



ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL SCIENCE

FOOD SCIENCE AND NUTRITION GRADUATE PROGRAM

EFFECTS OF TRADITIONAL FOOD PROCESSING METHODS ON NUTRIENT
COMPOSITIONS AND ANTI-NUTRITIONAL FACTORS OF GRASS PEA (*Lathyrus
sativus* L.) FOODS CONSUMED, IN ETHIOPIA

BY

GASHAW GOBEZIE

ADDIS ABABA

ETHIOPIA

June, 2010

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UNDER THE SUPERVISION OF Mr. KELBESSA URGA AND Dr. GULELAT DESSE

A thesis submitted to Graduate Studies Program of Addis Ababa University in partial
fulfillment of the requirements for the Degree of Master of Science in Food Science and
Nutrition

June, 2010

Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other University, and that all sources of materials used for the thesis have been duly acknowledged.

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List of abbreviations and acronyms

AR = analytical reagent grade

CSA = Central Statistical Agency

DAP =DL- α , β -diaminopropionic acid

EHNRI = Ethiopian Health and Nutrition Research Institute

IPGRI =International Plant Genetic Resources Institute

NRCS = Natural resource conservation service

OPT =Orthophthalaldehyde

TI =Trypsin Inhibitor

TIA =Trypsin Inhibitor Activity

USDA = United States Department of Agriculture.

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Abstract

The study was conducted to determine the effects of various traditional processing methods such as roasting, boiling, preparing unleavened bread (*kitta*) and sauce (*shiro wott*) on nutrient composition, and the level of anti-nutritional factors of grass pea (*Lathyrus sativus*). Mean moisture, crude fiber, crude protein, crude fat, total ash and crude carbohydrate contents of raw grass pea were 8.26, 8.57, 22.81, 0.61, 2.75 and 65.26%, respectively. The changes in moisture, crude protein, crude fat, total ash and crude carbohydrate contents were found to be significant for the processing methods. There were 9.47 % and 10.39% increased in protein content of roasted and boiled while there were 6.22 % and 8.46 % of lowered value of crude protein in unleavened bread and sauce. The increase in fat content of roasted, boiled and unleavened samples was found to be significant but not sauce samples. Roasted, boiled and sauce samples were decreased in ash content by 8.36 %, 16.36% and 16 %, respectively. The Ca contents increased in boiled and roasted samples. 38.45 % and 14.34 % of calcium content were decreased in unleavened bread and sauce. The P and Zn contents of grass pea samples almost in all processing methods showed significant difference. There was no a significant difference ($P < 0.05$) in the iron content due to roasting, boiling and sauce making. In unleavened bread and roasted samples, the Fe and Zn contents were increased. The results indicated that the processing methods had considerable effect on the nutrient composition and mineral contents. The raw grass pea seeds contained considerable amounts of phytic acid, (579.76 mg/100 g), tannin (352.04mg/100g and β -ODAP (421.18mg/100g). These were reduced significantly ($P < 0.05$) due to the traditional processing methods. The phytate

content was decreased by 33.21 %, 29.76 %, 70.98 % and 74.32 % respectively in roasted, boiled, unleavened bread and sauce samples. Tannin content was also decreased by 54.06 %, 33.21 %, 91.67 % and 83.11 % in roasted, boiled, unleavened bread and sauce samples respectively. Similarly, the content of β -ODAP was reduced by 12.03 %, 38.27 % and 47.22 % after roasting, boiling and preparing sauce respectively. 52% of the total phosphorus was present as phytate phosphorus in the unprocessed grass pea seeds. The traditional processing methods resulted in a significant decrease in phytate phosphorus with a corresponding marked increase in non-phytate phosphorus. Preparing sauce was found to be the best processing method, followed by unleavened bread, boiling and roasting seeds for reducing the content of phytate phosphorus. The processing method decreased the phytate: iron and phytate: zinc molar ratio and increased the calcium: phytate molar ratio. Boiling and preparing sauce (*shiro wott*) of grass pea were found to be the best cooking methods for healthy eating.

Key words: Grass pea, nutrient composition, anti-nutritional factors, phytate, tannin, β -ODAP, traditional food processing methods

Chapter 1

Introduction

1.1. Background

Plants are the most important sources of food for human beings. Among plant sources, legumes and legume products are important sources of protein in the diets of millions of people in the world (Ramakrishna *et al.*, 2006).

The leguminosae constitute the second largest family of plants (second only to the Gramineae), with about 650 genera and more than 18,000 described species (Delwiche, 1978). The genus *Lathyrus* in the family *Leguminosae* (*Fabaceae*) is large with 187 species and subspecies recognized. However, only one species *Lathyrus sativus* (grass pea) is widely cultivated as a food crop, while other species are cultivated to a lesser extent for both food and forage. Over the past decade, grass pea has received increased interest as a plant that is adapted to arid conditions and contains high levels of protein, a component that is increasingly becoming hard to acquire in many developing areas (Campbell, 1997).

The contribution of legumes to the nutrition of the consumer is limited principally due to the presence of anti-nutritional factors (Ramakrishna *et al.*, 2006). Manifestations of toxicity from the consumption of legumes containing anti-nutritional factors range from severe reduction in food intake and nutrient availability or utilization, to profound neurological effects and even death (Bhat and Raghuram 1993). Some of the known natural constituents, which affect their nutritional quality, are specific enzyme activity inhibitors, haemagglutinin,

saponins, tannins, anti-vitamins and phytic acid. The presence of phytic acid interferes with mineral element absorption and utilization and reacts with proteins to form complex products, which have an inhibitory effect on peptic digestion. The presence of tannin has been associated with lower nutritive value and lower biological availability of macromolecules, such as proteins and carbohydrates (Elsheikh, *et al.*, 2000).

Grass pea is an important crop of economic significance (Campbell, 1997) and was already cultivated in the Balkans around 8000 BC. In archaeological excavations in Turkey and Iraq, seeds of *Lathyrus* species were found as collected or cultivated items. Grass pea is also known as chickling pea, khesari, guaya or sabberi. It is a major crop in parts of Asia (Bangladesh, China, India, Nepal, and Pakistan) and to a lesser extent in the Middle East (Iraq, Iran, Afghanistan, Syria, and Lebanon), in southern Europe (France and Spain) and in northern Africa (Ethiopia, Egypt, Morocco, Algeria, and Libya) (Marian. *et al.*, 2001).

This plant is endowed with many properties that make it an attractive food crop in drought stricken, rain-fed areas where soil quality is poor and extreme environmental conditions prevail. Despite its tolerance to drought, it is not affected by excessive rainfall and can be grown on areas subjected to floods. It has a very hardy and penetrating root system and therefore can be grown on a wide range of soil types, including very poor soils and heavy clays. This hardiness, together with its ability to fix atmospheric nitrogen, makes the crop one that seems designed to grow under adverse conditions. Compared with other legumes, grass pea is resistant to many pests including storage insects (Campbell, 1997).

The main anti-nutritional factors occurring in grass pea include protease inhibitors (trypsin inhibitors), phytic acid, tannins, and β -ODAP. The major physiological effect of trypsin inhibitor is to inhibit the action of the enzyme trypsin found in the digestive tract of humans and animals and to cause the enlargement of the pancreas and secretion of excessive amounts of pancreatic enzymes much of which is lost to the animal in feces (Urga *et al.*, 2005). Phytic acid binds trace and macro-elements such as zinc, calcium, magnesium, and iron, in the gastrointestinal tract and making dietary minerals unavailable for absorption and utilization by the body. It can also form complexes with proteins, proteases and amylases of the intestinal tract, thus inhibiting proteolysis. Moreover, the phosphorus in phytate has been considered largely unavailable to the organism because of the limited capacity of monogastric species to hydrolyze phytate in the small intestine. Tannins are polyphenolic compounds of plant origin. Phenolic compounds are responsible for the bitterness and astringency of many foods and beverages. (Ramakrishna *et al.*, 2006).

1.2. Statement of the problem

The production of plant products has increased significantly during the last decades. Thus, there is an increasing demand for use of food legumes especially grass pea seeds in animal and human nutrition. Grass pea has high potential in the provision of high levels of protein, carbohydrates, and minerals for humans. The main limitation is the presence of various anti-nutritional factors and the neurotoxin β -ODAP, which could greatly undermine the potentials. Grass pea is very tasty, nutritious, easily cultivated and hardy crop. It tends to replace the staple cereal- based diet of the people of rural north and central Ethiopia during times of acute food shortage. Because of its drought-tolerant nature, it is extensively

cultivated in the highlands of north and central Ethiopia where cases of lathyrism are seen both sporadically and in the form of epidemics (Lambein *et al.*, 2007). In Ethiopia, there was a strong epidemiological association between excessive consumption of grass pea and the development of an irreversible crippling disorder of the legs, known as neurolathyrism in humans and animals (Urga and Gebretsadik, 1993).

Very little attention was being given to its improvement for varietals development. Four decades of plant breeding research to eliminate this non-protein amino acid β -ODAP from the seed has produced large numbers of “low-toxin” varieties of grass pea and in Ethiopia a new variety ‘*wassie*’ is released recently, but did not yet result in the development of “toxin-free” varieties. A major problem in this endeavor is the high variability of the β -ODAP biosynthesis. Environmental factors such as drought, zinc deficiency, iron oversupply and the presence of heavy metals in the soil can considerably increase the level of β -ODAP in the seeds grown in farmers’ fields as compared to more optimal experimental fields. The presence of cadmium in the soil can increase the β -ODAP level up to six-fold (Lambein *et al.*, 2007).

The production of grass pea in Ethiopia is not produced for export (the neurotoxin has constrained commercial releases) and not incorporated in the list of Ethiopian Seed Enterprise. In a region where grass pea is used as staple food, protein energy malnutrition and lathyrism appear frequently. One of the reasons is due to the presence of anti nutritional factors, which inhibits the digestibility of food and the bioavailability of essential minerals and trace elements. The other more serious reason is that when the grass pea is consumed by the people as staple food for 3-4 months it causes lathyrism (Malek *et al.*, 1995). The

valuable crop but becoming the threatening crop when it is consumed as staple food will remain as neglected crop in the production until toxin-free lines are released. Therefore, the aim of this research was to evaluate the various traditional processing methods on nutrient composition of grass pea and select the efficient processing method, which makes the food more safe that can prevent lathyrism and protein energy malnutrition.

1.3 The scope of the study

Most studies on the quality of grass pea in Ethiopia have centered mainly on its agronomic characters, resistance to disease, pest, high yield, adaptation, and content of β -ODAP in selected germplasm collections and prevalence of neurolathyrism (Urga *et al.*, 2005). Information on nutrient composition and anti-nutritional components of grass pea is very limited. New cultivars of peas are continually being developed and released from agricultural research centers. When new genotypes come into application information is needed to identify its nutritional benefits as well as the processing methods for reduction/elimination of anti-nutrients. The economic values of new cultivars depend on not only its low β -ODAP, resistance to disease, its yield, rate of maturity, and seed size but also its nutrient composition, quality and the flavor and the texture of the cooked food.

Very little was known about the nutrient composition and quality, anti-nutrients and their methods of reduction via different processing techniques of grass pea varieties released from Debre Zeit and Adet Agricultural Research Centers. This gap did not allow intensive utilization of grass pea varieties as a value-added product (food/feed) efficiently.

Several methods for the determination of β -ODAP in grass pea were carried out in different laboratories mainly in connection with development of low β -ODAP varieties, for large-scale cultivation. Different traditional processing methods including roasting, boiling, preparation of sauce and unleavened bread food samples were collected and assayed for β -ODAP levels (Teklehaimanot *et al.*, 1993). The effect of soaking time and soaking solution on the nutritional quality of grass pea seeds were investigated (Urga and Gebretsadik 1993). The effect of cooking, roasting, autoclaving and fermentation on the content of β -ODAP in the whole seeds and flour of grass pea were determined at different levels of temperature, time, pH, degree of soaking and moisture content (Akalu *et al.*, 1998). Physico-chemical and functional properties of starch and fiber in raw and processed grass pea seeds were evaluated (Akalu *et al.*, 1998). The nutritional and anti-nutritional factors of twenty-five grass pea germplasm accessions were analyzed (Urga *et al.*, 1995). The proximate composition, mineral contents and anti-nutritional components of local grass pea land-races collected from farmers' fields in different regions had been evaluated (Urga *et al.*, 2005). Effect of different processing techniques (Extrusion, fermentation, germination and autoclaving) on the nutritive value of grass pea had also studied (Ramachandran, and Ray. 2008). But the nutritional composition of grass pea foods prepared in traditional methods (boiling, roasting, preparing of sauce '*shirowott*' and unleavened bread '*kitta*') were not studied.

When grass pea is processed, the protein inhibitor and other anti nutritional factors, which inhibit the protein digestibility and chelate the mono, di and trivalent metal ions and form insoluble complexes will be degraded to a smaller molecular form and release the protein and the essential elements. The food processing methods including soaking, germination,

decortications, fermentation and cooking greatly influence the nutritive values of legumes. Of these, cooking and germination plays an important role as it influences the bioavailability and utilization of nutrients and improves palatability, which incidentally may result in enhancing the digestibility and nutritive value (Ramakrishna *et al.*, 2006). Therefore, data on the effect of traditional processes on the nutrient composition, mineral contents and anti-nutritional factors could be evaluated. As a result considering the importance of grass pea and the unparalleled contribution of improved varieties in the productivity of the crop, the present study was pursued with the following primary and specific objectives.

1.4 Objectives

1.4.1. General objective

The overall objectives of this research were to investigate the effects of traditional food processing methods on nutrient compositions and anti-nutritional factors of grass pea foods consumed, in Ethiopia.

1.4.2. Specific objectives

1. Study the effect of traditional food processing methods (roasting('kolo'), boiling ('nifro'), preparing 'shiro wott' and unleavened bread backing or 'kitta') on
 - The proximate composition (crude protein, extractable fat, total carbohydrate, Crude fiber, total ash and moisture content)
 - Mineral contents (Ca, Fe, Zn and P) and

- The anti-nutritional factors (phytic acid and tannins) and toxic substances (β -ODAP) of grass pea foods
2. Select the appropriate food processing methods and consumption pattern, which increase the absorbability of nutrients and decrease in a significant amount of anti-nutritional factors and toxic substances of grass pea food products.

Chapter 2

Literature review

This review discusses the area, production and yield of pulses; taxonomy, classification, status, production, utilization, nutritional value and chemical composition, production constraints, nutritional inhibitors and toxic factors of grass pea.

2.1 Production of legumes (pulses) in Ethiopia

Pulses are among the various crops produced in all the region of the country after cereals. Pulses are grown in different volumes across the country as indicated in Table 1 (CSA, 2009).

Pulses grown in 2008/2009 covered 14.4 % (1.58 million hectares) of the grain crop area and 11.48 % (more than 19.6 million quintals) of the grain production. Faba bean, haricot bean, field peas and grass pea were planted to 4.81 % (more than 538 thousand hectares), 2.38 % (more than 267 thousand hectares), 2.06 % (about 230 thousand hectares) and 1.43 % (more than 159 thousand hectares) of the grain crop area. The production obtained from faba beans, chickpeas, haricot beans and grass pea was 4.07 % (about 6.9 million quintals), 1.82 % (about 3.1 million quintals), 1.93 % (3.29 million quintals) and 1.18 % (about 2.03 million quintals) of the grain production in that order (CSA, 2009).

Table 2.1: Area, production and yield of pulses for private peasant holding for meher season 2008/2009 (CSA, 2009).

Crop	Number of holders	Area in hectares	Production in quintal	Yield (quintal/ha)
Pulses	7090955	1585236	19646301	
Faba bean	3841587	538820.5	6959836.9	12.92
Field peas	1658355	230749.2	2670932.5	11.58
Haricot beans	2457059	267069.2	3297753.2	12.35
Chick-peas	1025690	233440.4	3120800.3	13.37
Lentils	700213	94945.5	947734.03	9.98
Grass pea	774051	159731.5	2031255.5	12.65
Soy beans	61751	6236.04	78988.92	12.67
Fenugreek	621894	33773.59	376588.64	11.15
Gibto	113453	20469.4	172411.38	8.43

Source: CSA (2009)

2.2 Nutritional value of the legumes in the world

Of the thousands of legume species potentially useful as human food, only comparatively few have seen general acceptance. The fact that legumes have been part of the cropping system of virtually every society, but are invariably only part of the system with cereal grains providing the main caloric input, is a reflection, at least in part, of nutritional factors. The common recognition of the legume as the “poor man’s meat” emphasizes a cultural

preference for meat (or fish) as protein supplement to a cereal diet, a preference that is probably for both palatability and nutritional values (Delwiche, 1978).

The limitations of grain legumes as food have three principal sources, not necessarily related and all presumably susceptible to genetic modification (Delwiche, 1978). One of these is the common occurrence of various toxic substances in legumes. These constitute a long roster including antitryptic factors, hem-agglutinins, goiterogens, cyanogenic glycosides, estrogenic factors, toxic amino acids, vitamin antagonists, and others (Delwiche, 1978). Aside from known “poisonous” legumes, serious human disorders have been clearly associated with legume diets only in a few cases. Perhaps the most striking and best-documented syndrome is that of neurolathyrism, a debilitating and usually irreversible paralysis resulting from the consumption as a principal dietary constituent (usually in times of famine) of the grass pea (Delwiche, 1978).

A second limitation is the general use of legumes as a food is the problem of digestibility. Nutritionists generally agree that many grain legumes are less readily digested by humans than are many other protein foods. This probably stems both from the presence of proteins, which are intrinsically resistant to digestion (particularly some of the legume globulins) and from the antitryptic factors (Delwiche, 1978).

The third factor tending to lessen the usefulness of legumes as a human protein source is amino acid composition. The quality and distribution of amino acids vary greatly with species and strain as well as with growing conditions. Most legume seeds tend to be low in some of the amino acids essential to human nutrition, particularly the sulfur-containing

amino acids, methionine and cysteine. Other essential amino acids, such as lysine and tryptophan, are present in amounts greater than in cereals, and the supplementation of cereal diets with legume proteins tends to alleviate the nutritional deficiencies of a limited cereal diet. Amino acid composition and other nutritional factors vary greatly with cultivar, soil management and other factors; of course, these generalizations are not rigid (Delwiche, 1978).

2.3. Grass pea (*Lathyrus sativus* L.)

2.3.1. Taxonomy and names of the species

The scientific classification of *Lathyrus* has the following pattern.

Kingdom: *Plantae*

Subkingdom: *Tracheobionta*

Superdivision: *Spermatophyta*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Subclass: *Rosidae*

Order: *Fabales*

Family: *Fabaceae*

Subfamily: *Papilionoideae*,

Tribe: *Vicieae*

Genus: *Lathyrus* L.

Species: *Lathyrus sativus* L.

Source: (USDA).

2.3.2. Status of grass pea

Grass pea is an annual vine closely resembling field pea (*Pisum sativum L.*) in growth habit, but its leaflets are long and grass shaped, hence its name grass pea. In Ethiopia, grass pea is known by different local names as: *guaya* in Amharic, *sebere* in Tigrigna and *gayo* in Afan Oromo (Tsegaye *et al.*, 2007). In India, Nepal and Bangladesh grass pea is commonly called *khesari*. Its unique feature of drought tolerance makes it a golden crop for the arid regions of the world. Nevertheless, the presence of a toxic chemical in the seed, which causes paralysis of the limbs when consumed excessively, makes it a threatening crop (Tsegaye *et al.*, 2007).

In Ethiopia, grass pea is produced in the off-season under residual moisture. It is planted from the end of August to the beginning of October depending on the amount of the main season rainfall (Tsegaye *et al.*, 2007). Harvesting is carried out 4-5 months after planting in January or February when the leaves begin turning yellow and before the pods get completely ripen to avoid loss of seeds in the field. The crop is usually grown in Ethiopia after teff or barley, the common rotation being: teff- grass pea-teff, but noug and finger millet can also be used as preceding crops. In all grass peas -producing countries including Ethiopia, there is no application of fertilizer, intensive ploughing or weeding unlike other crops (Tsegaye *et al.*, 2007).

2.3.3. Production and utilization of grass pea in the world

Grass pea has been cultivated in South Asia and Ethiopia for over 2500 years and is used as food and feed. The legume is one of the many crops that have its primary genetic diversity in Ethiopia (Urga *et al.*, 2005). Grass pea is valued for its high protein content (26-32 %), high

degree of adaptability under extreme conditions, disease resistance and low input requirement for its cultivation. Despite its tolerance to drought, grass pea is not affected by excessive rainfall and can be grown on land subjected to flooding, including very poor soils and heavy clays (Urga *et al.*, 2005).

2.3.3.1. Utilization of Grass pea

Grass pea is cultivated in a number of countries for human food, animal feed and fodder. The crop is well adapted to areas that are suited for rice growth as it tolerates flooding at early stages and drought later in the season (Tsegaye *et al.*, 2007). The major uses of grass pea includes: green fodder, pasture, and seed as feed and as human food. In South Asia, Ethiopia and China the crop has a dual purpose and in other regions, it is mostly used as fodder or feed (Tsegaye *et al.*, 2007).

In Ethiopia, the grass pea seeds are processed in various forms. The most common method of preparation of grass pea is soaking in water and then boiling until the grains are soft ('*nifro*'). Other traditional methods include sprouting and boiling of sprouted seeds, roasting of soaked seeds (*kollo*) (Urga and Gebretsadik, 1993; Teklehaimanot *et al.*, 1993). When alternative crops are scarce grass pea is used alone or mixed with other cereal flours for making unleavened bread. In time of famine, the diet of the population of grass pea cultivating regions is forced to consist of only grass pea. When harvest is plentiful, grass pea flour is mixed with other legumes to prepare flour for sauce (*shiro wott'*) (Urga and Gebretsadik, 1993). In India, Pakistan, Bangladesh and Nepal the most common use of grass pea is a *dhal* (a soup-like dish). In India, the grains are sometimes boiled whole, but are most

often processed through a *dhal* mill to obtain split *dhal*. *Dhal* is the most common method of retailing the crop in the Indian subcontinent (Tsegaye *et al.*, 2007).

2.3.3.2. Production and distribution of Grass pea in Ethiopia

Grass pea is widely grown in Ethiopia. However, its production is more common in the north-western (58 %), the central (16 %) and the north-eastern (13 %) parts of the country. The northern as well as southern parts of the country account for the remaining 13% of the area under grass pea (Tsegaye *et al.*, 2007).

In Ethiopia, grass pea ranks fifth among legumes in terms of area planted and production (CSA, 2009). Its production area increased from 70,400ha in 1992 to 159,731.5 ha in 2009 with an average annual growth rate (in terms of area planted) consistently positive (CSA, 2009). The factor underlying this growth is the relatively good performance of grass pea under adverse environmental conditions, such as moisture stress, water logging, insufficient soil fertility, diseases, and pest damage (Tsegaye *et al.*, 2007).

Table 2.2: Area, production and yield of grass pea for private peasant holding for Meher season 2008/2009 (CSA, 2009).

Region (zone)	Number of holders	Area in hectares	Production in quintal	Yield (quintal/ha)
Tigray Region	50335	10519.4	110161.69	10.47
Central Tigray	10362	1099.22	12161.79	11.06
East Tigray	17998	3754.12	*	*
South Tigray	18342	5253.33	56788.19	10.81
Amhara Region	429776	87080.19	1054035.9	12.1
North Gondar	41021	6866.66	88432.27	12.88
South Gondar	54577	11755.84	132920.03	11.31
North Wolo	35337	4437.2	57641.23	12.99
South Wolo	94132	13890.26	202087.28	14.55
North shewa	29042	5781.81	76030.71	13.15
East Gojjam	93862	21070.12	314113.91	14.91
West Gojjam	80180	23203.87	181613.53	7.83
Oromia Region	286164	61469.09	848165.08	13.8
West Welega	21842	623.47	5457.4	8.75
North Shewa	66673	17606.51	265321.19	15.07
East Shewa	44129	8001.44	130129.16	16.26
Arsi	25176	3750.16	45759.33	12.2
South west Shewa	86068	20525.08	315642.61	15.38
S.N.N.P Region	7745	662.81	8892.79	13.42
Silitie	6630	535.54	8010.63	14.96

Note:-Those area and production designed by “*” in the table could not be reported because of high coefficient of variation (i.e. they are less reliable). However, they are consolidated in the total estimates.

Source: CSA (2009)

2.3.4. Nutritional composition of grass pea

Grass pea is nutritious, rich in protein (28-32 %) and contains good quantities of essential amino acids except the sulfur containing amino acids (Urga *et al.*, 2005), and is an important

food crop in Asia and the Middle East where the whole seed is used in soups and ground to make unleavened bread. In Canada uses would be for high protein livestock feed and as a green manure crop or cover crop. In addition to being important source of protein and calories, grass pea is rich in minerals. The seeds have a higher concentration of magnesium and phosphorus followed by calcium (Urga *et al.*, 2005).

The chemical composition of grass pea may vary according to varieties/ genotype, geographical region of their growing and maturity and environmental factors (soil fertility, nitrogen nutrition, temperature, and water stress and soil pH). There appears to be few studies on the nutritional aspects of grass pea. The nutritional aspect and the composition of four samples grown in Manitoba, Canada were studied by Rotter *et al.* (1991) and Campbell (1997) Table 2.3. Selected lines of three *Lathyrus* species have crude protein averaged 32.5 % (of dry matter) compared with *L. cicera* at 29.5 %. Ash content varies from 3.5 to 3.56% for advanced lines and crossing progeny of *L. sativus* (Aletor *et al.*, 1994).

Table 2.3. Composition of four samples of grass pea seeds

Component	Range
Water (%)	7.5-8.2
Starch (%)	48.0-52.3
Protein (%)	25.6-28.4
Acid detergent fibre (%)	4.3-7.3
Ash (%)	2.9-4.6
Fat (%)	0.58-0.8
Calcium (mg/kg)	0.07-0.12
Phosphorus (mg/kg)	0.37-0.49
Lysine (mg/kg)	18.4-20.4
Threonine (mg/kg)	10.2-11.5
Methionine (mg/kg)	2.5-2.8
Cysteine (mg/kg)	3.8-4.3

Source: Rotter *et al.* (1991)

The composition of four prepared foods of grass pea was reported by EHNRI (1997). The collected samples information such as type of variety, methods of preparation, storage time, cooking time and cooking temperature were not mentioned (Table 2.4).

Table 2.4: Composition of grass pea food commonly used in Ethiopia

Food & Description	Composition in terms of 100g of edible portion																
	Food Energy	Moisture	Nitrogen	Protein	Fat	CHO (including fiber).	Fiber	Ash	Calcium	Phosphorus	Iron	B-caroten equivalent	Thiamin	Riboflavin	Niacin	Tryptophan	Ascorbic acid
	Calories	%	Gram	Gram	Gram	Gram	Gram	Gram	Milligram	Milligram	Milligram	Microgram	Milligram	Milligram	Milligram	Milligram	Milligram
Grass pea; flour	360.50	9.30	4.02	22.10	0.90	66.00	2.40	1.70	136.00	174.00	4.20	0.00	0.32	0.26	1.30	-	3.00
Grass pea; souce	124.10	79.60	0.42	2.30	9.30	7.80	0.90	1.00	18.00	24.00	1.60	0.00	0.01	0.00	0.60	-	2.00
Grass pea; dried	347.00	10.70	4.10	22.60	1.40	61.00	8.20	4.30	336.00	273.00	108.20	Trace	-	0.17	-	290.00	-
Grass pea; boiled	177.70	55.30	1.90	10.50	0.50	32.80	3.80	0.90	94.00	85.00	4.70	0.50	0.13	0.08	1.50	-	3.00

2.3.4.1. Protein

Protein content of grass pea ranged from 27.29 % to 31.98 % and there were no significant differences in protein content by area of cultivation (Urga *et al.*, 2005). The nutritional value of a protein depends largely upon the pattern and concentration of essential amino acids. The amino acid composition of grass pea protein does not differ considerably from one variety to another. Grass pea proteins are well balanced in the essential amino acids, but lack a sufficient amount of the essential amino acids methionine and cystine. Methionine deficiency needs to be considered when the proteins are used for nutritional purpose. The high lysine content of grass pea protein makes them useful for supplementing cereal proteins, which are low in lysine.

2.3.4.2. Ash, crude fiber, fat, and carbohydrate

Ash and crude fiber concentrations ranged from 3.56 and 1.2 % to 8.62 and 4.14 %. However, crude fiber and ash content was significantly different by area of cultivation. The fat and carbohydrate content of grass pea ranged from 0.92 % and 55.05 % to 1.47% and 85.17 % respectively. The fat, carbohydrate and energy contents did not vary significantly by area of cultivation (Urga *et al.*, 2005).

2.3.4.3. Minerals

The mineral content of grass pea determined as ash, constitutes major and minor elements. Calcium, magnesium, phosphorus and iron in the seed samples were generally higher than the corresponding manganese, copper and zinc levels. The levels of calcium ranged from a low 82.01mg/100g to a high 118.97mg/100 g, whereas, magnesium and phosphorus values

ranged from a low 98mg/100 g and 242mg/100 g to a high 178 mg/100 g and 432mg/100 g in different cultivation areas. The seeds have a higher concentration of magnesium and phosphorus followed by calcium. The ratio of the content of grass pea of calcium to magnesium ranges from 1:0.53 to 1:1.02 and that of calcium to phosphorus ranges from 1:0.22 to 1: 0.43. Zinc content ranged from 2.74±0.5 mg/100 g to 4.52 mg/100 g with a significant variation. Although not much data are available for contents of minerals in grass pea foods from Ethiopia or other countries, results of different study indicate that the levels of minerals obtained falls within the usual range for most grain legumes (Urga *et al.*, 2005).The nutritional composition of *L. Sativus* and *L. cicera* (two closely related species) is similar to that of other feed grain legumes such as field pea (*Pisum sativum*), faba bean (*Vicia faba*), lupine (*Lupinus angustifolius*) (Hanbury *et al.*, 2000; Poland *et al.*, 2003; White *et al.*, 2001).

2.3.5. Anti-nutritional and toxic factors in grass pea

Anti-nutrients have been defined as substances, which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals (Francis *et al.*, 2001).

In common with those of other grain legumes, grass pea seeds contain a variety of anti-nutritional factors. The most frequently occurring anti-nutritional substances in grass pea are protease and amylase inhibitors, lectins, tannins, saponins, alkaloids, phytates, and lathrogens (Ramachandran and Ray, 2008).

2.3.5.1. Trypsin inhibitors

Trypsin inhibitors (TI) have a wide distribution in the plant kingdom and are present in most legume seeds and cereals (Francis *et al.*, 2001). In comparison with most edible legumes, trypsin inhibitor activity (TIA) in grass pea is high which had activities of about 23 g/ kg (Aletor *et al.*, 1994). Trypsin inhibitors inhibit growth by interfering with the digestion of protein in the intestine of animals thereby causing hypertrophy of the pancreas (Oke, 2007). The major physiological effect of trypsin inhibitor is to cause the enlargement of the pancreas and secretion of excessive amounts of pancreatic enzymes much of which is lost to the animal in faeces (Aletor *et al.*, 1994).

2.3.5.2. Phytates

Phytate (hexaphosphates of myo-inositol) is common in plant seeds. They can chelate with mono, di- and trivalent mineral ions such as Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Zn^{2+} , Cu^{3+} and Fe^{3+} resulting in these ions becoming unavailable for consumers. Since non-ruminants cannot break down phytates, their occurrence in feed reduces the availability of phosphorus to these animals. Phytates also form sparingly digestible Phytate–protein complexes, thus reducing the availability of dietary protein (Francis *et al.*, 2001).

2.3.5.3. Saponins

Saponins are a heterogeneous group of naturally occurring foam-producing steroid or triterpenoid glycosides found in many legumes (Francis *et al.*, 2001). Extracted commercially from soapwort (*Saponaria officinalis*) or soapbark (*Quillaja saponaria*) and used as foam producer in beverages and fire extinguishers, as detergents and for emulsifying

oils, bitter in flavor (Bender, 2006). Among the better-known biological effects of saponins is their capacity to cause lysis of erythrocytes and to make the intestinal mucous membrane permeable (Muzquiz *et al.*, 1999). Simultaneous consumption of saponin and tannin resulted in the loss of their individual toxicity to rats. This is considered to be due to chemical reactions between them, leading to the formation of tannin–saponin complexes, inactivating the biological activity of both tannins and saponins. Saponins might also increase the digestibility of carbohydrate-rich foods because of their detergent-like activity, which reduces viscosity and thus prevents the normal obstructing action of such foods against movement of digesta in the intestine. Because of the high solubility of most saponins in water, aqueous extraction would remove most saponins from feed ingredients (Francis *et al.*, 2001).

2.3.5. 4. Tannins

Tannins are secondary compounds of various chemical structures widely occurring in plant kingdom (Francis *et al.*, 2001). They are defined as high-molecular-weight polyphenolic compounds that have the ability to bind with protein and preserve animal hides. However, the term “tannin” is commonly used to refer polyphenolic compounds. Tannins are generally divided into hydrolysable (glucose polyesters of Gallic or hexahydroxydiphenic acids) and condensed tannins (proanthocyanidins) (Bender, 2006; Maxson and Rooney, 1972). Their anti-nutritional effects include interference with the digestive processes either by binding the enzymes or by binding to food components like proteins or minerals. Tannins also reduce the absorption of vitamin B₁₂. Contrary to condensed tannins, the hydrolysable tannins are easily degraded in biological systems, forming smaller compounds that can enter the blood

stream and over a period of time cause toxicity to the organs (e.g., liver and kidney). Tannins are also known to interact with other anti-nutrients. For example, interaction between tannins and lectins removed the inhibitory action of tannins on amylase, and interactions between tannins and cyanogenic glycosides reduced the deleterious effects of the latter (Francis *et al.*, 2001). Condensed tannin levels in grass pea lines ranged from zero to 4.38 g/kg. Tannins are strongly astringent (owing to their protein-binding properties), a depression of feed intake, which lowers animal productivity, would be expected. Although astringency seems to be the major cause of lower feed intakes, several other factors may contribute to the lower feed efficiency ratios of tannin-containing diets. These include the formation of tannin/protein complexes that make the protein unavailable, inhibition of the digestive enzymes, increased synthesis of digestive enzymes due to inadequate enzyme digestion, and increased loss of endogenous proteins such as the mucoproteins of the gastrointestinal tract (Cambell, 1997).

2.3.5. 5. Lectins

Plant lectins or phytohaemagglutinins are found in many legume seeds and are able to bind reversibly to carbohydrate moieties of complex glycol-conjugates present on membranes. Even though they are proteins, they are at least partially resistant to proteolytic degradation in the intestine. Their common biological effects include disruption of the small intestinal metabolism and morphological damage to the villi. Irritation caused by lectins to the intestinal membrane resulting in over secretion of mucus may impair the enzymatic and absorptive capacity of the intestinal wall (Francis *et al.*, 2001). Because lectins are capable of agglutinating red blood cells, intake of improperly cooked legumes is deleterious.

Phytohaemagglutinins are known to exert a non-selective adverse effect on the absorption of nutrients from the intestinal tract rather than a direct effect on the digestive process (Francis *et al.*, 2001). Their deleterious effect may be more potent when present along with other anti-nutrients (Francis *et al.*, 2001). The toxicity of lectins is characterized by growth inhibition in experimental animals and diarrhoea, nausea, bloating and vomiting in humans (Muzquiz *et al.*, 1999).

2.3.5.6. Lathyrogens

Lathyrogens are toxic compounds found in certain *Lathyrus* plant species, including the flat-podded vetch (*L. cicera*), Spanish vetch (*L. clymenum*), and the *L. sativus*. Lathyrogens include β -amino propionitrile and the neurotoxic amino acid β -N-oxalyl-L- α , β -diaminopropionic acid. Consumption of lathyrogens in humans causes a disease called lathyrism; the toxicity symptoms including skeletal lesions, retarded sexual development and paralysis (Tacon, 1995).

2.3.6. Production constraints

The main constraint of grass pea is the presence of neurotoxins, the most abundant of which is β -N-oxalyl-L- α , β -diaminopropionic acid (β -ODAP), responsible for the irreversible, crippling disease neurolathyrism in humans after prolonged consumption (Hanbury *et al.*, 2000; Smulikowska *et al.*, 2008,). However, there are other numerous constraints, which hinder the exploitation of grass pea's potential (Tsegaye *et al.*, 2007).

Some of these are as follows:

- Poor productivity of the traditional varieties
- Non-availability of quality seed to grass pea growers
- Low income status of farmers in grass pea growing areas
- Absence or ineffectiveness of rhizobium strains in soils
- Unawareness of input utilization
- Non-availability of grass pea production technology
- Poor cultivation practices
- Pre and post harvest mechanization difficulties
- Lack of trained man power in research institutes and extension departments
- Marketing and storage problems.

2.3.7. Lathyrism

Lathyrism is a motor neuron disease affecting population in Indian sub-continent, Africa and China (Tsegaye et al., 2007). The causative agent has been identified as β -N-oxalyl-L- α , β -diaminopropionic acid (β -ODAP) or β -N-oxalyl amino alanine (BOAA) (Tsegaye *et al.*, 2007).

Epidemics of neurolathyrism usually occur during times of food shortages, such as drought, flooding, or wars. Epidemics of neurolathyrism occurred during the Spanish Civil War (1936-1939), in China, Bangladesh, and India during the 1970s, and in Ethiopia during the 1970s and 1990s (Getahun *et al.*, .2002).

Lathyrism and its relation to grass pea have been known in Ethiopia for many years. The disease has been given descriptive ethnic names such as ‘*Sebere*’ and ‘*ye guaya beshita*’, ‘*guaya*’, all with implications of a ‘leg-breaking’ (paralytic) nature (Teklehaimanot et al., 1990). The distribution of neurolathyrism in Ethiopia is fairly widespread (throughout the arable parts) of the country with the greatest number of cases being reported in the northern and central highlands, particularly in the administrative regions of Tigray, Gondar, Gojam, Wollo and Shoa (Urga *et al.*, 2005; Tsegaye *et al.*, 2007). The highest incidence of lathyrism was recorded in the Fogera and Dembiya plains of the Gondar region and west Gojam zone (Tsegaye *et al.*, 2007), North Wello (Delanta Dawint District) (Getahun et al., 1999; 2003) and South Wello (Legambo district) (Teklehaimanot et al., 2005).

Chapter 3

Materials and Methods

3.1. Materials

3.1.1 Sample

Gray-mottled seed coats of grass pea seeds grown and harvested in 2008/2009 (Meher season) was obtained from Debrezeit Agricultural Research Centre.

3.1.2. Chemicals

Reagents used for analysis were purchased from Sigma Aldrich Company. All chemicals and reagents used were analytical reagent grade except H₂O₂, which was laboratory reagent grade.

3.1.3. Sample preparation

The seeds were cleaned manually to remove foreign matters, immature and damaged seeds.

a) Preparation of raw sample

The cleaned seeds (1Kg) were washed with tap water, rinsed with distilled water and immediately dried in drying oven (model: DHG-9055A) at 55 °C for 12 h, under air circulation, and then grind by grinder (model no.: NM-8300, Electrical Grinder-NIMA) to pass through a 0.425 mm sieve, packed in air tight bottle and stored at room temperature (in the shelf) until analysis.

3.2. Traditional processing methods

(The methods are adopted from Teklehaimanot et al., 1993 and then modified)

3.2.1. Boiling (*nifro*)

Whole cleaned seeds (1Kg) were washed with tap water, rinsed with distilled water, soaked with distilled water (1:2 w/v seed to water) for 3 h., decant the soaking water and washed with another distilled water, placed in 2L of distilled boiling water at 96 °C and cooked for 60 min. (until soft) and immediately dried in drying oven (model: DHG-9055A) at 55 °C for 12 h, under air circulation, and then grind by grinder (model no.: NM-8300, Electrical Grinder-NIMA) to pass through a 0.425 mm sieve, packed in air tight bottle and stored at room temperature (in the shelf) until required for analysis.

3.2.2. Wet roasting (*kolo*)

Whole cleaned seeds (1Kg) were washed with tap water, rinsed with distilled water, soaked with distilled water(1:5 w/v seed to water) at 28⁰c (using water bath)for 20 h and then roasted at 200 °C for 40 min in baking oven (model name: SEVERIN) placed in a baking try and turning with a fork, and then grind by grinder (model no.: NM-8300, Electrical Grinder-NIMA) to pass through a 0.425 mm sieve, packed in air tight bottle and stored at room temperature (in the shelf) until required for analysis.

3.2.3. Preparation of *shiro* flour

Whole cleaned seeds (1Kg) were washed with tap water, rinsed with distilled water, added to 2 liter of boiling distilled water on the stove and removed after five minutes, soaked with

distilled water(1:5 w/v seed to water) for 12 h and dried in the sun for 4hs and in an oven at 55 °C for 12 h under air circulation then roasted at 150 °C for 25 min in baking oven (model name: SEVERIN) placed in a baking try and turning with a fork, dehulled and splitted by using slicer, separating the hull and then grind by grinder (model no.: NM-8300, Electrical Grinder-NIMA) to pass through a 0.425 mm sieve, packed in air tight bottle and stored at room temperature (in the shelf) until required for processing and analysis.

3.2.4. Sauce (*'shiro wott'*)

When one litre of distilled water was boiled 200 gm of the prepared flour was dispersed and cooked with stirring at 96⁰Cfor 15 min., dried in the drying oven at 80⁰C for 13 hrs and then spread on the plastic sheet and dried in the sun for 4hrs. Pound with a mortar and pestle to pass through a 1.0 mm sieve and stored in an airtight plastic container until analysis.

3.2.5. Unleavened bread (*'kitta'*) flour

Whole cleaned seeds (1Kg) were washed with tap water, rinsed with distilled water, dried in an oven at 55 °C for 12 h under air circulation, dehulled and splitted by using slicer, separating the hull and then grind by grinder (model no.: NM-8300, Electrical Grinder-NIMA) to pass through a 0.425 mm sieve, packed in air tight bottle and stored at room temperature (in the shelf) until required for processing and analysis.

3.2.6. Unleavened bread (*'kitta'*)

300gm of the prepared flour was mixed with 200mL of distilled water and the moisten flour was kneaded to make a dough, baked in baking oven (model name: SEVERIN) at 200⁰C for

30 min. dried in an oven at 80 °C for 12 h under air circulation, pound with a mortar and pestle to pass through a 1.0 mm sieve and stored in an air tight plastic container until analysis.

3.3. Chemical analysis

3.3.1. Determination of moisture content

Moisture of the seed flours were determined according to AOAC (2000) using the official method 925.09. The empty aluminum dish and its lid were dried in drying oven at 100⁰C for 1h and cooled in a desiccator. The dried and cooled dish together with the lid was weighed. About 5g of the prepared grass pea samples (raw, roasted, boiled, unleavened bread and *shiro wott*) were weighed and dried in drying oven with air circulation at 105⁰C for 3hrs, cooled in a desiccator and then weighed. The amount of moisture was calculated by using the following formula. The result is shown in Table 4.1.

$$Moisture(\%) = \frac{w_2 * 100}{w_1}$$

Where W₁= weight (g) of sample

W₂=loss of weight (g)

3.3.2 Determination of ash content

The ash content was determined by AOAC (2000) using the official method 923.03. The cleaned crucible with its lid were dried in a muffle furnace at 550⁰C for 1h and cooled in a desiccator for 30 min. About 5g of the prepared grass pea samples (raw, roasted, boiled, unleavened bread and *shiro wott*) were weighed with and with out lid and charred on a hot

plate until the smokes disappeared. The charred sample was put in the muffle furnace at 550⁰C and ashed for 3hrs and then left in the muffle overnight. The ashed sample was cooled in a desiccator for 1h and weighed when it cools. The amount of ash was calculated by using the following formula. The result is shown in Table 4.1.

$$Ash(\%) = \frac{w_2 * 100}{w_1}$$

Where W₁= weight (g) of sample

W₂=weight (g) of ash

3.3.3. Determination of crude protein

Protein content was determined according to AOAC (2000) using the official method 979.09. In a cleaned Tecator flask about 0.5g of sample was weighed, 6mL of concentrated sulphuric acid (AR) was added and let to stand for 24hrs. After 24 hrs 3.5mL of H₂O₂ (30%) was added step by step. When the violent reaction stopped it was shaken and left in the rack. 3g of accelerated reagent (a mixture of copper sulphate pentahydrate and anhydrous potassium sulphate) was added and left for 15 minutes. The mixture was digested in a digest stove (HYP-1008 eight holes) at 370⁰C for 4hrs. After digestion it was cooled in the hood on the rack, 25mL of distilled water was added to dissolve the precipitate, 25 mL of 40% NaOH was added to the digested sample and placed in the distiller (KDN-102F, nitrogen analyzer distillation device). 25mL of H₃BO₃ (saturated solution), 25mL of distilled water and 3 drops of methyl red were added in the 250mL conical flask and placed in the distiller (KDN-102F, nitrogen analyzer distillation device). In the distillation when about 150-200mL distillate was collected it was titrated with 0.1N HCl and the amount of HCl was recorded. The amount of protein was calculated by using the following formula and the result is shown in Table 4.1.

$$\text{Crude protein (\%)} = \frac{(V2 - V1) * N * 14.01 * 6.25}{10W}$$

Where V1= volume (ml) of hydrochloric acid solution required for the blank test

V2= volume (ml) of hydrochloric acid solution required for the test sample

N= normality of hydrochloric acid

W= weight of sample

14.01= equivalent weight of nitrogen

6.25= conversion factor for legumes

3.3.4. Determination of crude fat content

The crude fat was extracted according to AOAC (2000) official method 4.5.01. The cleaned flask (cylinder) and boiling chips was dried in the drying oven at 100⁰C for 1h, cooled in the desiccators for 30min and weighed. Two grams of sample was weighed in thimble containing fat free cotton. The thimbles were placed in the thimble holders, 50mL of petroleum ether (boiling range of 60-90⁰C) was poured in the flask, the thimble was immersed in the petroleum ether (in the flask) and heated at 80⁰C in the fat determinator (SZC-C fat determinator) for 1hr, hanged the thimble and heated at the same temperature for 2hrs and then the solvent was recovered for 15 min. The heater was switched off, the flask was dried in the drying oven at 90⁰C for 30 min, cooled in the desiccators for 15 min and then weighed the flask with the extract. The amount of extractable fat was calculated by using the following formula and the result is shown in Table 4.1.

$$\text{Weight of fat } (W_f) = W_a - W_b$$

where, W_a = weight of extraction flask after extraction

W_b = weight of extraction flask before extraction

$$\text{Crude fat content (\%)} = \frac{w_f \times 100}{W}$$

where, W = Sample weight

3.3.5. Determination of crude fiber

Crude fiber analysis was conducted using the method of AOAC (2000) official method 962.09. About 1.5g sample was transferred into a 600 ml beaker and about 200 ml 1.25% sulfuric acid was added and boiled for 30 minutes. Recording took place by placing a watch glass over the mouth of the beaker. After 30 minutes heating by gently keeping the level constant with distilled water, 20 ml of 28% KOH was added and again boiled gently for further 30 minutes, and then the solution was filtered through sintered glass crucibles. Subsequently, washing was conducted with hot distilled water, 1% sulfuric acid, 1% NaOH solution and finally with acetone. Then, filtered and dried it in the electric oven (memmert 854 Schwabach, West Germany) at 130°C for 2hrs. Furthermore, it was cooled at room temperature for 30 minutes in a desiccator and weighed, then transferred it to crucible to muffle furnace (GALLENKAMP, Model FSL 340-0100, U.K.) for 30 minute ashing at 550 °C. Finally, it was cooled again in a desiccator and re-weighed. The crude fiber content was determined by using the formula and the result is shown in Table 4.1.

$$\text{Crude fiber content (\%)} = \frac{(w_1 - w_2) * 100}{w_3}$$

Where, w_1 = crucible weight after drying

w_2 = crucible weight after ashing

w_3 = weight of sample

3.3.6. Determination of crude carbohydrate

The crude carbohydrate was calculated by difference. The mathematical expression is as follow:

$$\text{Crude carbohydrate (\%)} = 100 - (\% \text{ crude fiber} + \% \text{ crude protein} + \% \text{ crude fat} + \% \text{ total ash})$$

Gross energy (in kilo Calories)

Gross energy was calculated according to Osborne and Voogt (1978):

The gross energy content in raw and processed sample was calculated as follow:

$$\text{Gross energy (\%)} = (9 \times \text{crude fat \%}) + (4 \times \text{crude protein \%}) + (4 \times \text{crude carbohydrate \%})$$

3.3.7. Mineral analysis

Calcium, zinc and iron were determined using atomic absorption spectrophotometer method of Osborne and Voogt (1978). The ash obtained after dry ashing at 550 °C was treated with 5 ml of 6N HCl to wet it completely and carefully dried on a low temperature hot plate. 7 ml of 3N HCl was added and the dish was heated on the hot plate until the solution just boils. Then, it has been cooled and filtered through a filter paper in to a 50mL volumetric flask. Again 7 ml of 3N HCl was added to the dish and heated until the solution just boils. Finally, cooled and filtered into the volumetric flask. For the determination of calcium, lanthanum chloride (1% w/v) was added to both standards and samples to suppress interference from phosphorus. Using atomic absorption spectrophotometer (Varian, spectra-10/20, Australia) a calibration curve was prepared by plotting the absorption or emission values against the metal concentration in mg/100g. Reading was taken from the graph, which depicted the metal

concentrations that correspond to the absorption or emission values of the samples and the blank. The metal contents were calculated by using the formula and the result is shown in Table 4.2.

$$\text{Metal content} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{[(A - B)XV]}{10w}$$

Where, W = weight of samples (g)

V = volume of extract (ml)

A = concentration of sample solution ($\mu\text{g/ml}$)

B = concentration of blank solution ($\mu\text{g/ml}$)

3.3.8. Determination of phosphorus

The phosphorus was determined calorimetrically by the method Fiske and Subbarow (1925). The ash obtained after dry ashing at 550°C was treated with 5 ml of 6N HCl to wet it completely and carefully dried on a low temperature hot plate. 7.5 ml of 3N HCl was added and the dish was heated on the hot plate until the solution just boils. Then, it has been cooled and filtered. Again, 7.5 ml of 3N HCl was added to the dish and heated until the solution just boils. Finally, cooled and filtered into the 50mL volumetric flask and then filled with distilled water. 5 ml of the sample dilution, 0.5 mL molybdate reagent and 0.20 mL aminonaphtholsulphonic acid reagent were added in to a test tube and let stand for 10 minutes. The absorbance at 660 nm was read using spectrophotometer (BECKMAN, DU-64, and Japan) against distilled water. The standard (A_s) was prepared by mixing standard phosphorous solution, molybdate reagent and aminonaphtholsulphonic acid. The blank (A_B) was prepared by mixing molybdate reagent, aminonaphtholsulphonic acid and deionized

water. The concentration of phosphorus was calculated first by subtracting the blank from all other reading and by using the following formula:

$$\frac{mg\ P}{100g} = \frac{Abso - blank}{Slope \times s.wt} \times dilution\ factor$$

Where, s.wt=weight of sample

Abs= absorbance

The molybdate reagent was prepared by taking 75 ml of 10 N H₂SO₄ in a 250 mL volumetric flask and dissolving 6.25 g of ammonium molybdate in a beaker in about 50 mL water and then transferring the beaker solution into the volumetric flask. The aminonaphtholsulphonic acid reagent was prepared by dissolving 100 mg of 1, 2, 4 aminonaphtholsulphonic acid in 39 mL of sodium bisulphate solution, and then adding 1 mL of sodium sulphite solution. Sodiumbisulphite 15 % and Sodium sulphite 20 % solution was prepared by dissolving 7.5 gm sodiumbisulphite in 50 mL of water and 2 gm sodium sulphite in 10 mL of water respectively. The standard phosphorous solution was prepared by dissolving 438.8 mg of KH₂PO₄ (water free) in some water in a 100 mL volumetric flask, adding 1 mL conc. H₂SO₄ and diluting to the mark with water. The standard curve was made by taking 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the standard solution and diluting to 100mL.

3.3.9. Determination of anti-nutritional factors

3.3.9.1. Determination of phytic acid

Phytic acid was determined by using Latta and Eskin (1980) as modified by Vaintraub and Lapteva (1988). About 1g of sample was extracted with 10 ml 2.4% HCl for 1hr at an

ambient temperature and centrifuged (3000 rpm/30min) (Nüve, bench-top centrifuge, NF 800R, 2001, Ankara, Turk). The supernatant was used for phytate estimation. About 2 ml of Wade reagent (0.03% solution of FeCl₃.6H₂O containing 0.3% sulfosalicylic acid in water) was added to 3 ml of the sample solution and centrifuged. The absorbance at 500 nm was read using spectrophotometer (BECKMAN, DU-64, Japan). The phytate concentration was calculated from the difference between the absorbance of the control (3ml of water + 1ml of Wade reagent) and that of assayed sample. The concentration of phytate was calculated using phytic acid standard curve (by preparing a series of standard solution containing 3.9, 7.8, 15.5, 23.4, 31.2 and 39µg/ml phytic acid in 0.2N HCl) using water to zero the spectrophotometer, and results were expressed as of phytic acids in mg/100gm dry weight by the following formula. The result is shown in the Table 4.3.

$$\text{Phytic acid (ug / g)} = \frac{\text{Abso} - \text{Intercept}}{\text{Slope} * \text{density} * \text{wt.sample}} \left(\frac{10}{3}\right)$$

Where, Abso= Absorbance

Density= density of the solvent

Phytate phosphorus and non-phytate phosphorus

Phytate phosphorus was calculated by assuming 28.18% of phytate (C₆P₆O₂₄H₁₈) is phosphorus.

That is, Phytate phosphorus (mg/100g) =content of phytate (mg/100g) x28.18%

The non-phytate phosphorus was determined from the difference between total phosphorus and phytate phosphorus

3.3.9.2. Determination of tannins

The amount of condensed tannin was determined by the Vanillin assay of Burns (1971) as modified by Maxson and Rooney (1972). About 1g of sample was weighed, extracted with 10mL 1% HCl in methanol, at room temperature in mechanical shaker for 24 h. The mixture was centrifuge at 1000G for 10 minutes. 1mL supernatant was mixed with 5mL of vanillin-HCl reagent in another test tube. When the reaction was completed (after 20 minute), the absorbance was read at 500nm using spectrophotometer (BECKMAN, DU-64, Japan). D-catechen was used as standard value of tannin in mg D-catechen per gram of sample. 40mg D-catechen was dissolved in 100mL of 1%HCl in methanol and from this 0, 0.2, 0.4, 0.6, 0.8 and 1ml was taken in a test tube and the volume was adjusted to 1mL with 1% HCl in methanol. 5mL of vanillin-HCl reagent in each test tube was added. After 20 minutes the absorbance was read at 500nm. The absorbance of the blank was subtracted from the absorbance of the corresponding vanillin-containing sample. A standard curve has been constructed (Absorbance vs. D-catechin) and the linear portion of the curve was extrapolated to produce the standard curve. Finally, the tannin contents were calculated. Values of tannins were expressed in milligram of D-catechin equivalent per gram of sample by the following formula. The result is shown in Table 4.3.

$$Tannin(mg / 100g) = \frac{Abso - Intercept}{Slope * density * wt.sample} (dilutionfactor)$$

3.3.9.3. Determination of β -ODAP content

The β -ODAP concentration in the raw and processed grass pea samples was determined spectrophotometrically using the o-phthalaldehyde method (Rao 1978) as modified by

Agriculture Canada Research Station at Morden (Campbell, 1993), with a sodium tetraborate buffer as standard procedure with slight modification, which involves a neutralization step prior to addition of the chromogenic reagent. 500mg sample was extracted with 10ml of 60% (v/v) ethanol for 22h in an orbital shaker. and centrifuge the mixture at 4000rpm for 30 min. 2ml of aliquot of the extract was mixed with 4ml of 3M KOH, hydrolyzed in a test tube (screw cap) hold in a boiling water bath for 30 min and brought to cool the tubes to room temperature. The base was neutralized by adding 4ml of 3N HCl. The following controls were taken to account for any interference from the reaction between the OPT reagent and other constituents of the extract.

- a. 0.25mL of unhydrolyzed extract was mixed with 0.75mL of distilled water and 2ml of OPT (OPT blank)
- b. 0.25mL of unhydrolyzed extract was mixed with 0.75mL of distilled water and 2mL of tetraborate buffer (sample blank)
- c. 0.25mL of hydrolyzed extract was mixed with 0.75mL of distilled water and 2mL of OPT (sample)
- d. 0.25mL of hydrolyzed extract was mixed with 0.75mL of distilled water and 2mL of tetraborate buffer (buffer blank)

The absorbance was measured at $\lambda_{\max} = 426\text{nm}$ using spectrophotometer (UV-Visible Spectrophotometer, UV-7804C) after 30 min. (when the solution was changed into yellow in colour) against a reagent blank and DAP.HCl was used as standard.

The general equation for calculating the concentration of β -ODAP was as follow (Addis and Narayan, 1994):

$$C = \frac{(c - d) - \frac{1}{3}(a - b) - y \text{ intercept}}{\text{slope}}$$

Where, C is the concentration of β -ODAP in the extract. The y intercept and the slope were the extrapolated from the regression line calculated from absorbance reading of DL- α , β -diaminopropionic acid (DAP). The concentration of β -ODAP was calculated from a calibration curve and assuming 100% conversion of β -ODAP to DAP at the given conditions of hydrolysis and a conversion factor from diaminopropionic acid (DAP) to β -ODAP of 1.69 (Aletor et al., 1994). The result is shown in Table 4.3. O-phthalaldehyde reagent was prepared by mixing 100mg orthophthalaldehyde (OPT), 1mL of 96% ethanol, 200 μ L mercaptoethanol and 99mL sodium tetraborate buffer. The sodium tetraborate buffer was prepared by dissolving 9.535g of sodium tetraborate decahydrate in 250mL and the pH was adjusted to 9.9. The standard solution for the standard curve was prepared by dissolving 0.01g of DAP.HCl in 500mL (20ppm) of distilled water and from this eight different concentrations (2ppm, 4ppm, 6ppm, 8ppm, 10ppm, 12ppm, 14ppm, and 16ppm) were prepared. A reagent blank was prepared by taking 1mL of distilled water and 2mL of OPT reagent.

3.3.10. Statistical analysis

Data were analyzed by the analysis of variance (ANOVA) procedures using SPSS/15.0 software for windows. Least significant differences (LSD) and Duncan were used for multiple mean comparison tests. Significance was accepted at 0.05 level of probability ($p < 0.05$).

Chapter 4

Result and Discussion

The effects of processing (roasting, boiling, preparing unleavened bread (*kitta*) and sauce (*shiro wott*) on the nutrient compositions and levels of certain anti-nutritional factors (phytic acid, tannins, and β -ODAP) pertaining to the seeds of local variety grass pea that was obtained from Debrezeit Agricultural Research Center was studied. The grass pea seeds were produced in 2008/2009 and harvested in the spring (*mehre*) season.

4.1. Nutrient composition

The results of analysis of nutrient composition of raw and processed grass pea is presented in Table 4.1.

4.1.1. Moisture content

The raw grass pea flour contained 8.26 % moisture. The result was in agreement with the finding of Hanbury et al. (2000) (8.1 to 12.4 %), Campbell (1997) and Rotter *et al.* (1991) (7.5 to 8.2 %). Some variation could be due to variety, storage conditions and temperature during harvesting.

The Moisture contents of roasted, boiled, unleavened bread and sauce of grass pea had 5.69, 7.30, 4.16, and 7.18 % respectively. There was a significant difference ($P < 0.05$) in the moisture content of processed with raw grass pea.

Table 4.1: Nutrients composition of raw and processed grass pea*

Sample type	Moisture (%)	Crude fiber (%)	Crude protein (%)	Crude fat (%)	Total ash (%)	Crude carbohydrate (%)	Gross energy (Kcal/100g)
Raw	8.26±0.01 ^a	8.57±0.12 ^a	22.81±0.51 ^a	0.61±0.02 ^a	2.75±0.02 ^a	65.26±0.39 ^a	357.77±0.30 ^a
Wet roasted	5.69±0.06 ^b	8.61±0.08 ^a	24.97±0.32 ^b	1.17±0.01 ^b	2.52±0.02 ^b	62.73±0.27 ^b	361.33±0.29 ^b
Boiled	7.30±0.025 ^c	8.55±0.04 ^a	25.18±0.06 ^b	1.00±0.01 ^c	2.30±0.04 ^c	62.97±0.07 ^b	361.58±0.03 ^b
Unleavened bread (<i>Kitta</i>)	4.16±0.04 ^d	3.31±0.12 ^b	21.39±0.27 ^c	0.95±0.02 ^d	2.78±0.01 ^a	71.57±0.42 ^c	380.37±0.44 ^c
<i>Shiro wott</i>	7.18±0.38 ^c	6.20±0.22 ^c	20.88±0.32 ^c	0.60±0.01 ^a	2.34±0.006 ^d	69.98±0.55 ^d	368.82±0.85 ^d

Means in the same column followed by different letters are significantly different (P< 0.05)

Means in the same column followed by the same letters are not statistically significant using one-way ANOVA test (P<0.05)

* mean value ± standard deviation and n=3

4.1.2 Protein

In the present study minor changes were noted in the proximate composition of grass pea after cooking with regard to crude protein. The protein content of raw grass pea was found 22.81 %, which was in agreement with the finding of Urga *et al.* (1995). Campbell (1997) and Rotter *et al.* (1991) reported 25.6 % to 28.4 % of protein. However, Hanbury *et al.* (2000) and Urga *et al.* (2005) reported much higher protein values (26 to 35 %). The variations in protein content may be attributed to the variety, soil and agronomic practices.

When we see the effect of the processing methods there was a significant difference ($P < 0.05$) in the crude protein content of processed with raw grass pea. There were 9.47 % and 10.39% increasing in protein content of roasted and boiled respectively. The observed increase in protein content of roasted and boiled samples might be due to loss of soluble solids during soaking. It might also be attributed to a net synthesis of enzymatic protein during soaking and also might be from the release of protein from the protein complexing agent. Similar result due to soaking was reported by Urga and Gebretsadik (1993). There was no significant difference ($P < 0.05$) in the crude protein content of roasted and boiled grass pea.. There were 6.22 % and 8.46 % lowered values of crude protein in unleavened bread and sauce. This might be due to loss during dehulling and probably dependent upon the leaching out of some amino acids (the sulfur containing amino acids) (Bhatty, *et al.*, 2000).

4.1.3. Crude fat

Grass pea was found containing 0.61% of crude fat a value lower than previously reported by Urga *et al.* (1995; 2005) (0.92 to 1.42%) and Hanbury *et al.* (2000) (0.9 to 5.3%).

However, the present finding is in agreement with Campbell (1997) which range from 0.58 to 0.8 %. These variations could be due to variety differences.

When we compare the results of the present study, there was a significant difference ($P < 0.05$) in the crude fat content of roasted, boiled and unleavened bread samples with raw grass pea. There were 91.80 %, 63.93% and 55.74 % increasing in fat content of roasted, boiled and unleavened bread samples respectively. The increased result might be due to soaking and boiling of the sample that led to the cleavage of the protein-lipid or carbohydrate-lipid linkages thereby, facilitating the easy extraction of the oil by the extracting solvent (Esenwah and Ikenebomeh, 2008). Similar result was reported on African locust bean by Esenwah and Ikenebomeh (2008).

4.1.4 Crude fiber

It was observed that raw grass pea contained 8.57 % crude fiber, which is agreed with the finding of Uрга *et al.* (1995; 2005) (3.56 to 8.62%). Hanbury *et al.* (2000) reported higher range of crude fiber content (5.3 to 10 %) and Akalu *et al.* (1998 reported much higher value 17.5% crude fiber (soluble 2.1% and 15.4% insoluble dietary fiber). Such variations were probably due to varying extent of dehulling and variety of grass pea.

When we see the processed samples, roasted, boiled, unleavened bread and sauce had 8.61, 8.55, 3.31 and 6.20 % crude fiber respectively. The processing method (roasting and boiling) applied in this study have no a significant difference ($P < 0.05$) in the crude fiber content. Akalu *et al.* (1998) reported that the total dietary fiber (TDF) content of the processed samples ranged between 16 % and 20 %, consisting of predominantly insoluble dietary fiber (14 % to 18 %) and it was also reported that there was no significant ($p = 0.01$)

difference in the dietary fiber content of processed sample as compared to that of raw samples. There was a significant difference ($P < 0.05$) in the crude fiber content of unleavened bread and sauce of grass pea. There were 62.56 % and 31.88 % decrease in fiber content of unleavened bread and sauce respectively from raw grass pea. The decrease might be due to dehulling and the removal of some water-soluble oligosaccharides such as the raffinose families and the indigestible carbohydrates.

4.1.5 Total ash

The total ash content of raw grass pea was found 2.75 %. This result was comparable to Urga *et al.* (2005) (1.28 to 4.14%) and Hanbury *et al.* (2000) (2.6 to 3.9%). However, Campbell (1997) and Rotter *et al.* (1991) reported in the range of 2.9 to 4.6 % ash content. These variations were probably due to variations in the soils and extent of dehulling.

When we compare the processing effect, there was a significant difference ($P < 0.05$) in the total ash content. There were 8.36 %, 16.36% and 16 % decreased in total ash content of roasted, boiled and sauce respectively. Loss in ash might be due to leaching of soluble inorganic salts into the processing water during the soaking of the sample followed by processing (roasting, boiling and cooking) (Esenwah and Ikenebomeh, 2008). There was no a significant difference ($P < 0.05$) in the total ash content of unleavened bread with that of raw grass pea.

4.1.6 Crude carbohydrate

Raw grass pea has crude carbohydrate 65.26 %, which was comparable to the finding of EHNRI (1997). Uрга *et al.* (1995; 2005) reported a lesser value, which was in the range of 52.4 to 65.17 %.

Due to the traditional processing methods there was increasing and decreasing effect on the content of crude carbohydrate. There were 9.86 % and 3.51 % decreased in crude carbohydrate content of roasted and boiled respectively. Loss in carbohydrate during soaking and then roasting and boiling might be due to leaching of soluble carbohydrates like sugars into the soaking and cooking water (Esenwah and Ikenebomeh (2008)). Similar result was reported on African locust bean by Esenwah and Ikenebomeh (2008). The increases recorded in the crude protein and ether extract may be due to the reduction in the carbohydrate content and may be regarded as apparent increase in both the protein and fat contents to complement the decrease in carbohydrate (Esenwah and Ikenebomeh (2008)). There were 9.67 % and 7.23% increased of crude carbohydrate in unleavened bread and sauce respectively. There was no a significant difference ($P < 0.05$) in the crude carbohydrate content of roasted with that of boiled grass pea.

4.1.7 Gross energy

The gross energy of raw grass pea was 357.77 Kcal/100g, which was in agreement with the finding of Uрга *et al.*(2005) (347.47 to 384.93 Kcal/100g). However, Uрга *et al.* (1995) reported in the range of 316.8 to 351.5 Kcal/100g and (Ramachandran (2004; 2008) reported 417.40Kcal/100g

There was a significant difference ($P<0.05$) in the gross energy content due to the traditional processing methods. The processing effect showed that 361.33, 361.58, 380.37 and 368.82 Kcal/100g respectively of roasted, boiled, unleavened bread and sauce. There was no a significant difference ($P<0.05$) in the gross energy content of roasted and boiled grass pea

4.2. Mineral composition

Calcium, phosphorus, iron and zinc contents of the raw and processed sample are presented in Table 4.2.

Table 4.2: Mineral content of processed and raw grass pea*

Sample type	Ca mg/100g	Zn Mg/100g	Fe mg/100g	P mg/100g
Raw	242.76±2.54 ^a	3.00±0.04 ^a	4.84±0.16 ^{ab}	189.79±2.8 ^a
Wet roasted	254.40±3.80 ^a	3.09±0.02 ^b	4.94±0.28 ^b	195.56±0.93 ^b
Boiled	301.34±14.54 ^b	2.93±0.03 ^c	4.60±0.04 ^b	217.96±1.31 ^c
Unleavened bread (<i>Kitta</i>)	149.42±7.10 ^c	3.33±0.01 ^d	5.34±0.01 ^c	176.66±0.38 ^d
<i>Shiro wott</i>	207.94±5.42 ^d	3.06±0.01 ^b	4.71±0.01 ^{ab}	207.52±5.07 ^c

Means in the same column followed by different letters are significantly different ($P<0.05$)

* mean value ± standard deviation and n=3

4.2.1 Calcium

The raw grass pea calcium content was comparable with the result of Hanbury *et al.* (2000) (120 to 280mg/100g). Campbell (1997) and Rotter *et al.* (1991) reported the calcium content

in the range 90 to 120mg/100g. Urga *et al.* (1995; 2005) were reported in the in the range of 79 to 200mg/100g.

Comparing the processing effect there was a significant difference ($P<0.05$) in the calcium content of boiled, unleavened bread and sauce with that of raw grass pea. 38.45 % and 14.34 % of calcium content were decrease respectively in unleavened bread and sauce. Loss in calcium content might be due to dehulling as minerals are more concentrated in the testa rather than in the cotyledon (Agbede and Aletor, 2005). Similar results were reported on *Canavalia ensiformis* and *Mucuna pruriens* seed flours by Agbede and Aletor, (2005). There was no a significant difference ($P<0.05$) in the calcium content of roasted and raw grass pea.

4.2.2. Phosphorus

The raw grass pea flour contained 189.97mg/100g phosphorus, which was much lower than results in the literature. Hanbury *et al.* (2000) summarized the content of phosphorus in the range of 260 to 440mg/100g. Campbell (1997) and Rotter *et al.* (1991) reported in the range 370 to 490mg/100g of phosphorus content. Urga *et al.* (1995; 2005) found in the range of 242.26 to 512mg/100g respectively.

. The study had shown a significant difference ($P<0.05$) in the phosphorus content due to processing effect. There were 2.94 %, 14.73 % and 9.24% increasing phosphorus content of roasted, boiled and sauce samples respectively.

4.2.3 Iron

The raw grass pea flour contained 4.84 mg/100g iron, in agreement with the finding of Urga *et al.* (2005). Hanbury *et al.* (2000) and Urga *et al.* (1005) reported that the raw grass pea contained iron in the range of 6.3 to 9.5mg/100g and 6.6 to 18.4mg/100g.

There was no a significant difference ($P < 0.05$) in the iron content of roasted, boiled and sauce as compare to raw grass pea. However, the content of iron in unleavened bread was increased by 10.12 %.

4.2.4. Zinc

The zinc content of raw grass pea flour was 3.00 mg/100g in agreement with the finding of Urga *et al.* (2005) (2.74 to 4.52mg/100g of zinc). Hanbury *et al.* (2000) reported that the raw grass pea contained 2.7mg/100g of zinc.

The Processing methods have significant effect on the zinc content. There were 3.00%, 11.0% and 2.0% increasing zinc content of roasted, unleavened bread and sauce respectively from raw grass pea. In contrast, zinc content of boiled grass pea slightly decreases as compared to the raw grass pea. There was no a significant difference ($P = 0.05$) in the zinc content of roasted and sauce of raw grass pea.

4.3. Anti-nutritional factors

The anti-nutritional factors (phytate, tannin and β -ODAP) contents of the raw and processed sample are show in Table-4.3.

Table-4.3: Anti-nutritional factors content of processed and raw grass pea*

Sample type	Tannin mg/100g	Phytate mg/100g	β-ODAP mg/100g
Raw	579.76±3.16 ^a	352.04±0.88 ^a	421.18±7.82 ^a
Wet roasted	266.37±3.16 ^b	235.14±0.84 ^b	370.5±3.90 ^b
Boiled	387.21±2.10 ^c	247.26±1.66 ^c	260.0±7.80 ^c
Unleavened bread (<i>kitta</i>)	48.30±2.10 ^d	102.16±0.86 ^d	431.58±15.58 ^a
<i>Shiro wott</i>	97.92±1.05 ^e	90.40±1.27 ^e	222.3±24.70 ^d

Means within the same column followed by same letter are not statistically significant using one-way ANOVA test (P<0.05)

* mean value ± standard deviation and n=3

4.3.1. Phytate

Raw grass pea contained 352.04 mg phytate per 100 g of sample (Table 4.3). This result was lower than the result reported by Uрга et al (1995; 2005) (525 to 1098.62mg/100g and Ramachandran (2004) (6520mg/100g).

The processing methods reduced the content of phytate to varying extents. Roasting, boiling, unleavened bread and sauce lower the level of phytate to the extent of 33.21, 29.76 %, 70.98 % and 74.32 % respectively over the control value. The loss in phytates in roasted, boiled and sauce samples of grass pea may be due to leaching of phytate ions during soaking into the soaking water under the influence of a concentration gradient (difference in chemical potential) which governs the rate of diffusion. In addition, it may be ascribed to the activation of the endogenous phytase during the long soaking treatment and possible

enzyme action continued during drying step. The differences in the loss of phytate contents in unleavened bread could probably be partly due to either formation of insoluble complexes between phytate and other components, such as phytate–protein and phytate– protein– mineral complexes, or hydrolysis of inositol hexaphosphate to penta and tetraphosphates. Similar results for reduction in phytate in the soaked and cooked peas have been reported in common other legumes (*Bauhinia purpurea* L. and *Prosopis chilensis* seeds) by Vijayakumari *et al.* (1997; 2007) and in pigeon pea (*Cajanus cajan*) by Duhan *et al.* (2002).

4.3.2 Tannins

The raw grass pea flour contained 579.76mg/100g tannin, which was in agreement with values reported by Urga *et al* (1995; 2005) (452.53 to 866.29mg/100g and 500 to 856mg/100g). Campbell (1997), Aletor *et al.* (1994) and Grela *et al.* (2001) reported that the condensed tannin levels in grass pea ranged from 0 to 550mg/100g.

The higher content of tannins is known to inhibit the activity of the digestive enzymes and, thus, interfere with the digestion and absorption of dietary proteins, carbohydrates, minerals and other nutrients, such as vitamin B₁₂. They may also cause damage to the mucosa of the digestive tract and hence, they are undesirable for human consumption from a nutritional point of view (Vijayakumari *et al.*, 2007).

The processed grass pea samples showed a significant difference ($P<0.05$) in the tannin content. In the present study tannin content decreased by 54.06 %, 33.21 %, 91.67 % and 83.11 % in roasted, boiled, unleavened bread and sauce respectively. Loss of tannin may be due to solubility in water and its sensitivity to heat. Since polyphenolic compounds are water-soluble in nature and mostly located in the seed coat, the decrease in the level of

phenolics and tannins during soaking may be attributed to leaching into the soaking medium (Vijayakumari *et al.*, 2007). Similar reduction in tannin content was recorded during processing of legumes by Vijayakumari *et al.* (1997; 2007) and Esenwah and Ikenebomeh (2008).

4.3.3. β -ODAP

Several methods for the determination of β -ODAP in grass pea were carried out in different laboratories mainly in connection with development of low β -ODAP varieties and for large scale cultivation. Traditionally, different processing methods including roasting, boiling, preparing unleavened bread, sauce and fermentation of grass pea were evaluated for the content of β -ODAP (Teklehaimanot *et al.*, 1993; Akalu *et al.*, 1998).

In this study raw grass pea flour contained 421.18mg/100g of β -ODAP, which was in the range of the result reported by Teklehaimanot *et al.* (1993) (370-1040mg/100g), Aletor *et al.* (1994) (360 to 589mg/100g) and Fikre *et al.* (2008) (20 to 540mg/100g (0.02 to 0.54%)). Hanbury *et al.* (2000) summarized the result of different researchers of the raw grass pea content of β -ODAP (mg/100g) as 160 to 250 (Spain), 70 to 750 (Syria), 40 to 760 (Australia), 450 to 1400 (Bangladesh), 280 to 1500 (India), 180 to 520 (Chile), and 80 to 990 (China). Other researchers also reported different values: Rotter *et al.* (1991) (130 to 270mg/100g), Urga *et al.* (2005) (618.29 to 1001.49mg/100g), Urga *et al.* (1995) (172 to 354mg/100g) and Grela *et al.* (2001) (94.8mg/100g) of β -ODAP. The variability in β -ODAP content can be attributed to the different germplasm collection of grass pea and might be influenced by the environment, growing conditions and locality (Campbell, 1997).

The result of this study on the β -ODAP content showed a significant effect. The content of β -ODAP was significantly ($P=0.05$) reduced by 12.03 %, 38.27 % and 47.22 % after roasting, boiling and preparing sauce respectively, which was comparable to finding of Akalu *et al.*(1998). The reason for this reduction could be leaching of β -ODAP with the water used for soaking and cooking (Akalu *et al.*, 1998). Even though, it was not significantly different from the raw, higher levels of β -ODAP were observed in the unleavened bread (*kitta*) and similar result was reported by Teklehaimanot *et al.* (1993).

4.4. Effects of processing on phytate phosphorus and non-phytate phosphorus.

The contents of phytate phosphorus and non phytate phosphorus are shown in Table 4.4.

Table 4.4 Contents of phytate phosphorus and non phytate phosphorus of raw and processed grass pea*

Sample type	Phytate phosphorus**		Non phytate phosphorus**	
	(mg/100g)	%of TP	(mg/100g)	%of TP
Raw	99.21±0.25 ^a	52.27	90.70±2.55 ^a	47.73
Wet roasted	66.26±0.24 ^b	33.88	129.30±0.69 ^b	66.12
Boiled	69.68±0.47 ^c	31.97	148.28±1.78 ^c	68.03
Unleavened bread	28.79±0.24 ^d	16.30	147.87±0.62 ^c	83.70
Shiro wott (sauce)	25.47±0.36 ^c	12.27	182.05±5.42 ^d	87.73

Means within the same column followed by same letter are not statistically significant using one-way ANOVA test ($P<0.05$)

* mean value \pm standard deviation and $n=3$

**phytate phosphorus was calculated by assuming that phytate contains 28.18% phosphorus and the non phytate phosphorus was the difference between total phosphorus and phytate phosphorus.

The percentage of phytate phosphorus to total phosphorus is very important since the phytate phosphorus cannot be utilized by human being. Fifty two percent of the total phosphorus was present as phytate phosphorus in the unprocessed grass pea seeds. The traditional processing methods resulted in significantly decreased in phytate phosphorus with a corresponding marked increase in non-phytate phosphorus (Table 4.4). Preparing sauce was found to be the best processing method, followed by unleavened bread, boiling and roasting seeds for reducing the content of phytate phosphorus. Cleavage of phosphorus from the phytic acid that is, increasing phosphorus utilization may explain the increased level of non-phytate phosphorus in the processed grass pea seed. Similar results were reported by Duhan *et al.* (2002).

4.5. Effects of processing on calcium: phytate, phytate: Iron and phytate: Zinc molar ratio

The effects of processing on calcium: phytate, phytate: iron and phytate: zinc molar ratio is shown in Table 4.5

Table 4.5 Molar ratio of calcium: phytate, phytate: iron and phytate; zinc *

Sample type	Calcium: phytate** (molar ratio)	Phytate: Iron** (molar ratio)	Phytate: Zinc** (molar ratio)	$\frac{[\text{Calcium}]x[\text{phytate}]}{[\text{Zinc}]}$
				(mol/kg)
Raw	11.38±0.15 ^a	6.06±0.18 ^a	11.49±0.14 ^a	0.70±0.02 ^a
Wet roasted	17.86±0.21 ^b	4.09±0.025 ^b	7.48±0.04 ^b	0.48±0.01 ^b
Boiled	20.12±1.10 ^c	4.54±0.000 ^c	8.28±0.04 ^c	0.62±0.04 ^c
Unleavened bread	24.14±1.35 ^d	1.63±0.02 ^d	3.02±0.02 ^d	0.12±0.01 ^d
Shiro wott (sauce)	37.95±0.45 ^e	1.64±0.03 ^d	2.91±0.04 ^d	0.16±0.01 ^e

Means within the same column followed by same letter are not statistically significant using one-way ANOVA test (P<0.05)

* mean value ± standard deviation and n=3

**mg of calcium/molecular weight of calcium: mg of phytate/molecular weight of phytate

** Mg of phytate/molecular weight of phytate: mg of iron/molecular weight of iron

** Mg of phytate/molecular weight of phytate: mg of zinc/molecular weight of zinc

Phytic acid exerts its inhibitory effect on the absorption of zinc and iron by forming insoluble complexes in the gut under physiological condition. The formation of such chelates depends on the ratio of the content of zinc, iron or calcium relative to that of phytate in the food (Umeta et al., 2005). When Phytate: zinc molar ratios is greater than fifteen, it is indicative of poor zinc bioavailability. Similarly when the phytate: iron molar ratio is greater than 0.15, regarded as indicative of poor iron bioavailability (Umeta et al., 2005). In this study as the processing method decreased the phytate, the molar ratio of calcium: phytate, increased and the phytate: iron and phytate: zinc decreased. The calcium: phytate molar ratio was above the critical molar ratio of 6:1 in raw and processed grass pea. The high calcium content of grass pea may jeopardize bioavailability of iron and zinc (Umeta et al., 2005). The ratio of $[Ca] \times [Phytate]: [Zn]$ is a better predictor of Zn availability and if the value were greater than half mol/kg, there would be interferences with the availability of Zn. (Umeta et al., 2005). In this result, $[Ca] \times [Phytate]: [Zn]$ values were greater than 0.50 mol/kg in raw and boiled grass pea while other $[Ca] \times [Phytate]: [Zn]$ values were lower than to 0.50 mol/kg. That is to say, using this indicator, Zn availability would be enhanced due to processing in grass pea except boiling.

Chapter 5

Conclusion and Recommendation

5.1 Conclusion

Although legumes constitute one of the most abundant and least expensive sources of protein in human/animal diets, their utilization is limited largely due to the presence of anti-nutritional/anti-physiological compounds (Vijayakumari *et al.*, 2007). The raw seeds of grass pea exhibit low biological value; however, processing could significantly improve the true protein digestibility and net protein utilization. The seeds of grass pea are also reported to contain many anti-nutritional compounds such as tannins, phytic acid, trypsin inhibitors, saponins and oligosaccharides (Ramachandra and Ray, 2008).

Processing methods for grass pea is very important primarily due to the high content of anti-nutrients and the difficulty in their digestion. Effective utilization of quality protein in grass pea as a source of food/feed, especially in the Ethiopia is also important.

The higher crude protein and ether extract obtained for the roasted and boiled grass pea are improvement on its nutritional quality. In addition, tannin and phytic acid were significantly reduced by the processing methods.

Traditional processing methods were found to be beneficial for lowering the phytate content and improving the bioavailability of dietary essential minerals in grass pea. Among the processing methods, preparing sauce was found to be the best method to reduce phytate, followed by unleavened bread, roasting and boiling grass peas seeds. Regarding the tannin

content, preparing unleavened bread is the best method, followed by sauce making, roasting and boiling. In relation to β -ODAP, sauce making is the best, followed by boiling and roasting.

Traditional processing methods especially boiling and preparing sauce significantly decreased the β -ODAP. However, Teklehaimanot et al. (1993) suggested that it would not be easy to set a standard of β -ODAP content that could be safe for consumption, because there is a large range of variability in β -ODAP concentration in germplasm collection of grass pea. Due to the absence of a well defined toxic level of β -ODAP, we can perhaps rely on the very low levels given in homeopathy to protect against paralysis of the legs (Lambein, 2000). The survey by Getahun et al. (2003) suggested that any addition of spices or condiments to grass pea can make it less toxic. The condiments added to the gravy form may improve the micronutrient balance, and its complex processing may wash out the water-soluble toxin. It was also reported that use of cereal and grass-pea flour mixtures reduced the risk of paralysis if they contained more than a third cereal. The addition of wheat and maize into grass-pea preparations could compensate for the deficiency of methionine and cysteine, as well as diluting the concentration of toxin (Getahun et al. (2003).

Hence, for proper utilization of grass pea, especially in developing countries, these simple and economic household processing and cooking methods should be followed, as they not only save time, energy and fuel consumption but also enhance the nutritional quality of the grass pea by lowering the content of anti-nutrients and increasing the bioavailability of minerals.

Overall, it can be concluded that when we compare the processing effect on the nutrient composition, mineral content and anti-nutrients, boiling, preparing sauce and roasting respectively are the most appropriate methods to consume grass pea. Although, scale-up and applicability of these processing, to large- or commercial-scale should be overlooked.

5.2 Recommendation

Legumes have to be cooked prior to their consumption as they are not acceptable to the human palate in raw form and, also, cooking improved the digestibility of carbohydrates and proteins. The consumption of diets based on legumes, without animal products could well be a cause of malnutrition. However, traditional household practices can decrease the anti-nutritional factors significantly, and thus need to be encouraged.

Based on the previous research results and this study the following recommendation should be considered.

- To consume as a snack the grass pea should be soaked at least for 3 hours with plenty of water and then boiled until the seeds soft.
- As other legumes, grass pea is low in sulfur containing amino acids, it is better consume with cereals to obtain well essential amino acids and probably to prevent lathyrism.
- Though grass pea is widely consumed in Ethiopia, its nutritional potential is not yet elucidated implying that isolation of protein and subsequent determination of its digestibility is important. In addition, evaluation of in vitro protein digestibility and biological values of processed grass pea seeds are needed.
- Education to the people on the dangers of consuming improperly processed foods especially legumes which are reported to contain very high concentrations of anti-nutritional factors is very important.

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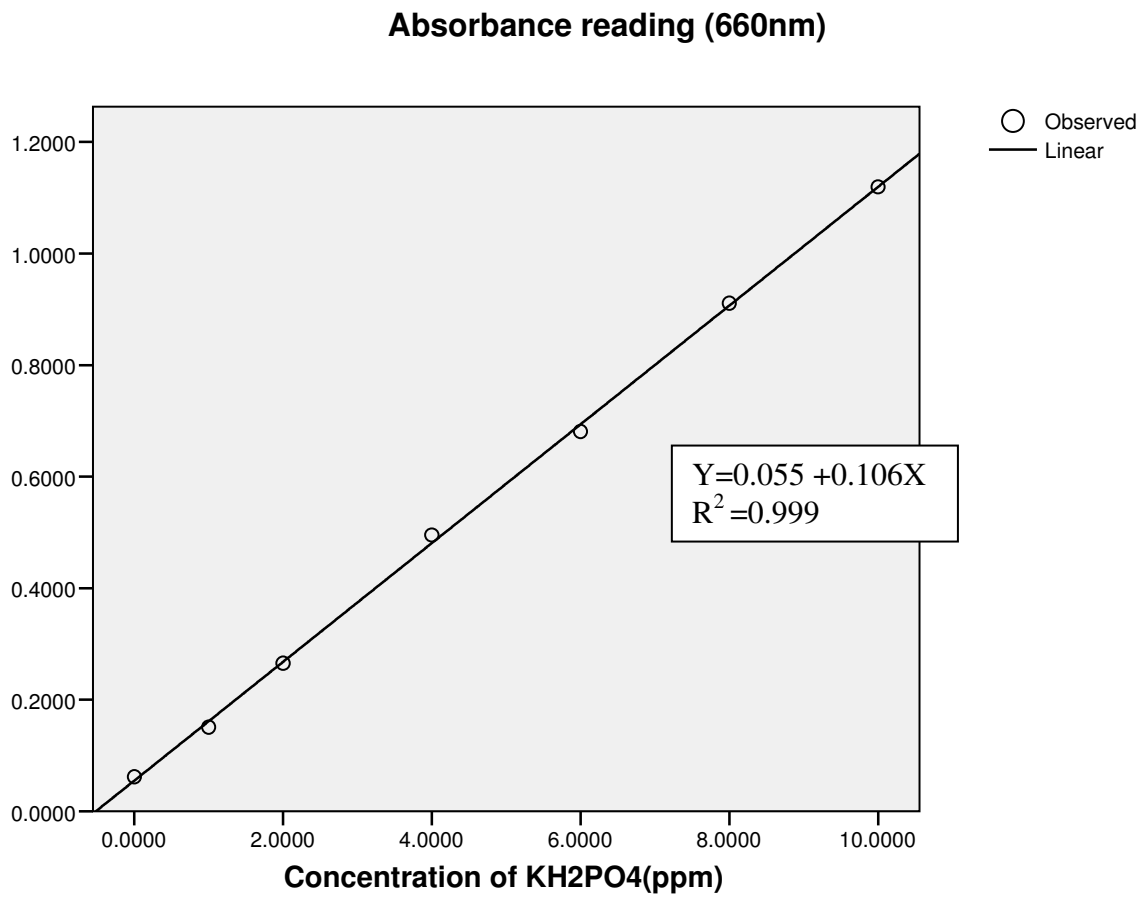
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Annex:

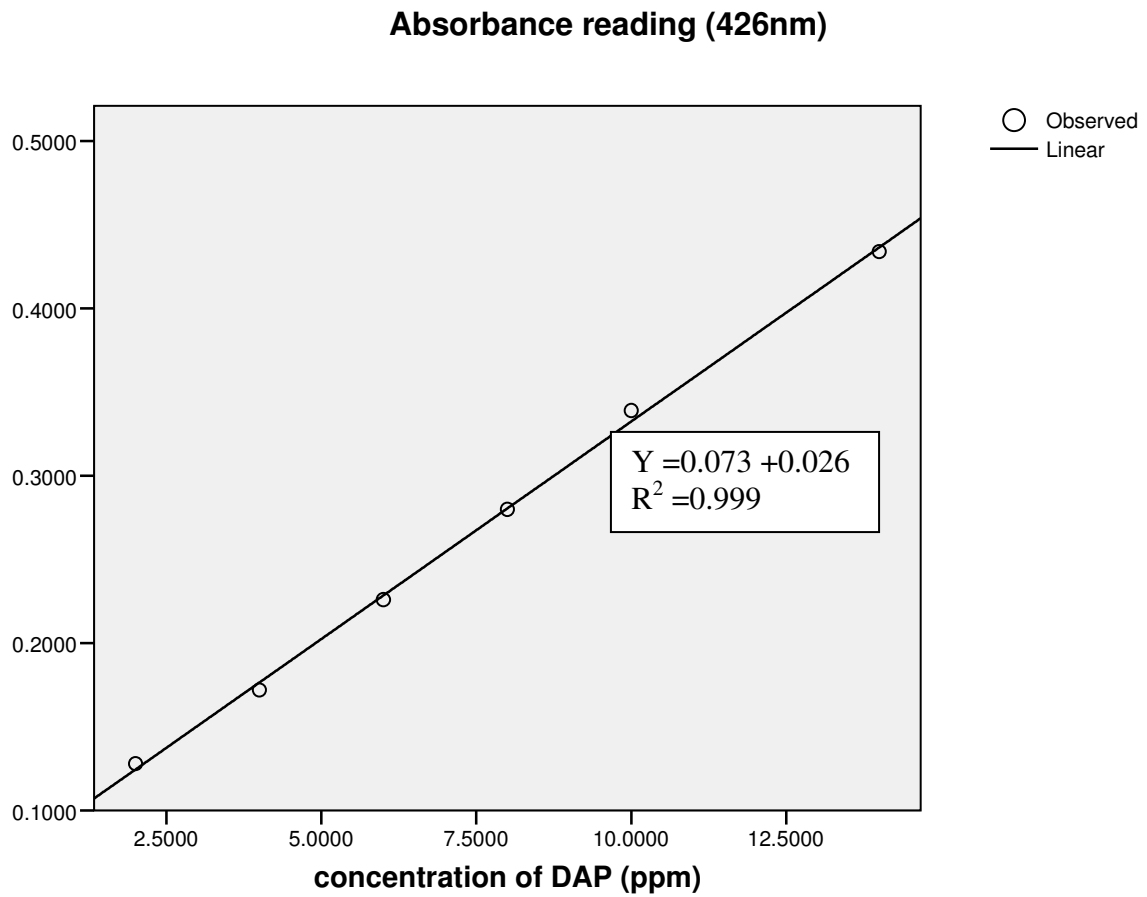
Absorbance values of phosphorous standard at 660nm in different concentration of KH_2PO_4 ($\mu\text{g/mL}$)

Concentration (ppm)	1	2	4	6	8	10	Blank
Absorbance	0.146	0.266	0.494	0.682	0.905	1.114	0.061
	0.156	0.265	0.497	0.680	0.917	1.125	0.063
Mean	0.151	0.2655	0.4955	0.681	0.911	1.1195	0.062
Net absorbance	0.089	0.2035	0.4335	0.619	0.849	1.0575	0.000



Absorbance values of DAP measured at 426nm in different concentration of DAP ($\mu\text{g/mL}$)

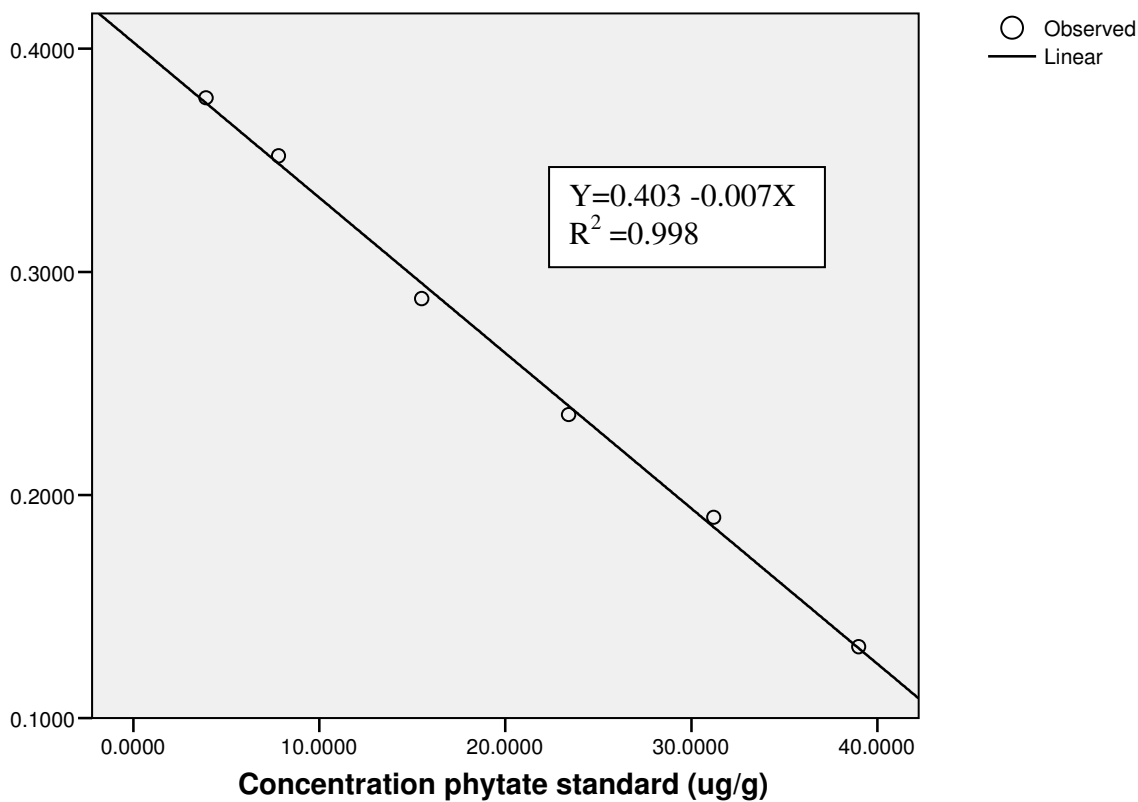
Concentration (ppm)	2	4	6	8	10	12	14	16
Absorbance	0.128	0.172	0.226	0.280	0.339	0.365	0.434	0.563



Absorbance reading of phytate standard (500nm)

Concentration (µg/g)	3.9	7.8	15.5	23.4	31.2	39
Absorbance	0.378	0.346	0.290	0.235	0.191	0.134
	0.377	0.358	0.287	0.236	0.188	0.130
Mean	0.378	0.352	0.288	0.236	0.190	0.132

Absorbance reading (500nm)



Absorbance of tannin standard (D-catechen) (500nm)

Concentration (mg/100g)	12	24	36	48	60
Absorbance (500nm)	0.073	0.151	0.227	0.297	0.364
	0.074	0.149	0.227	0.295	0.363
Mean	0.074	0.150	0.227	0.296	0.364

Absorbance reading (500nm)

