	$14.7^{a} \pm 0.8$
Sr	$0.15^{c} \pm 0.01$	$16.9^{cd} \pm 0.5$	$5.1^{a} \pm 0.4$	$14.00^{ab} \pm 0.05$
Sw	$0.21^{c} \pm 0.04$	$21.6^{b} \pm 0.7$	$3.9^{bcd} \pm 0.2$	$12.9^{ab} \pm 0.7$
G	$4.9^{a} \pm 0.5$	$18.4^{c} \pm 0.1$	$3.9^{bcd} \pm 0.2$	$12.2^{bc} \pm 0.2$
Gr	$3.8^{b} \pm 0.2$	$12.1^{e} \pm 0.7$	$3.3^{d} \pm 0.1$	$10.3^{c} \pm 0.3$
Pacw	$0.29^{c} \pm 0.07$	$17.6^{cd} \pm 0.4$	$4.5^{abc} \pm 0.3$	$12.2^{bc} \pm 0.2$
Pacwo	$0.16^{c} \pm 0.01$	$15^{d} \pm 2$	$4.3^{abc} \pm 0.6$	$13^{abc} \pm 2$
Pcw	$0.24^{c} \pm 0.01$	$17^{cd} \pm 2$	$4.5^{abc} \pm 0.2$	$13.2^{ab} \pm 0.1$
Pcwo	$0.4^{c} \pm 0.2$	$12.08^{e} \pm 0.03$	$3.75^{cd} \pm 0.04$	$11.7^{bc} \pm 0.4$

R-raw grass pea seeds, **Ro**-roasting, **Swo**-soaking and discarding the soaking water, **Sr**-soaking followed by roasting, **Sw**-soaking and keeping the soaking water, **G**-germination, **Gr**-germination followed by roasting, **Pacw**-soaking followed by pan-cooking while keeping the soaking water, **Pacwo**-soaking followed by pan-cooking while discarding the soaking water, **Pcw**-soaking followed by pressure-cooking while keeping the soaking water, **Pcwo**-soaking followed by pressure-cooking while discarding the soaking water. Values marked by different letters in a column are significantly different ($p < 0.05$).

soaking water could reduce the mineral concentration of grass pea. However, the minerals are added either from the tap water or from the surfaces of materials used for cooking and roasting.

As expected from other studies (Lopez et al., 2002; Greiner et al., 2006), the bioaccessibility of Fe was low. The highest Fe bioaccessibilities were obtained with the germinated (G) and the germinated and roasted (Gr) grass peas. The observed increase in Fe bioaccessibility is well correlated to the reduction in phytate when applying the indicated processes. The results are in good agreement with those obtained by Luo et al. (2014). They observed a 5-fold increase in Fe bioaccessibility by germination of fava beans and soybeans and a 3-fold increase by germination of rice. Even if no decline in phytate was observed, pan and pressure cooking of soaked grass pea seeds resulted in higher Fe bioaccessibilities compared to raw seeds (Table 6). Feitosa et al. (2018) also reported an increased bioaccessibility of Fe for soaked black beans cooked with a pressure cooker by keeping the soaking water without observing a reduction in phytate content. The Fe introduced into the grass pea matrix from the surface of the pan or pressure cooker might be more accessible than the Fe of the grass pea itself. Soaking by discarding the soaking water exhibited a slightly higher Fe bioaccessibility compared to the raw seed, an observation that correlates well with the small decrease in phytate content using this process. Thus, an improvement in Fe bioavailability by processing grass peas seems to be linked to a reduction in phytate content.

The bioaccessibility of Zn from pulse was reported to be higher compared to the bioaccessibility of Fe (Hemalatha et al., 2007b). This behavior is in good agreement with the results obtained in this study. The highest Zn bioaccessibilities were found in soaked and germinated grass pea seeds (Table 6). The improvements in Zn bioaccessibility could be attributed to a reduction in phytate content when applying the two processes. The observed reduction in Zn accessibility compared to the raw grass pea seeds when applying a heat treatment (pan or pressure cooking, roasting) was already reported by Hemalatha et al. (2007a) for other pulses such as chickpea, green gram, and black gram and explained by an interaction of Zn with sulfur-containing amino acids of proteins.

None of the household processing practices applied exhibited higher Ca bioaccessibility compared to raw seed (Table 6). Processing of grass peas seeds might have affected their content of insoluble dietary fiber as already shown for other pulses (Azizah and Zainon, 1997; Ramulu and Rao, 1997; Veena et al., 1995). In addition, an increase in inorganic P occurred when phytate was dephosphorylated during processing. Interaction of Ca with insoluble dietary fiber and inorganic P likely resulted in precipitation of Ca and, therefore, in lower measurable Ca bioaccessibilities.

The majority of the plant-derived phosphorus is organically-bound and not readily available for humans. Therefore, processes resulting in the dephosphorylation of phytate such as germination are expected to improve the bioaccessibility of P. The missing correlation of P bioaccessibility and the concentration of inorganic P in the processed grass

peas could be explained by an interaction of the inorganic P with the Ca present in the processed seeds resulting in precipitation of inorganic P as calcium phosphate.

4. Conclusion

This study suggests that fermentation and household processing practices are capable of reducing the content of phytate and β-ODAP in grass peas as well as improving mineral bioaccessibilities. The observed improvements in Fe and Zn bioavailabilities by processing grass peas could be attributed to a processing-induced reduction in phytate. As expected, Zn bioaccessibilities were higher compared to Fe bioavailabilities irrespective of the processing practice applied. The present study therefore clearly showed that fermentation and household processing practices are capable of reducing toxic component and antinutrients of grass pea seeds. All household processing practices require little energy, time, and are not cost-intense. Therefore, they can be used even by more impoverished populations in developing countries. Fermentation in the presence of phytase, however, might be more applicable to bakery industries.

Declarations

Author contribution statement

Meseret Bekele Buta, Ralf Greiner: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Clemens Posten, Shimelis Admassu Emire: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ann-Katrin Meinhardt, Alexandra Müller: Performed the experiments; Wrote the paper.

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Data availability statement

The data that has been used is confidential.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Akalu, G., Johansson, G., Nair, B.M., 1998. Effect of processing on the content of β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) in grass pea (*Lathyrus sativus* L.) seeds, and flour as determined by flow injection analysis. J. Food Chem. 62 (2), 233–237.

Aletor, V.A., El-Moneim, A.A., Goodchild, A.V., 1994. Evaluation of the seeds of selected lines of three *Lathyrus* spp for β-N-oxalylamino-L-alanine (BOAA), tannins, trypsin inhibitor activity, and certain in-vitro characteristics. J. Sci. Food Agric. 65 (2), 143–151.

Arslan, M., 2017. Diversity for vitamin and amino acid content in grass pea (*Lathyrus sativus* L.). Legume Res. 40 (5), 803–810.

Azizah, A., Zainon, H., 1997. Effect of processing on dietary fiber contents of selected legumes and cereals. Malays. J. Nutr. 3, 131–136. http://nutriweb.org.my/mjn/publication/03-2/d.pdf.

Bekele, M., Admassu, S., Posten, C., Andrée, S., Greiner, R., 2019. Reduction of β -ODAP and IP_6 contents in *Lathyrus sativus* L. seed by high hydrostatic pressure. Food Res. Int. 120, 73–82.

Bell, E.A., O'Donovan, P.A., 1966. The isolation of α-and β-oxalyl derivatives of α, β-diaminobutyric acid from seeds of *Lathyrus latifolzus*, and the detection of the α-oxalyl isomer of the neurotoxin oxalylaminopropionic acid, which occurs together with the neurotoxin. J. Phytochem. 5, 1211–1219.

Bolle, F., Brian, W., Petit, D., Boutakhrit, K., Feraille, G., van Loco, J., 2011. Tea brewed in traditional metallic teapots as a significant source of lead, nickel, and other chemical elements. Food Addit. Contam. 28 (9), 1287–1293.

Feitosa, S., Greiner, R., Meinhardt, A.-K., Müller, A., Almeida, D.T., Posten, C., 2018. Effect of traditional household processes on iron, zinc, and copper bioaccessibility in Black Bean (*Phaseolus vulgaris* L.). Foods 1–12.

Fikre, A., van Moorhem, M., Ahmed, S., Lambein, F., Gheysen, G., 2011. Studies on neurolathyrism in Ethiopia: dietary habits, perception of risks, and prevention. Food Chem. Toxicol. 49 (3), 678–684.

Greiner, R., Konietzny, U., 2006. Phytase for food application. Food Technol. Biotechnol. 44 (2), 125–140.

Greiner, R., Konietzny, U., Jany, K.-D., 2006. Phytate-an undesirable constituent of plant-based foods? Journal für Ernährungsmedizin 8 (3), 18–28.

Hailu, D., Abera, S., Abera Teka, T., 2015. Effects of processing on nutritional composition and antinutritional factors of grass pea (*Lathyrus Sativus* L.): a Review. Food Sci. Qual. Manag. 36, 61–71.

Hanbury, C.D., White, C.L., Mullan, B.P., Siddique, K.H.M., 2000. A review of the potential of *Lathyrus sativus* L. and *L. cicera* L. grain for use as animal feed. J. Anim. Feed Sci. Technol. 87, 1–27.

Haros, M., Rosell, C.M., Benedito, C., 2001. Fungal phytase as a potential breadmaking additive. Eur. Food Res. Technol. 213, 317–322.

Hemalatha, S., Platel, K., Srinivansan, K., 2007a. Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. J. Trace Elem. Med. Biol. 21, 1–7.

Hemalatha, S., Platel, K., Srinivasan, K., 2007b. Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India. J. Food Chem. 102, 1328–1336.

Hooda, S., Jood, S., 2002. Effect of soaking and germination on nutrient and antinutrient contents of fenugreek (*Triwnella foenum graecum* L.). J. Food Biochem. 27, 165–176.

Hussain, B., Khan, S., Ismail, M., Sattar, A., 1989. Effect of roasting and autoclaving on phytic acid content of chickpea. Food Nahrung 33 (4), 345–348.

Igzaw, Y., Gorton, L., Solomon, T., Akalu, G., 2004. Fermentation of seeds of teff (*Eragrostis teff*), grass-pea (*Lathyrus sativus*), and their mixtures: aspects of nutrition and food safety. J. Agric. Food Chem. 52, 1163–1169.

International Organization for Standardization, 1978. Meat and Meat productsDetermination of Nitrogen Content (Reference Method) (ISO Standard No. 937). Technical Committee ISO/TC 34, Agricultural Food Products. Switzerland.

Jain, P., 2018. Effect of cooking utensils on the iron content of the food. J. Public Health Catalog 1 (4), 1–4. http://www.alliedacademies.org/public-health-nutrition/.

Jiang, S., Cai, W., Xu, B., 2013. Food quality improvement of soy milk made from short-time germinated soybeans. Foods 2, 198–212.

Kebede, B., Urga, K., Nigatu, A., 1995. Effect of processing methods on the trypsin inhibitor, tannins, phytic acid, and ODAP contents of grass pea seeds. Ethiop. J. Health Dev. 9 (2). Ethiopian Nutrition Institute P.O.Box 5654, Addis Ababa, Ethiopia. https://ejhd.org/index.php/ejhd/article/view/1088.

Khandare, A.L., Kumar, R.H., Meshram, I.I., Arlappa, N., Laxmaiah, A., Venkaiah, K., Toteja, G.S., 2018. Current scenario of consumption of *Lathyrus sativus* L. and *lathyrism* in three districts of Chhattisgarh State, India. Toxicon 150, 228–234.

Kumari, V., 2001. Field evaluation of grass pea (*Lathyrus sativus* L.) germplasm for its toxicity in the northwestern hills of India. *Lathyrus* Lathyrism Newsl. 2, 82–84.

Kuo, Y.-H., Bau, H.-M., Quemener, B., Khan, J.K., Lambein, F., 1995. Solid-state fermentation of *Lathyrus sativus* seeds using *Aspergillus oryzae* and *Rhizopus oligosporous* sp T-3 to eliminate the neurotoxin β-ODAP without loss of nutritional value. J. Sci. Food Agric. 69 (1), 81–89.

Lagardo, M.J., Cilla, A., Barbera, R., 2016. Bioaccessibility of calcium in legumes. In: Preedy, V.R. (Ed.), Calcium: Chemistry, Analysis, Function and Effects, Food and Nutritional Components in Focus No, first ed., Vol. 10, pp. 237–253.

Lambein, F., Khan, J.K., Kuo, Y.-H., 1992. Free amino acids and toxins in *Lathyrus sativus* L. seedlings. Planta Med. 58 (4), 380–381. http://hdl.handle.net/1854/LU-206637.

Lopez, H.W., Krespine, V., Guy, C., Messager, A., Demigne, C., Remesy, C., 2001. Prolonged fermentation of whole-wheat sourdough reduces phytate level and increases soluble magnesium. J. Agric. Food Chem. 49 (5), 2657–2662.

Lopez, H.W., Leenhardt, F., Coudray, C., Remesy, C., 2002. Minerals and phytic acid interactions: is it a real problem for human nutrition? Int. J. Food Sci. Technol. 37, 727–739.

Luo, Y., Xie, W., Jin, X., Wang, Q., He, Y.-J., 2014. Effects of germination on iron, zinc, calcium, manganese, and copper availability from cereals and legumes. J. Food 12 (1), 22–26.

Menezes-Blackburn, D., Gabler, S., Greiner, R., 2015. Performance of seven commercial phytases in an *in vitro* simulation of poultry digestive tract. J. Agric. Food Chem. 63 (27), 6142–6149.

Mondal, M.M.A., Puteh, A.B., 2014. Spectrum of variability in seed size and content of protein and ODAP in grass pea (*Lathyrus sativus* L.) germplasm. Legume Res. 37 (5), 479–482.

Padmajaprasad, V., Kaladhar, M., Bhat, R.V., 1997. Thermal isomerisation of β-N-oxalyl-L-α, β-diaminopropionic acid, the neurotoxin in Lathyrus sativus, during cooking. J. Food Chem. 59 (1), 77–80.

Pastor-Cavada, E., Juan, R., Pastor, J.E., Alaiz, M., Vioque, J., 2011. Nutritional characteristics of seed proteins in 15 *Lathyrus* species (fabaceae) from Southern Spain. LWT-Food Sci. Technol. 44 (4), 1059–1064.

Piecyk, M., Wołosiak, R., Druzynska, B., Worobiej, E., 2012. Chemical composition and starch digestibility in flours from Polish processed legume seeds. J. Food Chem. 135, 1057–1064.

Požrl, T., Kopjar, M., Kurent, I., Hribar, J., Janeš, A., Simčič, M., 2009. Phytate degradation during bread-making: the influence of flour type and bread-making procedures. Czech J. Food Sci. 27 (1), 29–38.

Raes, K., Struijs, K., van Camp, J., 2014. Role of processing on bioaccessibility of minerals: influence of localization of minerals and antinutritional factors in the plant. Trends Food Sci. Technol. 37, 32–41.

Ramachandran, S., Bairagi, A., Ray, A.K., 2008. Improvement of nutritive value of grass pea (*Lathyrus sativus*) seed meal in the formulated diets for rohu, Labeo rohita (Hamilton) fingerlings after fermentation with a fish gut. Bioresour. Technol. 465–1472.

Ramulu, P., Rao, P., 1997. Effect of proccesing on dietary fiber content of cereals and pulses. Plant Foods Hum. Nutr. 50, 249–257.

Rao, S.L.N., 2001. Effect of high-pressure and ultrasonic processing of foods on rheological properties: do we need more research on neurolathyrism? *Lathyrus Lathyrism* Newsl. 2–4.

Reale, A., Konietzny, U., Coppola, R., Sorrentino, E., Greiner, R., 2007. The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. J. Agric. Food Chem. 55, 2993–2997.

Sarkar, A., Emmrich, P.M.F., Sarker, A., Zong, X., Martin, C., Wang, T.L., 2019. Grass Pea: remodeling an ancient insurance crop for climate resilience. In: Kole, C. (Ed.), Genomic Designing of Climate-Smart Pulse Crops, pp. 425–469.

Starzyńska-Janiszewska, A., Stodolak, B., 2011. Effect of inoculated lactic acid fermentation on antinutritional and antiradical properties of grass pea (*Lathyrus sativus*) 'Krab' flour. Pol. J. Food Nutr. Sci. 61 (4), 245–249.

Steiner, T., Mosenthin, R., Zimmermann, B., Greiner, R., Roth, S., 2007. Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. Anim. Feed Sci. Technol. 133, 320–334.

Stodolak, B., Mickowska, B., Starzyn, A., 2004. The effect of germination on antioxidant and nutritional parameters of protein isolates from grass pea (*Lathyrus sativus*) seeds. Food Sci. Technol. Int. 73–77.

Stoica, M., Mihalcea, L., Borda, D., Alexe, P., 2013. Non-thermal novel food processing technologies, an overview. J. Agroaliment. Processes Technol. 19 (2), 212–217.

Tadelle, D., Alemu, Y., Nigusie, D., Peters, K.J., 2003. Evaluation of processing methods on the feeding value of grass pea to broilers. Int. J. Poultry Sci. 2 (2), 120–127.

Tamburino, R., Guida, V., Pacifico, S., Rocco, M., Zarelli, A., Parente, A., Di Marco, A., 2012. Nutritional values and radical scavenging capacities of grass pea (*Lathyrus sativus* L) seeds in Valle Agricola district, Italy. Aust. J. Crop. Sci. 6 (1), 149–156.

Tarade, K.M., Singhal, R.S., Jayram, R.V., Pandit, A.B., 2007. Kinetics of degradation of ODAP in *Lathyrus sativus* L. flour during food processing. Food Chem. 104 (2), 643–649.

Urga, K., Fita, A., Kebede, B., 1995. Nutritional and antinutritional factors of grass pea (*Lathyrus sativus* L.) germplasm. Bull. Chem. Soc. Ethiop. 9–16.

Urga, K., Fufa, H., Biratu, E., Husain, A., 2005. Evaluation of *Lathyrus sativus* L. cultivated in Ethiopia for proximate composition, minerals, β-ODAP, and antinutritional components. Afr. J. Food Nutr. Sci. 5 (1), 1–15.

Veena, A., Urooj, A., Puttaraj, S., 1995. Effect of processing on the composition of dietary fiber and starch in some legumes. Nahrung 39 (2), 132–138.

Walker, A.F., Kochhar, N., 1982. Effect of processing including domestic cooking on nutritional quality of legumes. Proc. Nutr. Soc. 41–51.

Wang, N., Lewis, M.J., Brennan, J.G., Westby, A., 1997. Effect of processing methods on nutrients and antinutritional factors in cowpea. J. Food Chem. 58 (l), 59–68.

Yan, Z.Y., Spencer, P.S., Li, Z.X., Liang, Y.M., Wang, Y.F., Wang, C.Y., Li, F.M., 2006. *Lathyrus sativus* (grass pea) and its neurotoxin ODAP. Phytochemistry 67 (2), 107–121.