EFFECTS OF HIGH HYDROSTATIC PRESSURE (HHP) AND CONVENTIONAL FOOD PROCESSING TECHNIQUES ON THE NUTRITIONAL QUALITY OF GRASS PEA SEEDS/PRODUCTS

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Summary

Grass peas (*Lathyrus sativus* L.) seeds are a highly proteinous, a source of energy and micronutrients, having remarkable health improving functional properties and considerable amounts of polyphenols and antioxidants. Grass pea seeds however, contain an endogenous neurotoxic non-proteinogenic amino acid, β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP), a major limiting factor-for their human consumption. Furthermore, they contain antinutrients such as phytate ($InsP_6$), in concentrations capable of hindering bioavailability of minerals such as iron (Fe), zinc (Zn), calcium (Ca), and other micronutrients from the seeds.

The concentration of β-ODAP of twenty-nine grass pea accessions/varieties from different regions in Ethiopia were found to be in the range of 51.9/100 g (GP-240038 accession) and 806.5 mg/100 g (GF1-Alemu, AK accession). $InsP_6$ quantification revealed concentrations from 95.5 mg/100 g (GF1-Alemu, AK accession) to 1391.3 mg/100 g (GP-240038 accession). Two accessions (GF1-Alemu, AK; GP-240038) and two varieties (Wassie, German variety) among all were selected for the in depth study. The selection criteria were β-ODAP concentration (high and low), origin (Germany, Ethiopia) and breeding technology (conventional, genetic modification). The concentrations of eighteen proteinogenic amino acids in the four selected grass pea samples were quantified in the study. Glutamic acid was found to have the highest concentration (3454.9 to 4535.2 mg/100 g) followed by aspartic acid, arginine, leucine and lysine. Methionine was found to be the amino acid lowest in concentration (91.9 to 200.5 mg/100 g) in all samples analyzed. The amino acid variations among the grass pea samples might be due to environmental condition and genotype differences. Grass pea seeds were also found to be a good source of iron (72.6 to 89.1

mg/kg), zinc (22.0 to 38.7 mg/kg), calcium (903.2 to 1655.0 mg/kg) and phosphorus (1588.1 to 4404.6 mg/kg). The concentrations obtained were found to be comparable to other legumes.

Studies on effects of high hydrostatic pressure (HHP) on cereals and legumes are limited and have not been investigated in much detail. There are also no reports on the effects of HHP on the nutritional, functional and structural properties of grass pea seeds available so far. Thus, one of the major objectives of this work was to elucidate the effects of HHP on the level of a toxic compound (β-ODAP), on the content of antinutrients such as $InsP_6$ and physico-chemical, functional and structural properties in grass peas. Besides HHP, conventional processing technologies such as fermentation in the presence and absence of phytase as well as household processing practices such as germination and cooking were also included in the study.

The grass pea accession exhibiting the highest β-ODAP content (GF1-Alemu, AK) was chosen in soaked and batter forms to elucidate the best HHP conditions in respect to β-ODAP reduction, considering soaking and holding time for soaked-HHP treatment and holding time for batter-HHP treatment using Central Composite Face Centered Design of experiments. Those conditions (600 MPa pressure, 9 h soaking time, 15 min holding time) for soaked seed and (600 MPa pressure, 15 min holding time) for batter were also applied during HHP treatment of the three other grass pea samples included in the study. In addition to β-ODAP, significant reductions of $InsP_6$ were also achieved at those best conditions for GF1-Alemu, AK accession as well as the three grass pea samples. Thus, reduction of β-ODAP and $InsP_6$ in GF1-Alemu, AK accession for those specific conditions were found to be (70.4% and 74.6%) for soaked-HHP and (34.0% and 97.0%) for batter-HHP samples respectively. Moreover, the β-ODAP and $InsP_6$ reductions were obtained to be in

ranges of (50.5% - 91.0%) and (19.6% - 34.7%) for soaked-HHP and (15.8% - 44.2%) and (12.5% - 25.7%) for batter-HHP samples for the three grass pea samples respectively.

The regression model and 3D of the response surface plot for the GF1-Alemu, AK accession exhibited that the combined incremental effect of pressure and soaking time resulted in a more significant ($p \leq 0.001$) reductions than the interaction of pressure with holding time ($p \leq 0.05$) for both compounds. The trends of the data also showed that the β-ODAP reductions were always higher for soaked compared to batter grass pea seeds. Thus, the conclusion can be drawn that pressure, soaking and holding time in combination exhibited higher effects on the reduction of β-ODAP and $InsP_6$ contents for the GF1-Alemu, AK accession in particular. Further investigation for other accessions and varieties are recommended.

The impacts of HHP on water holding capacity, color, microstructure and pasting properties of grass pea seeds and flours in soaked and batter forms compared to controls were investigated. Results from response surface (RSM) model exhibited that applying 600 MPa pressure to soaked grass pea seeds for 6, 9 and 12 h resulted in a 13.2%, 16.0% and 8.9% uptake of water. In addition, the results from color observation exhibited less lightness (L*), more reddish (a*) and less yellow (b*) in all HHP treated seed flours compared to untreated ones. “Well” and “highly noticeable” color differences having ΔE* value of 6.69 and 11.26 units at 600 MPa for soaked and batter HHP treated grass pea flours were observed. Microstructure properties from SEM images at 600 MPa showed disruption of protein matrices adhered to ellipsoid surfaced starch granules and fibrous and ridged nature of cell walls in soaked grass pea whereas deformed and swollen-hole bowled structure appeared in batter-HHP grass pea flours. The highest values of peak (1871.00 cP), trough (1409.00

cP), breakdown (509.00 cP), final (2083.00 cP) and setback (674.00 cP) viscosities of GF1-Alemu, AK accession at low pasting time and highest pasting temperature were obtained at 600 MPa whereas the reverse was observed in batter-HHP treated pea flours. Moreover, the peak and final viscosities of batter-HHP treated samples of the same accession (1086.00 cP, 1272.00 cP) were higher than the values obtained for Wassie (586.00 cP, 845.00 cP), the German variety (488.00 cP, 681.00 cP) and GP-240038 accession (473.00 cP, 714.00 cP) treated with 600 MPa held for 15 min. In conclusion, pressure treatment resulted in microstructure changes in food components of grass pea flours, high water uptake of the seed, higher swelling capacities of the flour (peak viscosity) and higher final viscosities of the flour. Thus, the changes and modifications of these properties may eventually have positive impact for the quality of a grass pea product. Nevertheless, consumer perception due to color deviation may have impact on product acceptability. Pigment degradation, browning reaction of phenols/proteins and Maillard condensation of amino components are some of the reactions that can be activated by pressure influencing color formation.

Grass pea (*Lathyrus sativus* L.) is commonly consumed cooked, fermented, and roasted 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 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. Germination resulted

also 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 folds) 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 antinutrient and toxic compounds, and improves the bioavailability of grass peas which could contribute to their more widespread utilization.

Zusammenfassung

Platterbsen (*Lathyrus sativus* L.) sind sehr proteinhaltig und stellen eine Energie- und Mikronährstoffquelle dar. Sie besitzen gesundheitsfördernde Eigenschaften und weisen beträchtliche Mengen an Polyphenolen und Antioxidantien auf. Platterbsen enthalten jedoch mit β-N-Oxalyl-L-α,β-Diaminopropionsäure (β-ODAP) eine endogene, neurotoxische, nicht-proteinogene Aminosäure. Diese stellt einen wichtigen limitierenden Faktor für den menschlichen Verzehr von Platterbsen dar. Darüber hinaus enthalten Platterbsen antinutritive Faktoren, wie z.B. Phytat ($InsP_6$), der in den vorliegenden Konzentrationen in der Lage ist, die Bioverfügbarkeit von Mineralstoffen wie Eisen (Fe), Zink (Zn), und Kalzium (Ca) sowie von Mikronährstoffen zu reduzieren.

Die Konzentration von β-ODAP in 29 Platterbsensorten aus verschiedenen Regionen Äthiopiens lagen zwischen 51,9 mg (GP-240038) und 806,5 mg (GF1-Alemu, AK) pro 100 g Trockenmasse. Die $InsP_6$-Quantifizierung ergab Konzentrationen von 95,5 mg (GF1-Alemu, AK) bis 1391,3 mg (GP-240038) pro 100 g Trockenmasse. Vier Platterbsensorten (GF1-Alemu, AK; GP-240038; Wassie, deutsche Sorte) wurden für weitergehende Untersuchungen verwendet. Als Auswahlkriterien wurden die β-ODAP-Konzentration (hoch und niedrig), die Herkunft (Deutschland, Äthiopien) und die verwendete Züchtungstechnologie (konventionell, gentechnisch verändert) herangezogen. 18 proteinogenen Aminosäuren in den vier ausgewählten Platterbsensorten wurden innerhalb der Studie quantifiziert. Es zeigte sich, dass Glutaminsäure, die Aminosäure mit den höchsten Konzentrationen (3454,9 bis 4535,2 mg/100 g) darstellte, gefolgt von Asparaginsäure, Arginin, Leucin und Lysin. Methionin war die Aminosäure, die in den niedrigsten Konzentrationen (91,9 bis 200,5 mg/100 g) vorlag. Die Unterschiede in den Aminosäurekonzentration der analysierten Platterbsensorten könnten auf Umwelteinflüsse und den

genetischen Hintergrund zurückzuführen sein. Platterbsensamen erwiesen sich auch als gute Quelle für Eisen (72,6 bis 89,1 mg/kg), Zink (22,0 bis 38,7 mg/kg), Kalzium (903,2 bis 1655,0 mg/kg) und Phosphor (1588,1 bis 4404,6 mg/kg). Die erhaltenen Konzentrationen an diesen Elementen, liegt in der gleichen Größenordnung, wir für anderen Hülsenfrüchten beschrieben.

Studien zu den Folgen der Anwendung eines hohen hydrostatischen Drucks (HHP) auf Getreide und Hülsenfrüchte sind noch immer nur begrenzt verfügbar. Es liegen bisher auch keine Berichte über die Folgen von HHP auf die ernährungsphysiologischen, funktionellen und strukturellen Eigenschaften von Platterbsen vor. Daher war eines der Hauptziele dieser Arbeit aufzuzeigen, welche Auswirkungen HHP auf die Gehalte der toxischen Verbindung β-ODAP und des antinutritiven Faktors $InsP_6$ sowie auf die physikalisch-chemischen, funktionellen und strukturellen Eigenschaften von Platterbsen hat. Neben HHP wurden auch konventionelle Verarbeitungstechnologien wie die Fermentation in Gegenwart und Abwesenheit von Phytase sowie haushaltsübliche Methoden wie Keimen und Kochen in die Studie einbezogen.

Die Platterbsensorte mit dem höchsten β-ODAP-Gehalt (GF1-Alemu, AK) wurde nach Einweichen und in pastöser Form eingesetzt, um HHP-Bedingungen in Bezug auf eine Reduktion des β-ODAP-Gehaltes zu optimieren. Als Parameter wurden bei den eingeweichten Platterbsen u.a. die Einweichzeit und die Druckhaltezeit und für die pastöse Form u.a. die Druckhaltezeit unter Verwendung des Central Composite Face Centered Design. Die erhaltenen besten Bedingungen: 600 MPa, 9 h Einweichzeit, und 15 min Haltezeit für eingeweichte Platterbsen und 600 MPa Druck und 15 min Haltezeit für die pastöse Form, wurden dann auch für die anderen ausgewählten Platterbsensorten verwendet. Neben einer β-ODAP-reduktion durch HHP, wurde auch eine

signifikante Reduktion der $InsP_6$-gehalte für alle vier Platterbsensorten erzielt. Für GF1-Alemu, AK lag die β-ODAP-reduktion bei 70,4% und die $InsP_6$-reduktion bei 4,6% für eingeweichte Platterbsen und bei 34,0% und 97,0% für die pastöse Form. Bei den anderen drei Platterbsensorten lagen die β-ODAP-reduktionen zwischen 50,5 und 91,0% und die $InsP_6$-reduktionen zwischen 19,6 und 34,7% für eingeweichte Platterbsen. Die entsprechenden Werte für die pastöse Form lagen zwischen 15,8 und 44,2% bzw. 12,5 und 25,7%.

Das Regressionsmodell und 3D der Response Surface Plot für GF1-Alemu, AK zeigte einen kombinierten inkrementellen Effekt von Druck und Haltezeit. Sowohl die einzelnen Parameter ($p \leq 0,001$), als auch ihre Kombination ($p \leq 0,05$) hatten einen signifikanten Einfluss auf die Reduktion von β-ODAP und der $InsP_6$. Die erhaltenen Daten zeigten auch, dass die β-ODAP-Reduktionen für eingeweichte Platterbsen immer über denen der pastösen Form lagen. Daraus kann geschlossen werden, dass Druck, Einweich- und Haltezeit in Kombination einen größeren Effekt auf die β-ODAP und $InsP_6$-gehalte insbesondere bei GF1-Alemu, AK hat. Weitere Untersuchungen für andere Platterbsensorten sind angezeigt.

Außerdem wurden die Auswirkungen von HHP auf das Wasserhaltevermögen, die Farbe, die Mikrostruktur und die Eigenschaften der pastösen Form untersucht. Das Response Surface (RSM)-Modell zeigte, dass 600 MPa für 6, 9 und 12 Stunden zu einer Wasseraufnahme von eingeweichten Platterbsen von 13,2%, 16,0% und 8,9% führte. Darüber hinaus zeigten die Ergebnisse, dass HHP zu Farbunterschieden (ΔE*) im Vergleich zu den unbehandelten Platterbsen führte: weniger hell (L*), rötlicher (a*) und weniger gelb (b*). Mit ΔE*-Werten von 6,69 und 11,26 war der wahrnehmbare Farbunterschied „gut“ bzw. „sehr gut“ für HHP-behandelte Platterbsen bzw. die

pastöse Form. Aus den REM-Aufnahmen konnte abgeleitet werden, dass 600 MPa zum Ablösen der Proteinmatrices von der Oberfläche der ellipsoiden Stärkekörnern, sowie von faserige Strukturen der Zellwände bei eingeweichten Platterbsen führen, wohingegen deformierte und gequollene Struktur in der pastösen Form auftraten. Die höchsten Viskositäten nach Behandlung mit 600 MPa wurden mit GF1-Alemu, AK Platterbsen erhalten, während die entsprechende pastöse Form die niedrigste Viskosität aufwies. Zusammenfassend lässt sich sagen, dass die Druckbehandlung zu Mikrostrukturveränderungen, zu einer erhöhten Wasseraufnahme und einem erhöhten Quellvermögen bei Platterbsenmehlen führte. Die HHP-Behandlung kann sich somit positiv auf die Qualität von Platterbsenprodukte auswirken. Dennoch können die beobachten Farbveränderungen die Verbraucherakzeptanz derartiger Produkte beeinflussen. Pigmentabbau, Bräunungsreaktion durch Maillard-Kondensation sind einige der Reaktionen, die durch Druck induziert werden können und die Farbbildung beeinflussen.

Platterbsen (*Lathyrus sativus* L.) werden in Äthiopien häufig gekocht, fermentiert und geröstet verzehrt. Die Effekte haushaltsüblicher Verarbeitungsprozesse auf den Nährstoffgehalt und die Gehalte an antinutritiven Faktoren und toxischen Verbindungen sind jedoch nicht ausreichend untersucht. Daher wurden die Folgen haushaltsüblicher Verarbeitungsverfahren und der Fermentation in Gegenwart und Abwesenheit einer Phytase auf den Gehalt an β-N-Oxalyl-L-α,β-Diaminopropionsäure (β-ODAP), *myo*-Inositolphosphaten, Protein, Mineralstoffen und ihre Zugänglichkeit in einem *in vitro* Verdausystems untersucht. Die Fermentation zeigte eine signifikante Reduktion an β-ODAP (13,0-62,0%) und Phytat (7,3-90,5%) unabhängig von der Anwesenheit einer exogenen Phytase. Kochen in der Pfanne nach Verwerfen des Einweichwassers und in einem Schnellkochtopf führten zu einer 27,0 und 16,2%-igen Verminderung des β-ODAP-

gehaltes. Während der Keimung und des anschließenden Röstens wurde eine 30%ige Reduktion an Phytat beobachtet. Nach dem Keimen wurde auch ein signifikant ($p<0,05$) höherer Proteingehalt gemessen. Keimen und Keimen gefolgt von Rösten, führte zur höchsten Zugänglichkeit von Fe (mehr als 25-fach), gefolgt von der Nutzung des Schnellkochtopfs und dem Einweichen. Die Verarbeitung verbesserte auch die Zugänglichkeit von Zn um 50,0 % (Eingeweichte Platterbsen und Verwerfen des Einweichwassers), 22,5 % (eingeweichte Platterbsen und Weiterverwendung des Einweichwasser zum Kochen) und 4,3 % (Keimung). Die angewandten Verarbeitungsverfahren waren folglich also in der Lage, den Gehalt an antinutritiven Faktoren und toxischen Verbindungen in Platterbsen zu reduzieren und ihre physiologische Qualität zu verbessern. Das könnte zu einer breiteren Verwendung von Platterbsen in der Humanernährung beitragen.

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1. INTRODUCTION

Legumes are one of the largest families of flowering plants extensively grown in countries of the Asia-Pacific regions and Africa. They can generally be classified into pulses and oilseeds. Pulses are edible seeds of plants in the legume family and harvested exclusively as dry grains which differentiate them from other crops harvested green (FAO, Sandberg, 2002). Hundreds of varieties of pulses are grown in 173 countries in the world; among them are lentils, dry beans, chickpeas, dry peas, faba beans, cowpeas, pigeon peas, lupins, grass pea. Pulses have been considered as the cheapest sources of protein and have traditionally been consumed by the rural poor family. Among these, grass pea (*Lathyrus sativus* L.) is one of the most economically important pulses for resource constrained population as for example in Ethiopia (Hailu et al., 2015; Fikre et al., 2011a).

Grass pea (*Lathyrus sativus* L.) is a drought tolerant pulse instigating from the Near East around the Mediterranean Sea and South-East part of Asia (Girma & Korbu, 2012). It is a pulse crop nutritionally rich, adjustable to adverse environments such as flood, drought, insect attack and climate change. Compared to other legumes, grass pea is highly proteineous (26-34%) and rich in lysine. Thus, grass pea can be considered as a complementary source to cereal proteins that are low in lysine, but high in sulfur containing amino acids (Urg et al., 2005; Arslan, 2017a). Grass pea is however, underutilized for human consumption due to the presence of a toxic compound and non-proteinogenic amino acid, β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) that leads to neurolathyrism. Neurolathyrism is a disease resulting in a permanent paralysis of the

lower limbs caused by the consumption of *Lathyrus sativus* as a major part of the diet for an extended period of time (Campbell, 1997). Phytate ($InsP_6$, *myo*-inositol(1,2,3,4,5,6)hexakisphosphate), a common natural compound and constituent of food derived from plants, is considered as an antinutrient due to its negative impact on minerals uptake from plant-derived foods. Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mn^{2+}, Mg^{2+}, and Cu^{2+} are considered as minerals of concern (Greiner & Konietzny, 2006b). Furthermore, the formation of phytate-protein complexes was reported to negatively affect *in vitro* protein digestibility (Cheryan & Rackis, 1980). Trypsin inhibitors and tannins are also well-known antinutritional factors having negative effects on protein digestibility in legume-based diets (Urga et al., 2005).

All proteinogenic amino acids are present in grass pea protein, glutamic acid being the prominent one and methionine the lowest in concentration (Fikre et al., 2008b). The low content of sulfur rich amino acids in grass pea-based diets may predispose neurons to the neurotoxic effects of β-ODAP (Kusama-Eguchi et al., 2011). Grass pea contains essential micronutrients such as Zn, Ca and Fe. Antioxidants and vitamins that could prevent cardiovascular disease, hypertension, and hypoxia are also present in grass pea (Arslan, 2017b). Despite having such qualities, different processing technologies are being suggested to reduce the toxic level to an extent that pose minimum/no threat to health (Srivastava & Khokhar, 1996) and to improve the nutritional qualities of grass pea seeds (Wang et al., 1997; Kebede et al., 1995).

Food processing encompasses procedures of altering raw materials into semi-finished and/or finished products that can be consumed or warehoused. Food can be processed at

home and industrial level. While processing legume seeds, changes in their nutritive value is expected. Food quality indicators can be categorized into internal factors which comprise microbial load, chemical and physical, and external factors including appearance, texture and flavor. Heat treatments are applied for inactivation of microbes, destroying of heat-labile compounds such as trypsin inhibiters and lectines and extension of food shelf life. Moreover, some of the modifications of sensory attributes induced by heat treatment are desired as they result in the generation of the specific appearance and taste of the final food. However, heat treatments also cause undesirable nutrient alterations such as vitamin degradation, as well as texture, flavor and appearance changes by overheating (Stoica et al., 2013). Non-thermal processing technologies are introduced as alternative technologies and have been recognized in retaining nutrients such as vitamins (thiamin, folic acid, vitamin C) and resulting in fresh-like product tastes and textures. Recent studies on degradation of heat stable antinutritional factors such as phytate and tannin, reduction of oligosaccharides (raffinose and starchyose), inactivation of lipoxygenase in legumes (Han & Baik, 2006; Gertrud et al., 2013; Lee et al., 2018) have increased the demand for non-thermal processing technologies. Energy saving and being eco-friendly are another advantages over thermal processing. Some of the non-thermal advanced processing technologies are high hydrostatic pressure (HHP), pulsed electric fields (PEF), irradiation, membrane filtration (Martín-Belloso et al., 2014), whereas some of the conventional non-thermal processing are soaking, germination and fermentation. Non-thermal food processing and/or a combination of any of those processes has/have key advantages in retaining nutrients (vitamins) and sensory attributes (color, flavor, texture), reduction of antinutrients (phytate, tannin) inactivation of

microbes and shelf life extension of food products. However, spore-forming microorganisms, which are highly resistant to non-thermal processing such as HHP, may require additional heat applications. Different processing methods had been carried out in order to reduce β-ODAP despite the efficiency among the processing techniques was observed to be different (Hailu et al., 2015).

The impact of HHP on proteins, amino acids and antinutrients has been reviewed by different authors (Boonyaratanakornkit et al., 2002; Lullien-Pellerin & Balny, 2002; Mozhaev et al., 1996). They illustrated the effectiveness of HHP treatment of legumes to reduce antinutritional factors such as phytate, trypsin inhibitors and oligosaccharides (Lee et al., 2018; Linsberger-Martin et al., 2013; Han & Baik, 2006) with a concomitant improvement in mineral bioaccessibility and protein digestibility. However, application of HHP on legumes and cereal grains is still limited compared to its application in fruits, vegetables, meat and milk.

1.1. Objectives and outline

Comparable to many field legumes, grass pea is a nutrient rich seed containing high levels of protein, micronutrients, starch, energy and essential and non-essential amino acids. However, grass pea contains relatively low amounts of sulfur containing amino acids. Its remarkable tolerance to stress, unique stamina to grow under adverse environment (from global warming viewpoint), and high nitrogen fixation activity increased its economic significance. In the developing world, hundreds of million poor and often illiterate farmers make their living on grass pea cultivation despite the presence

of a toxic compound, β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) that leads to neurolathyrism.

Conventional breeding was extensively applied in reducing the β-ODAP concentrations in grass pea seeds. However, the varying contents of this toxic component while planted in different weather conditions (rainfall and temperature) and locations such as altitude and soil type discouraged to perform further studies. The effect of methionine on the toxicity of grass pea had been assessed. Supplementation of methionine in grass pea based diets resulted in β-ODAP reduction due to the counteracting nature of the two amino acids (methionine and β-ODAP). It is hypothesized that the antioxidant activity of methionine restrains the oxidative stresses generated by various physiological activities of β-ODAP (Fikre, 2008a). Methionine enrichment in grass pea plant had been also addressed. Moreover, assessing environmental influences on β-ODAP concentration as the way for improving field management options was also studied previously.

Being a potential food source, more attention is needed in improving the grass pea plant using further approaches in order to increase the share of the seed in the diet of the population. Production at industry level is required to reach out the demand of the population for urban society. The role of food processing technologies to reduce the concentration of β-ODAP in grass pea seeds to an acceptable level for consumption could be a promising approach to fundamentally minimize the problems and utilize the crop. In this study, high hydrostatic pressure was considered among emerging technologies in order to evaluate the effect of those technologies on the nutritional quality of grass pea

seeds. As a matter of fact, HHP is a new technology for Africa and particularly for Ethiopia whichout any application within the continent. Thus, this particular study may help to introduce HHP in Africa and to pave the way for further studies. In fact, its applications in some developed coutries in respect to food safety and quality, the development of new food products with novel product qualities has identified HPP as an alternative for thermal processing. However, the huge investment needed and the questions of acceptability of the products by consumers are reasons for delays in using the technology.

This work, therefore, focused mainly on the impact of high hydrostatic pressure on β-ODAP and phytate reduction in soaked grass pea seeds and battered grass pea flours. The reason behind selecting the β-ODAP is that, it's presence in grass pea based diet is the prior bottleneck for the utilization of the seed; while phytate is one of the antinutrient that inhibit the availability of important micronutrient such as Fe and Zn that are found in relatively high amount in grass pea. The impact of HHP on functional, physical and structural properties such as water absorption capacity of the seed, microstructure of the grass pea flour, pasting properties of the flour that impact the final quality of grass pea products in paste forms and its effect on color after treatment had been also dealt. In addition, application of some conventional household processing technologies such as soaking, germination and cooking and their impact on contents of β-ODAP, $InsP_6$, mineral and *in vitro* mineral bioaccessibility in grass pea seed flours were included. The impact of phytase supplemented, sourdough, and yeast fermentations on β-ODAP and $InsP_6$ contents were also included in the study.

The overall task of this work is outlined below.

Chapter 2 of this work is outlined to state the theoretical aspect of the study including main concepts, the principles and mechanisms of the processing technologies used in the study. Earlier works within the scope of this dissertation and related works from adjacent fields are also briefly stated.

Chapter 3 provides the overall materials and methods in detail. Supplementary materials providing further information are appendixed in chapter 10.

Chapter 4 reports the levels of the endogenous neurotoxic non-proteinogenic amino acid, β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) in different accessions and varieties from Ethiopia and Germany. Furthermore, the contents of *myo*-inositol hexakisphosphate ($InsP_6$), proteinogenic amino acids and minerals are presented. Water absorption, microstructure (SEM image) and pasting properties of selected accession/varieties of grass pea seeds and flours are also given.

Chapter 5 reports the effect of high hydrostatic pressure (HHP) treatment on β-ODAP and $InsP_6$ contents of soaked grass pea seeds and battered grass pea flour. In a first study, the best HPP conditions in respect to β-ODAP reduction were elucidated with the grass pea accession exhibiting the highest β-ODAP content. Soaking time (h), pressure (MPa) and holding time (min) were considered by using response surface methodology, central face centered composite design. The best process conditions were then also applied to the other grass pea samples included in the study. .

In **chapter 6**, the effects of high hydrostatic pressure on water absorption capacity of grass pea seeds designed by RSM and CCRD model and color deterioration, microstructure alteration as well as changes on the pasting properties of soaked-HHP grass pea seeds and batter-HHP grass pea flours are discussed.

In **chapter 7**, the contents of β-ODAP and $InsP_6$ of grass pea or grass pea-maize blend flour before and after fermentation in the presence or absence of phytase from *A. niger* (Natuphos®) are given and discussed. Before supplementing the enzyme, the activities of intrinsic phytase(s) in grass pea and maize were determined. Thus, the effect of fermentation in the presence or absence of Natuphos®; fresh yeast and sour dough on β-ODAP and $InsP_6$ contents of grass pea flours are evaluated, compared and discussed. Moreover, in this chapter, the application of household processing methods on contents of β-ODAP, *myo*-inositol phosphate ($InsP_6$), mineral and *in vitro* mineral bioaccessibility in grass pea (*Lathyrus sativus* L.) are reported.

Chapter 8 draws conclusions based on the main findings of the research reported in **chapter 4**, **chapter 5**, **chapter 6** and **chapter 7**. Furthermore, the chapter spotlights some remarkable areas where future research studies should be carried out.

2. LITERATURE REVIEW

2.1. Features of *Lathyrus sativus* L. (grass pea) seeds

In the tribe *Viciae* of the Fabaceae family, the genus *Lathyrus* contains more than 150 species. *L. sativus, L. hirsutus, L. odoratus, L. latifolius, L. cicera, L. clymenum* are among the species which have commercial importance to mankind (Yan et al., 2006). Archaeobotanical and phytogeographical evidenced that the Balkan Peninsula is the origin of *Lathyrus sativus* (grass pea). Grass pea cultivation dated back to the prehistoric era. Ethiopia and South East Asia are traced as early cultivation sites along with or right after its domestication period. Grass pea is probably derived from the genetically nearest wild species *L. cicero* (Fikre et al., 2008b). At the beginning of the sixth millennium BC, grass pea was already cultivated in the early Neolithic Period. *L. sativus* is among the first crop domesticated in Europe as a consequence of agriculture expansion from the Near East. In Italy, grass pea regained attention as a local and traditional product. It became a fashionable and an exclusive food product in the market for which consumers are paying a higher price compared to other pulse products (Tavoletti et al., 2005).

Grass pea (*Lathyrus sativus* L.) is an important crop in Ethiopia and the country is considered as one of the primary centers of origin (IBC 2008). Its importance in the Ethiopian agriculture emanates from its unique nature to resist harsh environment. Some of the features that make grass pea stunning to consumers and growers are its adaptability to the arid regions such as drought-prone and marginal areas of Asia and Africa. It also grows under waterlogged conditions and tolerates drought stress. Moreover, cultivation of grass pea is sustainable, because few inputs such as fertilizer are required. It also

releases nitrogen back to the soil after harvest since its residues are easily broken down by microorganisms, so that it could help in sustaining the farming system (Campbell, 1997).

2.1.1. Genetic diversity of *Lathyrus sativus* L. seed

Lathyrus sativus are considered as underutilized or neglected plants, because a toxic component (β-ODAP) was perceived as the main constraint for its consumption. It is characterized by great differences of morphological traits, variations in nutrient contents and the presence of different antinutritional factors within the seeds of different accessions (Grela et al., 2010; Fikre et al., 2011b). In a broader sense, grass pea germplasms can be grouped as the Asian with small seed having average to high β-ODAP contents, and the Mediterranean, characterized by large seeds and low β-ODAP contents (**Figure 2-1**). Different collections of grass pea accessions have been identified according to their grain yield (>10 g/plant), β-ODAP content (< 0.2%), number of pods (> 50 per plant), earliness (<100 days) and seed size (> 12 g/100 seed) (Grela et al., 2010). In particular, biotic and abiotic stress tolerances and nutritional qualities are important target traits characterized in the *Lathyrus* germplasm (Lambein et al., 2019). The high variability found in the primary gene pool within the *Lathyrus* accessions cautioned for the improvement of yield, nutritional quality and adaptability of grass pea plant to different conventional and advanced approaches.

Figure 2-1. Diversity of *Lathyrus sativus* seed varieties. A) Bangladesh, b) China, c) Ethiopia, d) Canada, e) India, f) Nepal, g) Portugal, h) Poland, i) China (Lambein et al., 2019)

Grass pea accessions which are originated from particularly Europe, can also be grouped into two categories: accessions from the Mediterranean basin comprising the grass peas from Italy and Spain; and accessions from West-central Europe, including grass peas from Germany, Northern France and Poland. The grass pea plants from West-central Europe are taller in height and the seeds are smaller than the seeds originating from the Mediterranean basin. They also have white flowers with higher number of pods. The seeds of *Lathyrus* accessions from West-central Europe and the Mediterranean basin are characterized by low β-ODAP levels (average 8.47 g/100g) and no considerable difference in their nutrient or antinutrient contents were reported (Grela et al., 2010).

However, correlations between flower color intensity and tannin content were observed; flowers having white or cream color were associated with a low seed tannin level, while the colorful blooming accessions had higher seed tannin contents (Campbell, 1997; Grela et al., 2010).

Ethiopian grass peas of different varieties and accessions belonging to the same regions were pooled. Moderate genetic variability in grass pea populations resides within accessions whereas different regions harbor non-comparable levels of diversity. Among these regions, high levels of diversity were observed in Gojam, Gonder, Shewa and Welo regions, with Gonder region showing a higher number of different alleles whereas low levels of diversity were exhibited in Arsi and Hararge regions (Shiferaw et al., 2012).

2.1.2. Grass pea production (cultivation) and consumption

Grass pea is produced worldwide throughout the arid regions of the Near East, North Africa, West Asia, Indian, Eurasia, North America, temperate South America, and East Africa. It has been also reported to be cultivated in a small scale in South America, Canada and China (Kumar et al., 2011; Chakraborty et al., 2018). Farmers are encouraged to cultivate grass pea due to its huge productivity, low cost of production, easy cultivation process, and ability to grow under ecological conditions where other crops fail to grow (Yan et al., 2006; Xiong et al., 2015).

Grass pea can be cultivated at the high altitudes of Kashmir and Nepal as a summer crop as well as at the low altitude of Bangladesh as a winter crop. In India, it is grown up to

1300 m above sea level, whereas in Ethiopia it can be cultivated at a height of 2500-3000 m with an annual rainfall of 1000 mm in average. Grass pea grows well on many soil types including poorly fertile soil in marginal areas and waterlogged conditions. It can even grow in areas receiving only 380–650 mm of annual rainfall. It is also adapted to harsh local environments such as the highland volcanic soils of Ethiopia and heavy clay in the paddy rice (*Oryza sativa*) fields of Bangladesh (**Figure 2-2**) (Lambein et al., 2019).

The total acreage of grass pea is estimated to be 1.50 million hectares with an annual production of 1.20 million tons, mainly in South Asia and in East Africa (Sammour, 2014). In Ethiopia, according to the Central Statistical Agency 2014/15 report, 287,439 tons of total production was estimated at 136,883 hectares of land. The production of the crop has increased both in area coverage and production volume, from 185,490 tons in 2005 to 287,674 tons in 2015 with a productivity increment of 1.3 tons to 1.8 tons per hectare respectively (Lambein et al., 2019). According to the Central Statistical Agency (2017), major grass pea producing regions in Ethiopia are Amhara (62%), Oromia (33%), and Tigray (5%). The total production volume in this year was 2,970,972 tons having 2.0 tons of productivity per hectare. The production of grass pea was 1.8 and 3.7 times higher than the production of lentils and soybeans in 2017.

Figure 2-2. Grass pea plants in different soil types

Grass pea is a staple food during drought season unlike other legume foods in certain developing countries including Ethiopia. In various parts of the world, different traditional foods from grass pea seeds or the green shoot tips are prepared with condiments (herbs, spices, salt). Grass pea meals are commonly used for daily home consumption while some others are only used on specific festive occasions (Fikre, 2008a). Traditional grass pea meals commonly prepared in India and Bangladesh are: curry and khichuri-cooked grass pea seeds with vegetable, fish and rice; Bara-oil fried paste-ball mixed with onion and spices; chapatti-unleavened bread and different snacks

as a substitute of pigeon pea and chick peas. Cooked young and green vegetative parts of grass pea plant (4 to 6 cm length) are also common meals in Western part of Asia (Hussain et al., 1994; Campbell, 1997).

A variety of recipes from grass pea seed alone or mixed with other pulses exists in Ethiopia. Grass pea seeds can be processed and consumed in sprout-boiled and soaked-boiled ('*Nifro*'), soaked-roasted ('*Kollo*'), soaked-cooked and fermented (*Injera* and bread) forms. The grass pea seed flour (*Shiro*) is commonly used for the preparation of the traditional Ethiopian sauce called '*Shiro wott'*. Moreover, grass pea flour is commonly utilized together with other legume flours. For example, dry peas or chickpeas mixed with grass pea seed flour is used for the preparation of unleavened bread and '*Shiro wott'* (Girma et al., 2011; Hailu et al., 2015). A fermented-pancake (*Injera*), unfermented unleavened bread (*Kitta*), well-spiced and fermented roasted grass pea powder often consumed in a fasting period (*Elbe*t) are some of grass pea meals commonly consumed in Ethiopia. Furthermore, (*Eshet*) green grass pea seeds in pod before maturity and harvest is consumed by children during their farm field tending (Fikre et al., 2011a). However, the occurrence of an endogenous neurotoxic non proteinogenic amino acid, β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP), which has been identified as the cause of neurolathyrism, limits its use for consumption.

2.1.3. Nutritional composition, antinutrient and toxic level of grass pea seeds

Grass pea, besides being cheap source of proteins, a source of energy and micronutrients, is also rich in free amino acids. In general, pulses have an amino acid composition

complementary to the one of major cereals. Therefore, combined consumption of pulses and cereals increases the overall protein quality of the meal (Gan, et al., 2017). Grass pea extracts of different genotypes were shown to have high phenolic, flavonoid and proanthocyanidin contents with a considerable level of antioxidant and free radical scavenging activity (Fratianni et al., 2014; Patras et al., 2009) that could prevent cardiovascular disease, hypoxia, and hypertension (Dixit et al., 2016). Phytate, trypsin inhibitors and tannin are well known antinutritional factors in grass pea having negative effects on mineral bioavailability and protein digestibility (Urga et al., 2005). Phytate (IP_6, *myo*-inositol(1,2,3,4,5,6)hexakisphosphate), a common natural compound and constituent of food derived from plants (**Figure 2-3**), is formed during maturation of grains or plant seeds. It is considered as an antinutrient due to its negative impact on minerals uptake (Greiner & Konietzny, 2006b). The formation of phytate-protein complexes were reported to negatively affect *in vitro* protein digestibility and the extent of this effect depended on the protein sources (Cheryan & Rackis, 1980). The effects of phytate on the *in vivo* activity of digestive enzymes, particularly trypsin, and their effects on amino acid and protein utilization in pigs and poultry was reported to be dependent on many factors such as the animal species, age of animals, composition of the diet, source of protein, processing of the feed (Selle et al., 2000). The presence of phytate in grass pea is also expected to exhibit a significant negative nutritional impact preliminary on the bioaccessibility of minerals.

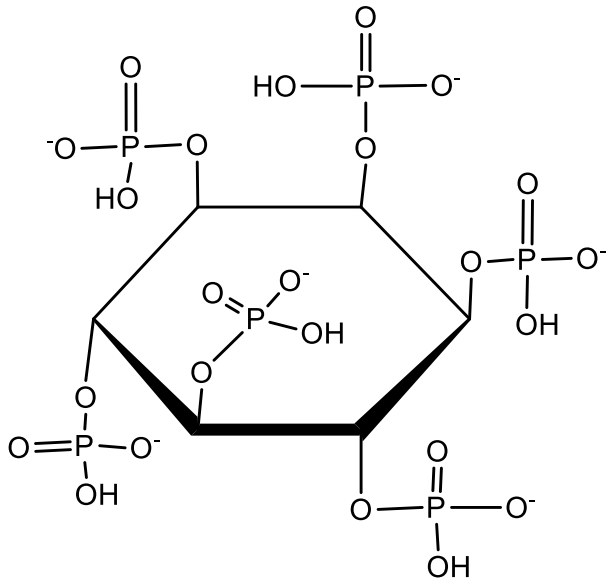

Figure 2-3. Structure of phytate

The presence of a toxic compound (β-ODAP) (**Figure 2-4**) in grass pea had been identified as the cause of neurolathyrism. Neurolathyrism is a disease resulting in permanent paralysis of the lower limbs caused by consumption of *Lathyrus sativus* as a major part of the diet for an extended period of time (Campbell, 1997).

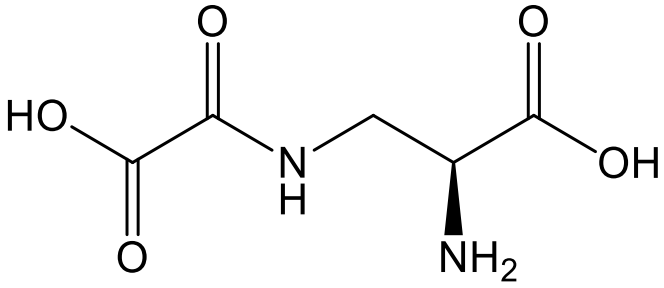

Figure 2-4. Structure of β-ODAP

2.1.4. Neurolathyrism

Neurolathyrism is an upper motorneurone disease causing lameness that possibly leads to muscle atrophy and paralysis of the lower limbs (Spencer et al., 1986). Typical features

of neurolathyrism are hard-touch of the heels to the ground, and knees contact while walking (Ludolph et al., 1988). About 500 mg of β-ODAP intake per day per person for a period of two to three months was considered as the maximum safe limit for human consumption (Rao, 2001; Campbell, 1997). Continual consumption of the pea and lack of using admixture of condiments or herbs (Getahun et al., 2005) is considered to lead to symmetric crippling of the legs. Neurolathyrism is a significant public health problem in developing countries where food insecure population is dwelled. Certain people living in India, Bangladesh, Nepal, part of China and Ethiopia are victims of neurolathyrism due to high consumption of grass pea meals (Haque et al., 2011).

There are four stages of neurolathyrism based on severity (Fikre, 2008a). The first stage is characterized as restricted ankle and joint movement that is more visible while running. Maintaining body balance is quite difficult in the second stage so that crutch might be needed to support the patient. The last two stages of neurolathyrism are the most severe stages where muscular rigidity and chronic paraplegia are developed so that the patient is obliged to rely on two sticks to walk.

From the studies carried out so far on physiological and biochemical activities of β-ODAP, the excitation of a subclass of glutamate receptors on the neuronal cells ascribed it best (Lambein et al., 2007). The activities prompt the release of the endogenous neuro-excitant glutamate, whereas β-ODAP inhibits the re-uptake of glutamate by the astrocytes in the central nervous system (CNS) that would ‘detoxify’ glutamate into glutamine. β-ODAP prolongs the excitation of the receptors during the neuronal signaling and it also

increases the production of nitric oxide (NO), a short-living molecule that is responsible for the production of reactive oxygen species (ROS) resulting in oxidative stress.

2.1.5. Environmental conditions in relation to levels of β-ODAP in grass pea

Environmental factors are responsible for the β-ODAP content of a given cultivar of grass pea (Xu et al., 2017). In addition, the genetic background of a cultivar seems to have an effect on its β-ODAP content. Last but not least, interactions of seed genotype and environmental factors such as drought, heavy metals and soil salinity affect the β-ODAP content. Cultivars having low β-ODAP contents may exhibit considerable variation when the cultivars were cultivated under different environmental conditions. Zinc deficiency and oversupply of iron in the soil; aluminum and cadmium contaminated soil; salinity as well as drought are environmental conditions which favor β-ODAP synthesis (Girma & Korbu, 2012, Haque et al., 2011).

In different grass pea lines, the level of β-ODAP was reported different. Substantial variations in β-ODAP content of the seeds have been revealed worldwide, ranging from 20 mg/100 g (Australian germplasm) to 2590 mg/100 g (some of the Indian germplasm), but no β-ODAP-free plants have ever been identified in either germplasm or wild species (Haque et al., 2011; Kumar et al., 2011; Lambein et al., 2019). Wassie, a genetically improved variety aimed to have a low β-ODAP content and an increased yield, has been officially released in Ethiopia. The initial β-ODAP content of this variety immediately after genetic modification was analyzed and reported to be <80 mg/100 g. The variety, however turned out to be higher in β-ODAP contents (>200 mg/100 g) when grown and

harvested in more drought and water deficit lowland areas (Fikre et al., 2008b). The presence of heavy metals, low levels of zinc and high levels of iron in Ethiopian vertisol soils could also be responsible for the higher levels of β-ODAP. In summary, the plant has a tendency to synthesize more of this toxic amino acid when stressed than when grown under optimum conditions (Girma & Korbu, 2012). Thus, it is quite difficult to control β-ODAP accumulation in grass pea.

2.1.6. Breeding/genetic engineering for grass pea seed improvement

Studies emphasized that β-ODAP is the major bottleneck to utilize grass pea for human consumption. Breeding programs for *Lathyrus sativus* L. were aiming in higher yields, lower β-ODAP contents and a better resistance to both biotic and abiotic stresses (Lambein et al., 2019). The effort made from national and international breeding initiatives resulted in improved grass pea cultivars with less than 100 mg/100 g β-ODAP. However, these long-term efforts ended-up non-promising as the levels of β-ODAP had been shown to increase while analyzed after different times of harvest (Jiao et al., 2011; Tadesse, 2003). Thus, processing grass pea seeds for safe consumption is becoming an inevitable alternative.

2.2. Emerging food processing technologies

Emerging and novel technologies can be classified into electrotechnologies and non-thermal processing. Electrotechnologies are those making use of novel methods to generate heat. Pulse electric field, radio-frequency (RF) heating, microwave heating, infrared heating, ohmic heating are some examples. Non-thermal technologies are pulse-

light, high hydrostatic pressure, oscillating magnetic field, irradiation, ozonization, plasma, osmotic treatment, etc. Some of these technologies are being used for commercial purposes whereas others are still under development (Ahmed et al., 2010). Emerging food processing technologies employed nowadays in industries of some developed countries are high hydrostatic pressure, pulsed electric fields, irradiation, membrane filtration, high-intensity pulsed light, microwave heating, infrared heating, ohmic heating etc. According to the responses of a survey conducted by North American and European groups in 2014, HHP and microwaves were identified as the technologies applied by the food industries and predicted to increase in application in the next 10 years due to their positive effects on food quality, food safety and shelf life (Jermann et al., 2015). Moreover, the survey pointed out that some technologies are deemed of more commercial importance in certain countries than others. For example, HHP and microwave heating are the two main technologies for commercial applications in all countries in the study. However, PEF is more popular in Europe, especially the Netherlands where a commercial scale unit exists. On the contrary, microwave technology seems to be popular in all countries but the Netherlands. UV and radiation are more important in North America than Europe. Pressure and CO_2 is only deemed to be of commercial importance in North America.

Thermal food process technologies such as pasteurization and sterilization have been used as common, efficient, reliable, economic and safe methods for food preservation. Thermal energy however, induces various biochemical reactions. This may result in quality deterioration by changes in nutrient and sensory characteristics in foods (Martens

& Knorr, 1999). Emerging and novel technologies however, can overcome some of the limitations of thermal processing. Most of these novel and emerging processes also focus on eco-friendly and energy-saving applications. As application of these technologies result in high-quality foods without compromising safety and create opportunities to develop new ingredients and products (Knorr 1999), food industries are becoming interested in such technologies. Since new technologies with better qualities incur high investment, the cost for unit product is also high. Moreover, the missing acceptability of such sophisticated technologies by consumers limits their applications. Nevertheless, researches on emerging technologies of foods and their applications have been growing worldwide (Elamin et al., 2015; Rastogi et al., 2007; Huang et al., 2017; Jermann et al., 2015).

High hydrostatic pressure (HHP) is an emerging food processing technology where food is subjected to elevated pressures with or without the addition of heat to improve food quality and to inactivate microorganisms. HHP mainly targets the cells of microbes unlike thermal processes that persuade to different biochemical reactions. HHP is also a reason for folding and corrugation of the cell wall which results in microstructure changes that in turn affect the textural properties due to loss of cellular firmness and cell wall integrity (Sila et al., 2004). It was suggested that changes in cell permeability are the main cause of microstructural or textural degradation (Prestamo & Arroyo, 1998; Kingsly et al., 2009). Cell permeability impacts a number of biochemical reactions along with texture degradation. Certain biochemical transformations on pectin, for example involving the action of endogenous enzymes such as pectinmethylesterase (PME) and

polygalacturonase (PG) had been highly correlated with the texture degradation of fruits and vegetables (Sila et al., 2005).

2.2.1. Principles of high hydrostatic pressure (HHP)

HHP employs pressure to over 800 MPa to foods without influencing covalent bonds. The process can inactivate yeasts and molds within a few minutes at a pressure ranging from 300 MPa to 400 MPa at room temperature. It also extends shelf life of food products since microbes are deactivated, and improves functional properties of foods. In addition, chemical reactions that cause the destruction of vitamins or produce off-flavors can be reduced under high-pressure conditions (Koutchma, 2014; Elamin et al., 2015). However, spore-forming microorganisms such as *Clostridium botulinum*, which are highly resistant to HHP, may require additional heat application or antimicrobial treatment to achieve a considerable reduction of the bacterial load in foods (Patazca et al., 2013).

High hydrostatic pressure is guided by two fundamental principles. Firstly, the *Le Chatelier* principle that states phenomena such as phase transition, chemical reaction and change in molecular configuration that lead to volume decrement favored by pressure. Secondly, pressure is distributed uniformly and simultaneously in all parts of foodstuffs irrespective of its size and shape (isostatic principle). Due to isostatic pressure, food is not crushed which is a benefit in some cases compared to thermal processing. The resultant pressure regulates subsequent biochemical reactions occurring in the treated products (Muntean et al., 2016).

HHP system consists of six major and basic components as demonstrated in (**Figure 2-5a**). They are high-pressure vessel, pressure generation system, a pressure transmitting fluid, a material handling system and supporting units such as heating and cooling components, and temperature and pressure monitoring systems. In HHP treatments, pre-packed foods (solid, semi-solid or liquid) are first placed in the perforated sample holding bascket. The pressure vessel is filled with a pressure transmitting medium (usually water). Prior to loading the food, the vessel is automatically deaerated by low pressure (fast-fill-and-drain) pump. High hydrostatic pressure is then generated by piston (direct) or pump (indirect) compression processes (**Figure 2-5b**) (Muntean et al., 2016). The pressure is transmitted to food samples immediately and uniformly via transferring medium from all sides (isostatic or Pascal principle). HHP can be conducted at ambient or below 4^{o}C thereby eliminating thermally induced off flavors. The product packaging must be able to withstand a change in volume approximately up to 15%, followed by a return to its original size. Depending on chemical composition of the foods and target pressure, the temperature of water increases by approximately 3°C per 100 MPa resulting in reversible compression due to adiabatic heating. However, if the food contains a significant amount of fat or highly compressible food ingredients, such as cream or butter, the temperature rise is greater (8 to 9°C/100 MPa). Foods cool down to their original temperature on decompression if no heat is lost to or gained from the walls of the pressure vessel during the holding stage. Once constant high pressure levels are achieved, holding time can range from milliseconds to over 20 min, and initial treatment temperatures can range from 0 to 20^{o}C (Rastogi, 2013).

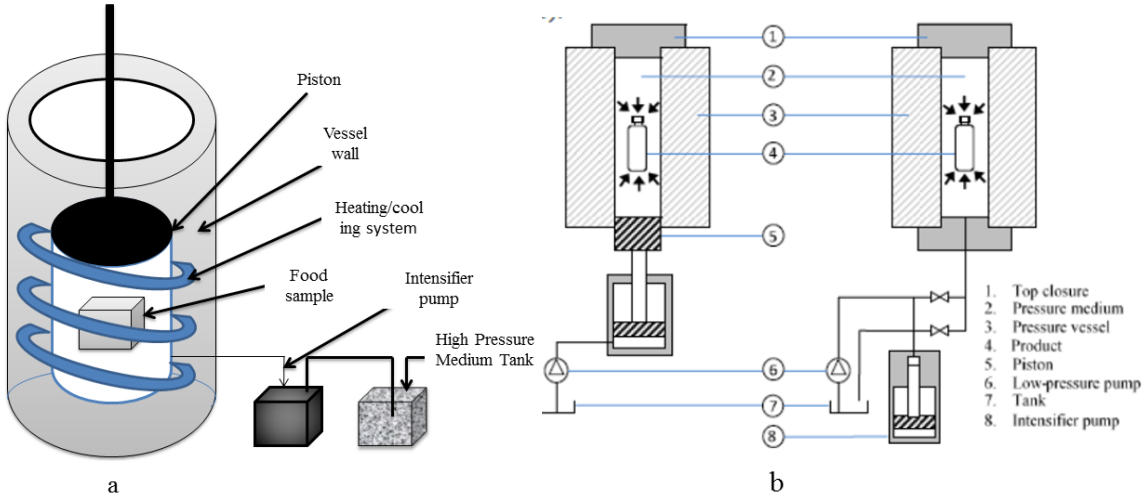

Figure 2-5. a) Basic components of HHP (Muntean et al., 2016), , b) Schematics of direct (left) and indirect (right) compression of HHP food processing techniques (Elamin et al., 2015) (partly re-drawn)

2.2.2. Effect of HHP on protein structure

HHP technology is an effective and safe method of modifying inherent conformational states of proteins that can lead to reversible or irreversible protein denaturation (Lullien-Pellerin & Balny, 2002; Bolumar et al., 2016). A general scheme of the changes taking place on proteins under pressure is presented in **Figure 2-6**. Generally, enzymes are active at low pressure and most of them are inactive after exposure to high pressure (Hendrickx, et al., 1998; Knorr et al., 2006). Exposure to around 200 MPa often affects only quaternary structure of proteins leading to the dissociation of oligomeric proteins into monomers. Quaternary structure is mainly held by hydrophobic interactions and thus it is very sensitive to pressure. The oligomers starts to rebuild (aggregate) at around 300-500 MPa. At around 400-600 MPa, microbial inactivation is occurring. At relatively high pressure, (more than 700 MPa) protein unfolding or irreversible protein denaturation may occure (Torrezan et al. 2007) depending on the magnitude and duration of the applied pressure, nature, concentration, pH and ionic strength of proteins and treatment

temperature (Galazka et al., 2000; Torrezan et al., 2007). Water is commonly used as a high pressure medium and it has a significant effect on protein structure under high pressure (Heremans et al., 2000). The properties of water encircled proteins are considerably affected by HHP in such a way that the hydration sphere becomes well-ordered under HHP treatment.

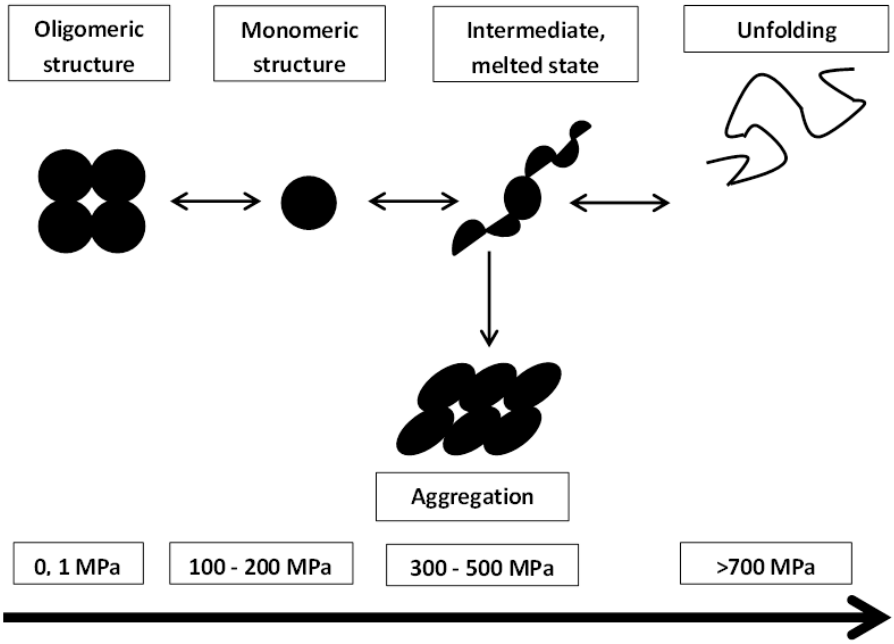

Figure 2-6. Scheme of protein structure modification by HHP. Adopted from (Bolumar et al., 2016) (Re-drawn)

2.2.3. Effect of HHP on enzymes

Enzymes are a special class of proteins in which biological activity arises from an active site. Small changes in the active site can lead to a loss of enzyme activity. HHP greatly affects the activities of endogenous enzymes with beneficial or harmful effects on foods (Martínez-Monteagudo & Balasubramaniam, 2016). Effect of HHP on enzymes can be categorized in two classes: activation of enzymes at low pressure and inactivation of

enzymes at higher pressure. In respect to pressure inactivation, four types of enzymes were distinguished based on their loss and recovery of activity. (i) completely and irreversibly inactivated, (ii) completely and reversibly inactivated, (iii) incompletely and irreversibly inactivated, and (iv) incompletely and reversibly inactivated (Hendrickx, et al., 1998). In most studies, enzymes can be irreversibly inactivated due to high high pressure-temperature (HP-T) treatment. The level of pressure and temperature stability is highly dependent on enzyme types and sources. The level of enzyme inactivation during HP-T treatment is affected by pressure level, temperature level and pressure-temperature holding time; enzyme concentration, enzyme purity, pH, presence of inhibitor/activator, composition of food medium, food matrix, etc (Swetha & Mukund, 2016).

2.2.4. Effects of HHP on nutritional quality of foods

HHP affects hydrogen, ionic, and hydrophobic interactions. Therefore, HHP processed product have a fresh-like appearance. The major consequences of applying HHP on foods are: changes in physical properties such as solubility and viscosity, effects on dissociation and ionization and effects on rates of processes.

2.2.4.1. Protein digestibility

Legume and native proteins such as intact globular proteins are poorly digestible under gastric conditions (Nielsen, et al. 2002). For better intestinal absorption of proteins, it is crucial to understand how processes improve protein digestibility so that nutritional values of proteins are increased. Modifying protein using HHP may improve digestibility by enhancing accessibility of the protein for proteolytic enzymes. Digestibility of

soybean whey protein and milk proteins showed a great digestibility improvement upon HHP treatment compared to non-treated. Vilela et al., (2006) reported HHP treatment at 550 MPa applied on whey protein isolates improved the digestibility of the isolates using pepsin and pancreatin. The hydrolysis of proteins from soybean whey by trypsin and pepsin (chymotrypsin) increased when a pressure of 200 MPa at 37^{o}C for 15 min were applied (Penas et al., 2004). Zeece et al., (2008) also reported that β-Lg treated in a pressure range of 600-800 MPa showed fast digestion with pepsin under simulated gastric condition. Yin et al., (2008) reported that pressure treatment of red kidney bean isolate exhibited slight protein solubility at 400 MPa, 25^{o}C. Significant protein digestibility increment was reported by Linsberger-Martin et al. (2013) to be 8.7% and 4.3% for beans and peas applying 600 MPa and 60^{o}C compared to untreated samples and higher than traditional cooking. Slight increase in *in vitro* digestibility of selected legumes (lentils, chickpeas, peas, and soybean) protein was reported by Han et al. (2007) at 621 MPa treated for 0.5 to 1 h. Higher *in vitro* protein digestibility of pea isolate solution at 600 MPa held for 5 min at pH 3.6 compared to untreated samples was reported by Laguna et al. (2017). Thus, protein digestibility improvement by HHP depends on sources of proteins, pressure treatment conditions, and type of enzymes. Nevertheless, limited studies are reported so far on protein digestibility of legumes processed by HHP.

2.2.4.2. Bioavailability of bioactive components

Improvement of bioaccessibility of bioactive compounds is highly related to the structure of food. HHP induces structural change in foods that influences bioaccessibility and bioavailability of components by enhancing their extractability (Barba, et al., 2015)

(**Figure 2-7**). Simulated gastric and pancreatic digestion of broccoli, green beans and carrots treated at 400-600 MPa for 2 min at 21^{o}C for example exhibited an increase in carotenoid bioavailability (McInerney et al., 2007). Disruption of plant cellular tissue upon exposure to high pressure has been hypothesized to influence carotenoid bioavailability (Knockaert et al., 2011)

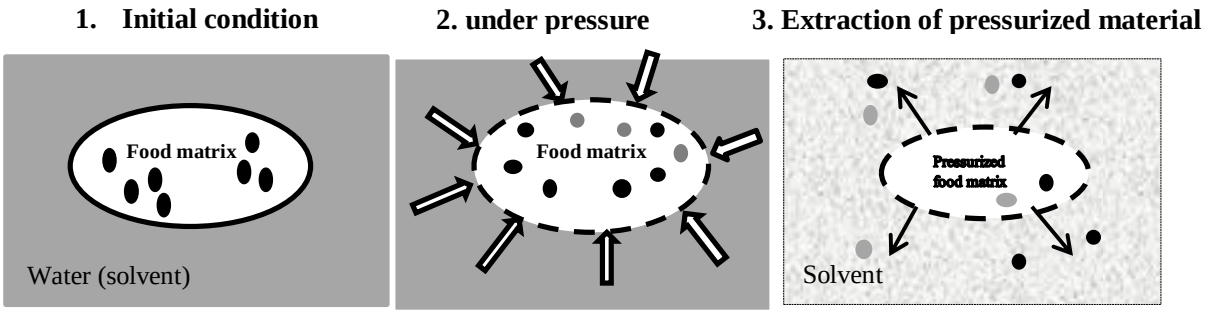

Figure 2-7. Schematic representation of HHP on food compound extractability. HHP is applied to obtain an enriched extract for food. The matrix is dispersed into a solvent in which the food is pressurized, and extractability into the solvent is determined after treatment. In step 2, change of membrane permeability can be one of the mechanisms behind change of extractability. It is more likely that some of the solvents in which the food matrix is dispersed will enter into the product during treatment. Black circles-compound of interest; gray or dashed circles-a solvent. (Source: Jung, 2016). * The picture is re-drawn.

2.2.4.3. Other food quality attributes

Direct consequences of the stability or instability of bioactive compounds such as vitamins, carotenoids, flavonoids, mono and polyunsaturated fatty acids, and sulfur containing bioactive compounds during HHP affect the functional properties of food products. Sensitivity difference among substances to temperature (activation energy) and pressure (activation volume) defines the ability of damage or withholding of the quality attributes of foods such as vitamins, enzymes, color, pigments and flavor compounds (Rastogi et al., 2007). The taste of pressure treated egg **(Figure 2-8)** reported to be closer

to that of raw egg because the flavor changing chemical reaction which occur in temperature-induced phenomena did not happened during HHP treatment (Muntean et al., 2016).

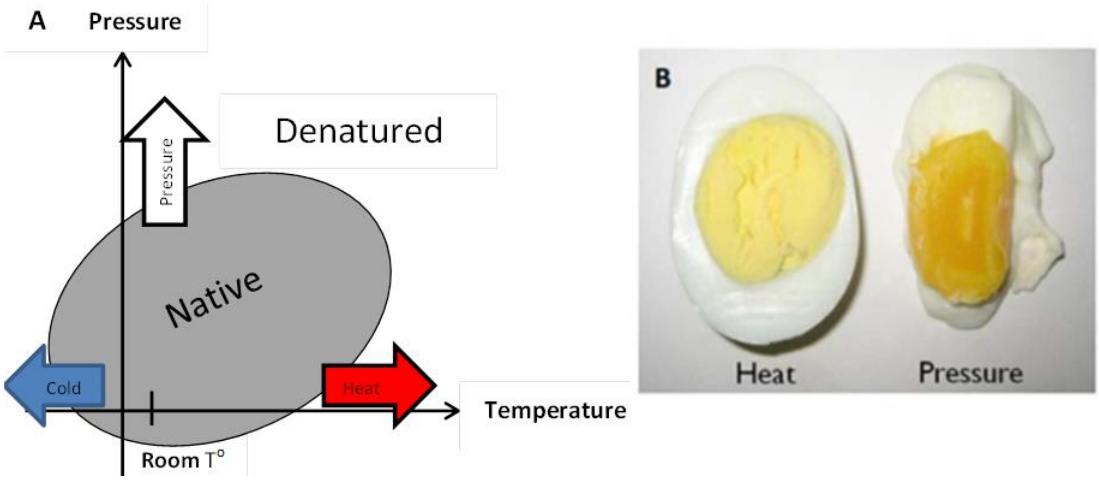

Figure 2-8. Schematic representation of the elliptic phase diagram of proteins illustrating pressure, heat and cold denaturation (A) and picture of denatured eggs (source: Muntean et al., 2016). *Pic A: re-drawn, Pic B: taken directly.

2.2.5. Application of HHP in food processing

Among different non-thermal food processing technologies, the application of HHP at low, ambient and high temperatures have been delivering different novel food products. Food product novelity is described by novel processes where the processes give rise to significant changes in the structure and composition of the food or food ingredient. These in turn have effects on nutritional qualities of food, metabolic effect or level of undesirable substances (Huang et.al., 2017). Demand of food industries to HHP applications for a number of foodstuffs is improved gradually. For example developed countries such as USA, China, Japan and some European countries in particular are using HHP technology. The global market for HHP foods was nearly $9.8 billion in 2015 and is

expected to be $ 54.77 billion in 2025 (Visiongain, 2015). Manufactures from North America, Japan and Europe are active participants in developing the commercial applications with around 500,000 tons of HHP products circulation around the world every year (Huang et al., 2017). Food industries in some European countries, Japan and US had been already launched conducting various researches, and products such as fruit sauces, salad dressings and fruit-yogurt have been introduced to the market since 1990 (Martínez-monteagudo & Balasubramaniam, 2016). Despite the high costs for HHP products, most fruit juice producers including Starbucks remain confident in HHP fruit juice that is claimed to be of high quality (Huang et al., 2017). They even plan to apply their successful marketing strategy previously used in the coffee industry to HHP fruit juices. In US, the demand for HHP processed products has been increasing after consumers' interest on HHP processed sauces, oysters and guacamole (Mertens & Deplace, 1993). The industrial applications of HHP, evidenced by the growing tendency of a number of HHP equipment installations, showed an increasing trend in 2014 compared to 2004. High pressure machines produced in 2014 was five times higher than in 2004 (Elamin et al., 2015).

The cost per HHP treated product is still high because the equipment is expensive. Many food companies are not able to manage applying the technology due to big capital investment it requires. This could be one reason for a gradual development of HHP technology (Elamin et al., 2015;Jermann et al., 2015). HHP manufactures also took options to market their services without the need to sell full HHP units. On the other hands, lack of knowledge to understand the technology creates high levels of consumer

concern and could be a barrier for accepting the technology. Products processed by such emerging technology rely on the consumer's sensitivity towards the impact the technology brought on health, taste, convenience, nutritional value and quality (Bruhn, 2016). Thus, advancement of the technology needs further tasks to be done to increase the demand. Because, if the demand to HHP treated products increases, mass production is encouraged that may lead to a price per product that is acceptable for both manufacturer and consumer. Morover, further tasks such as research on different HHP-food products, more survey on consumers' perception, developing and introducing regulations on HHP products, advertisement and awareness creation to the consumers are needed.

A number of successful utilizations of this technology in food products such as different fruits and vegetables, meat, fish and ham have been carried out. However, studies on the application of HHP in cereal grains and legumes processing are still limited (Estrada-Giron et al., 2005). No works on grass pea plant (seeds) are available. The development of off-flavor in soybean and other legumes due to the presence of for example, lipoxygenases and the reduction of phytate that inhibit the bioaccessibility of nutrients are some areas where HHP application had found places and needs further investigations (Estrada-Giron et al., 2005). Some examples of HHP applications in processing of cereal grains and legumes are also summarized in **(Table 2-1).**

Table 2-1. Application of HHP in some cereal and legume grains

Legumes/other grains	Pressure applied (MPa)	Sample treated	Effect	Author (s)
Soybean	300-400	Immersed in distilled water	Protein solubility increase, no apparent changes in shape, color & size compared to untreated	Omi et al., (1996)
Soybean	350-525	Immersed in distilled water	Inactivation of lipoxygenase	Ludikhuyze et al., (1998)
Rice	300-600	Soaked in distilled water	Partial destruction of the endosperm and allow starch granules to swell	Yamazaki & Sasagawa, (1998)
Barley and wheat flours	400-600	Slurries 25% (w/w)	Large increases in total soluble carbohydrate and reduced sugar content	Gomes et al., (1998)
Green bean extract	500	-	Inactivation of lipoxygenase	Indrawati et al., (1999)
Rice	100-400	Immersed in distilled water	Protein solubilization and release of allergenic proteins	Kato et al., (2000)
Soybean	400	Tofu	Reduction of microbial population	Prestamo et al., (2000)
Soybean	600	Soaked in distilled water	Inactivation of lipoxygenaze	van der Ven et al., (2005)

Chickpea, pea, Soybean	621	Soaked in distilled water	Reduction of oligosaccharides	Han & Baik, 2006)
Lentils, chickpeas, peas, and soybeans	621	Soaked in distilled water	Increased protein digestibility	Han et al. (2007)
Soybean	300	Sprout seed	Allergenicity reduction	Elena et al., (2011)
Soybean	650	Tofu	Inactivation of lipoxygenaze & m/o inactivation	Ghafoor et al., (2011)
Dry split peas & whole white beans	600	Immersed in distilled water	Reduction of oligosaccharides, phytic acid, trypsin inhibitors & increase in protein digestibility	Gertrud et al., (2013)
Buckwheat	600	Protein isolate	Allergenicity reduction	Lee et al., (2015)
Red bean	600	Soaked in distilled water	Reduction of phytate and trypsin inhibitors	Lee et al., (2018)

2.2.6. Effect of HHP on physical, functional and structural properties of legume seeds

Pulses are edible seeds of plants of the legume family and harvested exclusively as dry grains which differentiate them from other crops harvested green (FAO). They are staple foods in many developing countries of the world due to their low price and high protein content. Pulses are also a source of dietary phytochemicals, carbohydrate, insoluble and soluble fibers (Ahmed et al., 2016). The flours of pulses/legumes are also used as

ingredients in food formulations because of their functional properties (Kaur et al., 2009). Moreover, pulses have considerable amount of starch, which is commonly known as resistance starch that is resilient to digestion. Grass pea also acquires great attention to be considered as a good source of phytochemicals and starch. There are A-type starches that are found in cereals (e.g. maize, wheat and rice), B-type in tubers (e.g. potato) and C-type starch, containing both A-and B-type polymorphs in legumes (e.g. pea and faba bean). Grass pea starch could also be categorized as C-type.

The starch in legumes has a high potential of health benefits. It is evidenced that legume starches have lower glycemic index compared to cereal starches, thus helping in the dietary control of diabetes and arterial disease. Legume starches represent the main storage component of the seed ranging from 22-45%. However, they neither have been used by food industries nor were intensively studied. This is mainly due to their indigestibility due to raffinose family oligosaccharides, high rate of retrogradation and a challenge to isolate starches from legumes due to the presence of insoluble flocculent proteins and fine fibers (Hoover & Sbsuuki, 1990). The high retrogradation rates of legume starches could be reduced by chemical modification and processing technologies (Katopo et al., 2002). Different studies reported the impact of high hydrostatic pressure (HHP) treatment on gelatinization of legumes starch, considering the extent of pressure applied, pressurizing time, type and concentration of starch and temperature as the major factors (Oh et al., 2008). The effect of HHP on physicochemical, thermal and morphological properties of various A-type and B-type starches had been investigated by researches, whereas the information on C-type starch is scarce (Li et al., 2011; Hu et al.,

2011). Literatures also showed that starch gelatinized by HHP treatment is different from thermally treated, and exhibited a lower retrogradation rate than starch samples treated thermally (Ezaki & Hayashi, 1992; Knorr et al., 2006) (**Figure 2-9 a & b**). The lower retrogradation rate imparts value addition for starchy foods in properties such as texture in post processing. Furthermore, HHP was shown to alter the structure of proteins and starch (Katopo et al., 2002), to modify viscoelastic properties of starch (Vallons et al., 2011) resulting in improved functional properties, an effect that could be used to produce foods with novel textures. However, HHP also causes adverse color alteration of food products by activating enzymes at low pressure (Hendrickx, et al., 1998). This is attributed to the degradation of anthocyanins by enzymes such as β-glucosidase (β-GLC), polyphenoloxidase (PPO) and peroxidase (POD). It was proposed that anthocyanins are firstly hydrolyzed by the β-glucosidase, forming anthocyanidins which can be oxidized by polyphenol oxidase and/or peroxidase. The resulting oxidation products react with anthocyanins to form brown condensation products (Patras et al., 2009; Marszałek et al., 2017). High pressure processing (400-500 MPa) however, could also result in color loss. The color change was hypothesized to be due to the compacting and homogenizing effect of HHP (Swetha & Mukund, 2016). Moreover, pressure treatment at higher temperatures, can also cause bioactive compound degradation.

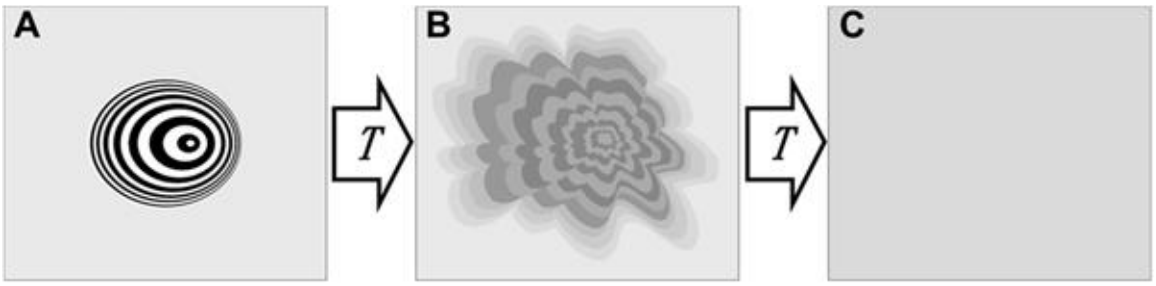

Figure 2-9a. Schematic diagram of heat gelatinization of a starch granule. **A**) Starch granule suspended in water: cross section of the granule presents lamellar structure of concentric ellipsoids. **B**) Heating induces swelling and disruption of the granule. Molecular motion is accelerated by heating and lamellar structure is lost by swelling. **C**) Further heating leads to complete gelatinization where the granular shape is not observed. The figure is re-printed from Yamamoto & Buckow (2016).

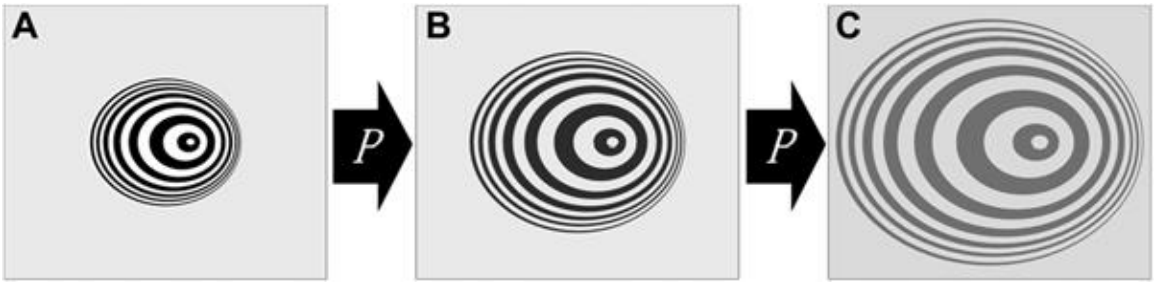

Figure 2-9b. Schematic diagram of pressure gelatinization of starch granule. **A**) Starch granule suspended in water. **B**) Starch granule can be partially gelatinized by HHP with swelling of the granule induced. **C**) Completely pressure-gelatinized starch granule can retain granular shape. The figure is re-printed from Yamamoto & Buckow (2016).

The functional properties of flours affect the characteristics of the prepared food/meal. Functional properties including water uptake of different legume seed flours obtained among others from black kidney beans (Sangnonis et al., 2002), adzuki beans (Ueno, 2015), chickpeas (Ibarz et al., 2004) and carioca beans treated by HHP have been studied previously (Sosa et al., 2019). HHP is proposed to accelerate hydration during soaking (Bello et al., 2004). The water uptake of legume grains is a key step in processing industries having a positive impact on the physical, chemical and nutritional quality of legumes and results in a modification of other functional properties and sensory attributes

of the legume flours (Shafaei, 2016). Water spreads slowly into the seeds and eventually reaches a constant level of moisture during the hydration process. Temperature influences water absorption capacities and rates in seeds (Sharanagat et al., 2018; Shafaei, 2016). Using warm water for instance increases water diffusivity in the seed leading to a higher hydration rates that result in diminished soaking time. Moreover, understanding microstructure of legumes could help in selecting suitable legume varieties for different processes and in extending its application from traditional to novel food products. It also provides information on milling property, chemical composition of seed and permeability of seed coat to water absorption.

Besides functional and structural properties, color is one of the vital quality attributes of food products that can be perceived as a measure of quality, mouth-feel and taste for a consumer. However, it can be affected by factors such as processing, packaging, storage etc (Ahmed & Ramaswamy, 2006) that could results in quality deterioration due to loss of ascorbic acid during processing as reported by Davey et al., (2000). HHP is responsible for a number of biochemical changes such as proteolysis, browning reactions, and inactivation of enzymes (Matser et al., 2000). These effects in turn affect the quality attributes of foods.

2.3. Conventional processing technologies in foods

2.3.1. Phytate degrading enzymes in foods

In food industry, enzymes play a great role in controlling the production and yield of final food products. They have impact on food quality, food property modification and as processing aid in food and beverage processing (Aguilar et al., 2008; Osma et al., 2010).

Some class of enzymes such as phytase could be used in processing of foods for human consumption as they result in an enhancement of mineral bioavailability. Phytase is a special kind of phosphatase capable of releasing phosphate from phytate (*myo*-inositol(1,2,3,4,5,6)hexakisphosphate) step-wisely. Phytate is a common constituent of plant-derived foods and considered as the primary storage form of phosphate and inositol in grains and plant seeds (Greiner & Konietzny, 2006b). Different research studies indicated that phytases have great potential in feed and food processing and production (Haefner et al., 2005; Duliński et al., 2020; Rosenfelder-Kuon et al., 2020; Handa et al., 2020). Nearly two decades ago, a phytase product was introduced to the market which assisted reducing phosphorus excretion from monogastric animals by up to 50% (Haefner et al., 2005; Handa et al., 2020). Food processing industries can use phytases for phytate reduction, to improve mineral bioavailability of a given food as well as to produce functional foods (Konietzny & Greiner, 2004). However, in spite of their huge potential in food processing and production industries, no product using phytase has found its way to the market (Duliński et al., 2020).

Phytate behaves in a broad pH range as a highly negatively charged ion and hence has a huge affinity to food components such as minerals, trace elements and proteins with positive charges. The formation of insoluble mineral-phytate complexes at physiological pH values is regarded as the major reason for the poor mineral bioavailability, because these complexes are essentially non-absorbable from the human gastrointestinal tract. In addition to this, human small intestine has very limited phytate hydrolyzing ability due to the lack of endogenous phytate-degrading enzymes and the limited microbial population in the upper part of the digestive tract (Konietzny & Greiner 2004; Lopez et al., 2002).

During food processing and preparation, adjusting optimal conditions for inherent plant and microbial phytases should be distinguished from the supplementation of exogenous For instance, phytate hydrolysis during soaking, germination, cooking, and fermentation is resulting from phytate degrading activities naturally present in plants and microorganisms. It is good to note that the ability to dephosphorylate phytate varies highly among various plants and microbial species because of differences in their native phytate degrading activities and properties of enzymes such as protein stability and optimum conditions for degradation of phytate (Greiner & Konietzny, 2006b).

2.3.2. Household processing procedures

2.3.2.1. Soaking

Soaking is one of the pre-processing techniques for whole legumes and cereal grains which allow the seed to absorb water, and the best way to soften the texture of seed for easy decortication or cooking (Jeong et al., 2019, Oghbaei & Prakash, 2016). Soaking

time, soaking temperature, pH, soaking medium, seed size and storage condition are however, factors which affect properties such as texture and water absorption of the seed (Taiwo, 1998). Short period soaking lasts about 15 to 20 min wheras long period soaking may require 16 to 20 h. Increasing the temperature of soaking water is one way to increase the rate of water imbibition that may minimize the time needed for soaking. The temperature of soaking water should not necessarily be high (80 to 100°C) as reported by Taiwo et al., (1994). For instance, soaking cowpea at 45°C for one hour is sufficient to hydrate the beans and attain the required weight increase of 80-100%.

Long time soaking and discarding the soaking water had been found to result in a loss of minerals (Huma, et al., 2008). Authors reported different effect of soaking on mineral contents of legumes. Elmaki et al. (2007) found that increasing the soaking length of beans or discarding the soaking water resulted in greater loss of minerals. Chopra & Sankhala (2004) reported reduction of phytate and tannin contents of horse gram and moth bean by soaking; but calcium and magnesium contents were not reduced significantly whereas the bioavailability of both minerals was increased. Aranda et al. (2004) also found reduction of tannin and phytate contents by soaking and discarding the soaking water, which improves iron bioavailability. Though minerals are lost leached out into the discarded water, soaking and discarding increases the bioavailability of the minerals remaining in the legumes (Elmaki et al., 2007). This is attributed to the removal of antinutrients during the treatment that chelate minerals (Fernandes et al., 2010) and the interaction of minerals with all components in the diet (Lopez et al., 2002; Raes et al.,

2014). Hence, studies on legumes in general had similar observation regarding mineral bioavailability improvement by soaking, especially when the soaking water is discarded.

Effect of soaking on enzyme activity

Soaking triggered the activation of various metabolic processes such as the synthesis of hydrolytic enzymes which resulting in the degradation of storage molecules present in the food matrix (Awatif & Alaaeldin, 2017). For example, phytate is enzymatically hydrolyzed by endogenous plant phytases (Greiner & Konietzny, 2006b).

2.3.2.2. Germination

Germination is another simple and inexpensive technique 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 & Jood, 2002; Jiang et al., 2013). It is widely used for legumes and cereals at the household level. It increases their palatability and nutritional qualities, particularly through the breakdown of certain antinutrients, such as phytate and protease inhibitors (Greiner & Konietzny, 2006b). Nowadays, the consumption of germinated legumes in the form of flours, culinary and as health foods is increasing worldwide (Kuo et al., 2004). Reported effects of germination on nutritional qualities of legumes are summarized in (**Table 2-3**).

Effect of germination on enzyme activity

Many biochemical changes occur during germinating that affect digestibility, structure, bioactivity, stability and flavor of the food product. Germination triggers the enzymatic activity of sprouting seeds, leading to the breakdown of proteins, carbohydrates and lipids into simpler forms (Awatif & Alaaeldin, 2017). Germination also activates proteases resulting in protein degradation, thereby increasing nutrient bioavailability. Hydrolytic enzymes are activated and they decompose proteins and starch which leads to the increase of amino acids and oligosaccharides (Singh et al., 2015).

Effect of germination on proteins

During germination, proteins are degraded to increase the soluble protein content. Increment of free amino acid content due to the degradation and conversion of protein after germination was reported (Tian et al., 2010). An increase in trypsin activity by the effect of germination is resulted in *in vitro* protein digestibility. Tannin has been also found to inhibit digestive enzymes and thus lower digestibility of proteins and other nutrients. In addition, germination leads to an increase in protein content (Gan et al., 2017). The increment of protein concentration due to germination is attributed to the losses of dry weight via respiration during malting (Tian et al., 2010). Tannin that trenders the availability of proteins, vitamins and minerals by inhibiting hydrolytic enzymes had been shown to decrease during germination (Butler et al., 1984). The reduction of tannin during germination is attributed to the formation of hydrophobic associations of tannin with seed protein and enzymes. Flatulence-producing α-galactosides, trypsin and chymotrypsin inhibitors, which affect the digestion of proteins,

are also reduced after germination (Kuo et al., 2004). Germination was also reported to improve the contents of nutrients such as vitamins and polyphenols (Omary et al., 2012; Gan et al., 2017).

Effect of germination on food attributes

Germination is an effective method for adjusting the flavor of grains, and in particular the subsequent heat treatment process is an important factor for flavor formation (Heinio et al., 2001). During germination, the alkaline pH promotes the formation of compounds yielding a caramel like odor. Free amino acids and sugars act as flavor precursors for the odor-active compounds. Germination process is also one of methods used to improve the functionality of seed protein. The decomposition of the high molecular weight polymers during germination leads to the generation of bio-functional substances and the improvement of the organoleptic qualities due to softening of texture and increase of flavor (Singh et al., 2015).

As long time periods (6-10 days of germination) are needed to improve nutrient bioavailability, the approach is more useful for household level than industrial applications as it is not economical method for industrial food processing (Greiner & Konietzny, 2006b, Omary et al., 2012). The oveall process of germination is also prone to fungal contamination unless adequate control measures are ensured.

Table 2-3. Summary of the effect of germination on nutritional qualities of different legumes

Cereal/legumes	**Conditions/time, temperature, etc.**	**Effects of germination**	**References**
Australian sweet Lupin	1-9 day at 25^{o}C	Increased fat, decreased Phytic acid & dietary fiber	(Rumiyati et al., 2012)
Lentils and faba beans	6 days, 20 ^{o}C	Reduced thiamine amounts; increased riboflavin and niacin	(Wang et al., 2014), (Prodanov et al., 1997)
Mung bean, Chickpea, Cowpea	12-20 h, 36-60 h, 16-24h	Increased crude protein content, fiber & decrease carbohydrate	(Uppal & Bain, 2012)
Green gram, cowpea, lentil, chickpea	(Soaking in water for 12 h at 22–25°C, and germination for 24 h)	Increase in protein content, Decrease in antinutrients: oxalate, tannin, trypsin inhibitor, & phytates; improved starch & protein digestibility	(Ghavidel & Prakash, 2007)
Red kidney beans	(Soaking in water for 6 hr at room temperature,	Decrease in cyanide, tannins, polyphenols, & phytic acid	(Yasmin et al., 2008)

	germination for 4 days at 22°C		
Kidney, mung beans, soybean, and peanuts	(soaking 6 h & germination until emergence of radical at maximum 5 mm)	Increase in total sugars & total dietary fibers	(Megat et al., 2016)
Soybean sprout (*Glycine max* cv. HeiNong48), Mung bean sprout (*Vigna radiata* cv. Sulv3)	2-10 days	Increase in B and C vitamins	(Shohag et al., 2012)
Black mung bean sprout (*Vigna radiate*)	1-5 days	Increase in C vitamin	Gan et al., 2016)
Germinated chickpea (*Cicer arietinum*), Germinated cowpea (*Vigna unguiculata* L.)	1-5 days	Increase in C vitamin	(Masood et al., 2014)
Germinated lupin (*Lupinus albus*)	2-9 days	Decrease in Ɣ-tocopherol	(Frias et al., 2005)
Mung bean sprout (*Vigna radiata* L. Wilczek)	5 days	Increase in total phenolic content	(Pajak et al., 2014)
Germinated chickpea	1-4 days	Increase antioxidant capacity	(Wu et al.,

(*Cicer arietinum* L.), Germinated black soybean (*Glycine max* L.) Germinated kidney bean (*Phaseolus vulgaris* L.)		from soluble extract	2012)
Mung bean sprout (*Vigna radiata*)	1-9 days	Increase antioxidant capacity from soluble and bound extracts	(Guo et al., 2012)

2.3.2.3. Fermentation

Fermentation is one of the oldest food processing and food preservation methods commonly utilized at industry as well as household level. Fermentation is claimed to have a number of benefits such as improving shelf-life of agricultural products, improving quality and digestibility of proteins, increasing mineral bioavailability, and reducing certain toxic substances including cyanide, hemoglutinins, and neurotoxins (Adams, 1990; Igzaw et al., 2004). It is also known to reduce heat-stable antinutritional factors such as phytate, tannins, raffinose and saponins.

Effect of fermentation on enzyme activation

Fermentation activates endogenous enzymes such as α-amylase, phytase, and glucosidases (Nkhata et al., 2018). These enzymes are responsible for degrading antinutritional factors and breaking down complex macronutrients to their simple and

more digestible forms. Protein digestibility, for example is improved either in natural fermentation or fermentation using starter culture (Pranoto et al., 2013).

Effect of fermentation on proteins

The effect of feremtnation on proteins and amino acids exhibited inconsistent results which might be due to the initial protein or amino acid profile of foods (Nkhata et al., 2018). The increase in protein may be due to degradation of complex protein by microorganism thereby liberating amino acids and peptides, and partly due to loss of dry matter during fermentation (Pranoto et al., 2013; Hamad & Fields, 1979). Nevertheless, it is reported that fermenting microorganisms also uses amino acid (s) which could lower the protein content and quality of some fermented food (Osman, 2011). Fermentation increases the digestibility of plant proteins (Alka, et al., 2012, Pranoto et al., 2013). Plant proteins are known having poor digestibility compared to animal protein which may cause gastrointestinal upset. Improvement of protein digestibility by fermentation is due to the partial breakdown of complex storage proteins into more soluble forms. Fermentation followed by cooking however, is more effective in increasing grain protein digestibility (Osman, 2004), probably due to partial pre-digestion of grain proteins by fermenting bacterial and destruction of trypsin inhibitors (protease).

Effects of fermentation on minerals (bioavailability)

Minerals from plant sources have very low bioavailability, because they are found complexed with non-digestible material such as cell wall polysaccharides (Torre et al., 1991) as well as phytate. Fermentation is one of the processing methods that are applied

to release these complexed minerals and make them readily bioavailable (Pranoto et al., 2013). The increase in mineral bioavailability is likely due to degradation of phytate and oxalates upon fermentation. In addition, fermentation increases the contents of minerals such as iron, zinc and calcium. This might be due to loss of dry matter upon fermentation for microbes degrade protein and carbohydrates (Day & Morawicki, 2018) as well as concomitant with the decrease of phytate.

Effect of fermentation on other quality attributes

Fermentation process ends up with the development of flavor, aroma and improved texture of foods due to the production of volatile compounds by the fermenting bacteria (Kiers et al., 2000). The degree of protein hydrolysis is an important factor in changing the texture and flavor of most fermented high protein products. Changes in these attributes are major stimuli for developing fermented products. The unpalatable beany flavor of the unfermented legume is eliminated due to the effect of fermentation in changing the texture and organoleptic properties of original legume seeds (Leejeerajumnean, 2003). During soybean fermentation for instance, protein is hydrolyzed to components such as peptides and amino acids due to the action of enzymes produced by bacteria that is favorable to the development of flavor, absorbability, digestibility, and functionality of traditional fermented soybean products used as seasoning. Glutamic acid is the most important flavor enhancing amino acid followed by glycine and alanine that give sweet flavor (Sarkar and Nout, 2014). Fermented food products generally have a very good safety record even in developing countries, where they are processed commonly by people who do not know the underlying principles of

fermentation (Marshall & Mejia, 2011). Fermented products prepared from rice, wheat, corn and other cereals are commonly consumed worldwide. However, consumption of fermented legume products is not as such common. Effect of fermentation of legumes on research level is listed in (**Table 2-2**). The microbiota in cereal and legume grains composed by lactic acid bacteria (LAB), molds, enterobacteria that compete for nutrients differ in type, depending on water activity, pH value, etc. in each fermented food products (Blandino et al., 2003).

Classification of fermentations

Fermentation can be classified as spontaneous by the action of endogenous microorganisms; back slopping by making use of batches of previous fermentation; and controlled by inoculating starter culture or particular strains (Adebo & Medina-meza, 2020). Solid state fermentation is another type of fermentation, used in food and pharmaceutical industries by making use of mainly fungi and yeasts (Ghosh, 2016). On the other hands, whenever enzymes such as phytase present in the plant are insufficient to eliminate the phytate in the substrate, commercial microbial phytases are being suggested by researches in food and feed industry applications (Greiner & Carlsson, 2006a).

Fermented food products are commonly consumed in Ethiopia. For instance, Injera (flat-bread) is a staple fermented baked food, made from an ancient whole grain cereal-tef (*Eragrostis tef*), which is indigenous to Ethiopia (Tamene et al., 2019). ‘Injera’ is a widely consumed fermented food in household level on average of twice in a day of the total food consumed by most society. Other home-made fermented products such as

'diffo'-home-baked thick bread, 'siljo'-fermented sauce, 'borde'-fermented drink are common products from cereals and legumes. Grass pea is also a staple pulse consumed in different forms such as a sauce 'Shiro wot' together with 'Injera'. It therefore has enormous importance to have good knowledge on different alternatives of fermentations such as exogenous phytase added fermentation and their impact on nutritional quality of particular food matrix.

Table 2-2. Summary of the effect of fermentation nutritional value of different legumes

Cereal/legumes (product)	**Conditions/time, temperature, fermentation type**	**Effects of fermentation**	**References**
Chickpea; mixture of chickpea & pseudocereals	*Lactobacillus plantarum* C48 & *Lactococcus lactis* subsp. lactis PU1	Increased free AAs and GABA concentrations; decreased starch hydrolysis index (HI); increased antioxidant activity; increased palatability & overall acceptability of bread	(Coda et al., 2010)
Grass pea	Lactobacillus plantarum	Decreased phytic acid concentration & trypsin inhibitory activity	(Starzynska-Janiszewska & Stodolak, 2011)
Bean, chickpea, grass pea, lentil, pea	*Lactobacillus plantarum* C48 and *Lactobacillus brevis* AM7	Increased phytase & antioxidant activity; increase of free AAs, GABA, soluble fibers, & total	(Curiel et al., 2015)

(local cultivars)		phenols concentrations; decreased raffinose & condensed tannins concentrations.	
Bean, chickpea, grass pea, lentil, pea (local cultivars)	*Lactobacillus plantarum* C48 & *Lactobacillus brevis* AM7	Release of lunasin-like polypeptides; inhibition of the proliferation of human adenocarcinoma Caco2 cells.	(Rizzello et al., 2015)
Cowpea, mottled cowpea, speckled kidney bean, small rice bean	Spontaneous fermentation; *Lactobacillus plantarum* (WCSF1 & ATCC 149170) & *Lactobacillus paracasei* ASCC 279	Increase of antioxidant activity	(Duenas et al., 2005; Gan et al., 2016)
Adzuki bean	*Lactococcus lactis* subsp. lactis & *L. rhamnosus* GG	Increase of GABA concentration	(Liao et al., 2013)
Faba bean	*Lactobacillus plantarum* DPPMAB24W	Increase of protein digestibility, nutritional indexes & resistant starch; no detrimental effect on pasta texture & cooking loss.	(Rizzello et al., 2017)
Cowpea	Spontaneous fermentation	Increase of lysine concentration & essential AAs concentration.	(Hallen et al. 2004)
Soybeans	48 h, 37^{o}C, Lactic acid bacteria	Decrease in phytosterols, glycosylated saponins, & tocopherols	(Hubert et al., 2008)

lupin flour	SSF with *Aspergillus sojae*, *Aspergillus Ficuum*	Phytic acid content & IVPD decreased, increased swelling capacity	(Olukomaiya et al., 2020)
Grass pea seed	SSF with *Rhizopus oligosporous* & *Aspergillus oryzae* in succession	Decrease of β-N-oxalyl-α,β-diaminopropionic acid (β-ODAP)	(Igzaw et al., 2004)
lupine (Lupinus mutabilis sweet)	*Rhizopus oligosporus* strain NRRL2710	Increased the protein levels & amino acids	(Villacres et al., 2020)
Grass pea seed	SSF with *A. oryzae* NRRL 1998 & *R. oligosporus* sp T-3 in succession	Decreased of β-N-oxalyl-α,β-diaminopropionic acid (β-ODAP) reduction	(Kuo et al., 1995)

2.3.2.4. Cooking

Legumes are widely grown and consumed as a source of plant proteins. However, their nutritional quality is hampered by certain compounds such as phytate, enzyme inhibitors, tannins and lectins. Legumes are often consumed after different processes such as soaking, germinating, cooking, roasting, milling and puffing. Cooking is the most common way to prepare edible legume products widely at household level. Soaking is a recommended procedure before cooking which is intended for increasing the water content in the seed to accelerate the cooking step. Soaked or dry seeds are boiled in water for an extended time of 1-2 h in an open pan cooker, whereas only 10-15 min are required in a pressure cooker (Güzel & Sayar, 2012). Pressure cooking has advantages over pan

cooking as it requires less time and energy since heat is distributed deeply, evenly and fast.

While cooking, development of aroma, improvement of overall acceptability of legumes and tender production is achieved in addition to increasing bioavailability of nutrients by inactivating the antinutrients and due to the changes in the legume matrix (Tharanathan & Mahadevamma, 2003; Chau et al., 1997). Nergiz & Gökgöz, (2007) reported a better reduction of phytate when dry beans are soaked and cooked in an open pan compared to applying pressure cooking. This effect was attributed to hydrolization by phytases during soaking. A similar tannin reduction was reported by the same authors. The losses are possibly originated from the diffusion of the tannins into the soaking water. The compounds that result in negative effects in respect to nutritional quality and organoleptic acceptability in legumes can be classified as heat-labile such as trypsin inhibitors and lectins, which disappear after proper heat treatment; and heat-stable (phytates, polyphenols, tannins, raffinose and starchyose) which resist heat. Heat-stable compounds can therefore be reduced by discarding the water after cooking (Silva, 1999 & 2000; Wang et al., 1997). Soaking followed by cooking was reported to be capable to reduce 40% of β-ODAP in grass pea (Padmajaprasad et al., 1997). β-ODAP is known to be a heat stable whereas a water-soluble compound (Wang et al., 1997).

Cooking improves the protein quality by denaturating proteins, by distruction of the heat-labile antinutritional factors and reduction of phytate and tannin (Mubarak, 2005; Drulyte & Orlien, 2019). Protein digestibility is increased by heat induced denaturation of the

proteins resulting in enhancing accessibility of susceptible sites to proteolysis. However, crosslinked, aggregated proteins are less accessible to digestive enzymes because of different localization of amino acid residues specific for protease action resulting in lack of protein digestibility improvement. It is also generally accepted that the abundance of antinutritional factors in plant protein sources contributes to the lowering of protein digestibility. Phytate, tannins, and polyphenols may interact with protein to form complexes by cross-linking with the proteins, resulting in decreased protein solubility and making these protein complexes less susceptible to proteolytic attack in the gastro intestinal tract. Trypsin inhibitors may interfere with the action of proteolytic enzymes in the gastro intestinal tract by forming inactive complexes of trypsin and chymotrypsin. However, since trypsin inhibitors are heat-labile compounds, thermal processing is known to be an effective method for reducing its activity. Tannins and phytate were also partly eliminated after various thermal treatments. Protein digestibility may also be influenced by factors such as cell wall rigidity and fiber content of the legume seed. A common conclusion was drawn by most studies that cooking with or without soaking water reduces protein content, but increases protein digestibility (Fernandes et al., 2010; Martin-Cabrejas et al. 2009). Pressure cooking resulted in protein reduction (Huma et al., 2008). The losses are attributed to partial removal of essential as well as non-essential amino acids with other nitrogenous compounds due to the denaturation of protein into water soluble amino acids by high temperature and pressure. Conformational changes in protein such as an aggregation of protein through increased hydrophobicity and disulfide bond formation induced by heat could impair susceptibility of protein to proteolysis (Park et al., 2010).

Loss of minerals and sugars from legumes was also exhibited due to leaching of minerals and the soluble sugars into cooking water (Huma et al., 2008). The loss of soluble solid components into the cooking water is higher during pressure cooking than during pan cooking (Güzel & Sayar, 2012). Cooking results in separation of certain cells rather than breaking so that the cell contents such as proteins, sugars and minerals are released to the surrounding media and eventually cause nutrient reduction (Kon, 1979). Eventhough soaking following cooking improves the nutritional quality of legumes, most of valuable nutrients such as minerals and vitamins are however considerably lost due to the process.

2.4. Concluding remark

Nowadays, products from legume seed/plants are considered as healthy foods and are being consumed worldwide. Grass pea is a low input legume with high production; highly nutritious and a staple seed for the poor society also during drought seasons. Moreover, there are possibilities to utilize the seed at industry level for daily consumption in towns. In Ethiopia, food industries and industry parks are booming. In relation to this, research centers owing product development are given attention by the government of Ethiopia. HHP is one of the promising non-thermal processing technologies recognized as value adding technology in various food industries for current and future development of sustainable food applications with improved quality attributes via direct reduction of energy compared to conventional food processing technologies, especially for the most energy consuming processing such as pasteurization, sterilization and extrusion cooking (Picart-Palmade et al., 2019). The high cost of HHP including initial capital cost, installation and mentainance costs was the major problems food processers encountered

that had been using HHP from the years of 1990s (Demetrakakes, 1996). A set of HPP equipment costs approximately $0.5 to 2.5 million depending on the capacity and operating parameter range of the equipment. HHP is a new technology in Ethiopia that could be introduced in different food industries for varieties of food products such as meat, fish, poultry, milk and milk products, fruits and vegetables as well as grains. HHP is an emerging technology and still novel and not applied in developing countries like Ethiopia. The technology is also not widely used even in developed countries and researches are on-going. It is one of the advanced instruments that could be an input for a number of research centers in different science and technology universities throughout the countries for research purpose.

Convensional food processing technologies are used for the household application as well as applied for industrial scale. Most low income societies are dependent on these technologies for their daily food preparation so that the technologies are good options for the society in developing countries like Ethiopia.

3. MATERIALS AND METHODS

3.1. Sample collection and preparation

Thirty accessions of grass pea (*Lathyrus sativus* L.) seeds collected from different regions of Ethiopia were obtained from Ethiopian Institute of Biodiversity and Debre Zeit Agricultural Research Centre (DZARC), which is part of federal centers within the Ethiopian Institute of Agricultural Research (EIAR) (**Table 3-1**). A variety from Germany (Not Registered, Ger.) was obtained from small gardeners (Blauetibelt, Bornträgert GmbH 67591 Offstein) and a variety (Not Registered, Eth.) was purchased from Merkato, a local market in Ethiopia (**Table 3-1**). Cleaned and graded raw seeds were kept at 4^{o}C before applying high hydrostatic pressure processing (HHP), household treatments and further analysis. Sample preparation, HHP, all household treatments and different analysis were performed at the Max Rubner-Institut, Karlsruhe, Germany in a controlled environment. Portions of raw seeds samples were milled (Rommelsbacher Gewürz und Kaffemühle, Germany) and passed through a 0.5 mm aperture sieve (Retsch testing sieve, Germany) and stored at 4 ^{o}C for further processing.

Table 3-1. *Lathyrus sativus* L. accessions collected from different regions of Ethiopia/Germany

No.	Code, #	Accession no.	Region/state	Wereda/district
1	421	240038	-	-
2	76	211511	Oromya. Eth	Nejo
3	201	IF_1-1348, DZ	Oromya, Eth	Debre-Zeit
4	205	GP-29, DZ	Oromya, Eth	Debre-Zeit

5	8	201509	Oromya, Eth	Ada'a-Chukala
6	125	IF_1-1332, DZ	Oromya, Eth	Debre-Zeit
7	27	GF_1-Alemu, AK	Oromya, Eth	Akaki
8	278	236656	Oromya, Eth	Gerar-Jarso
9	Wassie	Improved	- ------Eth	-
10	35	GF_1-Alemu, AK	Oromya, Eth	Akaki
11	36	GF_1-Alemu, AK	Oromya, Eth	Akaki
12	104	GP-25, AK	Oromya, Eth	Akaki
13	208	GP-66, DZ	Oromya, Eth	Debre-Zeit
14	116	GP-69, AK	Oromya, Eth	Akaki
15	216	GP-31, DZ	Oromya, Eth	Debre-Zeit
16	294	236672	Amhara, Eth	Enemay
17	398	238947	Amhara, Eth	Dega-Damot
18	205	IF_1-1322, DZ	Oromya, Eth	Debre-Zeit
19	221	GP-27, DZ	Oromya, Eth	Debre-Zeit
20	206	GP-39, DZ	Oromya, Eth	Debre-Zeit
21	24	GF_1-Alemu, AK	Oromya, Eth	Akaki
22	219	GP-25, DZ	Oromya, Eth	Debre-Zeit
23	4	IF1- 1936, AK	Oromya, Eth	Akaki
24	323	GP-236701	Amhara, Eth	Bahir-Dar
25	116	GP-69, DZ	Oromya, Eth	Debre-Zeit
26	118	IF_1-1351, DZ	Oromya, Eth	Debre-Zeit
27	325	236703	Amhara, Eth	Dera, Debub Gonder

28	33	GF_1- Alemu, AK	Oromya, Eth	Akaki
29	X	Not Registered	-----, Germany	Small garden
30	Y	Not registered	----------,Eth	Merkato Market

3.2. Chemicals and reagents

AG 1-X4 resin (Bio-Rad Lab, United States); 100 mM Na-acetate buffer, pH 5.5; 2.5 mM Na-phytate, acetone; (95-98%) sulfuric acid; 10 mM ammonium molybdate solution; citric acid; 2 M, 0.5 M and 0.025 M hydrochloric acid (HCl); bi-(distilled) water; PTFE-membrane; α,β-diaminopropionic acid (DAP)-standard; ethanol (95%); potassium hydroxide (KOH); potassium tetraborate ($K_2B_4O_7$); *O*-phthalaldehyde (OPT); mercaptoethanol; absolute ethanol (>99%); *Aspergillus niger* phytase (Natuphos®); fresh yeast and sour dough; ultrapure water; tryptophan standard solutions; bovine serum albumin; L-norleucine; amino acid standard solutions; anhydrous potassium sulphate; copper(II)sulphate; deionized water; sodium hydroxide; boric acid; HNO_3; 1 M sodium bicarbonate ($NaHCO_3$); 20 mM glycine-hydrochloric acid buffer, pH 2.0; pepsin; pancreatin.

3.3. Phytase activity assay

The phytase activity 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^{o}C for 10 min. Then, 10 µL of the phytase-

containing supernatant were added and incubated at 37^{o}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.

3.4. Processing technologies: High Hydrostatic Processing (HHP)

Grass pea accession (GF1-Alemu, AK), the one with the highest β-ODAP content among the accessions and varieties included in the study was selected for HHP treatment in order to perceive the highest possible reduction effect. The grass pea seeds were soaked in bi-distilled water (1:4 w/v) at room temperature for 6, 9, and 12 h. Thereafter, the water was discarded and the seeds were packed in high-density polyethylene bags (interscience, baglight) under vacuum and sealed with a thermosealer (Raypack, Hacona, CI-620, Deutschland). The packed samples were subjected to HHP treatment at 200, 400 and 600 MPa pressure for 5, 15 and 25 min in a high-pressure vessel (NC HYPERBARIC S.A, wave 6000/55, Burgos, Spain). Before HHP treatment, the temperature of the medium (water) was 11^{o}C. For non-fat samples such as grass pea a temperature increase of approximately 3°C for every pressure increase of 100 MPa was reported (Muntean et al., 2016). Thus, temperatures of the samples of 17, 23 and 29°C can be assumed after HHP treatment with 200, 400 and 600 MPa, respectively. The pressures remained constant isobarically for the indicated holding times and reached atmospheric pressure again upon decompression. Packed products are expected to change volumes up to 15% (*Le*

Chatelier principle) and return to their original volume upon decompression (Rastogi, 2013). Batter of grass pea flours were prepared by mixing the flour with bi-distilled water at a flour-water ratio of 1:2 (w/v) using a lab scale mixer (KRUPS 3Mix700, China) for 2 min. The batters were packed in polyethylene bags and subjected to 200, 400, and 600 MPa pressure for 5, 15, and 25 min at temperatures of 17, 23 and 29^{o}C respectively. Raw seeds were considered as control in soaked-HHP treated whereas untreated batter was used as a control for batter-HHP treated. The best HHP conditions in respect to β-ODAP reduction (600 MPa, soaking time: 9 h, holding time: 15 min (soaked grass peas); (600 MPa, holding time: 15 min (batter grass peas))) at 29^{o}C were also applied to the accession GP-240038, to the genetically improved and the German varieties. All treated and untreated samples were freeze-dried (MRI, Karlsruhe), milled, sieved with through a 0.5 mm aperture size sieve for further analysis. The flow diagram is shown in (**Figure 3-1**).

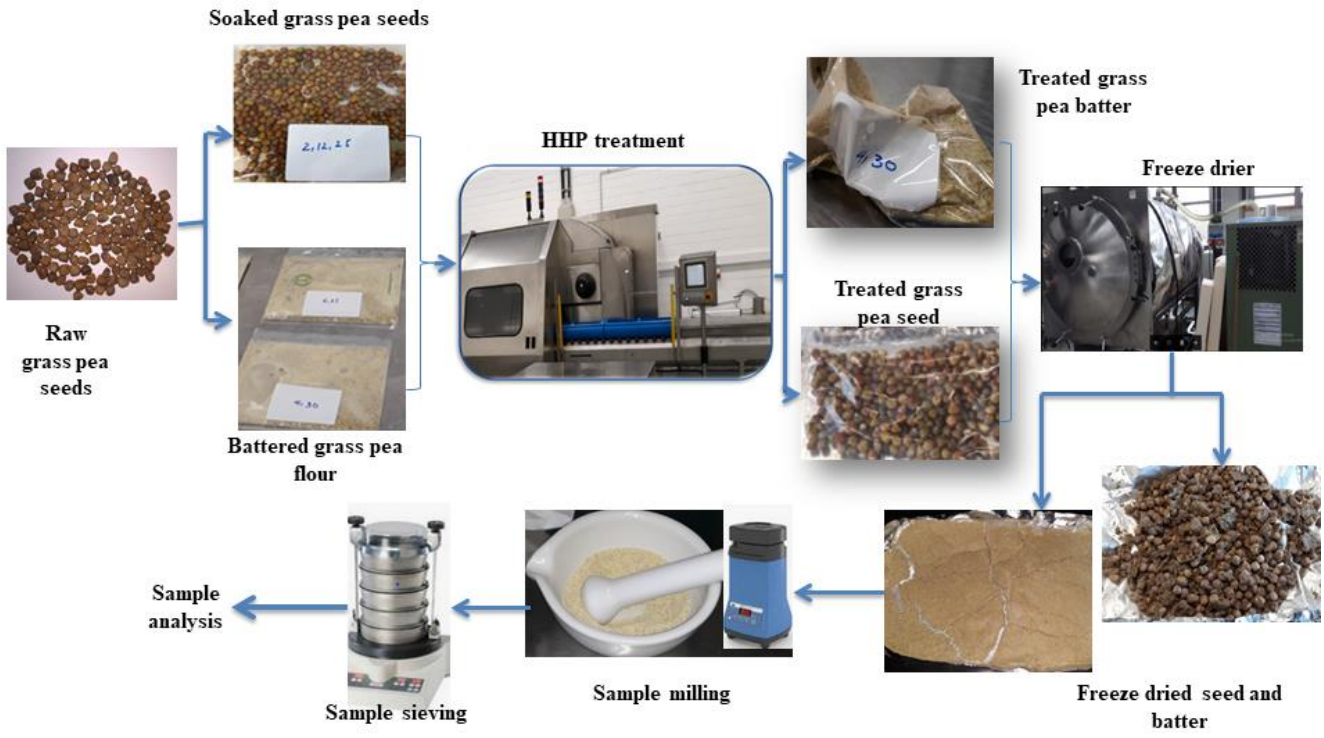

Figure 3-1. Flow diagram for HHP treatment

3.5. Processing Technologies: Conventional processing/Household Treatment

3.5.1. 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 3-2)**. 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^{o}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^{o}C) for 15 min after incubation in order to prepare the samples for

more effective freeze drying. 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.

Table 3-2. Experiment design for grass pea and blend 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

3.5.2. 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 (Sw) or freeze dried after discarding the soaking water (Swo). In order to prevent the seeds from sprouting the soaked samples were kept overnight at -20°C prior to freeze drying.

3.5.3. Pressure and pan cooking

Pressure and pan cooking were carried out with grass peas soaked as described in **3.4.2**. Both household processes were performed with or without discarding the soaking water. (Pcw: Pressure-cooking with the soaking water, Pcwo: pressure-cooking without the soaking water, Pacw: pan cooking with the soaking water, Pacwo: pan cooking without 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 100-150^{o}C and pan cooking for 25 min at a plate temperature of 200-250^{o}C. When discarding the soaking water, the same amount of tap water was added prior to cooking. 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 freeze-dried and kept under 4^{o}C until further analysis (**Figure 3-2**).

Figure 3-2. Pressure cooked and freeze dried grass pea seed

3.5.4. 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 (25^{o}C) for 24 h, 48 h and 72 h (**Figure 3-3**). Prior to freeze drying, germinated seeds were kept overnight at -20^{o}C in order to stop germination.

Figure 3-3. Germinated seed: **a.** day one, **b.** day two, **c.** day three, **d.** Freeze dried

3.5.5. Roasting

100 g of grass pea seeds were roasted on a plate heater adjusted to a temperature of 200-250^{o}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 appearance of a light brown color. The roasted samples were cooled down to 21^{o}C and freeze-dried.

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^{o}C until further analysis.

3.6. Quantitative analysis of neurotoxic compound, nutritional and antinutritional factors, and determination of physical, functional and structural properties of *Lathyrus sativus*. L seeds

3.6.1. Determination of β-ODAP

β-ODAP of grass pea flour were quantified according to (Hussain et al., 1994; Briggs et al., 1983) with modifications. A 0.5 g of the sample flour was mixed with 10 mL of 60 % ethanol and thoroughly agitated (Edmund Bühler E1, Germany) for 2 h at room temperature and centrifuged (Thermo Scientific™ Sorvall™ LYNX 6000, Germany) at 4000 rpm for 30 min. The extraction was repeated with 5 mL of ethanol and both extracts were combined. 2 mL of the extracts were mixed with 4 mL of 3 M KOH and hydrolyzed for 1.5 h at water boiling temperature. The cooled (room temperature) hydrolyzates (0.25 mL) and un-hydrolyzed extracts were mixed accordingly with bi-distilled water (0.75 mL) and 2 mL of OPT. The OPT was prepared by mixing 0.1 g OPA, 0.2 mL mercaptoethanol, 1 mL of absolute ethanol and 99 mL of 0.5 M potassium tetraborate buffer of pH 10.0 according to (Chen et al., 2000; Megías et al., 2015). The absorbance of the solution was determined at 426 nm after 2 h of incubation at RT. Calibration was performed with diaminopropionic acid (DAP) in a range from 1.0 to 8.0 g x 10^{-6} per mL (**Figure 3-4**). DAP was used, because β-ODAP is commercially unavailable (Briggs et al., 1983). DAP concentrations were converted to β-ODAP concentration using a conversion factor of 1.69 (Aletor et al., 1994).

Blank/samples	Concentration (µg/ml)	Absorbance (ΔE) (425nm)
1000µl H_2O + 2 ml OPT (Blank)	0	0.026
100µl DAP +900µl H_2O +2 ml OPT	1	0.047
200µl DAP +800µl H_2O +2 ml OPT	2	0.075
300µl DAP +700µl H_2O +2 ml OPT	3	0.097
400µl DAP +600µl H_2O +2 ml OPT	4	0.125
500µl DAP +500µl H_2O +2 ml OPT	5	0.151
600µl DAP +400µl H_2O +2 ml OPT	6	0.165
700 DAP +300µl H_2O +2 ml OPT	7	0.196
800µl DAP +200µl H_2O +2 ml OPT	8	0.221
900µl DAP +100µl H_2O +2 ml OPT	9	0.244
1000µl DAP +2 ml OPT	10	

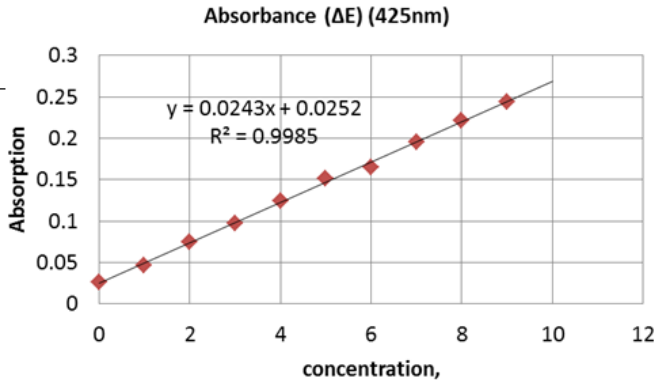

Figure 3-4. Calibration curve using Diaminopropionic acid (DAP)

3.6.2. *Myo*-inositol phosphate ($InsP_6$) analysis

Myo-Inositol phosphates analysis ($InsP_5$ and $InsP_6$) was carried out by High Performance Liquid Chromatography (HPLC) (Dionex Ultimate 3000, chromelion software) (Greiner et al., 2000). Briefly, 0.5 g of the samples was extracted by vigorous mechanical agitation (Edmund Bühler E1, Germany) with 20 mL of 0.5 M hydrochloric acid (HCl) for 2 h at 21°C. Thereafter, the extracts were centrifuged 15,000 g for 30 min (Thermo Scientific™ Sorvall™ LYNX 6000, Germany) and the supernatants were frozen overnight at -20°C. The samples were thawed at 21°C and the supernatants were diluted with bi-distilled water (1:3 v/v) and submitted to a column containing 0.5 g of AG 1-X4 resin (Bio Rad Laboratories, United States). Before applying the sample to the column, the column was equilibrated with 50 mL of bi-distilled water twice. The column was washed with 25 mL of water and 25 mL of 0.025 M HCl. Then, the *myo*-inositol phosphates were eluted with 25 mL of 2 M HCl. The eluent was evaporated to dryness using a vacuum evaporator and the dry residue was dissolved in 1 mL bi-distilled water. Then, the samples were ultrasonicated and filtered through a 0.45 µm Millipore membrane filter (Sartorius

Stedim Biotech GmbH, Göttingen, Germany). Finally, to quantify *myo*-inositol phosphates, 20 µL of the samples were chromatographed on Ultrasep ES 100 RP18 (2 x 250 mm). The column was run at 45^{o}C and 0.2 mL/min of an eluant consisting of formic acid:methanol:water:tetrabutylammonium hydroxide (44:56:5:1.5 v/v), pH 4.25. Standards were prepared by mixing the individual *myo*-inositol phosphate esters (IP_3-IP_6). Calibration was performed using $InsP_6$ as a standard.

3.6.3. 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^{o}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.

3.6.4. Determination of proteinogenic amino acids

3.6.4.1. Tryptophan

Tryptophan was analyzed using high performance liquid chromatography (HPLC) (Summit HPLC System, Dionex Corporation, USA). The analytical method was adapted according to (Dai et al., 2014; Çevikkalp et al., 2016). 100 mg pea powder was transferred in a 24 mL glass tube and hydrolyzed under nitrogen atmosphere with 10 mL 4.2 M sodium hydroxide solution at 105°C for 20 h. The resulting solution was centrifuged (5300 rpm, 15 min), filtered, and transferred to a 50 mL volume flask. The flask was brought up to 50 mL with ultrapure water. 5 mL of the resulting solution were diluted with 60 mL ultrapure water. The pH was adjusted to 6.3 using 2 M hydrochloric acid. Ultrapure water was added so that the combined volume equals 100 mL. 1 mL of the sample was filtered, transferred into a vial and 10 µL were injected into the HPLC. The HPLC system consisted of a SOR 100 solvent rack with an analytical 4 channel vacuum degasser, a P 680 pump, ASI 100 automated sample injector, TCC 100 thermostated column compartment and a RF 2000 fluorescence detector. Isocratic

chromatographic separations were performed on a Gemini-NX C18 column (150 x 3.00 mm, 3 µm) (Phenomenex Ltd, Germany). The mobile phase consisted of ammonium acetate buffer pH 6.3 and acetonitrile (9/1, v/v). Detection was obtained using fluorescence with excitation wavelength 280 nm and emission wavelength 340 nm. Eluent flow was 0.5 mL /min and column temperature 20 °C. The data were acquired using Chromelion software version 6.80 (Thermo Fisher Scientific, USA). Tryptophan standard solutions (Merck KGaA, Germany) were injected for systems calibration and tryptophan quantification using an external standard calibration. For recovery determination bovine serum albumin (Merck KGaA, Germany) was prepared the same way as the samples. All determinations were performed in duplicate.

3.6.4.2. Other proteinogenic amino acids

Amino acids were analyzed using HPLC (System Gold ®, Beckman Coulter Inc. USA). The analytical method was adapted according to (Domínguez et al., 2015; Weiss et al., 1998). 100 mg pea powder, 25 mg Tryptamine and 1.312 mg L-Norleucine (Merck KGaA, Germany) in 20 mM hydrochloric acid were transferred to a glass tube and hydrolyzed under nitrogen atmosphere with 5 mL 6 M sodium hydrochloric acid at 110°C for 24 h. The resulting solution was filtered, and transferred to a 200 mL volume flask. The flask was brought up to 200 mL with ultrapure water. 1 mL of the sample was filtered and stored at -20°C. 10 µL of the sample were subjected to derivatization using the ACCQ-Fluor Reagent Kit (Waters Corp. USA) according to the manufacturer's instructions, pipetted into an insert vial and transferred to the HPLC for analysis. The HPLC system consisted of a 126NM solvent module, 508 autosampler, a column thermostat (System Gold®, Beckman Coulter Inc. USA) and a RF10AXL fluorescence

detector (Shimadzu Corp. Japan). Chromatographic separations were performed on a Chromolith® Performance RP-18e (100 x 3.00 mm) (Merck KGaA, Germany). The mobile phase consisted of 20 mM sodium acetate in ultrapure water, 0.04 % trimethylamine, pH 5.6 (A) and 20 mM sodium acetate in ultrapure water, 0.04 % trimethylamine, pH 5.6 and acetonitrile (1/1, v/v) (B). The solvent was delivered to the column at a flow of 1.5 mL min-1 as follows: 0 - 10 min 1 % B, 10 - 33 min 1 % -13 % B, 33 - 68 min 13 % - 25 % B, 68 - 70 min 25 % - 100 % B, 70 - 80 min, 100 % B, 80 - 82 min 100 % - 1 % B. Column temperature was set at 10°C. Detection was obtained using fluorescence with excitation wavelength 250 nm and emission wavelength 395 nm. The auto-sampler was set to reagent addition at 20 °C. 90 µL of the sample were mixed with 10 µL Tris (2-carboxyethyl) phosphine hydrochloride (TCEP, Merck KGaA, Germany) solution (60 mg in 1 mL 20 mM hydrochloric acid). TECP is a reagent commonly used to accomplish the reduction of disulphide bonds as in cysteine (Alwael et al., 2010). Since the reduction reaction is reversible (Bazylewski et al., 2017), this step needed to be timed exactly and hence automated using the auto-sampler. After a waiting period of 15 min 20 µL of the mixture were injected into the HPLC system. The data were acquired using 32 Karat 7.0 (Beckman Coulter Inc. USA). Amino acid standard solutions (Merck KGaA, Germany and LGC Standards GmbH) were injected for systems calibration and amino acid quantification using an internal standard calibration with L-Norleucine as internal standard (IST). For recovery determination, bovine serum albumin (Merck KGaA, Germany) was prepared the same way as the samples. All determinations were performed in duplicate.

3.6.5. *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^{o}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.

3.6.6. 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 3-3** 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 3-4)** 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%.

Table 3-3. 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

Table 3-4. ICP-MS precision and accuracy of the method.

Elements	LOQ (µg kg^{-1})	Reference material measured value (mg kg^{-1})	Reference material certificate 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
P	27.8	4086 ± 138	3800 ± 300

3.6.7. Water absorption capacity

To determine the kinetics of water absorption, the method of Manejo et al. (2017) was used. Grass pea seeds were first graded according to size. In this respect 25 seeds were weighed and soaked in 100 mL of distilled water at three temperatures 25, 35 and 45 °C for 10 h. At given intervals of times, the seeds were removed from the water and blotted

dry of excess water and then weighted. Then, the moisture content of the seeds was calculated as the difference between the weight of the soaked seeds and dry seeds. The variation in the seed moisture content was then plotted against time. Moreover, 30 g of grass pea seeds were soaked with 120 mL of bi-distilled water for 6, 9 and 12 h at room temperature (the controls for the HHP treatments). According to the experimental design, the soaked seeds were treated with HHP (twenty runs). After soaking and HHP treatment, the water was drained and the seeds were wiped with dry absorbent paper to remove the water on top of the seed. The hydrated seed were weighed to determine the increase in mass after HHP treatment and soaking. Thereafter, the water absorption capacities were calculated as the weight of water absorbed per gram of seeds and expressed in (%).

3.6.8. Color measurement

The color of HHP treated (soaked and batter) and raw seed (control) samples were determined by using a portable color analyzer (spectrophotometer CM 600d, Conica Minolta, Inc, Japan) with a Silicon photodiode array detector. Calibration was performed using the standard white tile having approx. L*, a* and b* values of 98, -0.21, and 0.25 respectively. All measurements were carried out five times and the color values were expressed in terms of L* (whiteness or brightness/darkness), a* (redness/ greenness) and b* (yellowness/blueness) respectively. Moreover, results were expressed as total color difference (ΔE*) between untreated control samples and HHP treated ones based on the following equations (Maskan, 2001). $\Delta E^* = \sqrt{((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)}$; Where $L*$ ranges from 0 (black) to 100 (white), $a*$ ranges from +100 (redness) to −100 (greenness), and $b*$ ranges from +100 (yellowness) to −100 (blueness). A ΔE* of 0-0.5 was defined as ‘not noticeable’, 0.5-1.5 as ‘slightly noticeable’, 1.5-3.0 as ‘noticeable’, 3.0-6.0.as ‘well

visible' and 6.0-12.0 as 'highly visible' according to the method described by Cserhalmi et al., (2006).

3.6.9. Scanning Electro-Microscopy (SEM) analysis

Microstructure analysis of HHP treated soaked seed (600 MPa pressure, 9 h soaking time and 15 min holding time) and HHP treated batter (600 MPa pressure, 15 min holding time) as well as the respective controls were performed using Scanning Electron Microscopy (SEM) (FEI, Inspect 150, Czech Republic). All samples analyzed in SEM were prepared with the respective flours. The samples were mounted on circular aluminum specimen holders with carbon tapes. The images of the samples were taken with an acceleration voltage of 20 kV using a working distance of 11.3 mm. The raw grass pea and pressure treated flours were also viewed by Zeta image to view true color and (3D, 2D) composite image by Zeta-20 optical machine.

3.6.10. Pasting property

Pasting properties of raw grass pea seed, HHP-treated soaked seeds and HHP treated batter and the respective controls were determined using a Visco Rapid Analyzer (RVA, 4500, Australia). The sample was weighed into a dry canister, and distilled water was dispensed into the canister containing the sample using a robot dispenser. Depending on initial moisture content of the flours, the sample weighting about 3.5 g and distilled water of about 25 g dispensed into the canister to have total slurry weight of about 28 to 29 g and the base moisture content was set to 14%. The slurry was thoroughly mixed and the canister was well fitted into the RVA as recommended. Initially, the sample was

dispensed with the rapid stirring speed of 960 rpm for 10 s. The slurry was equilibrated at 50^{o}C for 1 min. The dispersion was heated at the rate of 12^{o}C/min to 90^{o}C, held for 2.7 min, cooled to 50^{o}C at the same rate and again held at 50^{o}C for 2 min with a constant paddle rotational speed (160 rpm) throughout the entire analysis. The total processing time was estimated to be 13 min. The paste properties examined were peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, peak time and pasting temperature with the aid of ThermoCline for Windows (TCW) version-3 software connected to a computer.

3.7. Experimental design and statistical data analysis

3.7.1. Experimental design

By investigating the effects of three independent variables (pressure, soaking time and holding time) in case of soaked-HHP treated and two independent variables (pressure and holding time) for batter-HHP treated samples, response surface methodology (RSM) was used on the response variables such β-ODAP, $InsP_6$, proteinogenic amino acids (Methionine, Lysine, Tryptophan and Glutamic acid), water absorption and color. A central composite face-centered design is the most popular RSM used to design the experiments and formulate model equations for individual response using Design Expert software (version 7.0.0) (Penas et al., 2010). For soaked-HHP treated samples, three levels of each independent variables (pressure, soaking time and holding time) and twenty combinations with six replicates of center points that estimated the system error were selected (**Table 3-4**). For batter-HHP treated samples, two levels of each independent variables (pressure and holding time) and thirteen combinations with five replicates of center points were taken (**Table 3-5**). All treatments were performed in

duplicate. The 1, 0 and -1 codes represent low, middle and high levels of each variable respectively.

Table 3-4. Central composite face-centered design for soaked-HHP treatment

Soaked-HHP treatment		
x_1	x_2	x_3
0 (400)	0 (9)	0 (15)
0 (400)	0 (9)	0 (15)
0 (400)	-1 (6)	0 (15)
1 (600)	-1 (6)	-1 (5)
1 (600)	1 (12)	1 (25)
0 (400)	0 (9)	-1 (5)
-1 (200)	1 (12)	1 (25)
-1 (200)	1 (12)	-1 (5)
1 (600)	1 (12)	-1 (5)
0 (400)	0 (9)	1 (25)
-1 (200)	0 (9)	0 (15)
1 (600)	0 (9)	0 (15)
0 (400)	0 (9)	0 (15)
0 (400)	0 (9)	0 (15)
0 (400)	0 (9)	0 (15)
0 (400)	1 (12)	0 (15)
-1 (200)	-1 (6)	1 (25)
-1 (200)	-1 (6)	-1 (5)
0 (400)	0 (9)	0 (15)
1 (600)	-1 (6)	1 (25)

Table 3-5. Central composite face-centered design for batter-HHP treatment

Batter-HHP treatment	
x_1	x_2
1 (600)	1 (25)
-1 (200)	1 (25)
0 (400)	1 (25)
1 (600)	-1 (5)
0 (400)	0 (15)
0 (400)	0 (15)
0 (400)	0 (15)
0 (400)	0 (15)
0 (400)	0 (15)
-1 (200)	0 (15)
-1 (200)	-1 (5)
1 (600)	0 (15)
0 (400)	-1 (5)

3.7.2. Statistical data analysis

Design expert (version7.0) software was used for statistical analysis. Results were averages of three independent determinations. The design of experiments was analyzed using a 2-factor interaction model with One-way analysis of variance ANOVA. The significance level ("Prob>F") was chosen to <0.05 for the model and individual terms of variables. Since the polynomial quadratic model fulfilled the required significance level, it was preferred as the best model compared to the others. "Lack of fit Tests" of the model was also not significant ("Prob>F" is the largest number of among the other models) which helped to choose the model as the best response predictor model. Moreover, the model was also selected and its adequacy was judged depending on its low

standard deviation, high “R-Squared” values and low “PRESS”. Other non-contributing variables (single, interaction and quadratic) were excluded from the model in such a way that their Prob>F or p-values were above the significance level. The software was used for those responses including some proteinogenic amino acids that fit the model to analyze the data statistically. For the rest of proteinogenic amino acids, Origin 2018 software was used and the results were graphically compared. One-way analysis of variance was performed for each accession and treated samples and the data were expressed as the mean value ± standard deviation. Mineral content and bioaccessibility analysis were conducted in triplicate whereas crude protein, β-ODAP and $InsP_6$ analysis were conducted in duplicate and expressed as ± standard deviation of three and two separate determinations respectively. ANOVA was performed using JMP Pro 13 for windows (version-13). Statistically significance differences comparison among raw and treated samples’ means ($p<.05$) were considered using the Student’s *t* comparison test at a 95% confidence level. Origin 2018 software was used for graphically compared results.

4. CHARACTERIZATION OF GRASS PEA SEEDS

In this chapter, the results of the characterized grass pea seeds of different accession/varieties are given. The β-ODAP, $InsP_6$, proteinogenic amino acids, minerals and some functional properties such as water absorption, and pasting properties, and microstructures of raw grass pea were presented. Different accession and/or variety resulted in variation in nutrient content, antinutritonal factor and β-ODAP content of grass pea seeds. In addition, understanding the variation of the seeds in nutrient and antinutrient contents, in their functional properties could help to select the best variety/accession for a specific application during product development.

4.1. β-ODAP and $InsP_6$ contents of grass pea seeds

Unpredictable levels of β-ODAP were recorded in a grass pea cultivar when it was tested in different years and grown at different locations (Girma & Korbu, 2012) due to the highly responsive nature of β-ODAP to environment and genetic background of grass pea. The deficiency and imbalance of micronutrients/macronutrients in the soil across different growing locations (Misra & Barat, 2016; Xu et al., 2006), stress due to drought and unusually high temperature during growth (Fikre et al., 2011a) are some of the possible reasons for the reported differences in β-ODAP levels. β-ODAP contents of the accessions and varieties used in this study ranged from 51.94 to 806.52 mg/100 g (**Table 4-1**). The results are in compliance with those obtained by Aletor et al., (1994), Chen et al., (2000) and Kumari (2001), but lower than the mean value of the grass pea germplasms collected from Syria (1050 mg/100 g) (Sammour et al., 2007). The

genetically improved variety, Wassie, was expected to have low β-ODAP levels, but contained with 475.16 mg/100 g, an intermediate β-ODAP level.

Table 4-1. β-ODAP content of the grass pea accessions and varieties used in the study

Accessions/Varieties	β-ODAP (mg/100g)
Gp-240038	$51.94^{h} \pm 0.31$
Gp-211511	$270.05^{g} \pm 4.33$
IF_1-1348, DZ	$278.54^{g} \pm 1.75$
Not Registered, Ger	$344.44^{fg} \pm 6.72$
GP-29, DZ	$348.29^{fg} \pm 1.54$
Gp-201509	$351.00^{fg} \pm 1.93$
IF_1-1332, DZ	$386.40^{efg} \pm 4.80$
$GF_{1,}$ 27 -Alemu, AK	$393.24^{efg} \pm 11.59$
Not registered, Eth.	$405.00^{efg} \pm 1.60$
Gp-236656	$413.48^{ef} \pm 6.55$
$GF_{1,}$35-Alemu, AK	$451.91^{cdef} \pm 1.31$
GP-66, DZ	$455.29^{cdef} \pm 7.00$
$GF_{1,}$ 36-Alemu, AK	$466.36^{cdef} \pm 6.17$
Genetically Improved (Wassie)	$475.16^{cef} \pm 0.29$
GP-25, AK	$483.34^{cdef} \pm 3.09$
GP-69, AK	$483.73^{cdef} \pm 0.39$
GP-31, DZ	$489.57^{cdef} \pm 10.38$
IF_1-1322, DZ	$503.57^{bcde} \pm 14.24$

GP-27, DZ	$506.06^{bcde} \pm 11.79$
Gp-236672	$506.49^{bcde} \pm 0.87$
Gp-238947	$507.80^{bcde} \pm 6.55$
GP-39, DZ	$534.27^{bcde} \pm 2.32$
$GF_{1,}$ 24 -Alemu, AK	$548.29^{bcd} \pm 1.54$
GP-25, DZ	$569.81^{bc} \pm 0.44$
IF_1- 1936, AK	$580.19^{bc} \pm 1.16$
GP-69, DZ	$601.59^{bc} \pm 8.11$
IF_1-1351, DZ	$603.44^{bc} \pm 1.75$
Gp-236701	$642.72^{b} \pm 5.49$
Gp-236703	$646.67^{b} \pm 3.93$
GF_1- Alemu, AK	$806.52^{a} \pm 2.31$

(Values are means ± standard deviations of three replicates). Different superscripts represent significant differences.

Accessions having the highest (GF1- Alemu, AK) and the lowest (GP-240038); β-ODAP content as well as the genetically improved variety and the variety from Germany were selected for phytate quantification. GF1- Alemu, AK (95.5 mg/100 g) exhibited the lowest and GP-240038 (1391.3 mg/100 g) the highest $InsP_6$ content (**Figure 4-1**). The German variety was shown to contain 907.7 mg $InsP_6$/100 g and Wassie 1098.2 mg $InsP_6$/100 g) (**Figure 4-1**). The results obtained are within the range reported by Ramachandran et al., (2008), Srivastava & Khokhar (1996), Urga et al., (2005) and Grela et al., (2001).

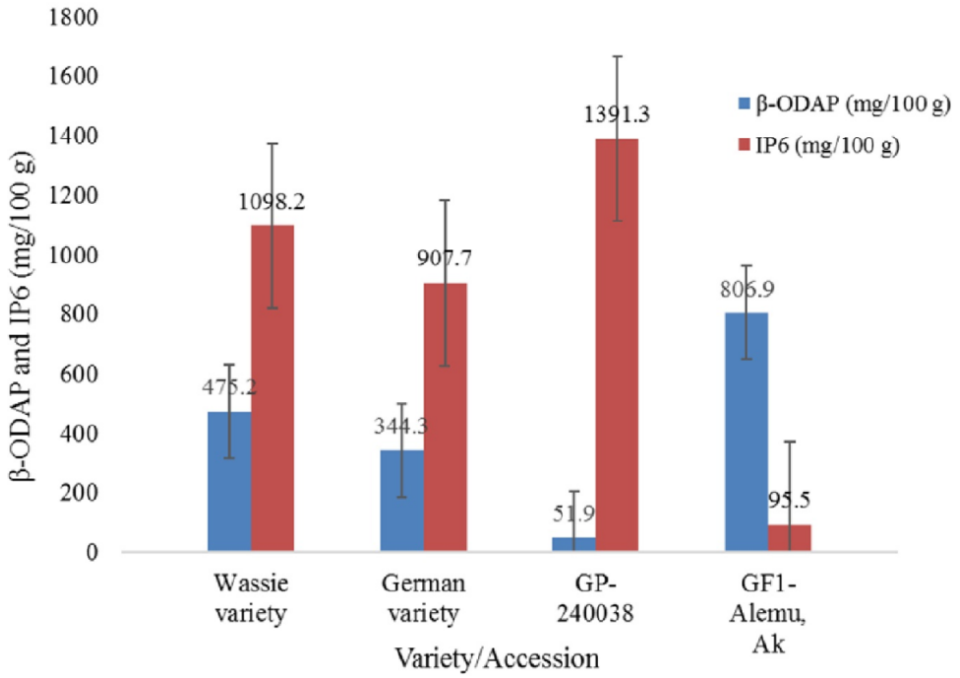

Figure 4-1. Content of β-ODAP and $InsP_6$ of different grass pea accessions and a grass pea variety.

4.2. Proteinogenic amino acids profile

The proteinogenic amino acid profiles of GF1- Alemu, AK and GP-240038 accessions and Wassie and German varieties are summarized in (**Table 4-2**). Significantly different ($p<0.05$) values of L-glutamic-acid, L-histidine, L-arginine, L-tyrosine, L-isoleucine, L-phenylalanine and L-lysine were observed among the accessions and varieties. All values of proteinogenic amino acids of GF1- Alemu, AK accession were significantly different ($p<0.05$) from the other accession and varieties. This variation may be related to genotype and environmental conditions interaction (Girma & Korbu, 2012). In all seeds of grass pea accessions and varieties analyzed, glutamic acid was found to have the highest concentration followed by aspartic acid, arginine, leucine and lysine. Methionine was found to be the amino acid lowest in content. The contents of the amino acids are in good agreement to those reported by Fikre, et al., (2008b).

Table 4-2. Proteinogenic amino acids of two accessions and two varieties.

Proteinogenic AAs (mg/100g)	Wassie variety	German variety	Accession 240038	Accession GF_1-Alemu, AK
L-Aspartic-acid	$3088.32^{a} \pm 15.8$	$2999.64^{b} \pm 1.9$	$3002.31^{b} \pm 6.4$	$2393.02^{c} \pm 56.8$
L-Glutamic-acid	$4535.16^{a} \pm 3.0$	$4339.36^{b} \pm 6.4$	$4489.01^{c} \pm 2.94$	$3454.86^{d} \pm 75.8$
L-Serine	$1458.96^{a} \pm 6.4$	$1442.44^{ab} \pm 2.1$	$1424.91^{b} \pm 4.4$	$1174.89^{c} \pm 59.0$
Glycine	$1021.90^{a} \pm 0.6$	$1025.83^{a} \pm 10.3$	$980.56^{b} \pm 7.4$	$833.12^{c} \pm 19.8$
L-Histidine	$730.52^{a} \pm 0.2$	$692.63^{b} \pm 8.3$	$660.86^{c} \pm 5.9$	$568.38^{d} \pm 7.3$
L-Threonine	$1043.23^{a} \pm 0.8$	$1063.01^{a} \pm 3.0$	$1011.43^{b} \pm 11.0$	$860.74^{c} \pm 52.3$
L-Alanine	$1106.10^{a} \pm 3.2$	$1066.46^{b} \pm 5.0$	$1069.49^{b} \pm 5.9$	$1023.86^{c} \pm 49.7$
L-Arginine	$2062.06^{a} \pm 10.8$	$1972.71^{b} \pm 3.1$	$1883.86^{c} \pm 0.07$	$1616.00^{d} \pm 38.3$
L-Proline	$1042.91^{a} \pm 4.1$	$984.16^{b} \pm 8.9$	$996.10^{b} \pm 5.4$	$846.27^{c} \pm 30.1$
L-Cystein	$210.82^{b} \pm 7.5$	$220.98^{a} \pm 12.8$	$241.82^{b} \pm 0.6$	$165.75^{c} \pm 7.6$
L-Valine	$1225.49^{a} \pm 3.8$	$1210.89^{a} \pm 2.5$	$1216.93^{a} \pm 10.9$	$1032.79^{b} \pm 64.9$
L-Tyrosine	$866.15^{a} \pm 2.1$	$797.46^{b} \pm 5.5$	$753.38^{c} \pm 9.7$	$621.93^{d} \pm 29.2$
L-Methionine	$191.83^{a} \pm 3.5$	$200.47^{ab} \pm 6.2$	$177.05^{b} \pm 1.2$	$91.92^{c} \pm 5.5$
L-Isoleucine	$1247.01^{a} \pm 8.9$	$1172.75^{b} \pm 7.5$	$1149.65^{c} \pm 10.3$	$940.46^{d} \pm 46.7$
L-Leucine	$1934.02^{a} \pm 5.4$	$1697.41^{b} \pm 8.2$	$1723.72^{c} \pm 2.8$	$1918.71^{a} \pm 49.2$
L-Lysine	$1785.42^{b} \pm 5.0$	$1734.95^{c} \pm 1.8$	$1894.07^{a} \pm 7.8$	$1518.22^{d} \pm 15.8$
L-Phenylalanine	$1136.34^{a} \pm 4.9$	$1052.99^{b} \pm 9.0$	$977.55^{c} \pm 13.5$	$902.35^{d} \pm 23.9$
L-Tryptophan	$288.90^{a} \pm 3.0$	$287.00^{a} \pm 3.5$	$300.40^{a} \pm 3.5$	$238.60^{b} \pm 2.4$

(Values are means ± standard deviations of three replicates). Different superscripts in a column represent significant differences ($p < 0.05$)

Among the grass pea accessions studied, accessions having high β-ODAP contents exhibited low levels in sulfur containing amino acids whereas accessions with low β-ODAP contents showed high levels of sulfur containing amino acids (**Table 4-1 & 4-2)**. However, Fikre et al., (2008b) reported comparable methionine contents in nine grass pea genotypes with high variations in β-ODAP contents. Furthermore, no significant differences in methionine levels between grass pea from India with high β-ODAP content and Canadian grass pea with low β-ODAP content have been observed. Therefore, the concentration of sulfur containing amino acids seems not to be linked to the β-ODAP concentration in grass pea. However, aggravation of neurotoxicity of β-ODAP is highly related to nutritional deficiencies with methionine and cysteine according to pharmacological studies (Xu et al., 2017). Thus, supplementation of sulfur containing amino acids in grass pea meals or increasing their amount in the plant itself was suggested to improve the nutritional value of grass pea and prevent neurolathyrism (Campbell, 1997; Fernand et al., 1994).

4.3. Mineral composition

Mineral compositions (iron-Fe, zinc-Zn, calcium-Ca and phosphorus-P) of two accessions and two varieties are shown in (**Table 4-3**). The Fe content of GF1-Alemu, AK, accession was comparable to the amount found in soybean (73.1 mg/kg) and slightly lower than in mung bean (71.7 mg/kg) and cowpea seed (66.0 mg/kg) whereas Fe content in GP-240038, accession, Wassie and variety form Germany were higher. Zn content of grass pea seed, GF1-Alemu AK accession in our study is lower than that of soybean (36.4 mg/kg), cowpea (37.8 mg/kg) and mung bean (28.1 mg/kg) (Lestienne et al., 2005),

however, the amount in GP-240038 accession and Wassie variety are higher. Zn contents of GP-240038 accession and Wassie variety are also higher than that of *Phaseolus vulgaris* (30 mg/kg), peas (*Pisum sativum*) (30.1 mg/100 g), chickpeas (35.4 mg/kg), lentils (37.3 mg/kg), soybeans (41.8 mg/kg) (Sandberg, 2002). The results reported from the same authors showed lower Ca contents in peas (*Pisum sativum*), chickpeas and lentils compared to the results presented in our study. The level of phosphorus in GF1-Alemu, AK, accession is lower than in other accessions and varieties in our study as well as the contents in chickpea (3980 mg/kg), pigeon pea (2660 mg/kg), green gram (2710 mg/kg) and black gram (4770 mg/kg) (Rao & Deosthale, 1981). Phosphorus content of grass pea varieties and accession other than GF1-Alemu, AK are higher than in chickpea (3777.0-4431.9 mg/kg), mung bean (3730.9-4056.3 mg/kg) and horse gram (2750.4-2844.9 mg/kg) (Sivakumaran et al., 2015). It can be concluded from the result that grass pea is a good source of trace elements such as Fe, Zn, Ca and P.

Table 4-3. Mineral composition of two accessions and two varieties

Pressure/soaking time/holding time	**Fe (mg/kg)**	**Zn (mg/kg)**	**Ca (mg/kg)**	**$P_{(Total)}$ (mg/kg)**
GF1-Alemu, AK, acc.	$72.58^{b} \pm 1.30$	$22.03^{e} \pm 0.15$	$1654.98^{a} \pm 13.98$	$1588.10^{d} \pm 7.73$
GP-240038, acc.	$86.13^{a} \pm 8.25$	$38.74^{a} \pm 0.10$	$1071.38^{bc} \pm 22.09$	$3665.54^{c} \pm 52.74$
Wassie, Var.	$88.38^{a} \pm 2.57$	$35.09^{b} \pm 0.21$	$1110.80^{b} \pm 58.51$	$4098.29^{b} \pm 77.15$
German, Var.	89.12 ± 0.43^{a}	28.41 ± 0.58^{d}	903.22 ± 13.20^{d}	4404.60 ± 6.08^{a}

(Values are means ± standard deviations of three replicates). Different superscripts in a column represent significant differences ($p < 0.05$)

4.4. Water absorption capacity of grass pea seed at different temperatures

It is good to understand the water absorption property of legumes during soaking as it highly influences the quality of final product for the succeeding processing such as cooking (Turhan et al., 2002). The water absorption capacity of grass pea seeds of different varieties/accessions at temperatures of 25^{o}C, 35^{o}C and 45^{o}C at different soaking time are shown in (**Figure 4-2)**. The water absorption rate of the Wassie variety at the beginning of the soaking process was lower than the rate of all the grass pea variety/accessions whereas, the water absorption rate of the variety from Germany used in the study was greater at all soaking temperature. However, it took shorter times for the Wassie variety to reach the saturation points of water absorption compared to others in the study. On the other hand, GP-240038 accession initially had slightly slower water absorption rates than the GF1-Alemu, AK accession. At the end of soaking process however, the water uptake was much higher compared to GF1-Alemu, AK accession at the same time. Water absorption capacity differences among varieties and/or accessions might be due to genetic variation of the seed as well as the agro-climatic condition of the area where the grass peas grow including the amount of rain, temperature, soil type and amount and proportion of nutrient in the soil (Kaur et al., 2009). In general, it is expected that at the beginning of the soaking process, water absorption was fast whereas at the end, the water uptake was slow. Because, the velocity of water uptake of the seed would be slower and slower when it gets closer to maximum. It was observed from the water absorption curve that the water absorption rate increased with increasing temperature. The reason could be the higher the temperature, the softer the seed so that the diffusivity

of the seed increases that lead to a higher water uptake of the seed. As a consequence, soaking the seed using hot water reduced the time for soaking.

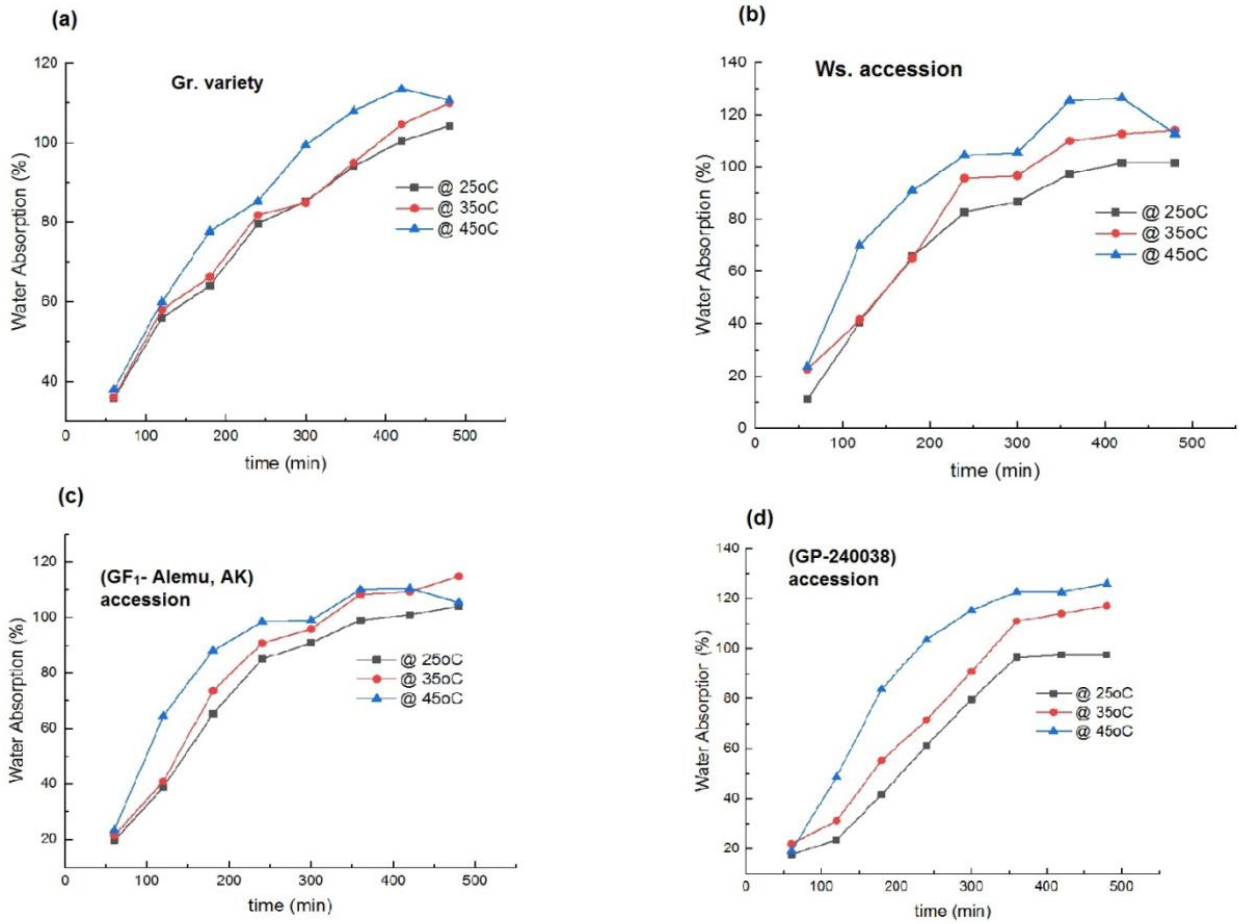

Figure 4-2. Water absorption capacity of grass pea seeds of different variety/ accessions at different temperatures

4.5. Scanning Electro-Microscopy (SEM) images

The images of grass pea flours of accessions (GF1-Alemu, AK and IF1-1936, AK) and a genetically modified variety (Wassie) subjected to scanning electron microscopy (SEM) are shown (**Figure 4-3**). The images of the flours were viewed at different magnification power (1000 to 4030x) and length ranges (100 to 30 µm). Depending on the type of starch, the shape of most legume starch granules are a mixture of simple and compound

granules having oval, spherical, elliptical, round and irregularly shaped with average size ranging from 4-85 µm (Hoover and Sosulski, 1990; Romano et al., 2018). The shape of starch granules of grass pea flours observed from SEM image were found to be oval, ellipsoidal and mixed shaped (**Figure 4-3**). The small irregular and spherical particles on the surface of the starch granules are found to be matrix of protein fragments, minerals and fibers (Romano et al., 2018; Ma et al., 2011) that could be disrupted while milling. GF1-Alemu, AK accession exhibited a deformed structure with swollen hole-bowled starch granules where the small protein matrix attached on the surface. Smoother surface with relatively insignificant hole-bowled-like structure was however observed for the Wassie variety. More disruption of protein was exhibited on IF1-1936, AK accession than on GF1-Alemu, AK accession and Wassie variety. SEM images of grass pea flours were scarcely studied, The image reported by Romano et al., (2018) however had similarity to the image obtained from Wassie variety in our study. The major factors that affect the structure of legumes/grains even in the same variety/accession are their chemical compositions and particle size distributions.

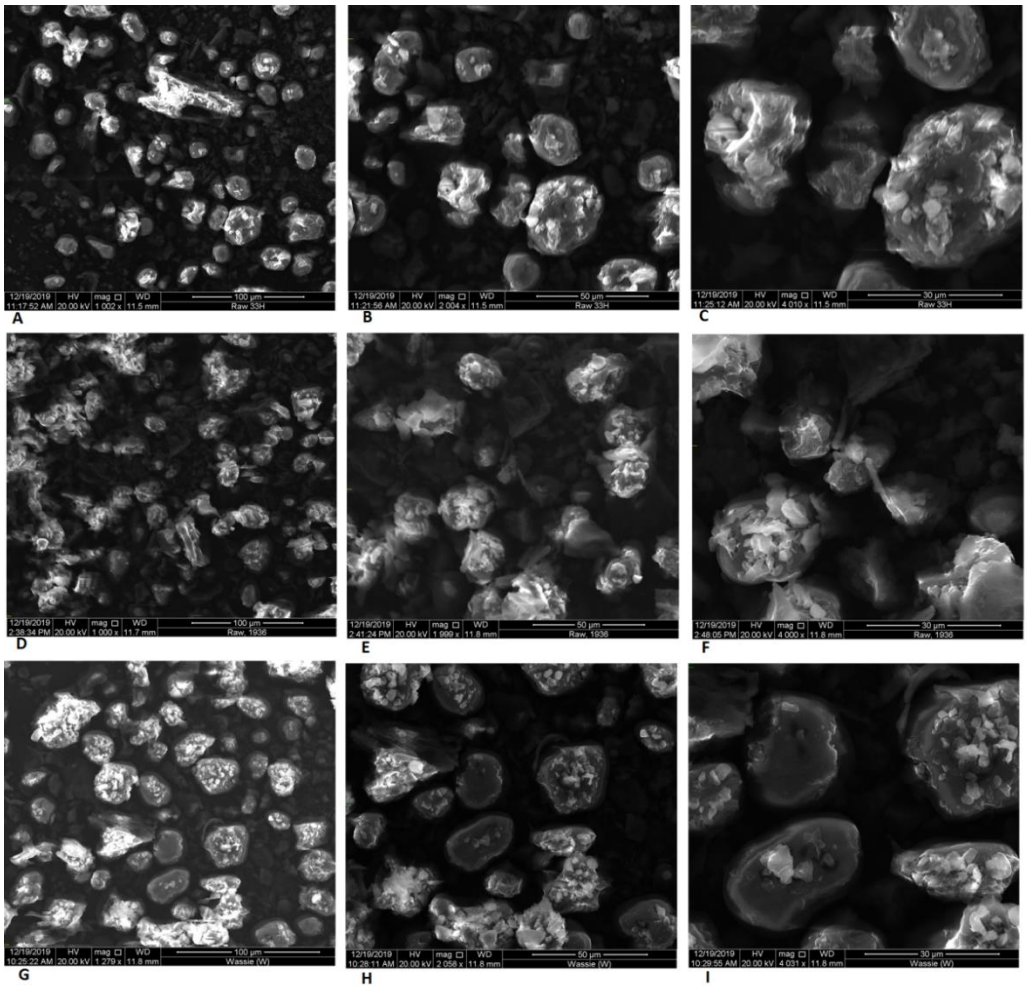

Figure 4-3. Scanning electron micrographs (SEM) of raw grass pea seed flours. A,B,C-GF1-Alemu, AK accession; D,E,F- IF1-1936, AK accession and G,H,I-Wassie variety

4.6. Pasting properties

Pasting properties of grass pea flours of Wassie variety, GF1-Alemu, AK and IF1-1936, AK accessions and the corresponding viscographs are given in (**Table 4-4**) and (**Figure 4-4**). Significant ($p < 0.05$) pasting characteristics differences were exhibited among the flours. The peak viscosities of the flours ranged from 470 to 670 cP where the highest peak viscosity was exhibited in the flour of IF1- 1936, AK accession followed by the GF1- Alemu, AK accession. The high peak viscosity could be attributed to high starch

contents and its water-binding capacity (Ahmed et al., 2013). Trough viscosities (hot paste viscosity) were in the range of 456 to 615 cP and, were also higher in IF1-1936, AK followed by GF1-Alemu, AK accessions and the Wassie variety. Trough viscosity is the holding strength at a maximum temperature of the RVA test, which is affected by the rate of amylose exudation, granule swelling, amylose-lipid complex formation (Wani et al., 2012). During the holding period, the granules undertake further disruption while the amylose molecules continue to leach out into the solution and undergo alignment that leads to viscosity reduction. The ability of the sample to withstand heating and shear stress is an important factor. Breakdown and setback viscosities ranged from (14.0 to 101.0 cP) and (110.0 to 192.0 cP) where the lowest breakdown viscosity was observed in the flour of Wassie variety whereas the highest setback viscosity was found in the flour of IF1-1936, AK accession. The low breakdown viscosity indicates restricted swelling of starch granules having high amylose content with high thermal stability, while high setback viscosity shows a higher tendency for retrogradation (Ovando-Martínez et al., 2011). The final viscosity of the flours ranged from 599.0 to 807.0 cP. The final viscosity of IF1- 1936, AK accession was significantly ($p<0.05$) higher than the GF1-Alemu, AK accession and Wassie variety. High final viscosity designates the ability of the material to form a more viscous paste due to the proper orientation of amylose (Kaur & Singh, 2005). This represents a higher removal of water from the exuded amylose that enables alignment of amylose chain to re-associate or retrograde to form a strong gel (Chung et al., 2008). Final viscosity commonly defines the quality of a particular sample. The pasting properties among accessions/varieties of legume flours can be attributed to the ratio of protein to starch and other constituents in their flours (Ahmed et al., 2013).

Figure 4-4. Pasting properties of grass pea flours of different accessions/varieties

Acce/Var	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Final viscosity (cP)	Setback Viscosity (cP)	Peak time (min)
GP-GF$_1$- Alemu, AK	657^{b}±2	556^{b}±3	101^{a}±1	666^{b}±3	110^{c}±1	4.87^{c}±0.04
Wassie variety	470^{c}±0.5	456^{c}±3	14^{c}±1.5	599^{c}±2	143^{b}±1.5	6.93^{a}±0.03
GP-IF$_1$- 1936, AK	670^{a}±1	615^{a}±1	55^{b}±2	807^{a}±0.5	192^{a}±1	5.27^{b}±0.04

(Values are means ± standard deviations of duplicates). Different superscripts in a column represent significant differences ($p < 0.05$)

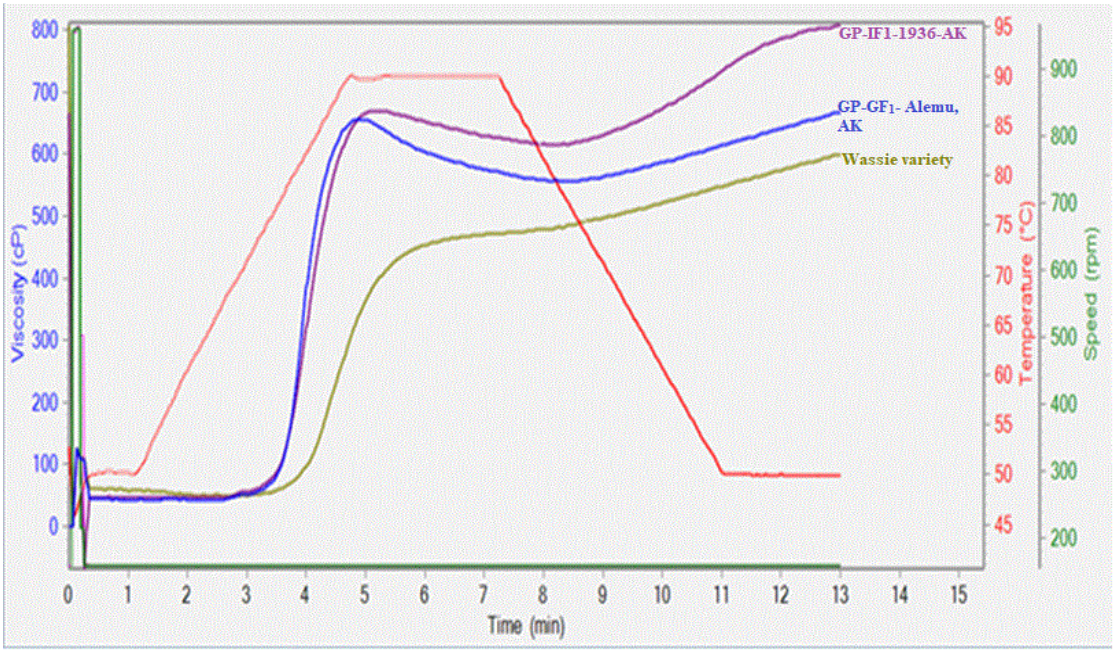

Figure 4-4. Viscogram of grass pea seeds**.** Gp-IF, 1936 stands for Gp-IF-1936, AK accession, Gp-33H stands for GF1- Alemu, AK accession and Gp-Ws stands for Wassie variety variety

4.7. Conclusion

The results in this chapter showed that the levels of β-ODAP of different accessions and varieties from different regions in Ethiopia were found to be in the range of 51.94 to 806.52 mg/100 g. Thus, considerable variations in the β-ODAP contents among the different accessions and varieties of *Latyrus sativus* L. seed were observed. That might possibly be due to effects of genotype, environment and their interaction. The Wassie variety was expected to have a relatively low β-ODAP level as it had been genetically modified targeting reduction of β-ODAP levels, but contained an intermediate β-ODAP level in our test.

Grass pea seeds were found to be good source of proteinogenic amino acids where glutamic acid was the highest in concentration (3454.86-4535.16 mg/100 g) whereas methionine was the least (92.00-200.47 mg/100 g). It was also observed that some accessions having high β-ODAP contents exhibited low levels in sulfur containing amino acids and vice versa. Nevertheless, due to the limited number of tests performed in our study, it is unlikely to draw conclusion between the link of β-ODAP concentration and sulfur containing amino acids in grass pea. In general, supplementing sulfur rich amino acids from cereal sources such as wheat and maize in grass pea meals or increasing their amount in the plant itself was suggested to improve the nutritional value of grass pea and prevent neurolathyrism. Moreover, grass pea seed was evidenced to be a good source of minerals having concentration of (72.58-89.12 mg/kg) iron-Fe, (22.03-38.74 mg/kg) Zinc-Zn, (903.22-1654.98 mg/kg) Calcium-Ca and (1588.10-4404.60 mg/kg)

Phosphorus-P. The mineral contents in grass pea seeds were also found to be comparable to other legumes.

It is good to understand the water absorption property of legumes during soaking as it highly influence the quality of final product after the succeeding processing such as cooking. Water absorption values are different among different varieties and accessions. In general, it was clearly observed that at the beginning of water soaking process, the water absorption rate was high whereas at the end, the water uptake was slow. The time consumed by hydration process can be enhanced by different technologies such as HHP, ultrasound and temperature. Temperature was found to accelerate the hydration process by softening the seed coat and cotyledon so that water permeability to the seed was increased and soaking time was reduced Having information on microstructure of flour and/or different flour types is an indication on their differences in nutritional and functional properties so that their sensitivity to different food processing might be assumed. Starch granules varying in shape from oval and ellipsoidal of different accession/variety were investigated in the study where small irregular matrix of proteins, minerals and fibers attached on the surface of starch granules. GF1-Alemu, AK accession exhibited slightly different structure with swollen-hole bowled starch granules. Considerable differences in pasting properties among the flours of grass pea accessions and a variety were exhibited. IF1- 1936, AK grass pea accession exhibited the highest final viscosity. Pasting properties are highly linked to starch swelling and water absorption that can influence the properties of legume food processing and defines the quality of a particular product.

5. IMPACT OF HIGH HYDROSTATIC PRESSURE ON β-ODAP and $InsP_6$ DEGRADATION

The effect of high hydrostatic pressure (HHP) had been elucidated on grass pea seeds in soaked and batter forms as there is no report on the effects of HHP on the nutritional properties of grass pea seed available so far. The major objective of the research in this chapter was therefore to reveal the effects of HHP on β-ODAP content and $InsP_6$ reduction in grass pea seeds. The results from this chapter may impart information for industrial application HHP for grass pea products, and products from other legume and cereals grains, in line with various application of HHP for food products such as meat, milk, fruits and vegetables in respect to nutrient retention, microbial inactivation and other food attributes.

5.1. Effect of HHP on β-ODAP and $InsP_6$ contents of grass pea seeds

High hydrostatic pressure treatments of grass pea seeds in soaked and batter forms considering pressure, soaking time and holding time as investigated factors were explained in this research chapter. The 3D of the response surface plot also demonstrated the significance of the factors. To study the effects of individual factors and their interactions on the response variables, response surface methodology is appropriate for it minimizes the time consumed to treat each factor separately. Following the experimental data (**Tables 5-1 & 5-2**), multiple regression analysis of the experimental data was performed. The analysis showed the quadratic polynomial equations and the significance of their coefficient at different p value for the soaked-HHP and batter-HHP treated grass pea seeds in respect to β-ODAP and $InsP_6$ content (**Tables 5-1 & 5-2**). The significance

of one term relative to another was also examined by the coefficients in the regression equation.

Table 5-1. Coefficients of the second-order polynomial model equation and significance of each model and response variables in soaked-HHP treated grass pea seeds

Source	**β-ODAP**	**$InsP_6$**
Intercept-b_0	331.76	39.78
Linear-b_1	-108.56*	-15.84*
b_2	-20.76*	-3.20*
b_3	-12.35*	-3.28*
Quadratic-b_{11}	16.88	1.68**
b_{22}	-	-
b_{33}	-	-1.60
Interaction-b_{12}	21.44*	1.92*
b_{13}	5.13**	0.82
b_{23}	-	-
Lack of fit (F-value)	2.62	2.2
R-squared	0.9973	0.9955
Adj. R-squared	0.9961	0.9928
Pred. R-squared	0.9934	0.9859
The final quadratic polynomial equation for the responses (coded)	$\beta\text{-ODAP} = b_0\text{-}b_1A\text{-}b_2B\text{-}b_3C+b_{12}AB+b_{13}AC+b_{11}(A)^2$	$InsP_6= b_0\text{-}b_1A\text{-}b_2B\text{-}b_3C+b_{12}AB+b_{13}AC+b_{11}(A)^2\text{-}b_{33}(C)^2$

*Significant at $p \leq 0.001$, **significant at $p \leq 0.05$

The quadratic polynomial equations in the tables expressed response variables as a function of independent variables, where b_0, b_1, b_2, b_3, b_{12}, b_{13}, b_{23}, b_{11}, b_{22}, b_{33} represent the coefficient of the model and A, B, C, AB, AC, BC represent the coded form of independent variables

Table 5-2. Coefficients of the second-order polynomial model equation and significance of each model and response variables in batter-HHP treated grass pea seeds

Source	**β-ODAP**	**$InsP_6$**
Intercept-b_0	583.81	17.97
Linear-b_1	-59.83*	-14.64*
b_2	-16.83*	-1.95*
Quadratic-b_{11}	15.11*	-
b_{22}	-10.17**	-
Interaction-b_{12}	8.82**	-1.86**
Lack of fit (F-value)	3.95	0.85
R-squared	0.9925	0.9970
Adj. R-squared	0.9872	0.9949
Pred. R-squared	0.9403	0.9855
The final quadratic polynomial equation for the responses (coded)	$\beta\text{-ODAP} = b_0 - b_1A - b_2B \pm b_{12}AB + b_{11}(A)^2 - b_{22}(B)^2$	$InsP_6 = b_0 - b_1A - b_2B - b_{12}AB$

*Significant at $p \leq 0.001$, **significant at $p \leq 0.05$
The quadratic polynomial equations in the tables expressed response variables as a function of independent variables, where b_0, b_1, b_2, b_3, b_{12}, b_{13}, b_{23}, b_{11}, b_{22}, b_{33} represent the coefficient of the model and A, B, C, AB, AC, BC represent the coded form of independent variables

5.1.1. β-ODAP level in soaked-HHP treated grass peas seeds

The model adequately explained the significance of the relationship between β-ODAP content and the independent variables because only 0.27 % of the variations were due to

other factors excluded in the model, i.e. 99.73 % of the variation for β-ODAP was covered by the model (**Table 5-1)**. Other indicators for the applicability of the model were also included in the table. The regression model and 3D of the response surface plot of β-ODAP degradation for soaked-HHP treated grass pea seeds exhibited that pressure, soaking time and holding time significantly ($p \leq 0.001$) affected β-ODAP degradation (**Figures 5-1a & b**), whereby pressure had the most pronounced effect. The combined incremental effect of pressure and soaking time resulted in a significant ($p \leq 0.001$) reduction of β-ODAP levels at a constant holding time (15 min) (**Figure 5-1a**). Pressure and holding time had also a significant ($p \leq 0.05$) effect on β-ODAP reduction at a constant soaking time (9 h) (**Figure 5-1b**). A 36.00 to 71.22 % reduction in β-ODAP was achieved by HHP treatment of soaked grass pea seeds. The observed reduction might be due to the isomerization of β-ODAP to α-ODAP and an enzymatic hydrolysis of β-ODAP (Ikegami et al., 1993) as observed by Kuo et al., (2000) and Starzy, (2008) during soaking of grass pea. An isomerization of β-ODAP in solution was reported to be facilitated by forces such as heat and iontophoresis (Bell & O'Donovan, 1966). As HHP is able to disrupt cell structures and cell membranes (Lullien-Pellerin & Balny, 2002) access of those enzymes to β-ODAP might be facilitated. In addition to isomerization and degradation by hydrolytic enzymes, β-ODAP was possibly detoxified by leaching after soaking the seed since β-ODAP is a water-soluble amino acid (Yan et al., 2006).

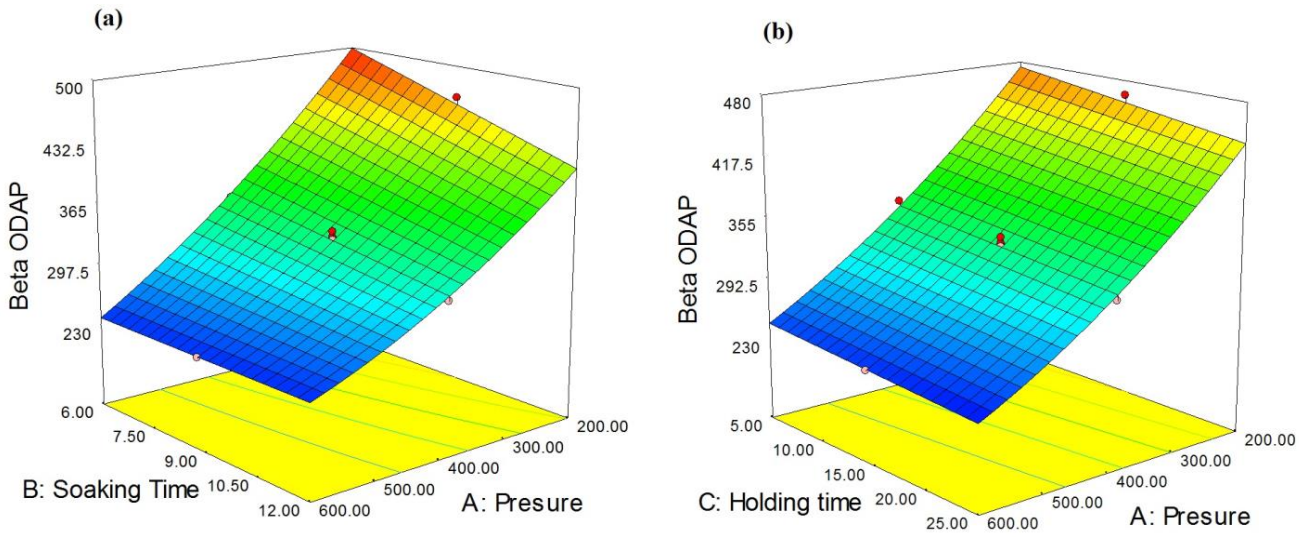

Figure 5-1. Response surface plot of degradation of β-ODAP in soaked-HHP treated grass pea seeds as a function of: (**a**) pressure and soaking time keeping the holding time constant (15 min); (**b**) pressure and holding time while soaking time is constant (9 h)

To the best of our knowledge, the effect of HHP on β-ODAP contents in grass pea was not studied so far. The amount of β-ODAP ranged from 232.11 to 515.45 mg/100 g for soaked-HHP treated grass pea seeds (**Table 5-3**). The β-ODAP contents after HHP treatment could be categorized in respect to their toxicity, especially taking into consideration that the accession selected for HHP treatment was the one with the highest β-ODAP content among the accessions included in the study. Kumari, (2001) reported from field evaluation of *Lathyrus sativus* L. germplasm that β-ODAP contents between 100 and 300 mg/100 g; 310 and 600 mg/100 g as well as >600 mg/100 g were considered to exhibit low, medium and high toxicity levels. Thus, all accessions and varieties included in the HHP treatment could be categorized as low or medium toxic in respect to their β-ODAP levels after the HHP treatment.

Table 5-3. Central composite face-centered design for soaked-HHP treatment and results

Soaked-HHP treatment			**β-ODAP (mg/100g)**	**$InsP_6$ (mg/100g)**
x_1	x_2	x_3		
0 (400)	0 (9)	0 (15)	333.94	39.07
0 (400)	0 (9)	0 (15)	332.48	41.26
0 (400)	-1 (6)	0 (15)	345.53	42.83
1 (600)	-1 (6)	-1 (5)	247.65	28.01
1 (600)	1 (12)	1 (25)	236.91	21.11
0 (400)	0 (9)	-1 (5)	348.94	41.51
-1 (200)	1 (12)	1 (25)	398.44	47.06
-1 (200)	1 (12)	-1 (5)	427.62	54.06
1 (600)	1 (12)	-1 (5)	245.00	25.48
0 (400)	0 (9)	1 (25)	313.21	34.31
-1 (200)	0 (9)	0 (15)	464.47	58.16
1 (600)	0 (9)	0 (15)	238.74	24.23
0 (400)	0 (9)	0 (15)	331,19	39.79
0 (400)	0 (9)	0 (15)	341.06	40.76
0 (400)	0 (9)	0 (15)	334.97	40.44
0 (400)	1 (12)	0 (15)	305.22	34.81
-1 (200)	-1 (6)	1 (25)	480.00	55.84
-1 (200)	-1 (6)	-1 (5)	515.45	64.95
0 (400)	0 (9)	0 (15)	331.48	39.81
1 (600)	-1 (6)	1 (25)	232.11	22.86

5.1.2. $InsP_6$ level in soaked-HHP treated grass peas

The ANOVA result from the second-order response model in (**Table 5-1)** showed that the model could explain 99.61 % of the variability of the response from the independent variables and it is a good fit. Pressure ($p \leq 0.001$), soaking time ($p \leq 0.001$) and holding time ($p \leq 0.001$) had significant effects on phytate reduction. In addition, the quadratic factor of pressure ($p \leq 0.0139$) and holding time ($p \leq 0.0479$) had a significant effect on IP_6 reduction, whereas the quadratic term of soaking time ($p=0.4397$) and its interaction with holding time ($p=0.3497$) was shown to be insignificant. Pressure had the most predominant effect on phytate reduction. The significant combination of pressure and soaking time as well as pressure and holding time on the reduction of $InsP_6$ were predicted from 3D response surface plot (**Figures 5-2a & b)**. It represented the reduction of $InsP_6$ as a function of: pressure and soaking time at a constant holding time (15 min) as well as pressure and holding time keeping soaking time constant (9 h). $InsP_6$ reduction achieved by HHP treatment of soaked grass pea seeds was 35.00 to 77.90 %. The reduction of phytate in plant based materials is mainly due to the intrinsic phytase activity in the plant seed (Greiner & Konietzny, 2006b). The better accessibility of phytate to phytase was possibly due a damage of internal cell structures and membranes caused by HHP.

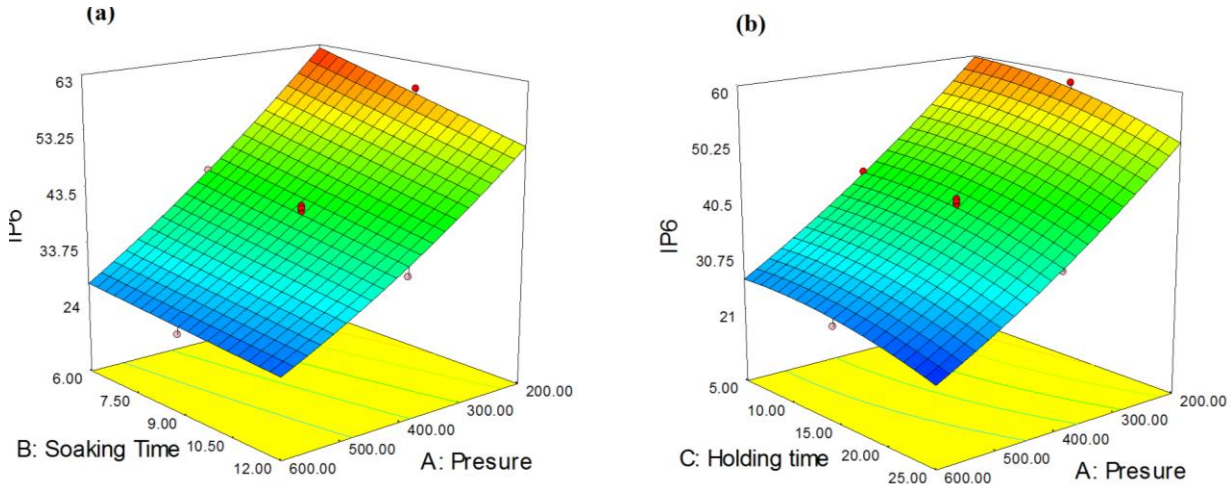

Figure 5-2. Response surface plot of degradation of $InsP_6$ in soaked-HHP treated grass pea seeds as a function of: (**a**) pressure and soaking time keeping the holding time constant (15 min); (**b**) pressure and holding time while soaking time is constant (9 h)

The $InsP_6$ content of soaked-HHP treated grass pea seeds ranged from 21.11 mg/100 g to 64.95 mg/100 g (**Table 5-3)**. No data are available so far on the effect of HHP treatment on phytate reduction in grass pea. However, Linsberger-Martin et al., (2013) reported that the content of phytate in pressure treated split pea was decreased by 36 % compared to untreated ones. Significant degradation of phytate during soaking and cooking was also reported by Máñez, et al., (2002). Phytate reduction from white kidney bean by up to 50% was also achieved by pressure cooking at temperature of 121 ^{o}C as reported by Zia-ur-Rehman & Salariya, (2005). Lee et al., (2018) reported that HHP treatment (400, 500, and 600 MPa) of red kidney beans was effective in reducing phytate content by 8.19 %, 8.33 % and 9.04 %. The maximum reduction was achieved using HHP at 600 MPa for 5 min.

5.1.3. β-ODAP and $InsP_6$ contents in soaked, soaked-HHP and batter-HHP treated grass peas

The model from the response surface plot (**Table 5-4**) and results from ANOVA showed that the R-squared value of 99.25 %, explained variability of the response from the independent variables whereas the insignificancy of the "lack of fit F-value" indicated that the model fitted well.

Table 5-4. Coefficients of the second-order polynomial model equation and significance of each model and response variables in batter-HHP treated grass pea seeds

Source	**β-ODAP**	**$InsP_6$**
Intercept-b_0	583.81	17.97
Linear-b_1	-59.83*	-14.64*
b_2	-16.83*	-1.95*
Quadratic-b_{11}	15.11*	-
b_{22}	-10.17**	-
Interaction-b_{12}	8.82**	-1.86**
Lack of fit (F-value)	3.95	0.85
R-squared	0.9925	0.9970
Adj. R-squared	0.9872	0.9949
Pred. R-squared	0.9403	0.9855
The final quadratic polynomial equation for the responses (coded)	$\beta\text{-ODAP}= b_0-b_1A-b_2B \pm b_{12}AB +b_{11}(A)^2-b_{22}(B)^2$	$InsP_6= b_0-b_1A-b_2B-b_{12}AB$

*Significant at $p \leq 0.001$, **significant at $p \leq 0.05$
The quadratic polynomial equations in the tables expressed response variables as a function of independent variables, where b_0, b_1, b_2, b_3, b_{12}, b_{13}, b_{23}, b_{11}, b_{22}, b_{33} represent the coefficient of the model and A, B, C, AB, AC, BC represent the coded form of independent variables

Table 5-5. Central composite face-centered design for batter-HHP treatment and results

Batter-HHP treatment		**β-ODAP (mg/100 g)**	**$InsP_6$ (mg/100 g)**
x_1	x_2		
1 (600)	1 (25)	524.36	0.00
-1 (200)	1 (25)	619.65	32.3
0 (400)	1 (25)	556.65	15.27
1 (600)	-1 (5)	540.11	7.47
0 (400)	0 (15)	585.15	17.67
0 (400)	0 (15)	580.41	19.00
0 (400)	0 (15)	584.49	17.98
0 (400)	0 (15)	588.35	18.47
0 (400)	0 (15)	580.41	16.99
-1 (200)	0 (15)	665.58	33.65
-1 (200)	-1 (5)	670.66	32.33
1 (600)	0 (15)	532.47	3.00
0 (400)	-1 (5)	590.84	19.48

The 3D graphic representation (**Figure 5-3a**) showed that pressure had the most pronounced effect on β-ODAP reduction in batter-HHP treated grass pea flour. The β-ODAP contents after HHP treatment ranged from 524.36 to 670.66 mg/100 g (**Table 5-5**). Thus, reductions from 16.84 % to 34.98 % were achieved. Moreover, the model elucidated significantly the relationship between $InsP_6$ and the independent variables for batter-HHP treated grass pea samples (**Table 5-4)**, because 99.70 % of the variation was

explained by the model. Pressure ($p \leq 0.001$), holding time ($p \leq 0.001$) and their combination (≤ 0.005) were shown to be significant. The highest $InsP_6$ reduction was achieved due to the interaction of pressure and holding time (**Figure 5-3b)**. The $InsP_6$ content after HHP treatment ranged from 33.65 mg/100 g to nil (**Table 5-5**). Thus, reductions from 61.37 % to 100 % were achieved. There is no information on the reduction of β-ODAP and $InsP_6$ of grass pea and other legume in batter form treated by HHP so far. As batters were found to be more resistant to deformation (Vallons & Arendt, 2010), higher pressures are required to break cell walls and membranes compared to the soaked legumes and the higher viscosity of the batter reduces the diffusion of the enzymes and their substrates.

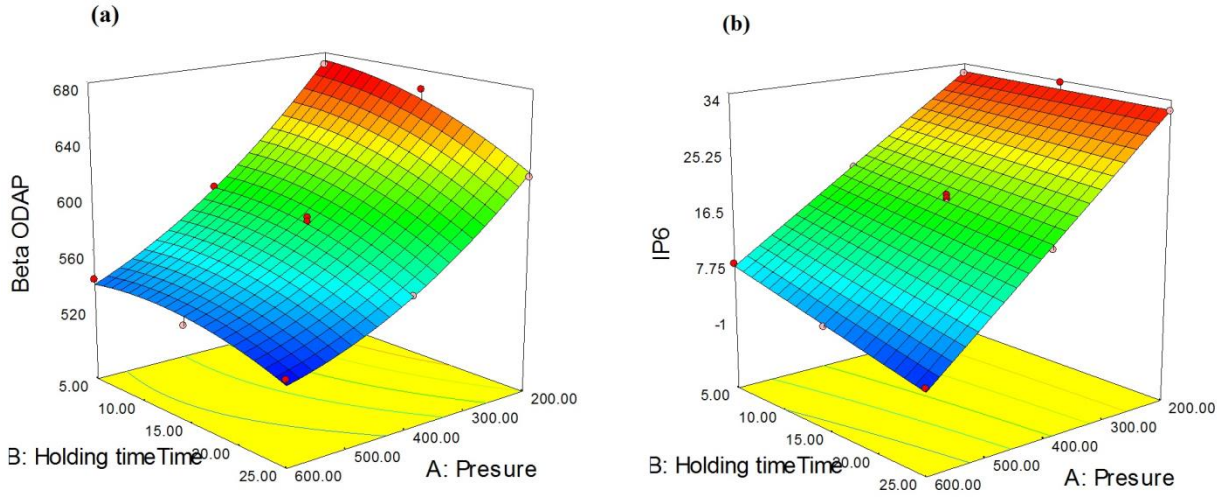

Figure 5-3. Response surface plot of batter-HHP treated grass pea seeds as a function of pressure and holding time for the degradation of: (**a**) β-ODAP; (**b**) $InsP_6$

The effects of soaking, soaked-HHP (600 MPa, soaking time: 9 h, holding time: 15 min)-treatment and batter-HHP (600 MPa, holding time: 15 min) treatment on β-ODAP and IP_6 contents of GF_1- Alemu, AK, GP-240038 accessions; the genetically improved

variety (Wassie) and the German variety (GR) were also compared (**Table 5-6)**. The best HHP conditions in respect to β-ODAP reduction obtained for the grass pea accession, GF1- Alemu, AK, the one with the highest β-ODAP content were selected. The highest β-ODAP reduction (73%) by soaking was found in GP-240038 accession. This is in good agreement with the 65-70% loss in β-ODAP content by soaking grass pea in boiled water as reported by Srivastava & Khokhar, (1996). In soaked-HHP and batter-HHP treatments, 91% and 44% reductions of β-ODAP contents respectively were obtained in the same grass pea accession.

Table 5-6. Comparison of β-ODAP and $InsP_6$ contents of raw, soaked, soaked-HHP and batter-HHP treated samples

Values reported in the table represent the mean ± SD of three independent replicates.

Sample type	GF_1- Alemu, AK*		GP-240038**		Not Registered, GR***		Genetically Improved (Wassie)	
	β-ODAP	$InsP_6$	β-ODAP	$InsP_6$	β-ODAP	$InsP_6$	β-ODAP	$InsP_6$
Raw	806.52^{a}± 2.31	95.5^{a} ± 8.90	51.94^{b} ± 00	1391.1^{a} ± 111.5	344.44^{a} ± 6.72	1154.00^{a} ± 55.5	579.02^{a} ± 8.05	1049.2^{a} ± 122.8
Soaked	404.68^{c}± 0.51	50.90^{bc}±1.43	14.15^{c} ± 2.39	1183.97^{c} ±0.35	199.7^{c} ± 3.61	804.73^{c} ± 3.45	384.51^{c} ± 10.1	936.04^{b} ± 1.08
S-HHP-6,9,15	238.74^{d}± 0.87	24.23^{d}±14.56	4.79^{d} ± 0.23	990.83^{d} ± 38.45	160.58^{d} ± 6.96	753.56^{d} ± 12.93	286.66^{d} ± 4.26	843.06^{d} ± 0.87
B-HHP-6,15	532.47^{b}± 2.81	3.15^{c}±2.30	28.97^{a} ± 1.28	1216.81^{b} ±0.26	248.11^{b} ± 0.26	856.93^{b} ± 24.88	487.44^{b}± 6.66	912.73^{c} ± 5.72

* Accession with high β-ODAP; ** accession with low β-ODAP; ***variety from Germany
Different letters (a, b,c,d) in the same column denote significant differences ($p < 0.05$), S-soaked, B-batter

5.2. Conclusion

The reduction of β-ODAP achieved by HHP treatment ranged from 36.00 to 71.22 % considering high β-ODAP-containing grass pea compared to the untreated grass pea samples. HHP treated seed at 600 MPa, 9 h soaking time and 15 min pressurization time was found to be the best combination parameters that result in the highest β-ODAP reductions. This reduction seems to be sufficient for most accessions and varieties to make grass peas safe for human consumption. Phytate content could also be reduced by HHP treatment (up to 100%). Therefore, mineral bioavailability of treated grass pea should also be improved. Thus, HHP is a promising technology for reduction of β-ODAP leading to minimization of neurolathyrism and moreover it is expected to improve the nutritional quality of grass pea seeds as it has a potential in reducing antinutrients. It is also revealed that soaking plays prominent role than batter prior to HHP treatment for degradation of β-ODAP and phytate. As it has been already mentioned in the experimental design, the best process parameters for HHP treatment were chosen according to the best β-ODAP reduction obtained from high β-ODAP-containing accession (GF1- Alemu, AK). Lower pressure and shorter holding time could be expected for other accessions having low β-ODAP concentrations, as it was achieved high reduction of β-ODAP (91%) from low β-ODAP-containing accession (GP-240038) using the best parameters selected in the study. Further studies are recommended for different accessions and grass pea cultivar having varying β-ODAP concentration. Process optimization to find out the optimum process conditions is addition task to be further investigated.

6. IMPACT OF HHP ON MICROSTRUCTURE, PASTING PROPERTIES, WATER ABSORPTION AND COLOR OF GRASS PEA (*LATHYRUS SATIVUS* L.) SEED AND FLOUR

6.1. Effect of HHP on microstructure of grass pea samples

Scanning electron microscopy might be helpful to elucidate structural, chemical and physical changes in food products during preparation and processing. Structural deformation of starch granules for example results in alteration of functional properties (Ahmed et al., 2016). The microstructure of soaked-HHP and batter-HHP treated grass pea samples and their controls was examined by scanning electron microscopy (**Figure 6-1D, E, F; A, B, C & D', E', F'; A', B', C'**). Discernible irregular and amorphous protein structure attached to oval and ellipsoid surface of starch micrograms were clearly observed in the image representing control samples. Slight differences were observed among the images of the controls where smoother structure appeared on the ellipsoid starch micrograms of untreated batter (**Figure 6-1A', B', C'**). Besides fragments of protein matrices, the particles attached to starch granules might also consist of fiber and minerals (Romano et al., 2018, Ovando-Martínez et al., 2011, Ma et al., 2011). The interaction and the changes in structure and composition occurring during processing are highly related to food product texture (Sila et al., 2008).

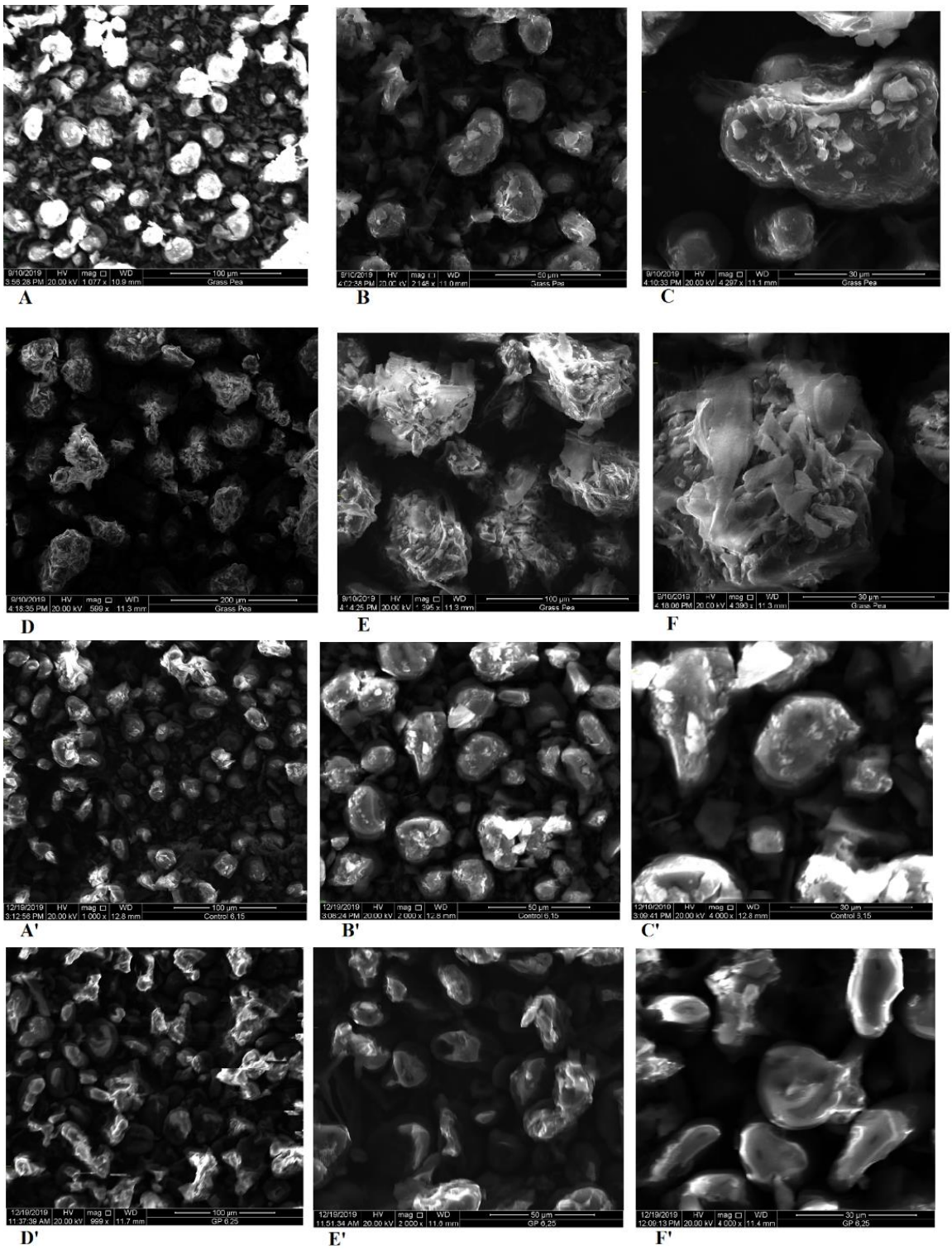

Figure 6-1. Microstructure of soaked-HHP (D, E, F) and batter-HHP treated (D', E', F') grass pea seeds and their controls (A, B, C) & (A', B', C'). Soaked-HHP SEM image (D, E, F) are at 600 MPa pressure, 9 h soaking time and 15 min holding time, and batter-HHP SEM images (D', E', F') are at 600 MPa pressure, and 15 min holding time, where control soaked seeds for 9 h are represented by (A, B, C) and control batter by (A', B', C')

When 600 MPa pressure was applied to soaked grass pea samples, a change in starch granules structure and disruption of protein matrices adhered to starch granules appeared (**Figure 6-1D, E, F**). Fibrous and ridged nature of cell walls was also visible. The starch, however kept its granular shape despite distinct interior structural changes, an observation already reported by Biaszczak et al., (2007). In batter samples treated at the same pressure, smoothly spread protein matrices attached to starch granules and 'hole' like bowled starch granules were observed (**Figure 6-1D', E', F'**). Denaturation of proteins and damage of major components of starch are the overall structural changes in flour-water suspensions caused by HHP treatment (Vallons et al., 2011). Changes in protein structure of the seed increase the rate of water uptake of the seed that results in shorter time meal preparation such as soaking and cooking time and ease decortication. It could be concluded from the images that HHP distorted starch granules and caused protein aggregation in grass pea flours. The extent of the structural changes of the food polymers in soaked-HHP and batter-HHP grass pea samples were found to be different as observed from SEM images.

6.2. Pasting properties of soaked and batter HHP treated grass pea samples

The pasting properties of soaked-HHP treated grass pea samples are summarized in **Table 6-1** and shown by viscographs (**Figure 6-2**). Significant differences of pasting characteristics were exhibited among raw, control and soaked-HHP treated flours. The minimum temperature required to cook the control flours was significantly different from the one required to cook soaked-HHP treated flours (**Figure 6-2**), whereas slight pasting time variations were observed among the raw and soaked-HHP treated pea flours (**Table**

6-1). The peak, trough and final viscosities of raw, control and treated samples ranged from 657.0 cP to 1871.0 cP; 556.0 to 1409.0 cP; and 666.0 to 2083.0 cP respectively. The maximum values of peak, trough, breakdown, final and setback viscosities, and the highest pasting temperature and lowest peak time were obtained at 600 MPa. The viscosity values were lower at the relatively low pressures (100 MPa and 200 MPa) than at the high pressures (400 MPa and 600 MPa) applied in the study. The viscosities of soaked-HHP samples increased as the pressure increased (**Table 6-1** & **Figure 6-2**). The highest peak, trough and final viscosities were observed in GF1-Alemu, AK accession soaked for 9 h at 600 MPa for 25 min (GP-600,12,25). All viscosity increments obtained in the study is not in agreement with the results reported by Li et al., (2015) for red adzuki bean starches and, Li et al., (2011) also reported a low peak viscosity, a low pasting temperature and a low peak time for mung bean exposed to high pressure.

There were no results previously reported that stated the impact of HHP on pasting properties of grass pea flours. Nevertheless, the final viscosity obtained from our study is in a good agreement to final viscosity reported by Katopo et al., (2002) from tapioca starch, which was found to be a C-type starch. It was reported to be associated with a cross-linking network between amylose and amylopectin that resisted the shear force and resulted in increased viscosity. The ratio of amylase to amylopectin was responsible for specific interactions which determine starch behavior (Pei-ling et al., 2012) in such a way that amylase had a role of stabilizing the structure of starch granules under HHP so that the starches with high amylase content required more energy during the course of gelatinization (Li et al., 2015). It could be revealed from our results that a complete gelatinization is expected to happen above 600 MPa and C-type starches were reported to

be less sensitive to pressure and require 600-1000 MPa for complete gelatinization (Błaszczak et al., 2005, Li et al., 2012). However, as those properties beyond 600 MPa for grass pea were not covered in our study, it would be recommended for further investigation. Peak, trough and final viscosities of grass pea flours of Wassie variety and Gp-240038 accession soaked for 9 h and pressured at 600 MPa for 15 min were lower than that of GF1-Alemu, AK accession treated using the same conditions.

The sample treated with higher pressure in our study showed lower pasting temperature and higher peak viscosity, because, HHP facilitated hydration and swelling of starch granules (Pei-ling et al., 2012). This may partly be attributed to the lack of amylose-lipid complex in grass pea starch that restrict swelling of molecules by holding amylopectin molecules together, as a result pressurized grass pea starch may lost considerable amount of crystallinity so that amorphous starch was apt to swell. Thus, HHP treatment increased the ability of the starch granule in soaked grass pea flours to swell freely that resulted in increased peak viscosity providing the thick starch.

Table 6-1. Pasting properties of soaked-HHP, control and raw grass pea flours

Sample type	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak time (min)
Gp-Raw	$657^{i} \pm 9$	$556^{h} \pm 8$	$101^{h} \pm 1$	$666^{i} \pm 6$	$110^{h} \pm 6$	$4.89^{abc} \pm 0.03$
GP-200,9,25	$1099^{h} \pm 9$	$919^{g} \pm 4$	$180^{ef} \pm 4$	$1188^{h} \pm 10$	$269^{g} \pm 4$	$4.93^{ab} \pm 0.09$
GP-400,9,25	$1510^{c} \pm 5$	$1126^{d} \pm 3$	$384^{c} \pm 5$	$1540^{de} \pm 4$	$414^{d} \pm 3$	$4.75^{c} \pm 0.10$
GP-100,9,15	$1118^{h} \pm 1$	$933^{g} \pm 2$	$185^{ef} \pm 3$	$1211^{g} \pm 3$	$278^{g} \pm 5$	$4.91^{abc} \pm 0.02$

GP-600,9,15	$1734^{b} \pm 2$	$1225^{b} \pm 3$	$509^{a} \pm 4$	$1768^{b} \pm 3$	$543^{b} \pm 3$	$4.73^{c} \pm 0.02$
GP-600,6,25	$1469^{d} \pm 4$	$1146^{c} \pm 3$	$323^{d} \pm 4$	$1534^{e} \pm 2$	$388^{e} \pm 4$	$4.83^{bc} \pm 0.04$
GP-600,12, 25	$1871^{a} \pm 2$	$1409^{a} \pm 8$	$462^{b} \pm 4$	$2083^{a} \pm 3$	$674^{a} \pm 3$	$4.80^{bc} \pm 0.03$
Control	$1220^{g} \pm 4$	$1039^{f} \pm 4$	$181^{f} \pm 4$	$1377^{f} \pm 4$	$338^{f} \pm 5$	$5.0^{abc} \pm 0.1$
Ws-600,9,15	$1325^{e} \pm 3$	$1132^{cd} \pm 2$	$193^{e} \pm 2$	$1606^{c} \pm 3$	$474^{c} \pm 3$	$5.20^{ab} \pm 0.15$
Lw-600,9,15	$1241^{f} \pm 1$	$1074^{e} \pm 3$	$167^{g} \pm 3$	$1552^{d} \pm 2$	$478^{c} \pm 4$	$5.2^{a} \pm 0.1$

$^{a-g}$All (Values are means ± standard deviations of duplicates). Different superscripts in a column represent significant differences ($p < 0.05$). **Gp**-Raw-raw grass pea four, (Gp-x, y, z) represents: x- x00 pressure applied, y-soaking time, z- holding time in the HHP

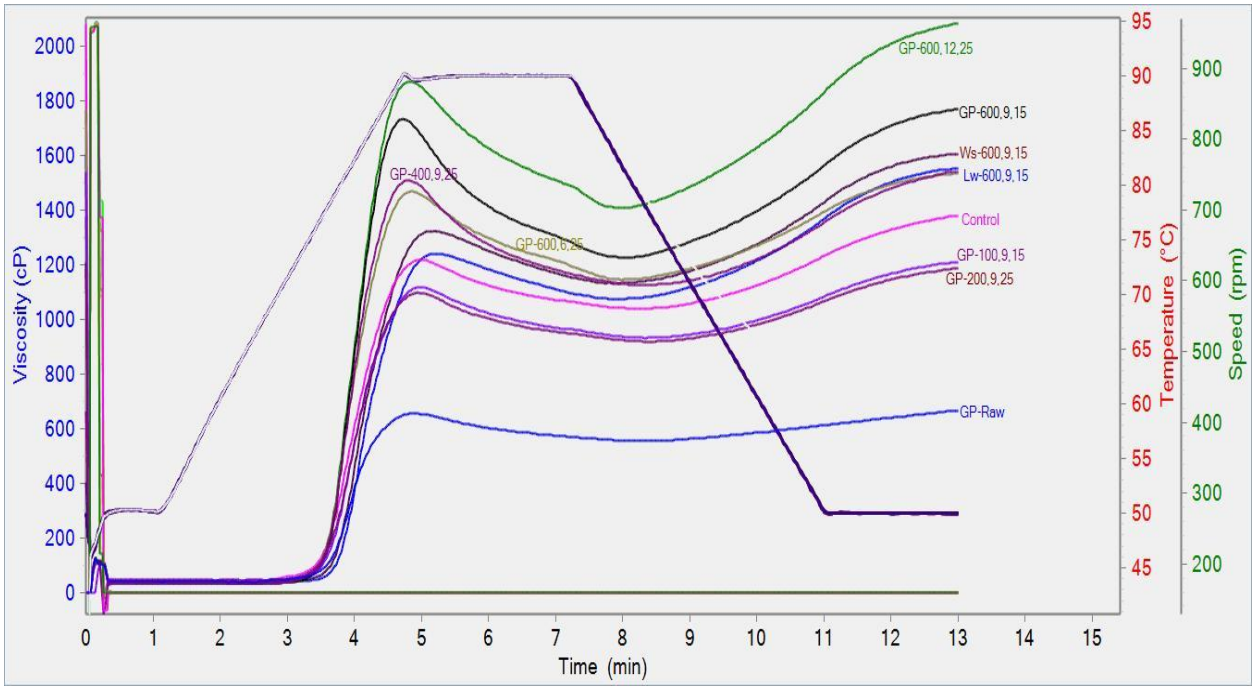

Figure 6-2. Vicrographs of soaked-HHP treated, raw and control (soaked) grass pea flours. Gp-Grass pea accession having high β-ODAP (GF1-Alemu, AK), Ws Wassie (genetically modified variety), Lw Grass pea accession having low β-ODAP (Gp-240038), Gr-A variety from Germany. In (100, 6, 15), (400, 6, 15), (600, 9, 25) etc., the first coordinates represent pressure; the second coordinates represent soaking time and the third coordinate represents holding time.

The pasting properties of batter-HHP treated grass pea flours are summarized in **Table 6-2** and demonstrated in **Figure 6-3**. All pasting properties of batter-HHP treated samples from GF1-Alemu, AK accession are significantly ($p<0.05$) different from the control and the accession and varieties included in the study. The peak and final viscosities for GF1-Alemu, AK accession decreased at all pressures applied and higher peak time was required compared to the control. However, peak, trough and final viscosities were higher at 600 MPa than that of 400 MPa (**Table 6-2**). The significant drop of peak viscosity is attributed to a complete gelatinization of starch granules at a given pressure (Ahmed et al., 2016). The control sample (unpressured batter), however, exhibited the highest peak and breakdown viscosities and lowest peak time than the treated accessions including GF1-Alemu, AK accession. The peak, trough and final viscosities of batter-HHP treated flours of Wassie variety, the variety from Germany, and Gp-240038 accession were lower than that of GF1-Alemu, AK accession treated at 600 MPa pressure and the control (**Table 6-2**). Our results are in good agreement with the data reported by Ahmed et al. (2016) and Li et al. (2012) for lentil seed and rice starches treated at 600 MPa respectively. The amylopectin molecules are possibly intertwined by amylase-lipid complex so that dispersion and swelling of starch granules are possibly restricted (Li et al., 2011). Batter-HHP treated grass pea seed treated by 600 MPa for 15 min (GP-600,15) presented the highest trough viscosity and the lowest breakdown viscosity. That indicated low tendency for retrogradation and thermal stability (Ovando-Martínez et al., 2011). The highest final viscosity was obtained in at 600 MPa. Batter-HHP-treated grass pea starch had a higher final viscosity and lower setback than control, indicating the stronger starch chain aggregation, lower swelling power, and retrogradation tendency than control (Li et

al., 2012; Ovando-Martínez et al., 2011). The final viscosities of batter-HHP similar to soaked-HHP were improved which indicate good quality for the final texture of grass pea seed product.

The values for peak, trough, breakdown, and final viscosities of the soaked-HHP treated samples were higher compared to the respective values of batter-HHP treated samples at 600 MPa. That might be partly due to the softening of the seed by soaking as well as the enhancement of water penetration into the seed by the pressure so that swelling of starch is achieved that in turn resulted to improved pasting properties. Moreover, sample preparation (soaked seed and batter) prior to HHP application might be different in their amylose-lipid complex that could bring amylopectin molecules together or apart which have impact on swelling of starch granules. Peak viscosity is an indicator of early and rapid swelling of starch granules with amylose leaching out of granules (Li et al., 2011). The peak viscosity suggested that the HHP treatment at 600 MPa in soaked seed allowed hydration and swelling during gelatinization. Significant differences were observed in the pasting properties of the different HHP treated samples within an accession, and among accessions treated by similar HHP treatment parameters. The pasting properties were greatly related to starch swelling and water absorption.

Table 6-2. Pasting properties of batter-HHP and control (untreated batter) grass pea flours

Sample type	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak time (min)
GP-600,15	$1086^{b} \pm 2$	$1079^{a} \pm 4$	$7.0^{g} \pm 0.5$	$1272^{b} \pm 2$	$193^{e} \pm 1$	$6.73^{d} \pm 0.02$
Ws-600,15	$586^{f} \pm 3$	$544^{f} \pm 2$	$42^{d} \pm 1$	$845^{e} \pm 2$	$301^{a} \pm 1$	$6.9^{bcd} \pm 0.1$
Lw-600,15	$473^{h} \pm 2$	$446^{h} \pm 3$	$27.0^{e} \pm 0.5$	$714^{f} \pm 2$	$268^{c} \pm 2$	$6.98^{abc} \pm 0.05$
GR-600,15	$488^{g} \pm 1$	$472^{g} \pm 2$	$16.0^{f} \pm 1.5$	$681^{h} \pm 2$	$209^{d} \pm 2$	$6.87^{cd} \pm 0.04$
Control	$1288^{a} \pm 3$	$912^{c} \pm 3$	$376^{a} \pm 3$	$1060^{c} \pm 2$	$148^{f} \pm 4.0$	$4.80^{f} \pm 0.05$
GP-600,25	$804^{d} \pm 3$	$730^{d} \pm 3$	$74^{b} \pm 3$	$1013^{d} \pm 2$	$283^{b} \pm 1$	$7.1^{a} \pm 0.1$
GP-400,15	$592^{e} \pm 2$	$562^{e} \pm 1$	$30^{e} \pm 2$	$690^{g} \pm 1$	$128^{g} \pm 2$	$5.87^{e} \pm 0.03$
GP-600,5	$1072^{c} \pm 1$	$1018^{b} \pm 3$	$54^{c} \pm 2$	$1314^{a} \pm 2$	$296^{a} \pm 3$	$7.00^{ab} \pm 0.05$

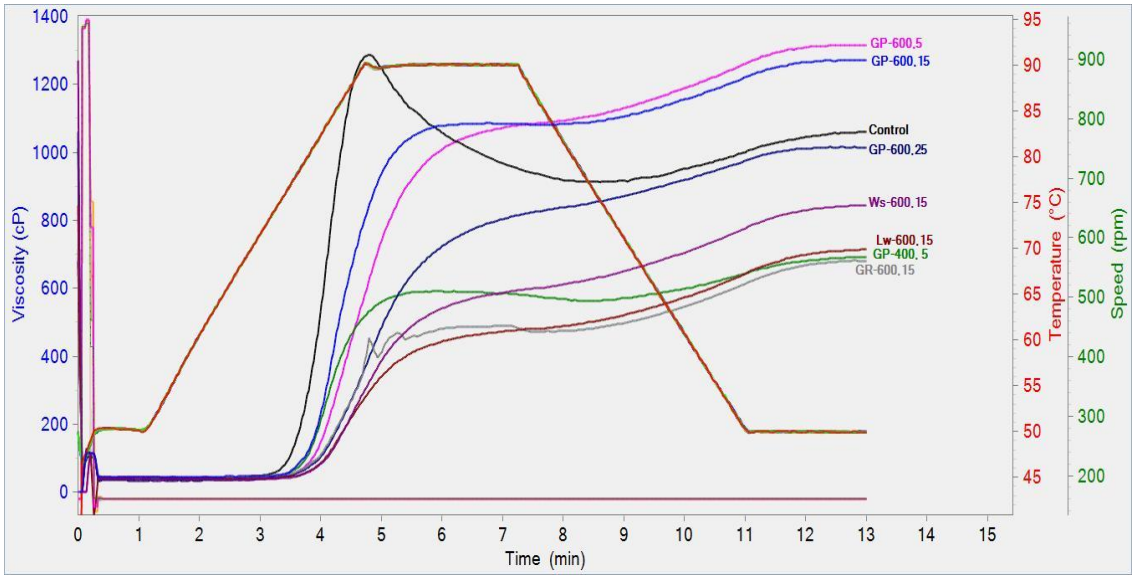

Figure 6-3. Vicrographs of batter-HHP treated, and control (untreated batter) grass pea flours. **Gp**-Grass pea accession having high β-ODAP (GF1-Alemu, AK), Ws Wassie (genetically modified variety), **Lw** Grass pea accession having low β-ODAP (Gp-240038), **Gr**-A variety from Germany. In (100, 5), (400, 15), (600, 25), the first coordinates represent pressure and the second coordinates represent holding time.

6.3. Effect of HHP on water absorption capacity of grass pea seed

The model from the response surface plot and results from ANOVA for the water absorption values before and after the HHP treatment are given in **Figure 6-4** and **Table 6-3**. The R-squared (0.9413), adj. R-squared (0.9143), pred. R-squared (0.7976) values showed that the model adequately explained the results obtained. The regression model for the water absorption showed that the linear term of pressure, soaking time ($p \leq 0.001$), the quadratic term of pressure and soaking time ($p \leq 0.05$) and their interaction ($p \leq 0.05$) were significant whereby pressure had the most prominent effect followed by soaking and holding time. The linear term of holding time ($p \leq 0.05$) was also significant (**Table 6-3**). The 3D response surface plot exhibited increasing water absorption as a function of pressure and soaking time at constant holding time (15 min) (**Figure 6-4**).

Table 6-3. Coefficients of the second-order polynomial model equation and significance of each model and response variables in soaked-HHP treated grass pea seeds

Source	**Water absorption capacity (WAC)**
Intercept-b_0	49.82
Linear-b_1	0.87*
b_2	1.13*
b_3	0.48**
Quadratic-b_{11}	-0.65**
b_{22}	-0.63**
b_{33}	-
Interaction-b_{12}	-0.52**

b_{13}	-
b_{23}	-
Lack of fit (F-value)	30.69***
R-squared	0.9413
Adj. R-squared	0.9143
Pred. R-squared	0.7976
The final quadratic polynomial equation for the responses (coded)	$WA= b_0+b_1A+ b_2B+b_3C-b_{12}AB-b_{11}(A)^2-b_{33}(C)^2$

*Significant at $p \leq 0.001$, **significant at $p \leq 0.05$, *** lack of fit (F-value is significant)

Water uptake of the control (seeds soaked for 6, 9, and 12 h) were found to be 41.30 %, 43.48% and 46.27% respectively. The water uptake of the seeds treated by HHP is given in **Table 6-4**. Percentage water absorption increments ranged from 8.8% to 13.2%; 11.3% to 16.0% and 7.4 to 8.9% after HHP treatment ranging from 200-600 MPa soaked for 6, 9 and 12 h obtained compared to the controls. The water uptake increased as a function of pressure and soaking time at constant holding time (15 min) (**Table 6-4** & **Figure 6-4**). However, pressure was observed to have a more pronounced effect than soaking time on water uptake. The increase in water uptake is linked to the enhancement of the swelling of starch granules by HHP in the flours (Pei-ling et al., 2012; Ahmed et al., 2016). Our result is in good agreement with data reported by Ahmed et al. (2016). They reported excessive water absorption of starch isolated from lentil seeds pressurized by 600 MPa. Ueno et al., (2015) reported a significant effective water diffusion coefficient increase for adzuki beans treated at 200 MPa for 10 min at 25 ^{o}C promoting mass transfer to the

cotyledon of adzuki beans due to structural changes by damaging about 30% of tight testa. The increased water uptake with pressure increase obtained in this study is possibly due to the disruption of microstructures for example cracking of seed coats, breaking of starch networks and protein structures in the cells (Raghupathy et al., 2015). Moreover, the breakdown of the middle lamella which is largely responsible for legume softening (Swanson et al., 1985) could be affected by HHP facilitating water uptake by the seed. Hydration in general is an important step in food processing affecting physico-chemical properties, nutritional quality and functional properties of legume flours positively.

Table 6-4. Central composite face-centered design for soaked-HHP treatment and results (mg/ 100 g)

Soaked-HHP treatment			Water absorption capacity
X_1	X_2	X_3	(%,WAC)
0 (400)	0 (9)	0 (15)	49.84
0 (400)	0 (9)	0 (15)	49.89
0 (400)	-1 (6)	0 (15)	48.86
1 (600)	-1 (6)	-1 (5)	47.83
1 (600)	1 (12)	1 (25)	50.40
0 (400)	0 (9)	-1 (5)	49.51
-1 (200)	1 (12)	1 (25)	49.69
-1 (200)	1 (12)	-1 (5)	49.19
1 (600)	1 (12)	-1 (5)	49.75
0 (400)	0 (9)	1 (25)	49.93

-1 (200)	0 (9)	0 (15)	48.40
1 (600)	0 (9)	0 (15)	50.38
0 (400)	0 (9)	0 (15)	49.69
0 (400)	0 (9)	0 (15)	49.75
0 (400)	0 (9)	0 (15)	49.66
0 (400)	1 (12)	0 (15)	49.97
-1 (200)	-1 (6)	1 (25)	46.75
-1 (200)	-1 (6)	-1 (5)	44.94
0 (400)	0 (9)	0 (15)	49.80
1 (600)	-1 (6)	1 (25)	49.30

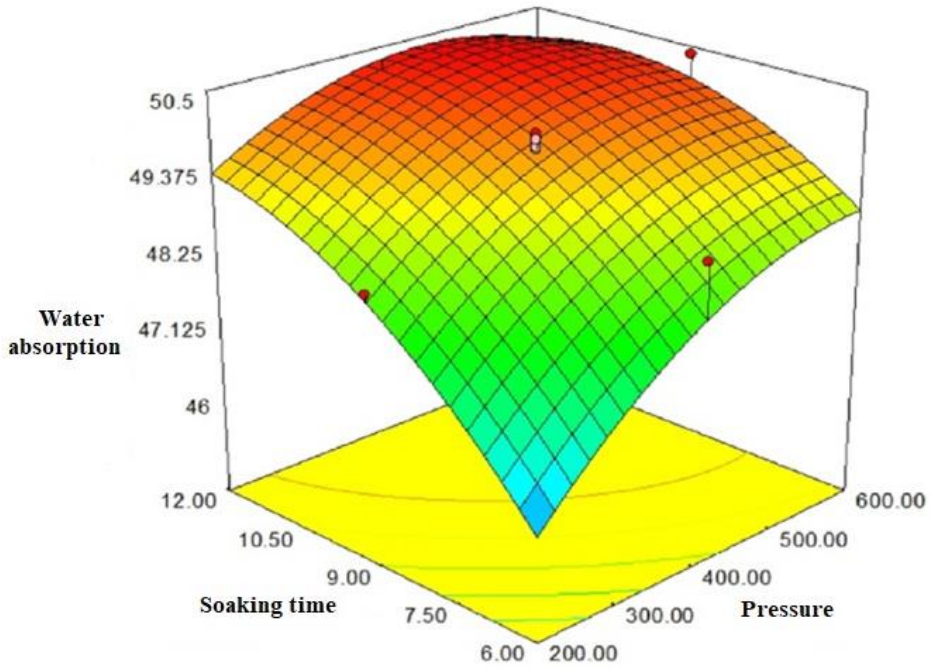

Figure 6-4. Response surface plot of water absorption capacity of grass pea seeds as a function of pressure and soaking time at constant holding time (15min)

6.4. Color change in soaked-HHP and batter-HHP grass pea samples

Color can be affected by different reactions such as pigment degradation, Maillard condensation of amino acids and hexoses as well as oxidation of ascorbic acid (Lee & Coates, 1997). Moreover, processing temperature, pH, acidity and duration are important factors that affect color (Skrede, 1985). Combined processes such as blanching-HHP, temperature-HHP and suitably designed packaging material and treatment equipment were reported to minimize color deterioration (Maskan. 2001). In this study, soaked-HHP and batter-HHP treated samples were considered.

The color (L*, a* and b*) values of untreated grass pea seeds was found to be 88.16, 1.89 and 19.07, respectively (**Table 6-5**). The L* and a* values were higher and the b* value was lower than that of grass pea variety from Italy (86.63, -1.12, 19.88) (Romano et al., 2018). Pattern of seed coat color and cultivar types were supposed to be important factors influencing color.

Table 6-5. Effect of HHP on color of untreated, soaked-HHP treated grass pea samples

Soaked-HHP	**Color parameters**			
Treatment	**L***	**a***	**b***	**ΔE**
00/00/00	$88.16^{a} \pm 0.82$	$-1.89^{h} \pm 0.13$	$19.07^{a} \pm 1.60$	0
100/9/15	$87.38^{b} \pm 0.13$	$-1.24^{g} \pm 0.08$	$16.49^{g} \pm 0.04$	3.73
200/6/5	$86.22^{c} \pm 0.07$	$-1.02^{f} \pm 0.04$	$16.15^{ef} \pm 0.17$	3.66
200/6/25	$85.34^{d} \pm 0.24$	$-1.02^{f} \pm 0.09$	$15.73^{fg} \pm 0.18$	4.51
200/12/5	$82.75^{fgh} \pm 0.39$	$-0.91^{ab} \pm 0.07$	$17.30^{b} \pm 0.28$	5.86

200/12/25	$82.48^{gh} \pm 0.64$	$-0.55^{ab} \pm 0.13$	$16.57^{de} \pm 0.03$	5.77
400/9/5	$81.79^{i} \pm 0.13$	$-0.54^{a} \pm 0.02$	$16.89^{cd} \pm 0.25$	6.28
400/6/15	$83.01^{efg} \pm 0.46$	$-0.62^{ab} \pm 0.08$	$16.83^{cd} \pm 0.46$	5.83
400/9/15*	$83.21^{ef} \pm 0.37$	$-0.64^{bc} \pm 0.02$	$17.32^{bc} \pm 0.45$	6.03
400/9/25	$83.31^{ef} \pm 0.30$	$-0.57^{ab} \pm 0.03$	$17.34^{b} \pm 0.40$	5.41
400/12/15	$83.43^{e} \pm 0.20$	$-0.78^{cde} \pm 0.03$	$17.28^{bc} \pm 0.14$	5.25
600/6/5	$82.49^{e} \pm 0.23$	$-0.84d^{e} \pm 0.04$	$17.59^{b} \pm 0.14$	5.08
600/6/25	$82.72^{fgh} \pm 0.32$	$-0.63^{ab} \pm 0.04$	$16.61^{de} \pm 0.26$	6.18
600/9/15	$82.17^{hi} \pm 1.03$	$-0.69^{abcd} \pm 0.15$	$16.25^{ab} \pm 0.57$	6.69
600/12/5	$83.33^{ef} \pm 0.21$	$-0.77^{ef} \pm 0.01$	$16.90^{ef} \pm 0.06$	5.31
600/12/25	$82.48^{gh} \pm 0.32$	$-0.70^{bcd} \pm 0.14$	$17.16^{bc} \pm 0.32$	6.18

*, **average value of six and five replicates of center points.
All measurements are average values ± SD of five times measurement determinations.
Averages in the same column with different letters are significantly different ($P < .05$).

The color of the grass pea flours was significantly ($p<.05$) affected by HHP for both soaked and batter forms compared to raw seeds (control) in all process combinations (**Table 6-5 & 6-6**). Generally, as pressure increased, lower L* and b*; higher a* values were exhibited in soaked as well as batter-HHP treated samples compared to the control (**Tables 6-5 & 6-6**). These results are in good agreement with those reported by Krebbers et al., (2002) for green beans treated at 500 MPa and those reported by Patras et al., (2009) for carrot purées treated at 500 and 600 MPa. The soaked-HHP and batter-HHP treated grass pea samples were less bright compared to raw samples in general. Seeds soaked for 6, 9 and 12 h and treated at 400 MPa for 15 and 25 min; and seeds soaked for

6 and 12 h treated with 600 MPa for 5 and 25 min exhibited clear declining trend in brightness. The treated samples were darker compared to the controls, but they were not significantly different among each other. The result complies with HHP treated soy-smoothie reported by Andrés et al. (2016). They observed that L* and b* values were progressively decreased as pressure increased. At 200 and 400 MPa, the brightness (L*) of batter-HHP flours were significantly different ($P<0.05$) from the brightness treated at 600 MPa. Nevertheless, the intervals of L* values from the batter-HHP treated flours and control were higher than intervals from soaked-HHP treatments and control. The flours of soaked and batter-HHP treated grass pea seeds exhibited ($p<0.05$) higher a* values (redness) than untreated ones. The trend that the redness (a*) values were high as the pressure, soaking and holding time increased could be derived from the results. However, the a* values were higher in batter-HHP treated samples than soaked-HHP flour (**Table 6-5 & 6-6**). Matser et al., (2000) reported the effect of pressures (600 and 800 MPa) in mushroom treatment exhibited higher a* values compared to the control. Yellowish (b*) values in both HHP treatments were lower than in the controls. Higher decrements were observed at lower pressures (100 and 200 MPa) and higher pressure (600 MPa) with some exceptions. The b* values of the samples were however, less uniform in soaked HHP than batter-HHP treatments. The results revealed that the brightness of treated grass pea samples was highly influenced by pressure, soaking time and holding time. The ΔE^* value of all HHP treated samples were > 3 (**Tables 6-5 & 6-6**) indicating a significant (visible) color differences between treated and control samples. However, the ΔE^* values for batter-HHP treated samples were much higher compared to the ΔE^* values for soaked-HHP treated ones. This might be attributed to the method of preparation (soaked

seed or batter form) and it could be explained as the incorporation of oxygen during batter preparation, and together with process time and process temperature during HHP treatment that might result in oxidation.

Table 6-6. Effect of HHP on color of untreated and batter-HHP treated grass pea samples

Batter-HHP Treatment Color parameters

Batter-HHP Treatment	**Color parameters**			
	L*	**a***	**b***	**ΔE**
00/00	$88.16^{a} \pm 0.82$	$-1.89^{i} \pm 0.13$	$19.07^{a} \pm 1.60$	-
100/15	$82.98^{b} \pm 0.40$	$0.23^{gh} \pm 0.00$	$16.13^{b} \pm 0.21$	6.39
200/5	$82.61^{b} \pm 0.27$	$0.49^{e} \pm 0.04$	$15.94^{bc} \pm 0.13$	6.87
200/25	$81.97^{bc} \pm 0.57$	$0.38^{f} \pm 0.00$	$15.48d^{ef} \pm 0.24$	7.87
400/5	$81.80^{c} \pm 0.10$	$0.17^{h} \pm 0.02$	$15.26^{efg} \pm 0.09$	7.76
400/15**	$81.27^{c} \pm 0.31$	$0.28^{g} \pm 0.03$	$15.66^{cd} \pm 0.12$	7.32
400/25	$80.76^{e} \pm 0.53$	$1.32^{d} \pm 0.08$	$15.57^{cde} \pm 0.32$	8.86
600/5	$79.84^{f} \pm 0.35$	$2.44^{b} \pm 0.03$	$15.11^{fg} \pm 0.23$	10.23
600/15	$80.22^{ef} \pm 0.30$	$2.25^{c} \pm 0.07$	$14.91^{g} \pm 0.22$	9.38
600/25	$79.14^{f} \pm 0.07$	$3.44^{a} \pm 0.02$	$15.12^{fg} \pm 0.01$	11.26

*, **average value of six and five replicates of center points. All measurements are average values ± SD of five times measurement determinations. Averages in the same column with different letters are significantly different ($P < .05$).

6.5. Conclusion

The results obtained from the study revealed that high hydrostatic pressure is able to modify starch in grass pea seeds. The fundamental and basic information obtained from the HHP treatment could invite for further investigation on functional properties of starch isolated from grass pea and other starch-rich legumes and the mechanism behind the changes. The increased water absorption capacity achieved by HHP (600 MPa) is linked to the change in microstructure of grass pea seeds due to the breaking of starch network and disruption of protein matrices by HHP. It was observed from the results that as pressure increased, the viscosity of the soaked seeds was also increased. That implies, the swelling capacity of starch granule was improved by HHP, ultimately resulted in an increased peak viscosity providing the thickening of starch. Batter-HHP treated grass pea flour exhibited also a higher final viscosity and lower setback viscosity than control, indicating stronger starch chain aggregation and lower retrogradation tendency. Thus, HHP increased the ability of a sample to withstand heating and shear stress in pasting process which is an important factor for thickening of sauce, soup, and different products in food processing. In conclusion, the results from functional, physical and structural properties provided fundamental information how and in what extent HHP modified food matrices in the seed. This therefore implies that HHP is one of the emerging technologies that could be used as an alternative in the process of starchy products preparation. The observed effect of HHP treatment on the color of soaked and batter grass pea samples may also negatively affect consumer acceptance because of the unexpected appearance of the final products.

7. EFFECTS OF PHYTASE-SUPPLEMENTED FERMENTATION AND HOUSEHOLD PROCESSING ON THE NUTRITIONAL QUALITY OF *LATHYRUS SATIVUS* L. SEEDS

7.1. 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, (2006b), 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).

7.2. Impact of fermentation on the β-ODAP and the $InsP_6$ content

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 (**Figures 7-1a and 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 7-1a**).

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 & 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 & 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 7-1a**). 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 7-1a**). 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.

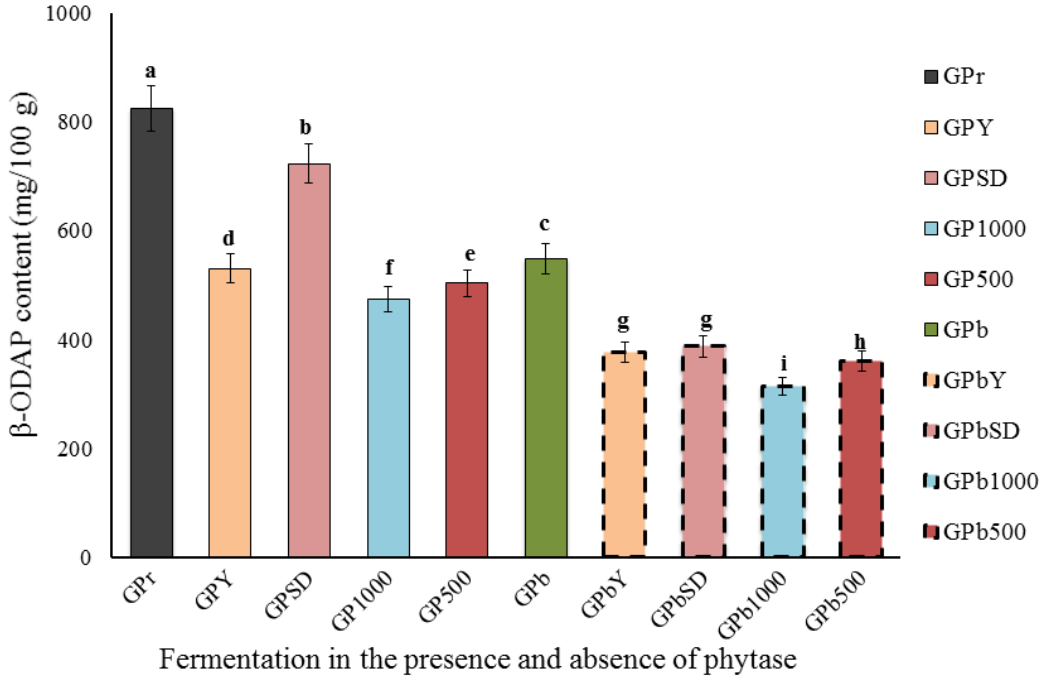

Figure 7-1a. β-ODAP content (mg/100 g DM) of grass pea or grass pea blend flour before and after fermentation in the presence and absence of Natuphos®. **GPr**-raw (unfermented grass pea), **GPY**-yeast fermented grass pea, **GPSD**-sourdough fermented grass pea, **GP1000**-yeast fermented grass pea 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®.

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 7-1b**). Yeast fermentation was observed to result in slightly lower $InsP_6$ levels compared to sourdough 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 & Konietzny, 2006b). 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 & Konietzny, 2006b; 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 7-1b**). 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.

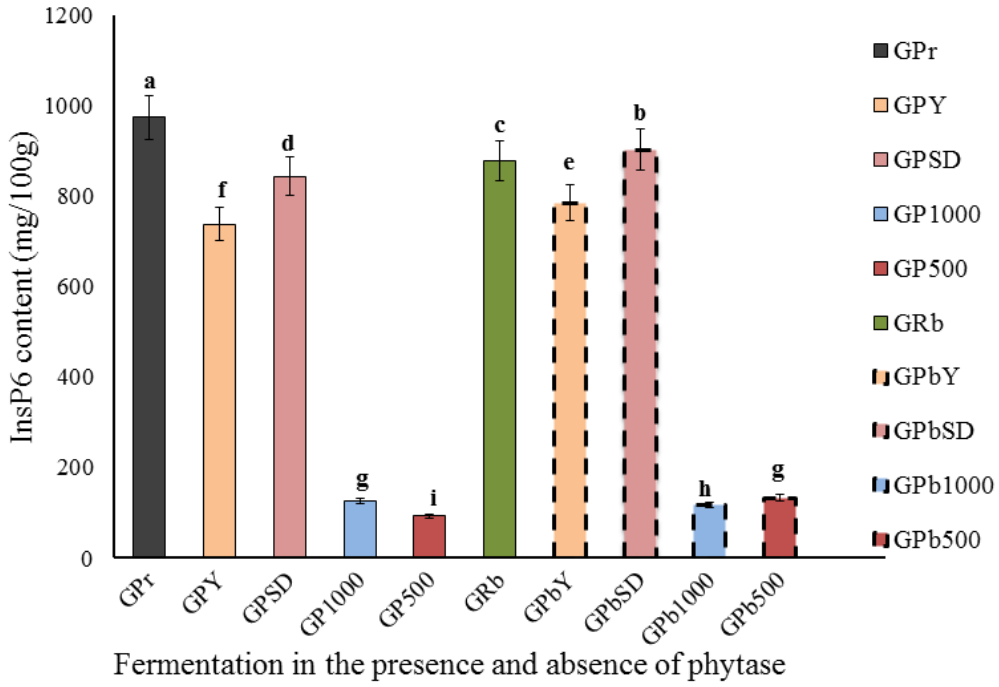

Figure 7-1b. $InsP_6$ content (mg/100 g DM) of grass pea or grass pea blend flour before and after fermentation in the presence and absence of Natuphos®. **GRr**-raw (unfermented grass pea), **GPY**-yeast fermented grass pea, **GPSD**-sourdough fermented grass pea, **GP1000**-yeast fermented grass pea in the presence of 1000 U/kg Natuphos®, **GP500**-yeast fermented grass pea in the presence of 500 U/kg Natuphos®, **GRb**-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®.

7.3. Impact of household processing on grass pea composition

7.3.1. 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 7-2)**. 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 & 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.

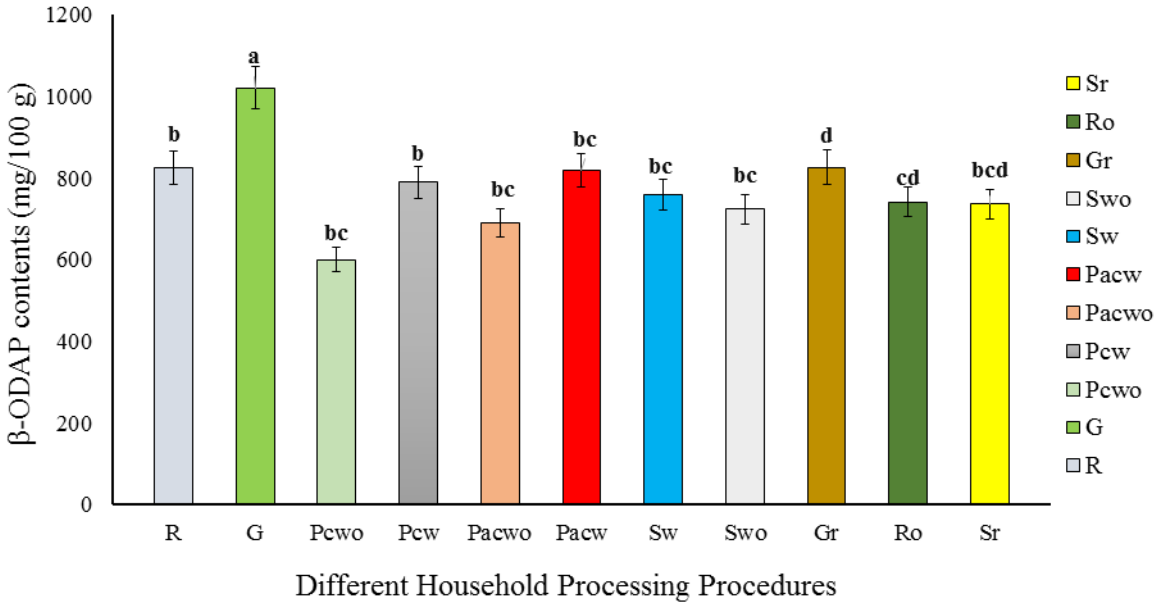

Figure 7-2. β-ODAP contents (mg/100 g DM) of raw and processed grass pea. Values marked by different letters are significantly different to each other ($p < 0.05$). **R**-raw grass pea seeds, **G**-germination, **Pcwo**-soaking followed by pressure-cooking while discarding the soaking water, **Pcw**-soaking 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

7.3.2. 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 7-3).** 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 & Konietzny, 2006b). 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.

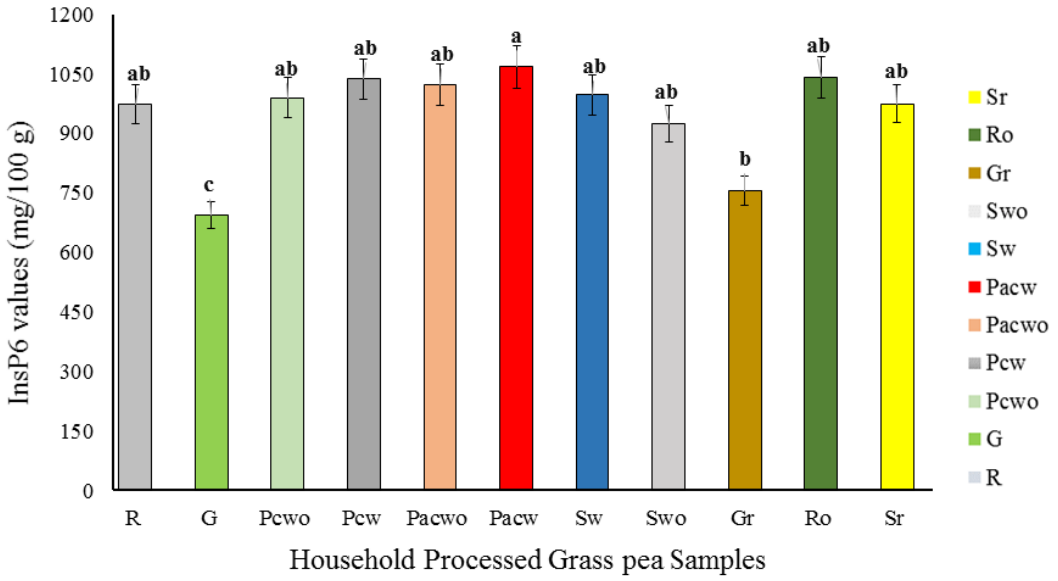

Figure 7-3. Phytate ($InsP_6$) contents (mg/100 g DM) of raw and processed grass pea. Values marked by different letters are significantly different to each other ($p < 0.05$). **R**-raw grass pea seeds, **G**-germination, **Pcwo**-soaking followed by pressure-cooking while discarding the soaking water, **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

7.3.3. Crude protein

The crude protein content of raw grass pea seed was determined to be 26.02 ± 0.04% DM (**Table 7-1)**. 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.

Table 7-1. Total protein concentration of household processed grass pea samples

Samples	**Protein (N*6.25), %**
R	26.02^{g} ± 0.04
Ro	26.60^{f} ± 0.06
Swo	27.60^{de} ± 0.08
Sr	27.87^{cd} ± 0.08
Sw	27.93^{cd} ± 0.08
G	28.70^{a} ± 0.03
Gr	27.3^{e} ± 0.1
Pacw	28.0^{c} ± 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 ($p < 0.05$)

7.3.4. *In vitro* mineral digestibility

Iron, zinc, calcium, and phosphorus contents of raw and different household processed grass pea seeds are shown in (**Table 7-2)**. 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 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.

Table 7-2. 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} ± 0.1	45.3^{e} ± 0.1	1537^{g} ± 5
Sw	63.1^{d} ± 0.4	43.5^{f} ± 0.1	1630^{e} ± 2
G	62.0^{ef} ± 0.4	47.8^{d} ± 0.4	2146^{b} ± 5
Gr	66.6^{b} ± 0.7	80.4^{a} ± 0.9	2270^{a} ± 10
Pacw	62.5^{de} ± 0.4	43.9^{f} ± 0.3	1709^{d} ± 7
Pacwo	63.06^{d} ± 0.04	48.88^{c} ± 0.04	2035^{c} ± 9
Pcw	79.8^{a} ± 0.1	45.04^{e} ± 0.03	1724^{d} ± 6
Pcwo	64.0^{c} ± 0.3	53.70^{b} ± 0.06	2050^{c} ± 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 ($p < 0.05$)

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 7-2**). 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. Furthermore, the transfer of

minerals such as iron from the kitchen utensils used might overcome the loss of the same mineral during soaking. Even if processing resulted in a loss of minerals, the absolute bioaccessible minerals are higher after processing in most cases (**Table 7-3**). 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.

Table 7-3. Absolute bioaccessibility (mg/kg) of iron, zinc, and calcium in raw and processed grass pea seeds with traditional household processes

Samples	**Fe, (mg/kg)**	**Zn, (mg/kg)**	**Ca, (mg/kg)**	**P (mg/kg)**
R	9.43^{c}	771.73^{de}	6517.64^{b}	66873.02^{c}
Ro	9.84^{c}	811.75^{cd}	6548.14^{b}	67820.84^{abc}
Swo	12.16^{c}	1151.45^{a}	7266.29^{ab}	77827.14^{a}
Sr	9.47^{c}	766.77d^{e}	7804.41^{ab}	73201.72^{ab}
Sw	12.98^{c}	940.10b^{c}	6357.00^{b}	67823.14^{abc}
G	304.28^{a}	881.10b^{cd}	8437.34^{a}	67079.58^{abc}
Gr	255.50^{b}	980.10^{b}	7387.22^{ab}	57365.56^{c}
Pacw	17.87^{c}	774.65^{de}	7707.58^{ab}	63771.39^{bc}
Pacwo	10.10^{c}	754.62^{de}	8723.78^{a}	68894.80^{ab}
Pcw	19.76^{c}	767.88^{de}	7828.62^{ab}	70030.23^{ab}
Pcwo	27.58^{c}	648.78^{e}	7709.90^{ab}	62620.55b^{c}

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 ($p < 0.05$).

Bioaccessibilities of Fe, Zn, Ca, and P from raw and household processed grass pea seeds are given in (**Table 7-4)**. 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., 2006c; 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 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.

Table 7-4. 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 ($p < 0.05$).

As expected from other studies (Lopez et al., 2002; Greiner et al., 2006c), 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 7-4**). 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 7-4**). 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 7-4**). Processing of grass peas seeds might have affected their content of insoluble dietary fiber as already shown for other pulses (Azizah & Zainon, 1997; Ramulu & 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.

7.4. 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.

8. CONCLUSIONS AND OUTLOOK (RECOMMENDATIONS)

8.1. Conclusions

The aim of this dissertation was to evaluate the effect of high hydrostatic pressure and some conventional processing technologies on some nutritional qualities of grass pea seeds. In this regard, the first task was to characterize the pea with some nutritional compositions and properties. Huge difference in β-ODAP concentration was obtained among accessions grown in Ethiopia. The same variation was exhibited on the contents of phytate on certain accessions and varieties. Grass pea is evidenced from the study as a good source of proteins, proteinogenic amino acids, and minerals equivalent to most legumes, even more compared to some of them. Difference in water uptake, pasting properties, microstructures was also exhibited among accessions and varieties.

It is revealed that high reduction of β-ODAP having 91% and 71% from low and high β-ODAP-containing accessions were achieved respectively, under the selected pressure (600 MPa), soaking time (9 h) and holding (15 min) time combinations. The grass pea treated by this process conditions also exhibited total removal of phytate. In addition to this, due to starch network breakage and disruption of protein matrices by HHP application resulted to increase water uptake in the seed. The high water uptake of the seed in turn resulted in increased peak viscosity. In general, it is revealed that HHP positively affect the functional, physical and structural properties of the seed which are crucial to develop novel final grass pea products. It was however, observed from the color indicators that HHP caused color deterioration in grass pea flours.

HHP is an emerging non-thermal technology that could be used as an alternative food processing technology in various food industries with its own pros and cons. The technology is costly, and its accesptability by consumers is still unclear. Application of the technology may have however, advantages for food industries in respect to nutrient retention, 'fresh-like' appearance, eco-friendly and energy saving processes compared to some energy intense thermal processing technologies such as sterilization, extrusion and pasteurization. In this regard, it would be highly beneficial to make use of the technology in food processing industries, incubation centers, higher science and technology institutions. Owing the equipment in research centers, not only help to produce various novel products and as a pretreatment, it also used to enable to build the capacity to nearby industries, to carry out various research activities to further develop and practice the technology and further investigations.

In respect to utilizing the grass pea seed in high capacity as it is highly nutritious as well as productive, the HHP technology is revealed to reduce the toxic component in the seed to an extent of safe consumption. The technology could also be used for massive production of various foods and research purpose despite its limitations. Cost, adaptability to the technology, consumer acceptance to the product after treatment might be some of the constraints that could challenge for further utilization of the technology in Ethiopia. However, its applicability for different foods, being used as hudle tehnology as well as toll survice that could provide may possibly compensate these challenges.

Conventional procedures such as soaking, germination, cooking and fermentation with and without exogenous phytase addition were also considered in the study. It is revealed that fermentation and household processing are able to reduce the β-ODAP and phytate content of the seed. Iron and zinc bioavailability are also improved which attribute to phytate reduction. Therefore, these household procedures and spontaneous fermentation are good alternatives for low income society. Phytase supplemented fermentation could be a favorable technology for countries like Ethiopia where the population is highly dependent on fermented products. Thus, fermentation technology for medium and large scale bakery industries as well as for clustered youngsters who are organized for doing businesses in small and medium scale food processing sectors can be an appropriate alternative.

8.2. Outlook and Recommendations

This study mainly investigated the effect that HHP brought to grass pea seed on the contents of toxic compound (β-ODAP) - a non-protein amino acid and an antinutrient - *myo*-inositol phosphate as well as some functional properties and microstructure of selected varieties/accessions of grass pea seeds in soaked and batter forms. Since the technology is emerging, especially in the application of legumes and cereals, it is quite researchable area and this study paves the way for further investigations on grass pea seed and other legumes and cereal grain seeds and products. Due to facility limitation, some missed tasks related to this research work to be further investigated are:

- ✓ Process optimization of HHP parameters on various grass pea accession and cultivars,

- ✓ Effects of protein denaturation, starch deformation and the impact that the technology imparts on phenolic compounds and free amino acids in depth in grass pea seeds.
- ✓ The impact of other antinutrients such as tannin, trypsin inhibitors and the effects they will bring on nutrient quality such as mineral bioavailability upon HHP treatment.
- ✓ Application of HHP on different grass pea products including sensory analysis compared to untreated fresh products,
- ✓ Detailed cost-benefit analysis to ensure the successful application of the technology to more food products,
- ✓ Comparing HHP technology with other emerging technologies,

Furthermore, potential toxic component (β-ODAP) in overall grass pea plant is believed by traditional knowledge as to be accumulated in evaporates from its flowers in the farm or while the seeds are cooked. Thus, evaporates of grass pea should be studied since there is no clear evidence in spite of the ingrained traditional conception. In line with this, indigenous knowledge from Ethiopia is also believes that consuming grass pea meal followed by whole milk aggravates neurolathyrism. The chemistry or the interaction of milk with grass pea meal could also be one research idea for further investigated.

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10. APPENDIX

10.1. Experimental set-up

10.1.1. High hydrostatic pressure

A

B

Figure 10-1. Grass pea seeds and batter treated in HHP (A) & Freeze dried and ready for analysis (B)

10.1.2. Spectrophotometric β-ODAP analysis

Figure 10-2. β-ODAP analysis: extraction to spectroscopic absorption detection

10.1.3. Procedure for *myo*-inositol-phosphate analysis by HPLC

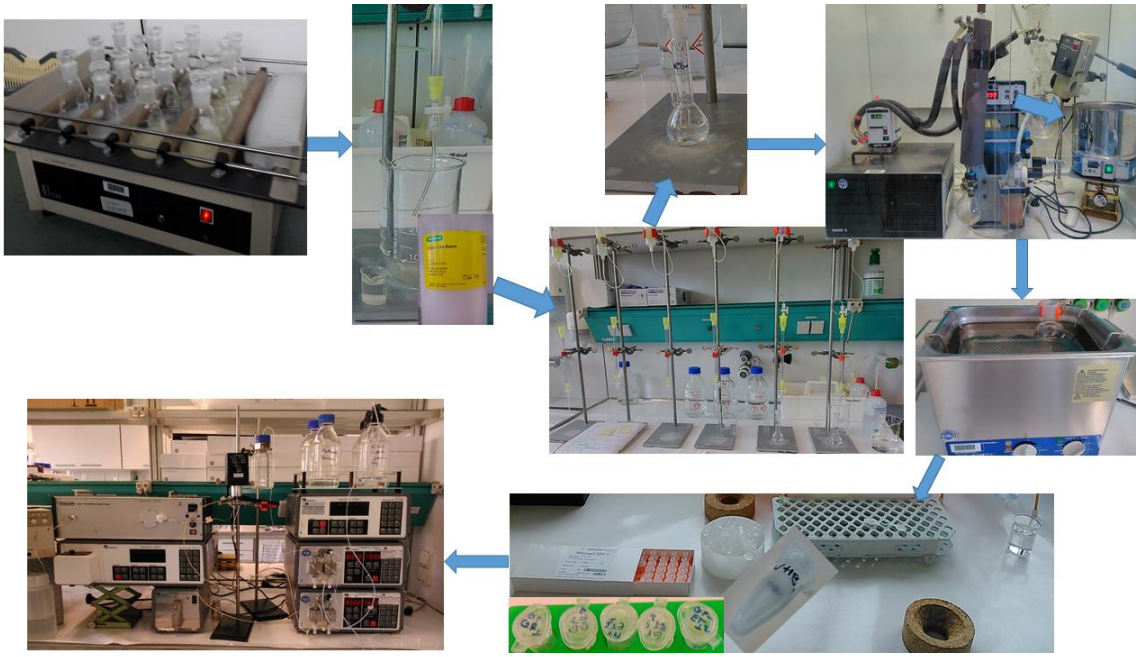


Figure 10-3. $InsP_6$ analysis: extraction to HPLC detection

10.1.4. *In vitro* mineral bioaccessibility and ICP-MS for mineral analysis

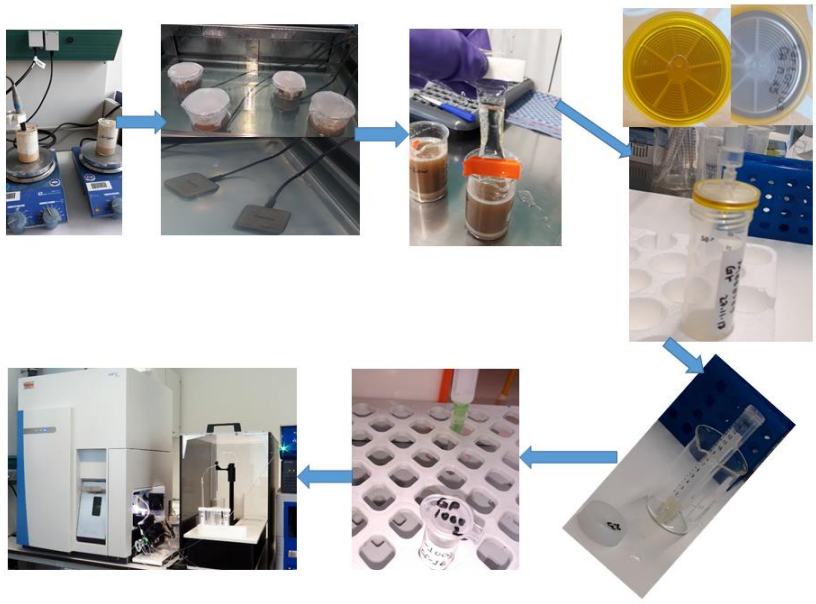


Figure 10-4. *In vitro* mineral bioaccessibility and ICP-MS mineral analysis

10.2. Sample data as supportive information

10.2.1. Calibration curve for β-ODAP quantification

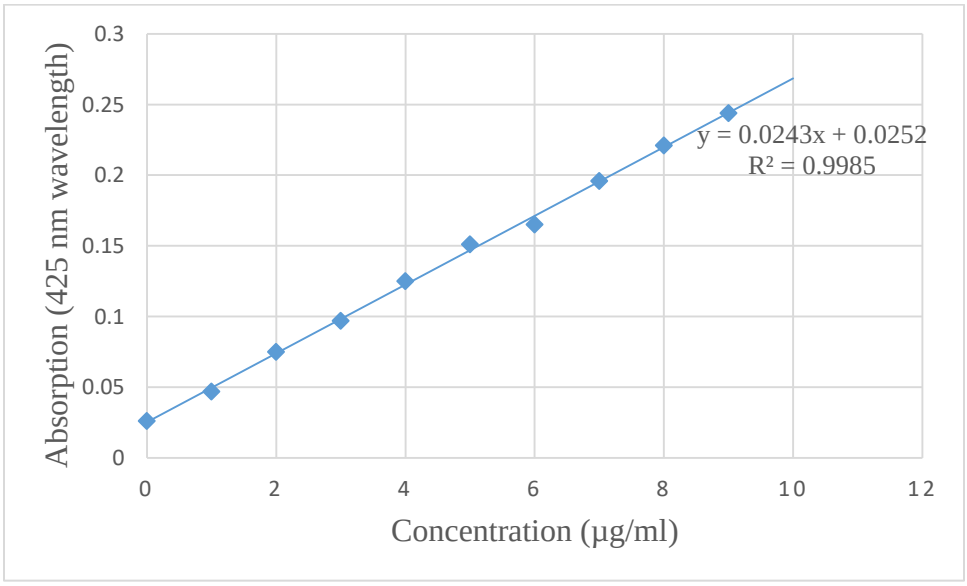


Figure 10-5a. Calibration curve for β-ODAP quantification

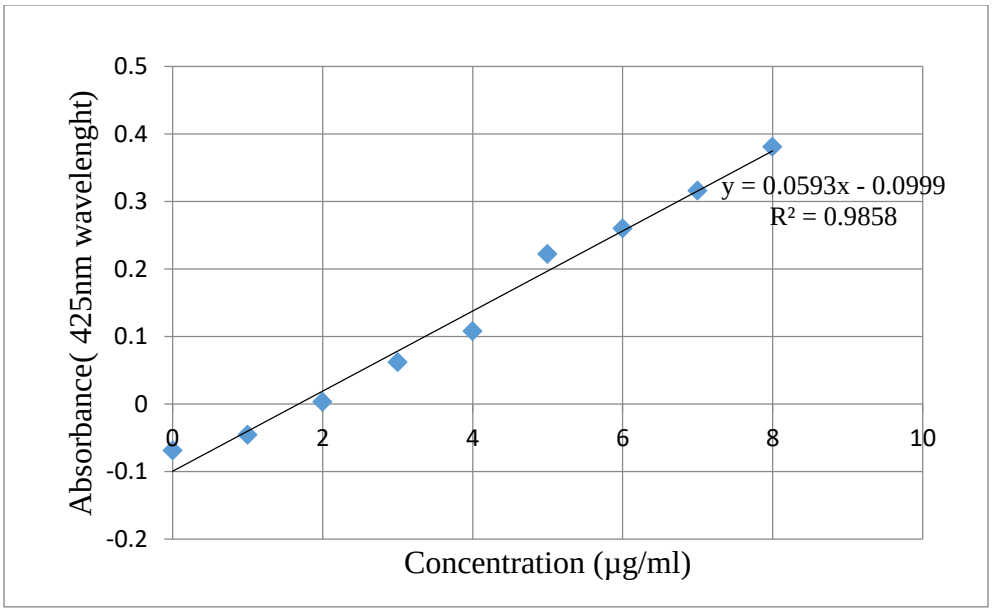


Figure 10-5b. Calibration curve for β-ODAP quantification

10.2.2. Chromatograms of proteinopgenic amino acids

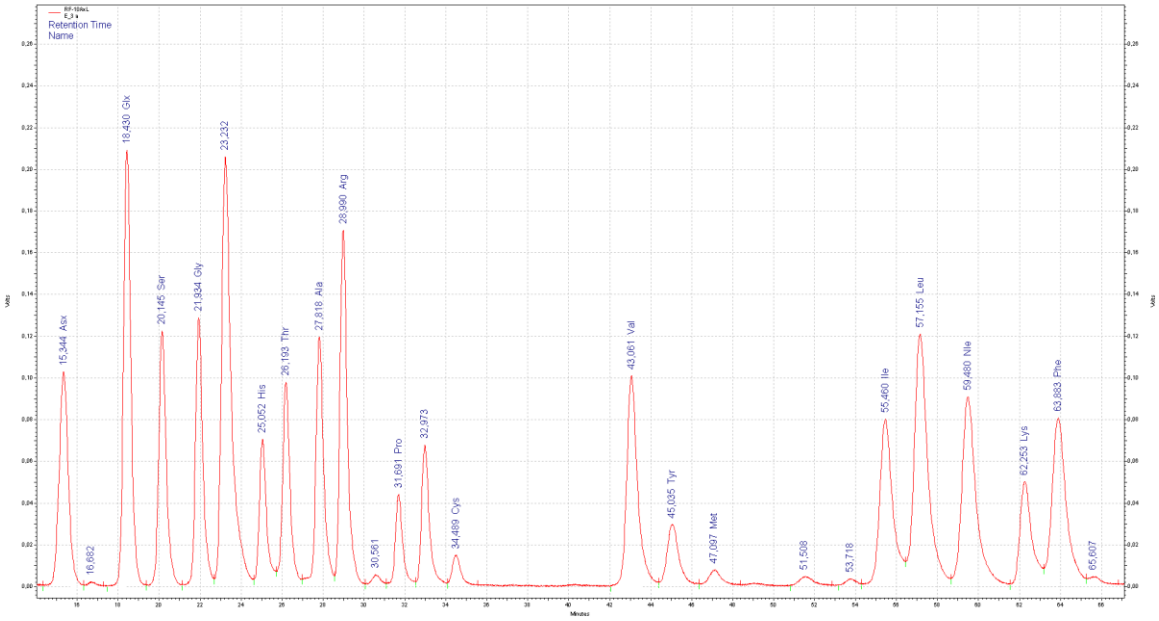


Figure 10-6a. Sample chromatograms of eighteen proteinogenic amino acids

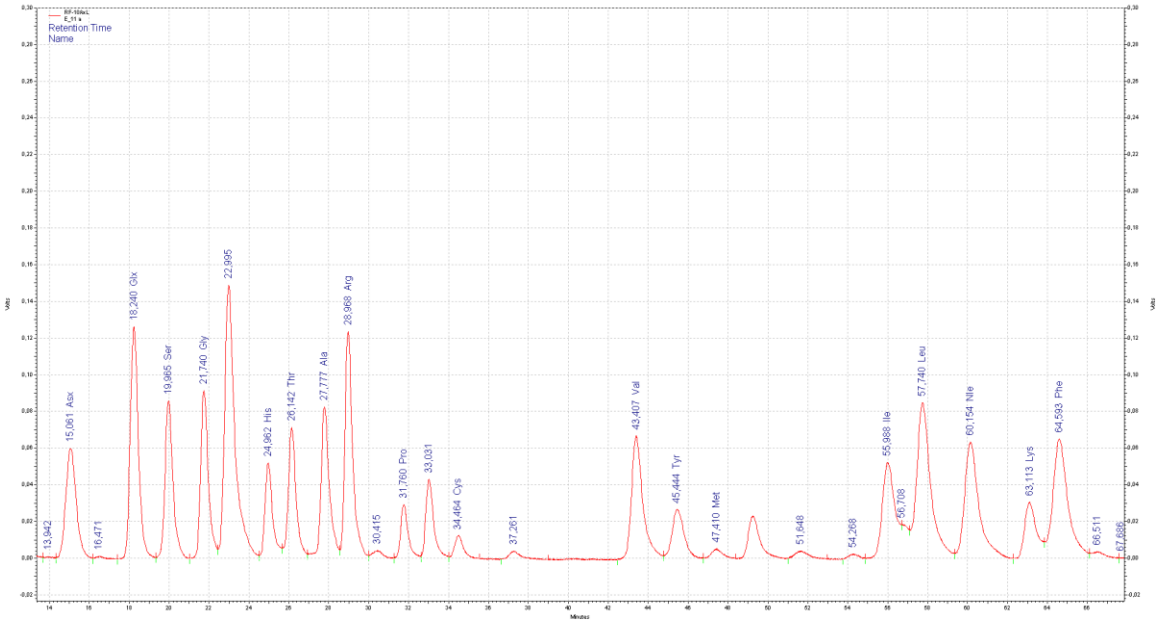


Figure 10-6b. Sample chromatograms of eighteen proteinogenic amino acids

10.2.3. Chromatograms of *myo*-inositol phosphates

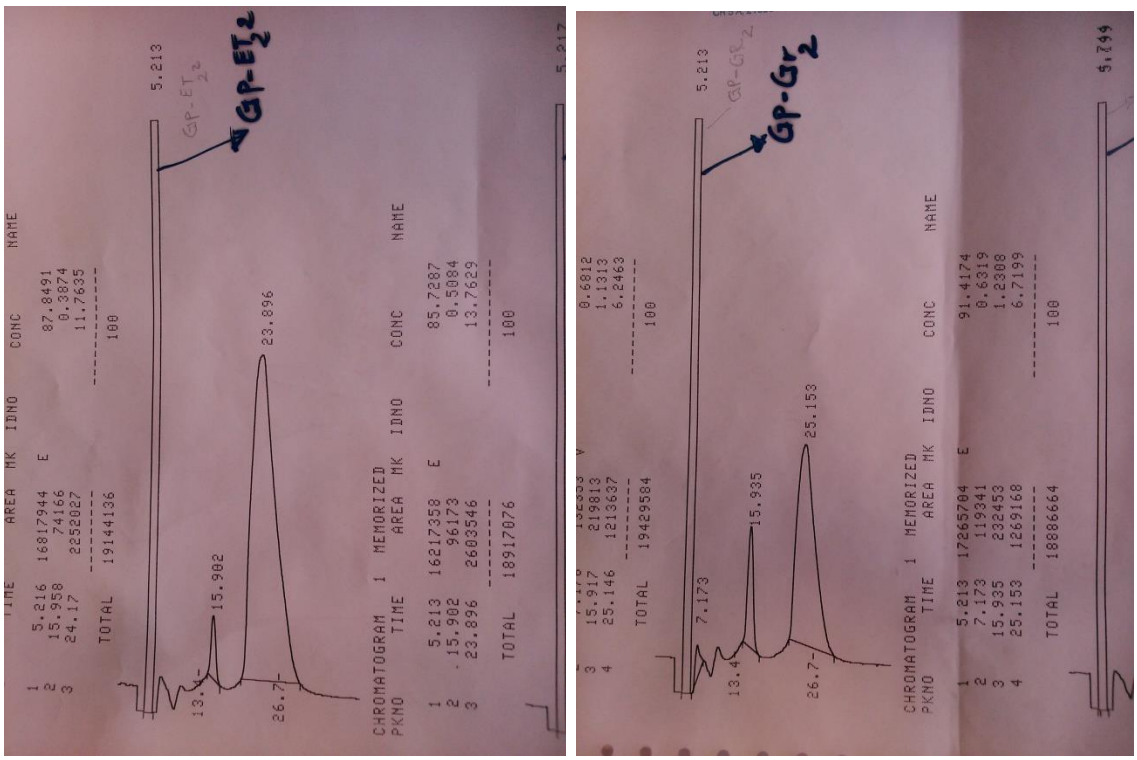


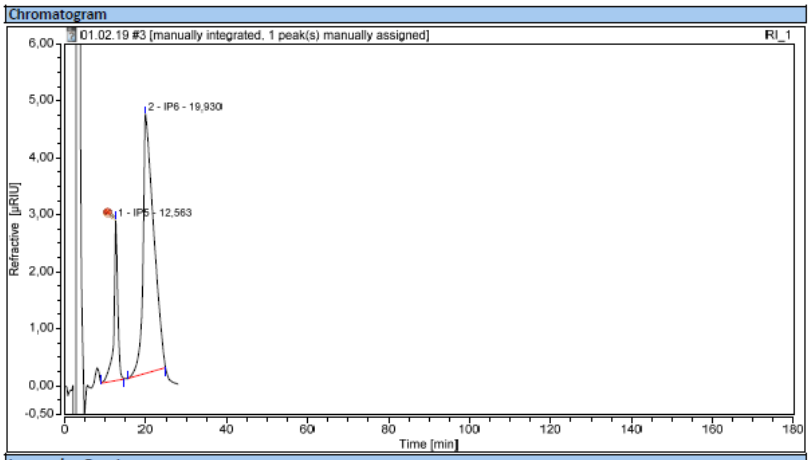
Figure 10-7a. Chromatograms *myo*-inositol phosphate

Chromatogram and Results

Injection Details

Injection Name:	**Rw2**	*Run Time (min):*	**28,00**
Vial Number:	**RA3**	*Injection Volume:*	**20,00**
Injection Type:	**Unknown**	*Channel:*	**RI_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**28min_Standard_Methode flow 0,25**	*Bandwidth:*	**n.a.**
Processing Method:	**IP Auswertung**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**01.Feb.19 13:41**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

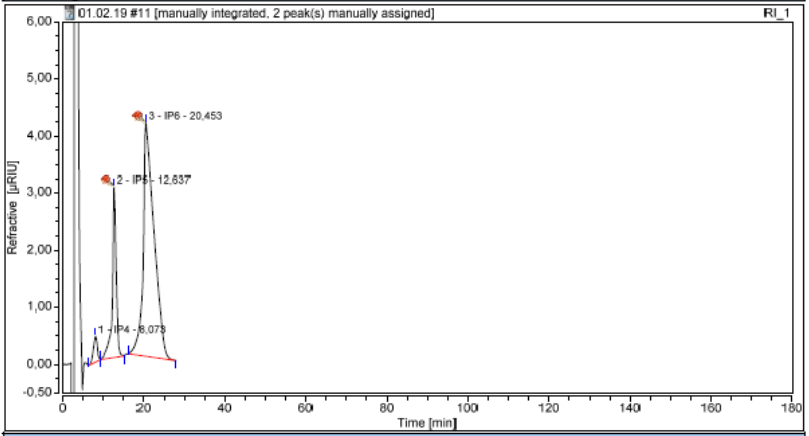
No.	Peak Name	Retention Time min	Area µRIU*min	Height µRIU	Relative Area %	Relative Height %	Amount
n.a.	IP3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
n.a.	IP4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	IP5	12,563	3,068	2,821	18,22	38,27	0,4801
2	IP6	19,930	13,765	4,551	81,78	61,73	1,4373
Total:			**16,832**	**7,372**	**100,00**	**100,00**	

Chromatogram and Results

Injection Details

Injection Name:	**Pcw2**	*Run Time (min):*	**28,00**
Vial Number:	**RB3**	*Injection Volume:*	**20,00**
Injection Type:	**Unknown**	*Channel:*	**RI_1**
Calibration Level:		*Wavelength:*	**n.a.**
Instrument Method:	**28min_Standard_Methode flow 0,25**	*Bandwidth:*	**n.a.**
Processing Method:	**IP Auswertung**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**01.Feb.19 17:31**	*Sample Weight:*	**1,0000**

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area µRIU*min	Height µRIU	Relative Area %	Relative Height %	Amount
n.a.	IP3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1	IP4	8,073	0,511	0,456	3,11	6,05	0,1370
2	IP5	12,637	3,244	2,990	19,72	39,70	0,4993
3	IP6	20,453	12,693	4,087	77,17	54,25	1,3444
Total:			**16,448**	**7,533**	**100,00**	**100,00**	

Figure 10-7b. Chromatograms *myo*-inositol phosphate

10.2.4. 2D and 3D images of HHH treated and untreated grass pea seeds

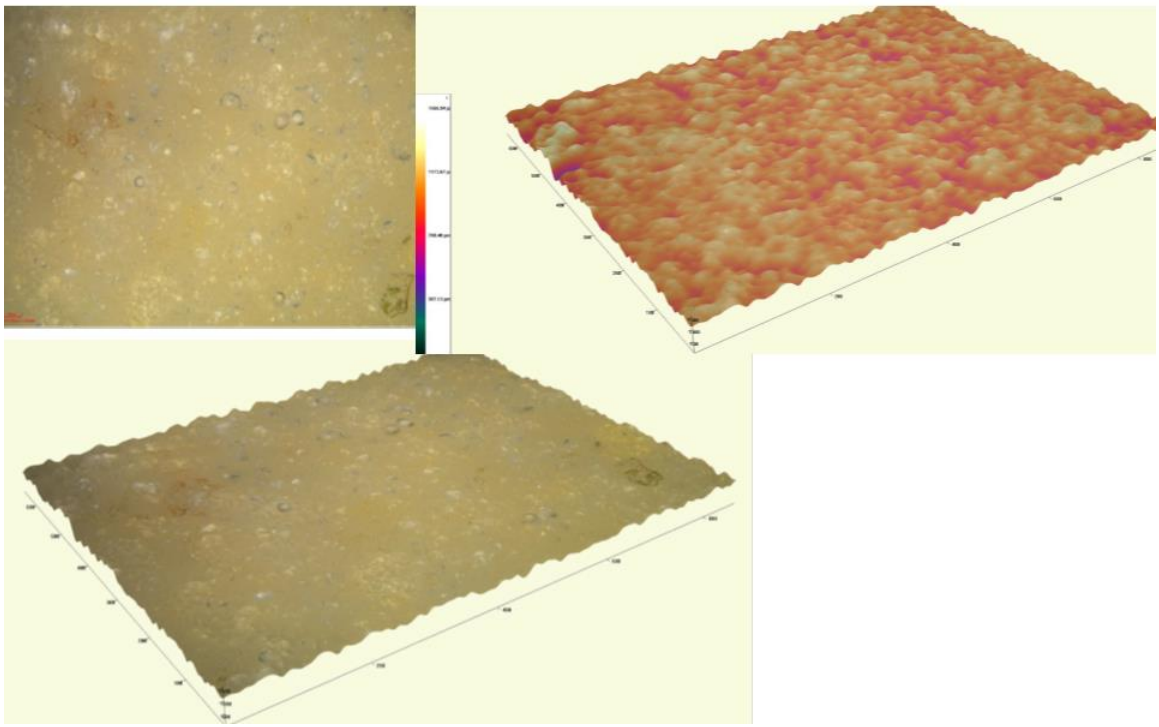


Figure 10-8a. Image of soaked grass pea seed

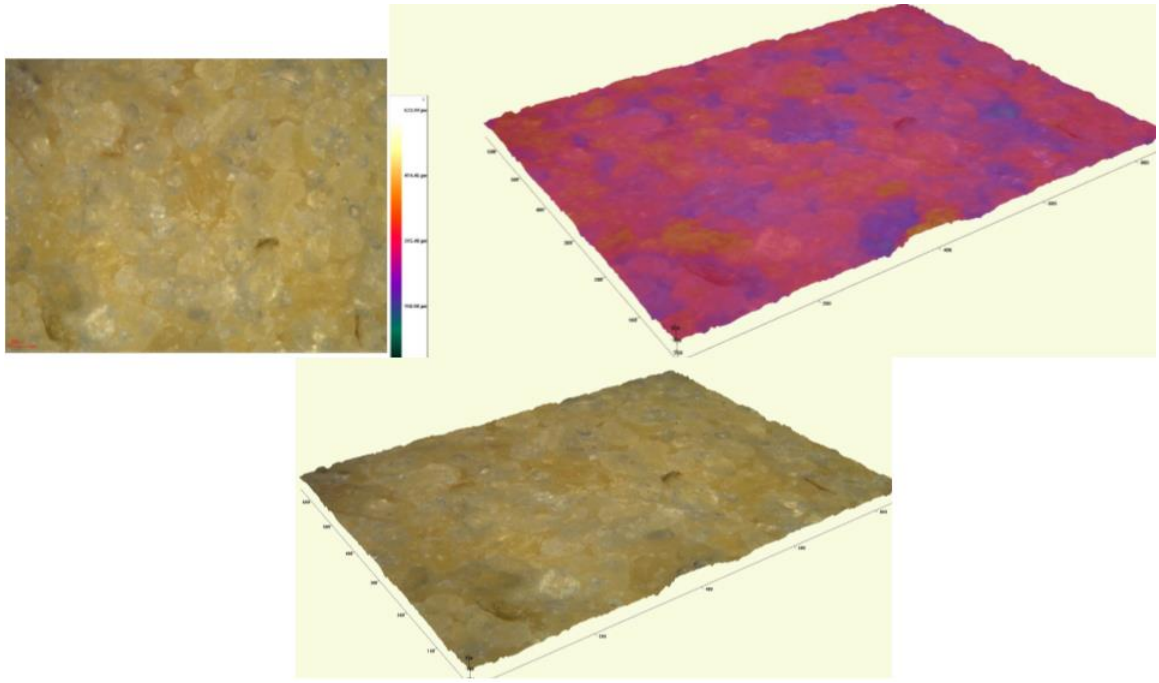


Figure 10-8b. Image of soaked-HHP treated grass pea seed

10.3. Symbols and abbreviations

10.3.1. Symbol

β	Beta
α	Alpha
L*	Whiteness or brightness/darkness
a*	Redness/ greenness
b*	Yellowness/blueness
ΔE	Total color difference

10.3.2. Abbreviations

β-ODAP	β-N-oxalyl-L-α,β-diaminopropionic acid
$InsP_6$	*myo*-inositol(1,2,3,4,5,6)hexakisphosphate
InsP5	*myo*-inositol(1,2,3,4,5)heptakisphosphate
HHP	High hydrostatic pressure
GP	Grass pea
AK	Akaki
GR	German
CCFCD	Central composite face centered design
NEAAs	Non-essential amino acids
EAAs	Essential amino acids
TAAs	Total amino acids
TNEAAs	Total non-essential amino acids

TEAAs	Total essential amino acids
RSM	Response surface methodology
BC	Before Christ
CNS	Central nervous system
NO	Nitric oxide
ROS	Reactive oxygen species
RF	Radio-frequency
DM	Dry matter
PEF	Pulsed electric field
NPA	Non-protein amino acids
FPA	Free protein amino acids
DZARC	Debre Zeit Agricultural Research Centre
EIAR	Ethiopian Institute of Agricultural Research
MRI	Max Rubner Institute
KIT	Karlsruhe Institute of Technology
HCL	Hydrochloric acid
DAP	Diaminopropionic acid
KOH	Potassium hydroxide
$K_2B_4O_7$	Potassium tetraborate
OPA	*o*-phthalaldehyde
AAM	Ammonium molybdate
HNO_3	Nitric oxide
R	Raw seed

Ro	Roasted seed
Pcwo	Pressure-cooked seed without soaking water
Sw	Soaked seed with soaking water
Swo	Soaked seed without soaking water
Sr	Soaked-roasted seed
G	Germinated seed
Gr	Germinated-roasted seed
Pacw	Pan-cooked seed with soaking water
Pacwo	Pan-cooked seed without soaking water
Pcw	Pressure-cooked seed with soaking water
ISO	International Organization for Standardization
HPLC	High performance liquid chromatography
SOR	Solvent rack
P	Pump
ASI	Automated sample injector
TCC	Thermostated column compartment
RF	Fluorescence detector
TCEP	Tris (2-carboxyethyl) phosphine hydrochloride
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LOQ	Limit of quantification
SEM	Scanning electron microscopy
ANOVA	One-way analysis of variance
Acc.	Accession

Var.	Variety
HMF	Hydroxymethyl)-2-furfural
WAC	Water absorption capacity

10.4. Publications and conferences

Peer-reviewed publication

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 Research International, 120, 73-82. https://doi.org/10.1016/j.foodres.2019.02.011

Buta, M. B., Posten, C., Emire, S. A., Meinhardt, A. K., Müller, A., & Greiner, R. (2020). Effects of phytase-supplemented fermentation and household processing on the nutritional quality of *Lathyrus sativus* L. seeds. Heliyon, 6(11), 1–10. https://doi.org/10.1016/j.heliyon.2020.e05484

Impact of High Hydrostatic Pressure on microstructure, pasting properties, water absorption and color of grass pea (*Lathyrus sativus* L.) seed and flour - **submitted** to journal of Food Research International (**Ms. Ref. No.: FOODRES-D-21-00027**)

Regional and international conferences

Buta, Meseret Bekele, Ralf Greiner, Clemens Posten, Astrid Beinhauer, S. A. (2018). β - ODAP and IP_6 quantification & degradation from *Lathyrus sativus* L. Arbeitstagung 2018, Lebensmittelchemische Gesellschaft, Regionalverband Südwest, 06.-07. März 2018, Gießen, 2018.

Buta Meseret Bekele, Ralf Greiner, Clemens Posten, Astrid Beinhauer, Emire Shimelis

Admassu. Degradation of β-ODAP and IP_6 from *Lathyrus sativus* L. Seed by high hydrostatic pressure. The Food System Approach: New Challenges for education . Research and industry, Book of Abstracts. Session 2: RESEARCH - Research and Innovation Across Baundories - Poster.