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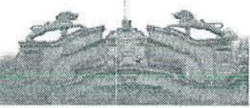

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
FOOD SCIENCE AND NUTRITION PROGRAM

Change in phytates and HCl-extractability of minerals due to processing of chick pea in Ethiopia variety

BY

YORDANOS FIKRE

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Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF FOOD SCIENCE AND NUTRITION

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A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Nutrition

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SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF FOOD SCIENCE AND NUTRITION

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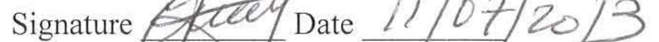
Approval of Board of Examiners

1. Advisors

Name: Mr. Kelbesa Urga


Signature ______ Date 11/07/2013

Name: Dr Melese Abdisa

Signature ______ Date 15 July 13

2. Internal Examiner

Name: Dr. Kaleab Baye

Signature ______ Date 03/07/2013

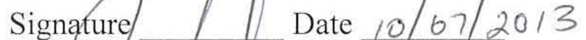
3. External Examiner

Name: Prof. Negussie Retta

Signature ______ Date ______

4. Chairperson

Name: Dr.Zelalem Debebe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Acronyms

FAO: Food and Agriculture Organization

LDL: Low-density lipoprotein

USD: United States dollar

CVD: Cardiovascular disease

GMO: Genetically modified organism

DNA: Deoxyribonucleic acid

PA: Phytic acid

SPSS: Statistical Product and Service Solution

ANOVA: Analysis of variance

CHO: Carbohydrate

AOAC: Association of Official Analytical Chemists

Rpm: Revolution per minute

Abstract

Change in phytates and HCl-extractability of minerals due to processing of chickpea grown in Ethiopia was studied Chickpeas contained a significant amount of phytic acid, i.e. 98.06±0. 136mg/100g When it was subjected to cooking methods , soaking (6, 12, 18 h), dehulling, autoclaving and sprouting (24, 36 and 48 h), a strong decrease in level of phytic acid with a remarkable increase in the HCl-extractability of calcium, zinc phosphorus, and iron occurred.

Combination of soaking and cooking was found to be the best method for decreasing the phytic acid content, that is. 48.89±0.152 mg/100g the control and significantly ($p< 0.05$) increasing the non-phytate phosphorus and HCl- extractable phosphorus. Some mineral losses were noticed when the legume was subjected to soaking, cooking, and germination but the maximum losses, occurred when the seeds were dehulled. However, HCl-extractability of Ca, P, Zn and Fe improved to a significant extent when the chickpea seeds were soaked, soaked- dehulled, cooked and sprouted which may have been due to decrease in the phytate content followed by processing.

Moisture content, crude protein, crude fat, crude fiber and total ash ranges were 5.04-6.88 %, 14.21- 23.86 %, 3.71-6.21%, 1.25-3.97% and1.01- 2.78% respectively. The range of iron, zinc, calcium and phosphorous were 1.34- 4.76, 1.72- 2.94, **,** *69.29 -162.37and 20.75-30.66 mg/100g respectively.*

Key words: Cooking, dehulling, sprouting, autoclaving, HCl-extractability, iron, calcium, Phosphorus, Phytic acid, soaking

1. INTRODUCTION

1.1. Background of the Study

Legumes play an important role in the traditional diets of many regions throughout the world. They are low in fat and are excellent sources of protein, dietary fiber, and a variety of minerals. Legumes useful as human and animal foods .Generally, legumes have been reported to have low nutritive value due to low amounts of sulfur-containing amino acids, low protein digestibility and presence of antinutritional factors including tannins, phytic acid, trypsin inhibitors and flatulence causing oligosaccharides. Among all the antinutritional components, phytic acid is one of prime concern for human nutrition and health management (Alonso, et *al* 1998).

Ethiopia is a primary producer of food legumes with production in 2003 estimated to be over one million metric tons produced on nearly 1.2 million hectares.The majority of the production consists of cool-season food legumes, such as chickpea (Pollan *et al.*, 1999).

Chickpea production works well in rotation with cereals such as wheat and teff and is widely grown in relatively well-drained black soils. Globally, chickpea is adapted to black soils in the cool semi-arid areas of the tropics, sub-tropics as well as the temperate areas. It is the third most important legumes grown in the world after dry bean and pea and constitutes 20% of the world's legumes production .Chickpea was first produced in the Middle East about 7, 000 years ago. At present, it is produced in over 40 countries represented in all continents. However, the most important chickpea producing countries are India, Turkey, Pakistan, Iran, Mexico, Australia, Ethiopia, Myanmar, and Canada. Chickpea is currently grown on about 10.7 million hectares worldwide with average annual production of 8.2 million tones. About 95% of chickpea cultivation and consumption is in the developing countries (Omer, 2009).

Chickpeas are a rich source of vitamins, minerals and phytoestrogens. They contain folate, thiamine, riboflavin, niacin, pantothenic acid, vitamins C, A and E. Chickpeas have a higher content of calcium and phosphorus than other legumes and are a good source of iron and zinc. Also contain magnesium, copper, manganese and selenium. Chickpeas are abundant in the isoflavones formononetin and biochanin A, phytoestrogens common to many legumes (Trugo, et al. 1990).

Chickpea is widely grown in Ethiopia and serves as a multi-purpose crop. It fixes atmospheric nitrogen in soils and thus improves soil fertility and saves fertilizer costs in subsequent crops. Also it improves more intensive and productive use of land, particularly in areas where land is scarce and the crop can be grown as a second crop using residual moisture. Chick pea reduces malnutrition and improves human health especially for the poor who cannot afford animal products. Chickpea is an excellent source of protein, fiber, complex carbohydrates, vitamins, and minerals. The growing demand of chickpea in both the domestic and export markets provides a source of cash for smallholder producers. Chickpea increases livestock productivity as the residue is rich in digestible crude protein content compared to cereals (Shiferaw, *et al.* 2007).

Chickpea seeds are usually cooked before being used in the human diet. This improves the protein quality by destruction or inactivation of the heat labile antinutritional factors. Chickpea seed is processed and cooked in a variety of forms for consumption. Different processing methods (soaking, sprouting, boiling, roasting, frying, steaming) remove anti-nutritional factors and increase digestibility of chickpea seed. The removal of undesirable components is essential to improve the nutritional quality of Chickpea. These processing methods could effectively be utilized to their full potential to prepare chickpea as human food. It is widely accepted that simple and inexpensive traditional processing techniques are effective methods of achieving desirable changes in the composition of seeds. In many instances, usage of only one method may not impart the desired removal of antinutritional compounds and a combination of two or more methods is required (Maluku *et al.*, 2005).

Phytic acid has been reported to be a strong chelating agent of divalent cations and interferes with mineral availability due to the formation of insoluble phytates. Phytic acid is the principal storage form of phosphorus in many plant tissues, especially chickpea seeds. Phytate is not digestible to humans or nonruminant animals minerals such as zinc, calcium, iron and phosphorus chelates and thus makes unabsorbable. For effective utilization, it is imperative to reduce the level of this antinutrient in chickpea seeds through processing and cooking methods (Osman , 2007).

In Ethiopia, chickpeas are processed and consumed in a variety of forms, depending on cultural and taste preferences. It used as 'kollo (soaked and roasted), Shero wet (hot chick pea flour sauce) shenbra asa we't (oven backed chick peas cookies simmered in spicy berbere sauce) and other. The most common domestic method of processing chickpeas include soaking, cooking, sprouting and dehulling, which may bring about changes in the contents of antinutritional factors and improve the bioavailability of dietary essential minerals. Extractable minerals in a food are those which are soluble in 0.03 N HCl, the concentration of HCl found in the human stomach. Extractability of minerals is an index of their bioavailability during processing and cooking of chickpea cultivars (Sadeghi *et al.*, 2008). Therefore, the purpose of this study is to report change in phytates and HCl-extractability of calcium, phosphorus, zinc and iron of soaked, dehulled, cooked, autoclaved and sprouted chick pea varieties in Ethiopia this would help in develop simple and cost effective processing options for developing countries in order to improve the nutritional value of chickpea.

1.2. Statement of the problem

The most serious threat to the survival of humanity is the ever-increasing gap between population growth and food supply. In order to arrest this situation, much attention has been centered on the use and utilization of plant materials for food. Much of the attention has been paid to seeds like chickpeas which have excellent sources of protein, dietary fiber, and a variety of minerals. Contribution of minerals to human nutrition is however limited due to the presence of antinutritional factors which render some of the nutrients unavailable thus leads to micronutrient deficiency.

Chickpea contain phytate that can affect the availability and absorption of the minerals. Phytate is known to interfere with metabolic processes such that the bioavailability of nutrients are negatively influenced and has the ability to form chelates with di-and tri-valent metallic ions such as calcium, phosphorus, zinc and iron to form poorly soluble compounds that are not readily absorbed from the gastrointestinal tract thus decreasing their availability. Therefore, investigation outcomes with optimized processing of cheakpea is justifiable as the

study of antinutritional factor and phytate naturally present in this crop could result to possibly reduce undesirable nutritional factors to safe level of consumption.

Consequently, this study aimed to benefit consumption of the legumes like chickpeas by enhancing decrease in phytate to prevent micronutrient malnutrition. It could propose ways to improve absorption of nutrients.

1.3. Significance of the study

Like other legumes, chickpeas offer countless health benefits and helpful source of minerals, protein and provide dietary phosphorus. In Ethiopia chickpea used as a food in many ways.

This study will benefit consumption of the legumes like chickpeas by enhancing changes in phytate to prevent micronutrient malnutrition. It can improve calcium, phosphorus, zinc and iron deficiencies. The finding may be used as source of information for the further research.

1.4. Objective

This investigation was conducted with the general objective to evaluate processing techniques for chickpea reduction of phytates and HCl-extractability of minerals due to processing of chick pea in Ethiopia.

The specific objectives were:

1. To determine the changes in phytates of soaked, dehulled, cooked, autoclaved and sprouted chickpea
2. To investigate the effect of soaking, dehuling, cooking, autoclaving and sprouting of chickpea on some antinutritional factors and HCl-extractability of some minerals
3. To assess possible interaction of the antinutritional factors with minerals.

2. LITRATURE REVIEW

2.1. Productions of Chickpeas in Ethiopia

Legume grains comprise an important part of the human diet in developing countries in tropical and subtropical areas, where their nutritional contribution is of paramount importance as a large segment of the populations in these areas have limited access to food of animal origin Chickpea is a high-value crop that is adapted to deep black soils in the cool semi-arid areas of the tropics, sub-tropics as well as the temperate areas (Canada and Australia). Chickpea is the most important leguminous food grain in the diets of people in South and West Asia and northern Africa. It is grown on about 10.3 million hectares worldwide and its annual production averages 7.9 million tones. India alone accounts for 63% of the total chickpea growing area. The other major chickpea producing countries are Pakistan, Iran, Canada, Turkey, Ethiopia, Mexico and Syria. Because chickpea is generally grown in drought prone areas, and derives most of its water requirements from residual stored soil moisture rather than from rainfall, chickpea yields tend to trail those of cereals and other legumes cultivated in more favorable areas (Maluku *et al*, 2005).

There are two main types of chickpea, distinguished by seed size, shape and color: one produces relatively small seeds with an angular shape, dark color and called *desi*, the other produces large, rounded, cream color seeds and is called kabuli. Kabuli chickpea seeds are grown in temperate regions, whereas the *desi* type is grown in the semi-arid tropics Both types of chickpea are grown in Ethiopia, Although most chickpeas are produced for human consumption, they provide the livestock industry with an alternative protein and energy feedstuff (Obizoba , 1991).

Ethiopia has suitable agro-climatic conditions for production of both Desi and Kabuli type chickpeas. But, the country has traditionally grown Desi chickpeas both for consumption and sale. Although the Food and Agriculture Organization of the United Nations (FAO) database indicated the recent total area cultivated under chickpeas in Ethiopia at about 170 thousand hectares, MoARD (2003) estimated the total annual production to be around 180 thousand tonnes (Table 1). Chickpeas are grown with the residual end of season soil moisture in

Vertisol areas where water-logging hinders agricultural practices at the height of the rainy season. The crop is traditionally grown with minimal external inputs and does not require thorough land preparation like for cereals. Given the high cost of fertilizers, cereal rotations with nitrogen-fixing chickpeas have traditionally been used for improving the productivity of the following cereal crop (Geletu, 2002).

Table 1. Production and marketing of chickpeas in Ethiopia

Year	Production (t)	Traded in domestic markets (t)	Total production (%)	Exported (t)	Total production (%)	Value of exports (USD)	Value (USD/t)
1997	129,588	129,555	99.97	33	0.03	17,000	515.15
1998	137,133	137,073	99.96	60	0.04	31,000	516.67
1999	138,837	138,810	99.98	27	0.02	14,000	518.52
2000	164,627	164,525	99.94	102	0.06	59,000	578.43
2001	176,313	147,519	83.67	28794	16.33	4,147,813	144.05
2002	179,821	130,992	72.85	48829	27.15	14,771,412	302.51

Source: Enhanced based on data compiled by MoARD (2003).

In Ethiopia, the earliest finding of chickpea is reported in 1520 BC .Ethiopia is the largest producer of chickpea in Africa accounting for about 46% of the continent's production. It is also the seventh largest producer worldwide and contributes about 2% to the total world chickpea production (Chavan *et al.*,2001)

The diverse biophysical and agro-climatic conditions in Ethiopia make it very suitable for growing a number of pulses and legume crops. Chickpea is one of the most important pulses grown widely across the highlands and semi-arid regions of the country. Chickpea is rich in proteins and serves as an economical source of nutritious food for many poor households. Because of its ability to withstand drought stress, smallholder farmers in Ethiopia grow chickpea at the end of the main rainy season using residual soil moisture. This permits farmers to grow a second crop and secure an additional source of income and protein through efficient use of the residual moisture in black soils at the end of the rains. This improves food security for the household while the nitrogen fixed by the crop enriches soil nutrients for the subsequent cereal c crops (Omer,2009).

The crop is widely grown in over 24 districts (*woredas*) of the country that contain deep black soils and is highly preferred by cash-constrained farmers who cannot afford to buy to commercial fertilizers for cereals that are rotated with chickpeas. This makes the crop highly integrated into the farming system, poor and highly ecologically friendly for growing in many areas that suffer from soil nutrient depletion. Recent data from Ethiopian sources shows that about 185 thousand hectares of land were cultivated under chickpea and the total production reached about 180 thousand tones, making Ethiopia the largest producer of chickpea in Africa and the 6th largest worldwide (Siddhuraju et *al*., 2002).

Ethiopia is a secondary center of diversity for chickpea. It is possible that these germplasm lines have developed certain degrees of adaptation to drought during many centuries of cultivation on residual soil moisture Chickpea (*Cicer arietinum* L.) is among the most Chickpea genotypes that are tolerant / resistant to important pulse crops grown mainly by small-scale farmers in the semi-arid tropics (Shiferaw *et al*, 2007).

2.1.1. Chickpea producing areas in Ethiopia

Although chickpea is widely grown in Ethiopia, the major producing areas are concentrated in the two regional states - Amhara and Oromia. These two regions Cover more than 90% of the entire chickpea area and constitute about 92% of the total chickpea production The top 9 chickpea producing zones (North Gonder, South Gonder, North Shewa, East Gojam, South Wello, North Wello, West Gojam, Gonder Zuria) belong to the Amhara region and account for about 80% of the country's chickpea production (see Table 2) (Maluku, U *et al*, 2005).

In Oromia region, the major producing zones are in West Shewa, East Shewa and North Shewa, which account for about 85% of the total area and production in this regional state.

Table 2 Chickpea producing zones in Ethiopia

Region/Zone/Special Wereda	Area (Ha)	Region/Zone/Special Wereda	Area (Ha)
AMHARA		SNNPR	
North Gonder	27,521	Gurage	2,569
South Gonder	19,885	Wolaita	735
North Wello	8,998	Gamo-Gofa	581
South Wello	11,277	Hadiya	550
North Shewa	16,049	Silte	520
East Gojjam	16,029	Burji SW	265
West Gojjam	7,541	Derashe SW	234
Waghemra	1,030	Dawro	90
Awi	490	Kembata/Tembaro	44
Oromia	613	Sub-total	5,588
Sub-total	109,432	ADDIS ABABA	
OROMIA		**Sub-total**	**2,668**
West Shewa	27,062	BENSHANGUL-GUMUZ	
East Shewa	13,670	Metekel	208
North Shewa	6,415	Asosa	154
Jimma	1,609	Kemashi	14
West Wellega	1,368	Sub-total	376
Bale	1,177	SOMALI	
Illubabor	1,151	Jijiga	410
West Hararge	1,028	Sub-total	410
Arsi	950	AFAR	
Borena	662	Zone 2	83
East Wellega	410	Zone 3	143
East Hararge	145	Sub-total	225
Sub-total	55,645	HARARI	
TIGRAY		Sub-total	153
West Tigray	3,417		
Central Tigray	3,292		
East Tigray	1,512		
Sub-total	8,221	**GRAND TOTAL**	**182,718**

*SW = special *wereda*

Source: Customs Authority of Ethiopia (2003)

2.2. Chickpea and its health benefit

Chickpea is a beige-colored legume commonly included in Middle Eastern and Indian food. The Chickpea is the primary ingredient in hummus the dense texture and nutty flavor of the chickpea provide a substance in food that often replaces meat and nuts in vegetarian meals. The dry chickpea is soaked prior to cooking and rinsing the canned chickpea prior to using it improves its digestibility. Including chickpeas in a dietary weight-loss plan provides several benefits. Chickpeas provide an excellent source of a good source of protein, as well as minerals such as iron, copper, zinc, and magnesium. As a good source of fiber, chickpeas can help lower cholesterol and improve blood sugar levels. This makes them a great food especially for diabetics and insulin-resistant individuals. When served with high quality grains, chickpea beans are an extremely-low fat, complete protein food (Sadeghi et al.,2008).

Chickpeas are high in fiber and protein, and they have a low glycemic index. Chickpeas in ones diet each day can help lower LDL (bad cholesterol) levels, which reduces the risk of heart disease. Chickpeas can be used in salads, soups, curries, chilies and stews. High-protein chickpea flour (also called garam) is gluten-free and can serve as a substitute for eggs in baked goods (Alajaji et al, 2006).

Chickpea (*Cicer arietinum* L.) is an important legume crop grown and consumed all over the world, especially in the Afro-Asian countries. It is a good source of carbohydrates and protein, and protein quality is considered to be better than other legumes. Chickpea has significant amounts of all the essential amino acids except sulphur-containing amino acids, which can be complemented by adding cereals to the daily diet. Starch is the major storage carbohydrate followed by dietary fiber, oligosaccharides and simple sugars such as glucose and sucrose. Although lipids are present in low amounts, chickpea is rich in nutritionally important unsaturated fatty acids such as linoleic and oleic acids. β-Sitosterol, campesterol and stigmasterol are important sterols present in chickpea oil. Ca, Mg, P and, especially, K are also present in chickpea seeds (Barampama *et al.*, 1995)

Chickpea is a good source of important vitamins such as riboflavin, niacin, thiamin, foliate and the vitamin A precursor β-carotene. As with other pulses, chickpea seeds also contain anti-nutritional factors which can be reduced or eliminated by different cooking techniques. Chickpea has several potential health benefits, and, in combination with other pulses and cereals, it could have beneficial effects on some of the important human diseases such as CVD, type 2 diabetes, digestive diseases and some cancers. Overall, chickpea is an important pulse crop with a diverse array of potential nutritional and health benefits (A .Richard *et al*, 2003).

2.3. Nutritional composition of Chickpea

Chickpeas are a very good source of carbohydrates and proteins Crude fiber, an important constituent of chickpeas is mostly located within the seed coat. Based on amino acid composition, the proteins of chickpea seed were found, on average, to be of higher nutritive value than those of other grain legumes. Chickpeas meet adult human requirements for all essential amino acids Chickpeas have a high protein digestibility and are richer in phosphorus and calcium than other pulses However; both types of chickpea can be used as potential energy and protein sources in ruminant nutrition (Grahame *et al*, 2000).

Although most chickpeas are produced for human consumption, they provide the livestock industry with an alternative protein and energy feedstuff. During the last decades there has been an increase in interest in their role in animal diets due to ban of animal origin proteins and dissemination of using genetically modified organism (GMO) products. Chickpea seed contains 29% protein, 59% carbohydrate, 3% fiber, 5% oil and 4% ash. Chickpea protein is rich in lysine and arginine but most deficient in sulphur-containing amino acids methionine and cystine Chickpea is also a good source of absorbable Ca, P, Mg, Fe and K (Sadeghi A, 2008).

2.4 Effect of processing

The removal of undesirable components is therefore essential to improve the nutritional quality of Chickpeas and effectively utilize their full potential as human food. Simple and inexpensive processing techniques are an effective method of achieving desirable changes in the seed composition and improving palatability. Various authors have reported that soaking and heat treatments improve the quality of legumes. Soaking removes some antinutritional compounds, which can be partly or totally soluble and eliminated with the discarded soaking solution. At the same time, some metabolic reactions take place during the soaking procedure, affecting the content and composition of the seeds (Messina, 1999).

Legumes are widely consumed throughout the world as a source of protein and other nutrients in human and animal diet. Supplementation of cereals with high protein legumes such as chickpea is potentially one of the appropriate solutions to protein-calorie malnutrition, especially in developing countries. Chickpea on average contains 21%protein and 61% carbohydrates. It is used in cooked or boiled form, whole grain or in split form. However, its usefulness is limited by several factors including low protein digestibility and higher contents of ingredients that cause, flatulence. Numerous toxic constituents are found in raw legumes and many of them may be destroyed by adequate soaking treatment. Heat treatments generally inactivate heatsensitive factors such as trypsin inhibitors and remove volatile compounds. The cooking water may be discarded and some other soluble compounds removed. In many instances, the use of only one method may not result in the desired removal of antinutritional compounds and the combination of two or more methods is required chickpeas are consumed in various forms as processed food. The effects of cooking, Sprouting, and fermentation to reduce the levels of these antinutritional factors. Boiling, cooking and germination affected the composition, antinutritional factors and nutritional quality of chickpeas (Anbreen *et al*, 1999).

2.5. Antinutrational factors and their removal by processing

Chickpeas legumes have been reported to have low nutritive value due to low amounts of sulfur-containing amino acids, low protein digestibility and presence of antinutritional factors. Cooking is usually done before the use of legumes in a human diet. This improves the protein quality by destruction or inactivation of the heat labile antinutritional factors. However, cooking causes considerable losses in soluble solids, especially vitamins and minerals Germination also enhances the nutritive value of legumes by inducing the formation of enzymes that eliminate or reduce the antinutritional and indigestible factors in legumes (Adawy , 2000).

The dry seeds can also be consumed whole or decorticated after cooking and processing in different ways. In addition to these uses, the flour of decorticated chickpea seeds is used in several dishes and as a supplement in weaning food mixes, bread and biscuit. Increasing the time and temperature of processing has been reported to reduce the nutritive value and available lysine of legumes Cooking of chickpea by microwave has not been extensively studied but it has been shown to reduce antinutritional factors and have positive effects on protein digestibility in others legumes A study on chickpeas cooked by microwave is thus needed to ascertain whether this treatment could improve nutritional quality and eventually replace traditional cooking or germination (Omer, 2009) .

2.5.1. Soaking of chickpea

Soaking is often used as a pretreatment to facilitate processing of legume grains and cereal seeds. Soaking may last for a short period, about 15 to 20 minutes, or for a very long period, usually 12 to 16 hours. In household situations cereals and legumes are typically soaked in water at room temperatures overnight. Because phytate is water soluble, a significant phytate reduction can be realized by discarding the soak water. In addition, action of endogenous phytases contributes to phytate reduction. Temperature and pH value have been shown to have a significant effect on enzymatic phytate hydrolysis during soaking. If the soaking step is carried out at temperatures between 45 and 65 °C and pH values between pH=5.0 and 6.0,

which are close to the optimal conditions for phytate dephosphorylation by the intrinsic plant phytases, a significant percentage of phytate (26–100 %) was enzymatically hydrolysed (Sadeghi *et al*,2008).

A soaking procedure usually is a prior step to legume cooking in which aroma and palatability is enhanced. It could be one of the processes to remove soluble antinutritional factors, which can be eliminated with discarded soaking solution. Moreover, some metabolic reactions can also take place during soaking, which may affect the content of some antinutritional compounds (Anbreen *et al.*, 1999).

This process may affect antinutritional factors in legumes in the variable manner. As far as studies on comparative reduction of antinutritional factors are concerned, soaking reduced the contents of phytate, polyphenols and trypsin inhibitors in various media to a significant extent within four lines of L. sativus. The losses were higher in freshly boiled water, alkaline and tamarind solutions than after soaking in drinking water. The highest losses (65-70%) were observed for β-ODAP in boiled water, followed by trypsin inhibitors (42-48%) and polyphenols (30-37%) (Siddhuraju et *al.*, 2002).

2.5.2. Dehulling of Chickpea

The cotyledon (endosperm) of chickpeas is coated by a single fibrous seed coat, which is called hull and having a wrinkled surface .The primary process for obtaining starch-rich and protein-rich fractions is called dehulling which is conversion of whole seed into *dhal* or dehulled seed. During dehulling, the seed coat is removed resulting in the reduction of antinutritional factors contained in seeds such as tannins in pigmented seed coats .Dehulling reduces the fiber content and improves the appearance, texture and palatability of chickpeas. Dehulling also reduces cooking time of legumes. A number of pre-treatment processing such as wetting, equilibrating and drying, improves seed coat removal. Increasing dehulling time results in the decrease of soluble sugar, protein and ash content of dehulled seed fraction but starch concentration increase (Haytowitz *et al.*, 1983).

2.5.2.1. Home-scale Methods

Home-scale methods are the oldest technique for dry dehulling. In this procedure, a small quantity of water is added to the seeds and mixed and/or dried in the sun for a few hours. Then seeds are pounded by mortar and pestle for several minutes. The mortar is made of wood or stone and is shallow and the pestle is made of wood too and has hollow metal shoes 2 to 3 cm long. The shearing action between the pestle and seeds and the scarification between seeds improve dehulling. An air stream is passed through the seeds to separate hull from cotyledons. Sieving also helps to remove the hull. The whole seeds are pounded again for more dehulling and splitting (Tabil *et al.* 1995).

In this method, water and sun drying help in splitting the cotyledons. This method is used for small quantity of grains (1 to 5 kg). For large quantity of grains hand-operated wooden, stone or emery-coated shellers are used instead of pounding in mortar and pestle. After mixing of seeds and water, sun drying follows and the seeds are passed through shellers. The rotating disc provides shearing action and resulting dehulling and splitting. The corrugated contact surfaces of the shellers and the weight of rotating top disc help in dehulling and splitting. Care must be used because excessive pressure can cause high breakage of cotyledons. When grains have excessive moisture, they are soft and breakage of cotyledons results. Thus, important parameters, to be considered, include the exact amount of moisture to be added, the length of sun drying, speed of disc rotation and feed rate (Duhan *et al.*,2002).

Dry Dehulling Method In the first stage, pulse grains pass through an emery-coated roller. Then 1% linseed oil is added and they are dried for 2 to 5 days. Later the seeds are added with 2 to 5% water, mixed and heaped at night. The seeds are passed on scarifier roller to remove hull. In this stage, the hull, which is about half of the seeds, is removed. The seed coat is separated from the seed using an aspirator and sieves are used to obtain split seeds. In order to remove the rest of the hulls, the non-split seeds are passed through the scarifier roller for the second time (Tabil *et al.* 1995).

2.5.2.2. Wet Dehulling Method

Wet processing, in home scale, consist of soaking the seeds in water for one to four hours, rasping and followed by separating the hulls by floatation. If the hulls are very resistant to soaking, the dry seeds are split or ground coarsely before soaking. Wet processing is used to prepare more highly purified protein and starch, where high amount of energy is spent for drying and refining of effluent. Dry processing such as pin milling and air classification, does not result in as pure protein fraction as wet processing, but this method is an effluent-free process and cheaper (Nonogaki *et al.* ,2010).

2.5.2.3. Cottage-Scale Methods

Cottage-Scale Methods The principles of this method is similar to home-scale methods but on a larger scale. Thefirst stage of dehulling includes: continuous sun drying until the hulls are removed; or using water, sun drying for several hours and tempering; or heating the seeds in a pan with or without sand with strong stirring; or a combination of these techniques the second stage is done by: pounding in mortar and pestle; or grinding in hand operated wooden or stone shellers; or grinding on a rough flat stone with a stone rolling pin, which usually operated, by legs. The hulls are removed by passing an air stream through the seeds (Bishnoi *et al.*, 1995).

2.5.2.4. Commercial Methods

The basic techniques are the same as mentioned in cottage-scale method but some operations like water addition, dehulling, splitting, aspiration and sieving are mechanized. The cleaned size-graded seeds are passed through the roller-machine resulting in 'pitting' and cracking of the hulls. This leads to the facilitating of water absorption. For loosening of chickpea hull, sun drying must be used. When the sun shines during the rainy season, the seeds are dried because even mild sun drying is enough. Dehulling and splitting are done in a roller machine, which are either simultaneous or separate operations. In separate operations, the water treated and sun dried dehulled seeds are split in sheller machines. In a typical commercial method,

the cleaned size-graded seeds are pitted in rollermachine, resulting in cracks in the hulls. After the hull and small broken seeds have been removed, the seeds are treated with 8 to 10% water in a warm mixing unit. This is followed by tempering the water-treated seeds for 2 to 5 hours and spreading the seeds for sun drying for two to three days. The amount of drying is determined by test grinding. The seeds are collected, cooled and dehulled in a roller-machine until 50 to 70% of the seeds are dehulled and some of them are split. The hulls and powder are sieved and aspirated and the dehulled splits are separated by sieving (Khattak *et al.*, 2008).

Finally, the hulled and dehulled unsplit grains are sun dried and dehulled repeatedly .In separate splitting operation; the seeds are dried to a lower moisture level because drying prevents splitting. The grains are passed through the roller-machine for dehulling. More moisture is added to the dehulled whole seeds and they are split after 3 to 6 hours of sun drying through the sheller machine (Tabil *et al.*, 1995).

2.5.2.5. Improved Dehulling Method

A hot air steam of 300°C is passed through the seeds to reach a critical point. This treatment makes seed coat brittle. Then the seeds are passed through the pulse-dehulling machine to remove the seed coat. This action is done by stone coated-rollers. From a nutritional point of view, the difference between dehulled seeds and whole seed in mineral composition is marginal except for calcium (Salunkhe et al. 1985; Chavan et al. 1986; Saxena and Singh 1987). Most of the seed calcium is located in the seed coat. Therefore, the consumption of whole seed would be useful in calcium-deficient diets. Chickpeas are also a good source of iron. They contain higher level of iron in comparison with other legumes ((Tabil et al. 1995).

2.5.3. Sprouting

Sprouting is a process widely used in legumes and cereals to increase their palatability and nutritional value, particularly through the breakdown of certain antinutrients, such as phytate and protease inhibitors. In non- sprouted legume grains and cereal seeds, with the exception

of rye and to some extent wheat, triticale and barley, only little intrinsic phytate-degrading activity is found, but during sprouting a marked increase in phytate-degrading activity with a concomitant decline in phytate content was observed Phytate is hydrolyzed during sprouting in a stepwise manner by phytases or a concerted action of phytases and phosphatases which do not accept phytate as a substrate to supply the nutritional needs of the plant without an accumulation of less phosphorylated myo-inositol intermediates.Because phytate is heat-stable (Anbreen *et al.*, 1999).

Sprouting has mobilizing secondary metabolic compounds which are thought to function as reserve nutrients. The phyticacid serves as an important reserve of phosphate generated by the action of phytase during seed sprouting for the developing seedling. However, this conversion depends upon the type of beans and Sprouting conditions. In lentils, Sprouting reduces phytic acid content at a higher rate than soaking or cooking. It can reduce the phytic acid content of chickpea and pigeon pea seeds by over 60% and that of mung bean, urd bean, and soybean by about 40% (Omer, 2009).

As far as the affect of sprouting on tannins is concerned, sprouting could make tannins move in the same way as that of soaking and cooking, and the fact that these compounds are heat and light sensitive make them more prone to destruction. As the sprouting period increased to six days, TIA and the PA contents decreased to a large extend, while amounts of tannins and catechins increased in two chickpea cultivars. Moreover, sprouting was considered as an effective treatment for destroying trypsin enzyme inhibitors than either phytates or polyphenols (Urbano, *et al.*, 1995).

2.5.4. Autoclaving/ Cooking

It has been found that the thermostability of antinutritional factors in legumes varies not only with legume source but also with the different conditions used during processing, such as pH, humidity, time, temperature, and pressure. Cooking treatment of legumes reduced the antinutritional factors but at variable rate. It was noted that cooking for 60 min at 100°C was sufficient to inactivate over 90% of TIA in *P. vulgaris* and eliminated completely on heating

soaked red gram seeds in boiling water for 5 min. TIA of pre-soaked faba beans decreased significantly after they were cooked for 35 min. Moreover, application of wet and/or heat processing reduced the trypsin inhibitor levels in faba beans down to 42 to 56% of the original (Haytowitz et al., 1983).

A complete destruction of TI after cooking pre-soaked chickpeas for 35 min was noted. Similarly, chickpea seeds were cooked for 40 min and complete elimination of TI was observed Overnight soaking chickpeas followed by boiling for 2 hrs completely abolished TIA. This complete inactivation action of trypsin inhibitor, potentially improves legume protein digestibility. TI isolated from faba bean seeds were inactivated after autoclaving. However heat treatments improved legume protein digestibility which is attributed to the removal of the heat-labile antinutritional constituents **It** has been observed that cooking of pre-soaked seeds were more effective in reducing the level of the contents of phytate, polyphenols, trypsin and amylase inhibitors than soaking (Omer, 2009).

Considerable phytate dephosphorylation during cooking only takes place either by discarding the cooking water or by enzymatic phytate hydrolysis due to the action of the intrinsic plant phytases during the early part of the cooking phase. Prolonged times at elevated temperatures lead to a progressive inactivation of the endogenous enzymes. Thus, providing plants with heat-stable phytases or addition of exogenous heat-stable phytases are seen as possibilities to improve phytate dephosphorylation during cooking (Walter *et al.*, 2002).

2.5.5. Bioavailability of minerals

Being a good source of minerals, legumes fulfill dietary requirements of human in adequate manner. Among different food groups, chickpea plants get minerals from their soil environment and deposit these to their seeds. Roots utilize specific and/or selective transport proteins to obtain minerals that are essential for plant growth and development including calcium (Ca), phosphorus (P), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), magnesium (Mg), potassium (K) and sodium Na) . These minerals collectively contribute towards ash fraction of the seed. These take part in many metabolic activities including

photosynthesis, respiration, chlorophyll synthesis, cell division and various responses to biotic stress. The concentrations of any given mineral in legume seeds vary depending on genotype and environmental constraints (Omer, 2009).

A number of studies have been carried out for the evaluation of processing impact on the fate of minerals. In general, mineral contents seem not to be affected during food processing. However, the loss of minerals on soaking and cooking may be attributed to their leaching out into discarded water which influences bioavailability of minerals during processing treatments. The minerals have a multiple and complex type of interactions within the food matrix. Processing usually exerts a positive impact through separation or partitioning of minerals, or through the destruction of inhibitors or the beneficial complex formation between food components and metal ions, thereby enhancing their availability (Singh *et al.*, 1987).

The bioavailability of a nutrient can be subdivided in to three constituent phase's i.e. availability in the intestinal lumen for absorption, absorption and/or retention in the body and finally utilization by the body. Food processing can only influence the first phase by determining the amount of minerals (content in raw materials minus losses) and the speciation of the metal ion in the product and in the intestinal lumen. However, other phases depend on the homeostatic regulation mechanisms and individual physiological needs of the body. As the minerals are extractable in 0.03N HCl, the concentration of HCl found in human stomach, is an index of its in-vitro bioavailability from foods(Manary *et al.*, 1998)..

The determination of HCl-extractability of minerals in processed chickpea showed a great improvement in this attributes. However, in soaked seeds in the absence of further treatments,the HCl-extractability of minerals was recorded at par with unprocessed seeds of chickpea and blackgram (Vigna rnungo). Comparatively, poor extractability of minerals when soaked in water might be due to leaching out of some minerals into discarded soaking water under the influence of concentration gradient. Hence, these minerals from chickpea seeds may be lost when water used during cooking was discarded. Haytowitz and Matthews (1983) reported that cooking in boiling water caused great losses of potassium (24%), copper

(15%) and iron (8%). Reduction in iron content of the soaked chickpea as compared to raw ones was also noted (pollan *et al.*, 1999)

The soaking and cooking treatments are found as effective techniques and caused improvement in the availability of both major and trace minerals in chickpea seeds In unprocessed chickpea seeds, the HCl-extractability of calcium, iron and phosphorus was 70.2, 78.0 and 33.4%, respectively whereas the soaking the seeds for 12 hrs enhanced the HCl-extractability of calcium, iron and phosphorous by 4, 8 and 13%, respectively, over the control values. Except for soaking and dehulling, the remaining processing and cooking methods did not lower the contents of total calcium, phosphorus and iron in chickpea seeds. HCl- extractability of dietary essential minerals was enhanced significantly when the chickpea seeds were processed and cooked with extended cooking time HCl-extractable ash and phosphorus were enhanced. High temperature such as pressure cooking showed the highest percentages of both extractable ash (94.93%) and phosphorus (4.36%) in vegetable chickpea (Omer, 2009).

Sprouting of seeds is a useful method in increasing the availability of minerals but their raw consumption is sometimes unacceptable from sensory point of view. Therefore, the consumption of cooked sprouts may be encouraged to enhance the availability of food legumes and to reduce the incidence of several prevailing mineral deficiencies particularly in developing countries. It has the most pronounced effect on increasing the HCl-extractability of calcium, phosphorus, iron, zinc, copper and manganese of primarily processed chickpea and black gram Further, sprouting followed by dehulling and soaking has been the best method for improving the extractability of calcium, phosphorous and iron in seeds of rice bean and faba bean. The husks of these legumes have significantly lower extractable levels of these minerals' HCl- extractability of calcium, iron and zinc in kidney bean (Reddy *et al.*, 1989).

Germination up to 48 hrs enhanced the HCl-extractability of zinc and copper to a remarkable extent. In case of pigeon pea, germinated for 36 hrs followed by pressure cooking, it increased up to 18–19% and 16-18% by 48 hrs germination. Copper extractability enhanced

to the maximum extent (3–4%) over the control when the seeds of pigeon pea cultivars were germinated for 36–48 hrs. Significant improvements in HCl-extractability of all minerals were observed after sprouting the seeds for 24 hrs. In rice bean seeds, maximum enhancement was noticed in phosphorus extractability followed by iron and calcium) Fermentation also improves HCl-extractability of all minerals. However, fermentation of the cooked seeds almost has no effect on contents of all major and minor elements studied (Omer, 2009).

2.6. Minerals in Chickpeas

Chickpea is an excellent nutritional choice as they are high in minerals that support the metabolic and digestive system. The most important minerals contained in chickpeas are calcium, phosphorus, magnesium, iron, copper, zinc, sodium and potassium. Chickpeas are versatile legumes that have a delicious nutlike taste and buttery texture. They come in varieties of black, green, red and brown, as well as the most commonly eaten beige colored beans. Chickpeas provide a good source of folate, manganese, copper, and the antioxidant Vitamin E, which promotes the ability of white blood cells to fight infections, as well as zinc, needed for healthy cell growth. They contain *protease inhibitors*, which halt the DNA-destroying action of cancer cells. Chickpeas are a good source of isoflavones, plant chemicals that are converted in the gut into a substance that mimics the hormone *estrogen*, helping to prevent estrogen-related conditions such breast cancer. These beans are further beneficial for women's health as they are very high in iron, and women in particular tend to be deficient in iron. High in fiber and flavonoids, chickpeas help to keep the digestive system health and stabilize blood sugar. When combined with whole grains such as brown rice, chickpeas provide a high quality protein (Bhaskarachary, 2010) .

2.6.1. Iron

Iron (Fe) is a major constituent of blood, being a component of haemoglobin, and thus plays a crucial role in oxygen delivery throughout the body. Due to its redox potential, iron is also involved in many heme- containing compounds or iron sulphur enzymes that are essential for electron transportation, respiration and energy metabolism. It is an essential element and

physiological losses of it must be compensated regularly. Three factors affect the iron needs: the total amount in the diet, the type of Fe compound, and the other diet components. Iron deficiency anemia is a worldwide health problem that is especially common in young women and in children. It is the most common form of malnutrition in the world, affecting more than 2 billion people globally and highly prevalent in less-developed countries. It remains a problem in developed countries (Johnson, 2012).

Iron deficiency is the primary cause of anemia, which affects roughly one-quarter of the world's population As the most prevalent micronutrient deficiency in the world, iron deficiency affects all age groups, with the most common being children between the ages of 0 and 5 Chickpeas are considered a good source of iron (Vijayakumari *et al.*, 1998)

2.6.2. Calcium

Calcium is an important component of a healthy diet and a mineral necessary for life. The National Osteoporosis Foundation says, "Calcium plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life." Approximately 99 percent of the body's calcium is stored in the bones and teeth. The rest of the calcium in the body has other important uses, such as some exocytosis, especially neurotransmitter release, and muscle contraction (Pointillart *et al*, 1999).

Calcium is an important mineral for human body. It primarily takes three forms in the body. First, it is part of the molecular composition of bone, forming a complex with other molecules. In the blood, it exists in two forms: one bound to proteins and other molecules, and the second as free electrically charged calcium atoms. In these forms, calcium helps perform a number of important functions. Long-term calcium deficiency can lead to rickets and poor blood clotting and in case of a menopausal woman, it can lead to osteoporosis, in which the bone deteriorates and there is an increased risk of fractures. While a lifelong deficit can affect bone and tooth formation, over-retention can cause hypercalcemia (elevated levels of calcium in the blood), impaired kidney function and decreased absorption of other minerals (Morris *et al.*, 1985).

High calcium intakes or high calcium absorption were previously thought to contribute to the development of kidney stones. However, a high calcium intake has been associated with a lower risk for kidney stones in more recent research. Vitamin D is needed to absorb calcium. Other possible benefits of using calcium for the human body include: help manage high blood pressure, cutting down risks of colorectal cancer, cholesterol reduction, help in weight loss prevention of stroke, and reducing tooth loss. Chickpea seeds contain 103±259 mg Ca/0.1 kg dry weight 70% of this is in the seed coat and are therefore a potential source of dietary Ca. Adequate Ca nutrition during childhood has important implications for bone growth and development, and is thought to reduce the incidence of osteoporosis in later life. (Pointillart *et al*, 1999)

2.6.3. Phosphorus

Phosphorous (or phosphate) is part of the phospholipids, an essential functional component of cell membranes, and is part of high energy phosphate compounds like e.g. adenosine triphosphate (ATP) and creatine phosphate, the biological energy conservation molecule which is essential to all vital processes. Phosphorus is also an essential component of hydroxyapatite, the main structural bone mineral. Deficiency of phosphorus is common in malnourished children and severe hypophosphatemia is associated with increased mortality in kwashiorkor (Manary *et al.*, 1998).

Phosphorus deficiency is also likely to cause rickets-like bone changes in malnourished children. Phosphorous is likely to be a limiting nutrient in treatment of children. Absorption of dietary phosphorus is high (55-70%), relatively independent of dietary composition, and does not appear to be up-regulated at low intakes. Dairy products, meat, poultry, eggs, fish, nuts, and legumes are generally good sources of highly available phosphorus. However, the main form of phosphorus from plant material is phytate which is resistant to digestion unless enzymatically degraded by phytase. Thus, phosphorus from phytate is only absorbed to a minor degree under normal conditions and the phytate fraction of phosphorous should therefore be discounted from the calculations of the total phosphorous requirements (Golden, 2009).

2.6.5. Zinc

Zinc is a micromineral needed in the diet on a daily basis the concentration of zinc in plants varies based on levels of the element in soil. When there is adequate zinc in the soil, the food plants that contain the most zinc are chickpeas and various seeds. Zinc deficiency is usually due to insufficient dietary intake, but can be associated with malabsorption, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses Nearly two billion people in the developing world are deficient in zinc. In children it causes an increase in infection and diarrhea, contributing to the death of about 800,000 children worldwide per year. The World Health Organization advocates zinc supplementation for severe malnutrition and diarrhea. Zinc supplements help prevent disease and reduce mortality, especially among children with low birth weight or stunted growth. However, zinc supplements should not be administered alone, since many in the developing world have several deficiencies, and zinc interacts with other micronutrients. Although zinc is an essential requirement for good health, excess zinc can be harmful. Excessive absorption of zinc suppresses copper and iron absorption. The free zinc ion in solution is highly toxic to plants, invertebrates, and even vertebrate fish (Maluku *et al*, 2005).

Zinc (Zn) plays a crucial role in energy metabolism, as it is involved in numerous catalytic, structural or regulatory processes .It prohibits oxidative damage to human body by acting as a co-factor of the superoxide dismutase enzyme involved in protection against oxidative processes. The metal is also required for DNA and RNA synthesis .It is vital to endothelial cell integrity and plays an important role in vascularendothelial barrier function. The total amount of this element in foods and its HCl-extractability jointly contribute towards net delivery of Zn.

The results for the effect of processing treatments on HCl-extractability of zinc in chickpea differed highly significantly. Soaking and autoclaving remained at par in respect to their effect on Zn HCl- extractability (56.99±1.71 and 52.05±1.38%, respectively). Controlled fermentation achieved higher HCl-extractability of Zn (79.94±3.78%). The results differed from raw samples by 27.06%. It was followed by HCl-extractability of Zn affected by natural

fermentation (75.90±2.47%) differing by 23.02 percentage points and germination (71.14±3.29%) showing the difference of 18.26 percentage points. On the other hand in chickpea, controlled fermentation, natural fermentation and germination did not differ significantly for their effect on the Zn HCl-extractability. The results for these treatments ranged between 77.78 to 81.25% extractability (Oberleas *et al.*, 1981).

2.7. Antinutritional factors

Although, legumes have been proven as a cheap source of protein, the nutritional quality of a legume could be affected by a few of antinutritional factors i.e. proteolytic inhibitors, phytates, compounds causing favism, polyphenols, phytohemagglutinins, lathyrogens, cyanogenetic compounds and saponins .Chickpea seeds, like many legumes, also contain a variety of chemical substances that cause digestive problems in human beings. Thus its nutritive value depends on its chemical composition, bioavailability of several essential nutrients and concentration of antinutritional substances occurring in food (Walter *et al.*, 2002).

2.7.1 Tannins

Tannins are polyphenol components prevalent in food legumes. Studies have shown that tannins interact with proteins, enzymes or nonenzymes, and form tannin-protein complexes, which decrease protein digestibility and protein solubility. This decrease in protein digestibility may be caused by either the inactivation of digestive enzyme or the reduction of the susceptibility of the substrate proteins after forming the complex. Chickpea seeds (whole seed) contain 78 to 272 mg tannins per 100 g seeds; while the cotyledons have only 16 to 38 mg per 100 g seeds. Tannins are mainly located in the seed coati (Maluku *et al*, 2005).

Tannins have been reported to occur in appreciable amount in legumes. The seed coat color of legumes has largely been associated with tannin content . These have the tendency to form stable or weak complexes with proteins, carbohydrates and vitamins. These can cross link with protein by reacting with lysine, threonine and tyrosine making them unavailable during digestiont . The degree of polymerization of these polyphenolic compounds plays an

important role in protein digestibility and availability of vitamins and minerals (Sadeghi *et al.*, 2008).

2.7.2 Phytate

Phytates are the principal storage form of phosphorus and are particularly abundant in cereals and legumes. These chelate divalent cations such as calcium, magnesium, zinc and iron, thereby also reducing their bioavailability. These can inhibit the activity of several enzymes and can also reduce the digestibility of legume proteins by forming the complexes with them (Omer. 2009).

Phytate, which is also known as inositol hexakisphosphate, is a phosphorus containing compound that binds with minerals and inhibits mineral absorption. The presence of phytate in foods has been associated with reduced mineral absorption due to the structure of phyate which has high density of negatively charged phosphate groups which form very stable complexes with mineral ions causing non-availability for intestinal absorption. Phytic acid binds trace elements and macro-elements such as zinc, calcium, magnesium and iron, in the gastrointestinal tract are 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 to be largely unavailable to the organism because of the limited capacity of monogastric species to hydrolyse phytate in the small intestine (Walter *et al.*, 2002).

3. MATERIALS AND METHODS

3.1 Chemicals and reagents Apparatus

Analytical balance, Fume hood, Measuring cylinder ,Pipette, Spatula ,Boric acid (H_3BO_3), ,Hydrochloric acid (HCl), H_2O_2,Methyl red ,Sodium hydroxide (NaOH), Sulfuric acid (H_2SO_4), Conc. ,Potassium sulfate (K_2SO_4), Cupric sulfate, Crucible ,Oven, Atomic Absorption Spectrophotometer, Ferric Chloride ($FeCl_3.6H_2O$), 0.03% , Sulfosalicylic acid, Wade Reagent .

3.2 Sample collection

The seeds of Harbu variety of Chickpea were obtained from Debire Zeit Agricultural Research Center. It is about 50 km, East of Addis Ababa. Debre Zeit Research Center was the major research entity.

3.3 Experimental setting

Samples were grouped into eight categories. Accordingly, the samples were either soaked, raw chickpea (RC), SO- soaked chickpea for (6, 12 and18 hr),dehulled chickpea(DE), un-soaked cooked chickpea (UNS- cooked), soaked cooked chickpea(SO- cooked), dehulled cooked chickpea (DE- cooked), autoclaved chickpea (AUT) and Sprouted chickpea for (24, 36 and 48hr).

3.4 Preparation of sample

The seeds were cleaned of dust, cracked and broken seeds, and other foreign material prior to processing treatments. Raw seeds were milled to powder (0.425 mm sieve) in an electric grinder packed in polyethylene bags and used as the control. The processing and cooking treatments, describe below.

3.4.1. Soaking

300g of seeds were soaked in distilled water for 6, 12 and 18 h at 30 ^{0}C The seed to water ratio used was 1:5 w/v. The soaked seeds were washed and rinsed with distilled water.

3.4.2. Sprouting

300g of soaked seeds (12 hr) were washed and rinsed with distilled water. The seeds were rolled in germination paper and kept in an incubator at 30 ^{0}C for 24, 36 and 48 h.

3.4.3. Dehulling

After soaking of 300g of seeds (12 hrs), hulls were removed manually.

3.4.4 Cooking

Seeds un-soaked, soaked (12 h) and soaked (12 h)-dehulled were cooked for 30 minutes. The amount of water used for cooking was three times the weight of the seeds. Seeds were cooked until soft when compressed between the fingers.

3.4.5 Autoclaving

300g chickpea seeds autoclaved at 121°C and 15 lb pressure for 15 min and cooled to ambient temperature. All the processed seeds were dried in oven at 60 ^{0}C to a constant weight then ground in an electric grinder using 0.425 mm sieve size. Ground samples were packed in polyethylene bags for further chemical anal

3.5 . Proximate analysis

3.5.1 Determination of moisture content

The moisture content of chickpea seeds can therefore be determine (AOAC 925.09, 2000) accurately by measuring the number or mass of water molecules present in a known mass of sample. Empty dishes and lids where dried using air drying oven for 1 hour at 1000C, transferred to the discator with granular silica gel, cooled for 30 minutes, and weighed. The prepared samples were mixed thoroughly and about 5.000g of fresh samples were transferred to the dried and weighed dishes. The dishes and their contents were placed in the drying oven and dried for 3 hr at 1050C, and then the dishes and their contents were cooled in a discator to room temperature and reweighed. The moisture content calculate

$$Moisture\ content = \frac{weight\ of\ wet\ sampie - weight\ of dried\ sample}{weight\ of\ wet\ sample} \times 100\%$$

3.5.2 Determination *of Crude* protein

Protein was determined by the Kjeldahl method (AOAC 979.09, 2000). All nitrogen is converted to ammonia by digestion with a mixture of concentrated sulfuric acid and concentrated orthophosphoric acid containing copper sulfate and potassium sulfate as a catalyst. The ammonia released after alkalinization with sodium hydroxide is steam distilled into boric acid and titrated with hydrochloric acid.

Digestion: About 0.5000g of fresh samples were taken in a Tecator tube and 6ml of acid mixture (5 parts of concentrated orthophosphoric acid and 100 parts of concentrated sulfuric acid) was added, mixed, thoroughly and 3.5ml of 30% hydrogen peroxide was added step by step .As soon as the violet reaction had ceased, the tubes were shaken for a few minutes and placed back into the rack. A 3.0000g of the catalyst mixture (ground 0.5000g of copper sulphate with 100 g of potassium sulfate) was added into each tube, and allowed to stand for about 10 min before digestion. When the temperature of the digester reached 370^0C, the

tubes were lowered into the digester. The digestion was continued until a clear solution was obtained, about 1 hr.

The tubes in the rack was transferred into the fume hood for cooling, a 15ml of de ionized water was added, and shaken to avoid precipitation of sulfate in the solution.

Distillation: A 250ml conical flask containing 25ml of the boric acid-indicator solution was placed under the condenser of the distiller with its tips immersed into the solution. The digested and diluted solution was transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5ml deionized water and the rinses were added into the solution. A 25ml of 40% sodium hydroxide solution was added into the compartment and washed down with a small amount of water, stoppered and the steam switched on. A 100ml solution of the sample was distilled, and then the receiver was lowered so that the tip of the condensers above the surface of the distillate. The distillation was continued until a total volume of 150ml is collected. The tip was rinsed with a few milli-liter of water before the receiver was removed

The amount of Nitrogen was calculated using the formula:

$$\%N = N\,HCl \times \frac{(volume\ of\ sample - volume\ of\ blank)L}{grame\ of\ sample} \times \frac{14gm}{mole} \times 100$$

Crude protein=6.25×%N

3.5.3. Determination of crude fat content

Crude fat was determined by (AOAC 925.09, 2000) semicontnous solvent extraction methods (soxhlet method). Accordingly, for all sample categories, 2gm of dried grounded samples will be placed in a porous cellulose extraction thimble and thimble was covered with fat free cotton. The thimble was placed in an extraction chamber which is suspended above a flask containing the solvent (50ml of diethyl ether) and below a condenser. The flask which had dried drying oven at 105 ^{0}C containing boiling chips was placed inside the extraction

chamber and heated at 55^0C and the solvent evaporates and moves up into the condenser where it converts into a liquid that trickles into the extraction chamber containing the sample .at the end of the extraction process, which typically lasts for 3hrs, the flask containing the solvent and lipid was removed , the solvent was evaporated in drying oven at 70^0C the mass of lipid remaining was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage . The crude fat in the initial sample was calculated as.

$$Fat\ content = \frac{weight\ of\ fat}{weight\ of\ sample} \times 100$$

3.5.4 . Determination of crude fiber content

Crude fiber was determined (AOAC 925.09, 2000) after digesting a known weight of Chickpea seeds by Refluxing 1.25% boiling sulfuric acid and 28% boiling potassium hydroxide. About 1.6000g of fresh sample was placed into a 600ml beaker,200ml of 1.25% H2SO4 was added, and boiled gently exactly for 30 minutes placing a watch glass over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot distilled water. After 30 minute boiling, 20ml of 28% KOH was added and boiled gently for a further 30 minute, with occasional stirring. The bottom of a sintered glass crucible was covered with 10 mm sand layer and wetted with a little distilled water. The solution was poured from beaker into sintered glass crucible and then the vacuum pump was turned on. The wall of the beaker was rinsed with hot distilled water several times; washings were transferred to crucible, and filtered the residue in the crucible was washed with hot distilled water and filtered (repeated twice). The residue was washed with 1% H_2SO4 and filtered, and then washed with hot distilled Water and filtered; and again washed with 1% NaOH and filtered. The residue was washed with hot distilled water and filtered; and again washed with 1% H_2SO4 and filtered. Finally the residue was washed with water- free acetone. The crucible with its content was dried for 2 hours in an electric drying oven at 130^0C and cooled for 30 min in the desiccator (with granular silica gel), and then Weighed. The crucible was

transferred to a muffle furnace (Gallenkamp, size 3) and incinerated for 30 min at 550^0C. The crucible was cooled in the desiccator and weighed.Then the fiber was calculated as a residue after subtraction of the ash:

Crude fiber g /100 g= (W1-W2) x100]/W3

Where: W1 = weight of (crucible +sample) after drying; W2 = weight (crucible +sample) after ashing; W3 = weight of fresh sample

3.5.5. Determination of total ash content

Ash was determined by (AOAC 925.09, 2000) incineration of known weights of the samples in a muffle furnace at 550^0C (Gallenkamp, size 3) until a white ash is obtained. Organic matter is burned off and the inorganic material remaining is cooled and weighed. Heating was carried out in stages, first to derive the water, then to char the product thoroughly and finally to ash at 550^0C in a muffle furnace. The ashing dishes (made of porcelain) were placed into a muffle furnace for 30 min at 550^0C. The dishes were removed and cooled in a desiccator (with granular silica gel) for about 30 minutes to room temperature; each dish was weighed to the nearest g. About 2.000g of flour sample was added into each dish. The dishes were placed on a hot plate under a fume hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes were placed inside the muffle furnace at 550^0C for 4 hours, and removed from the muffle and then placed in a desiccator for 1hr to cool. The ash was clear white in appearance. When cooled to room temperature, each dish. Weight of total ash was calculated by difference and expressed as percentage of sample.

$$\text{Ash content}=\frac{(W_2-W_1)}{W_3}\times 100$$

3.5.6 Determination of carbohydrate

Utilizable carbohydrate content was determined by difference. It was determined by subtracting the crude protein, crude fiber, total ash and fat from the total dry weight of the sample.

3.5.7 Determination of gross energy

Gross energy was determined by calculation from fat, carbohydrate and protein contents using the Atwater's conversion factors; 4 kcal/g for protein, 9 kcal/g for fat and 4 kcal/g for carbohydrates and expressed in calories.

3.6. Minerals Analysis

Minerals were determined using AOAC (1984) Atomic Absorption Spectrophotometer while phosphorous determine by colorimetric method using ammonium molybdate. After removal of organic material by dry ashing, the residue was dissolved in dilute acid. The solution was sprayed into the flame of Atomic Absorption and the absorption of the metal to be analyzed was measured at a specific wavelength. Standard solutions: The stock standard solutions of minerals (iron, zinc and calcium) were diluted with 0.3 N HCl The Atomic Absorption Spectrophotometer (AAS) used for mineral determination were calibrated using standard solutions and the reagent blank solution was run with the sample Ashes were obtained from dry ashing. The ash was wetted completely with 5ml of 6N HCl, and dried on a low temperature hot plate. A 7ml of 3N HCl was added to the dried ash and heated on the hot plate until the solution just boils. The ash solution was cooled to room temperature at open air in a hood and filtered through a filter paper into a 50ml graduated flask. A 5ml of 3N HCl was added into each crucible dishes and heated until the solution just boiled, cooled, and filtered into the flask. The crucible dishes were again washed three times with de-ionized water; the washings were filtered into the flask. A 2.5ml of 10% Lanthanum chloride solution was added into each graduated flask. Then the Solution was cooled and diluted to the mark (50ml) with de-ionized water. A blank was prepared by taking the same procedure as the sample Spectrophotometer

$$Metal\ content(^{mg}/_{100gm}) = \frac{(A-B) \times V}{10W}$$

Where:

W=weight of the sample

V=volume of the extract

A = concentration (µg/ml) of sample solution

B = concentration (µg/ml) of blank solution

Phosphorous is determined by Taussky & Shorr by the colorimetric method using ammonium molybdate. It was converte to phosphomolybdate, which is reduced to a blue 35 molybdenum compound by aminonaphtholsulphonic acid to give a blue molybdenum compound. A sample solution is obtained from mineral analysis (determination of Fe, Zn and Ca). 1 ml of the clear extract was taken from the sample solution and diluted to 100 ml with de-ionized water in a 100 ml volumetric flask. A 5ml (triplicates) of the sample dilution was added into test tubes. A 0.5ml of molybdate and a 0.20ml aminonaphtholsulphonic acid was added into the test tube (sample solution) and mixed thoroughly step by step. A 0.20ml aminonaphtholsulphonic acid was added into the test repeatedly each time until the solution becomes clear. The solution was allowed to stand for 10 minute. The absorbance (reading A) of the solution was measured at 660 nm against distilled water. Simultaneously with sample phosphorous, standard and blank analysis were carried out. Standard and blank solutions were prepared as above but 5 ml of working standard. And 5 ml of de-ionized water (reading B) in place of the sample dilution were used respectively. A standard curve was made from absorbance versus concentration and the slope was used for calculation.

$$P(^{mg}/_{100gm}) = \frac{(A-B) \times 50 \times 100}{Slope \times 10 \times W}$$

3.6.1. HCl-extractability

Minerals in the samples were extracted by the method of Chauhan and Mahjan (1988). About 1.0 g of samples were shaken with 10 ml of 0.03 N HCl for 3 hr at 37°C and then filtered.

The clear extract obtained was oven dried at 100°C and then place in a muffle furnace. At 550°C for 4hr. Thereafter, the samples were cooled and about 5ml of 5N HCl is added and boiled gently for 10min and then cooled dilute to 100ml with deionized water. Minerals were determined as describe above. Extractability of each element was calculated as a percentage of total amount of the element.

$$\text{Mineral extractability (\%)} = \frac{mineralextractable\ in\ 0.03\ NHCL}{total\ minerals} \times 100$$

3.7 Anti nutritional factor

Tannin content was determined by the method of Burns (1971) as modified by Maxson and Rooney (1972) and phytic acid was determined by method of Vantraub and Lapteva (1988).

3.7.1 Determination of phytate content

Phytate was determined by the method of by Vantraub and Lapteva (1988). About 0.1000g of fresh samples were extracted with 10ml 2.4% HCl in a mechanical shaker (Eberbach) for 1hour at an ambient temperature and centrifuged at 3000rpm for 30 minute. The clear supernatant was used for phytate estimation. A 2ml of Wade reagent (containing 0.03% solution of FeCl3.6H2O and 0.3% of sulfosalicilic acid in water) was added to 3ml of the sample solution (supernatant) and the mixture was mixed on a Vortex (Maxi Maxi II) for 5 seconds. The absorbance of the sample solutions were measure at 500 nm using UVVIS spectrophotometer (Beckman DU-64- spectrophotometer, USA). A series of standard solution will prepare containing 0, 5, 10, 20 and 40 μg/ml of phytic acid (analytical grade sodium phytate) in 0.2N HCl. A 3ml of standard was added into 15ml of centrifuge tubes with 3ml of water which were used as a blank. A 1ml of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 5 seconds. The mixtures were centrifuged for 10 minutes and the absorbance of the solutions (both the sample and standard) were measured at 500nm by using de-ionized water as a blank. A standard curve

was made from absorbance versus concentration and the slope and intercept were used for calculation.

Calculation:

Phytic acid in mg/100g = (absorbance-intercept) 3/(slope x ρ x wt. of Sample x10)
Where, ρ is density

3.7.1.1. Determination of phytate and non-phytate phosphorus

Phytate and phosphorous were determined by the above methods. Phytate phosphorus Was calculated with the following formula (Khetarpaul and Sharma, 1997).

Phytate phosphorous (mg/100g) = (A x28.18)/100
Where: A = phytate content (mg/100g)

Non-phytate phosphorus was calculated as a difference between the total phosphorus and phytate phosphorus

3.7.1.2. Phytate mineral molar ratio calculation

Phytate calcium molar ratio was obtained (mg of phytate/molecular weight of phytate divided by mg of calcium/molecular weight of calcium).

Phytate iron molar ratio was obtained (mg of phytate/molecular weight of phytate: mg of iron/molecular weight of iron).

Phytate zinc molar ratio was obtained (mg of phytate/molecular weight of phytate: mg of zinc/molecular weight of zinc).

Phytate x calcium/zinc millimolar ratio was obtained (mg of phytate/molecular weight of phytate) (mg of calcium/molecular weight of calcium)/ (mg of zinc/molecular weight of zinc) divided by 100.

3.7.2 Condensed tannin determination

Tannin content was determined by the method of Burns (1971) as modified by Maxson and Rooney (1972). About 2.0000 gram of chickpea flour was weighed in a screw cap test tube. The chickpea flour was extracted with 10ml of 1% HCl in methanol for 24 hours at room temperature with mechanical shaking. After 24 hours shaking, the solution was centrifuged at 1000rpm for 5 minutes. A 1ml of supernatant was taken and mixed with 5 ml of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol). D-catechin was used as standard for condensed tannin determination. A 40mg of Dcatechin was weighed and dissolved in 1000 ml of 1% HCl in methanol, which was used as stock solution. A 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution was taken in test tube and the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. A 5ml of vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of sample solutions and the standard solution were measured at 500nm by using water to zero the spectrophotometer, and the calibration curve was constructed from the series of standard solution using SPSS-16. A standard curve was made from absorbance versus concentration and the slope and intercept were used for:

Calculation

Concentration of tannin was read in mg of D-catechin per 100g of sample

Tannin in mg/100g = (absorbance-intercept)/ (slope x density x weight of samplex10)

3.8. Statistical analysis

All data were subject to one-way analysis of variance. Mean value ±standard deviation (SD) of duplicate. (Each duplicate was presented by a mean of two determinations). Analysis were executed using, SPSS 16.0. Significant mean differences were determined at the $p<0.05$ by the method of LSD.

4. RESULT AND DISCUSIION

4.1. Effect of processing techniques on proximate composition of chickpea

4.1.1 Moisture content

Moisture level determination is a fundamental part of the proximate composition analysis of the foods. Moisture content in chickpea flour determines its stability. The mean moisture content in raw and processed flours of chickpea is presented in Tables 3. Results show highly significant ($p<0.05$) of processing treatments on moisture level. The highest level of moisture was present in 48hr germination and 18hr soaked chickpea flour samples 6.88±0.089 and 6.79±0.112 respectively.

Moisture content of soaked chickpea flours were 5.43±0.181, 6.01±0.175 and 6.79±0.112 with soaking time of 6hr, 12hr and 18hr respectively. Water absorption of the seeds followed a different pattern but soaking significantly increased moisture content to 6.79±0.112 at 18 hr soaking time. The increase in water uptake with time is due to the increasing number of cells within the seed becoming hydrated (Nonogaki et al., 2010)

The mean values of moisture contents of the sprouts of chickpea flour were significantly different. Effect of soaking/germination significantly increases the moisture contents and our results agree with earlier reports of increased moisture content during germination of chick pea and other legume seed (Osman, 2007) and Khattak et al., (2008) reported that as germination proceeds, seeds take up water from the surrounding in order for the metabolic process to start. During cooking, dehulling and autoclaving moisture content showed no significance difference as compared to the raw seeds.

4.1.2 Crude protein

The data for crude protein in raw and processed chickpea flours are given in Table 3. There are significant differences ($p<0.05$) in crude protein content observed between processing techniques. During germination and dehulling protein content significantly increased. Whereas, the other methods decreased the content of protein. The crude protein content

during 24hr, 36hr and 48hr germination were 20.01±0.89, 20.47±0.04and23.86±0.15, respectively. This increase is related to increased water activity during germination due to hydrolytic enzymes and chemical changes that occur basically the breakdown of materials such as protein into a more usable form for the growing seed. Indrani, *et al.* (2001) and Nonogaki et al. (2010) noted that protein synthesis occurred during imbibitions and that hormonal changes play an important role in achieving the completion of germination. According to Obizoba (1991), increase in percentage of crude protein, total nitrogen, total non-protein nitrogen; protein nitrogen, true protein nitrogen also were observed.

Soaking of seed followed by dehulling significantly increased protein content 21.61±0.07. This can be attributed to quantitative reduction of antinutritional factor but dehulling followed by cooking reduced the protein content 17.67±0.01 due to the destruction or inactivation of certain essential amino acids by heat.

Soaking for more than 12 hr, cooking and autoclaving significantly decreased the crude protein content. The decrease in protein content during soaking might be attributed to the leaching of soluble proteins. Cooking time reduces the nutritive value of legumes as the levels of some essential amino acids are markedly decreased or during cooking methods the protein contents (Salunkhe et al., 1985).

4.1.3. Crude Fat

The crude fat content of raw and processed chickpea flours is shown in Table 3. Maximum fat level was observed in the raw (6.21±0.297). Whereas chickpea flours in the sprouts of 24hr, 36hr and 48hr of chickpea lower crude fat (4.53±0.099, 4.41±0.183and 3.71±0.265) content was observed, respectively. The crude fat concentration decreased with sprouting time. The reduction was caused by break down of fat by oxidation with fat begin used for energy purposes in embryo development these observation are in agreement with those reported by Idouraine *et al*. (1989) and Alonso *ea al* (1998).

Soaking and cooking treatments decreased the fat contents as compared to the raw these decreases might be attributed to their diffusion into the cooking water and due to total solid

loss during soaking. These results are in agreement with those reported by El-Beltagy (1996).

Up on dhulling no significant reductions in crude fat was observed (5.79±0.301) as compared to the raw sample due to a large portion of legumes lipids are stored in oil bodies or lipid containing vesicles, which are located in the cotyledons could be the reason (Salunkhe et al.1985).

Table.3 The effect of processing on proximate composition of chickpea

Treatment	Moisture Content %	Crude Protein%	Crude Fat%
RC	6.45 ± 0.068^{cb}	21.67 ± 0.18^{f}	6.21 ± 0.297^{f}
SO-6	5.43 ± 0.181^{a}	19.66 ± 0.23^{de}	6.04 ± 0.126^{f}
SO-12	6.01 ± 0.175^{dca}	16.22 ± 0.23^{b}	5.49 ± 0.049^{e}
SO-18	6.79 ± 0.112^{e}	14.21 ± 0.02^{a}	5.37 ± 0.040^{e}
SP-24	5.40 ± 0.062^{b}	20.01 ± 0.89^{e}	4.53 ± 0.099^{b}
SP-36	5.93 ± 0.297^{c}	20.47 ± 0.04^{e}	4.41 ± 0.183^{a}
SP-48	6.88 ± 0.089^{e}	23.86 ± 0.15^{f}	3.71 ± 0.265^{a}
DE	5.69 ± 0.281^{ac}	21.61 ± 0.07^{f}	5.79 ± 0.301^{f}
Uns -cooked	5.93 ± 0.011^{c}	19.08 ± 0.25^{d}	6.01 ± 0.121^{e}
SO-cooked	5.04 ± 0.213^{a}	13.56 ± 0.77^{a}	5.69 ± 0.132^{de}
DE- cooked	5.37 ± 0.238^{a}	17.67 ± 0.01^{c}	4.76 ± 0.050^{c}
AUT	6.07 ± 0.219^{c}	19.86 ± 0.39^{d}	5.30 ± 0.371^{d}

Values are means (± SD). Means not sharing a common letter in a column are significantly different at $p<0.05$ (LSD)

RC- Raw chickpea
SO- soaked chickpea for 6, 12 and18 hr
DE-dehulled chickpea
UNS- cooked- un-soaked cooked chickpea
SO- cooked- soaked cooked chickpea
DE- cooked- dehulled cooked chickpea
AUT - autoclaved chickpea
SP- Sprouted chickpeafor24, 36and48hr

4.1.4. Ash content

Ash contents are shown in Table 4. During socking the ash content decreased from 2.25±0.129, to 1.01±0.040 respectively as compared to the raw chickpea flours. The

reduction was highly significant during soaking for 18 hr 1.01±0.040 may be attributed to leaching. Also, on cooking the ash contents of chickpea was decreased due to cooking of seed with water the leaching of minerals occured but there is no significant changes in ash content due to autoclaving.

During sprouting the ash contents increased with increase in sprouting time. Ash was not significantly ($p > 0.05$) affected by germination of chickpea, the ash content slightly decreased with 24 hr sprouting and thereafter during 36hr and 48 hr germination it became at similar to the control. El-Adawy et al. (2002) reported significant increase in ash content during sprouting in mung bean, pea and lentil seed. However, dehulling decreased the ash content of chickpea due to the removal of hull. Singh *et al* (1987) also reported a considerable reduction of minerals during dehulling of chickpea cotyledons.

4.1.5 Crude fiber content

Crude fiber content significantly ($p < 0.05$) increased by cooking, autoclaving and soaking treatments as compared to the raw. This increase could have been due to protein-fiber complexes formed after possible chemical modification induced by soaking and cooking of dry seeds. These results are in agreement with those reported by El-Beltagy (1996) for legumes and no effect was found in chickpeas with cooking. This means that the inter conversion between insoluble and soluble fiber fractions does not always take place.

Sprouting of chickpea seeds resulted in a significant ($p < 0.05$) increase in crude fiber content. 48hr germinnation highly increased the crude fiber contents, with mean values of 3.25±0.152 in the control and 3.97±0.115 at 48 hr sprouting. Jimenez et al. (1985) also noted an increase in the fiber during sprouting of chickpea seeds. However, during dehulling crude fiber content a significant reduction was recorded this reduction was may be attributed to the removal of the seed hull since crude fiber is mainly concentrated in the seed coat.

Table.4 The effect of processing on proximate composition of chickpea

Treatment	Crude Fiber%	Total Ash%	Utilizable CHO%
RC	3.25±0.15^{d}	2.78±0.147^{e}	65.36±0.741^{c}
SO-6	3.87±0.31^{fg}	2.25±0.129^{c}	62.29±0.339^{b}
SO-12	3.78±0.42^{f}	2.15±0.045^{c}	66.94±0.476^{e}
SO-18	3.67±0.18^{f}	1.01±0.040^{a}	69.34±0.203^{f}
SP-24	3.07±0.13^{dc}	2.44±0.053^{dc}	68.51±0.145^{d}
SP-36	3.15±0.58^{d}	2.66±0.015^{de}	68.95±1.684^{b}
SP-48	3.97±0.11^{g}	2.74±0.440^{d}	58.41±0.134^{a}
DE	1.83±0.05^{b}	1.81±0.171^{b}	72.81±0.287^{f}
Uns cooked	2.94±0.17^{c}	2.36±0.024^{c}	69.60±0.523^{f}
SO-cooked	3.38±0.57^{c}	2.03±0.047^{b}	72.31±0.573^{ef}
DE- cooked	1.25±0.10^{a}	1.37±0.052^{a}	73.94±0.122^{f}
AUT	3.46±0.00^{e}	2.68±0.043^{e}	71.29±0.811^{e}

Values are means of (± SD). Means not sharing a common letter in a column are significantly different at $p<0.05$ (LSD)

RC- Raw chickpea
SO- soaked chickpea for 6, 12 and18 hr
DE-dehulled chickpea
UNS- cooked- un-soaked cooked chickpea
SO- cooked- soaked cooked chickpea
DE- cooked- dehulled cooked chickpea
AUT - autoclaved chickpea
SP- Sprouted chickpeafor24, 36and48hr

4.1.6. Carbohydrate

Table 4 shows carbohydrate content of raw and treated chickpea seeds. Carbohydrate content of germinated chickpea seeds significantly increased for 24 and 36 hr then a decrease was observed at 48hr of germination. These decreases could be attributed to use carbohydrate as an energy source to germination. When the germination period increase reduction in total carbohydrate was attributed to increase in amylatic enzyme activity that hydrolyzes starch in to simple absorbable sugar to the developing embryo similar results were reported by EL-Adway (2002).

No reduction in total carbohydrate contents were observed between cooking and dehulling treatments of chickpea seeds. These observations are in agreement with those reported by

Barampama and Simard (1995) for cooked common beans .However ,on soaking for 6hr chickpea seeds decrease of total carbohydrate was observed as compared to 12 and 18 hr soaking due to solubility during the soaking processing (Souci et al., 1989).

4.1.7. Gross energy

There are significant difference (P<0.05) in gross energy between processing techniques (Table 5). Energy contents were 404.13±0.438 raw chickpea and versus 420.78±1.865 in dehulled, 398.70±1.652 in germinated, 377.66±0.378 soaked and 404.36±1.686 autoclaved, respectively.

Table 5. The effect of processing on proximate composition of chickpea

Treatment	**Energy Kcal/(100g)**
RC	404.13±0.43[d]
SO-6	402.52±1.15[d]
SO-12	386.13±1.26[b]
SO-18	377.66±0.37[a]
SP-24	400.79±5.52[d]
SP-36	398.78±1.48[c]
SP-48	398.70±1.65[c]
DE	420.78±1.86[f]
Uns cooked	408.84±0.03[e]
SO-cooked	394.78±0.32[c]
DE- cooked	409.35±0.89[e]
AUT	404.36±1.68[d]

Values are means of (± SD). Means not sharing a common superscript letter in a column are significantly different at *P*<0.05 (LSD)

RC- Raw chickpea
DE-dehulled chickpea
SO- cooked- soaked cooked chickpea
DE- cooked- dehulled cooked chickpea
AUT - autoclaved chickpea
SP- Sproutedchickpeafor24, 36and48hr
SO- soaked chickpea for 6, 12 and 18 hr
UNS- cooked- un-soaked cooked chickpea

During germination and soaking decreasing of energy was observed may be attributed of chemical changes due to the hydrolysis by the enzymes, of complex macromolecules such as starch and portion into low molecular weight and more digestible molecules. On dehulling

energy content of chickpea seeds showed a significant increase compared to the raw and other processing methods.

4.2. Effect of processing on antinutritional factors of chickpea

4.2.1 Phytic acid

Phytic acid is known to be the major storage form of phosphorus in legumes and is considered to be an antinutritive factor (Table 6). Raw chickpea seeds contained considerable amount of Phytic acid (98.06±0. 136 mg/100g), which indicates that this anti-nutrient has a significant bring on the nutritional value of the legumes. Soaking 6,12and18hr, cooking as well as dehulling of the soaked seeds contributed significantly to lowering of phytic acid content.

Soaking for different time periods minimized the level of phytic acid below the control value during 6 hr soaking, the loss was 79.12±0.309 mg/100g with an increase in the periods of soaking, 12 and 18 hr, further reduction in phytic acid content was reached 65.82±0.328and 58 .95±0.329, respectively,. The loss in phytates during soaking of the samples may be due to leaching of phytate ions into the soaking water under the influence of a concentration of gradient (difference in chemical potential), which governs the rate of diffusion. Similar results for reduction in phytic acid in soaked legumes have been reported earlier(Desphande *et.al* ,1983).

Cooking is usually done before the use of legumes in a human diet. This improves the protein quality by either destruction or inactivation of heat-labile antinutrtional factors (Siddhuraju and Becker 2002). The effect of different cooking methods on the level of phytic acid content of chickpea seeds was shown in Table 6

Cooking of un-soaked, soaked and soaked-dehulled chickpea seeds brought about a significant decrease in the phytic acid content (Table 6). No doubt, cooking of unsoaked chickpea seeds lowered the phytic acid content, but the loss appeared to be less than in chickpea seeds cooked after soaking and soaking-dehulling. The extent of phytate loss

ranged from 89.25±0.377 to 54.75±0.379 mg/100g when the unsoaked, soaked and soaked-dehulled seeds of chickpea seeds were cooked.

On other hand, there was a significant reduction in phytic content in chickpea seeds after autoclaving for 15 min. It reduced phytate content by 74.04±0.489 mg /100gm for chickpea seeds, which cooked by autoclaving for 15 min. similar results, were obtained by Shimelis and Rakshit (2007). The apparent decrease in the content of phytic acid of legume seeds during cooking may be partly due to leaching into the cooking medium, degradation by heat or formation of insoluble complexes between phytate and other components, such as protein and minerals (Siddhuraju and Becker 2002).

A significant reduction ($p<0.05$) was observed in phytic acid content of chickpea seeds during germination (Table 6) as the period of germination prolonged for 36 and 48 hours successive reduction in phytic acid content was observed (65.80±0.340 and 63.94±0.148). Decrease in phytates observed with germination was probably the result of the combined effect of an increase in phytase activity during germination and the leaching out effect. kumar *et al.* (2010) stated that during germination of legumes, phytate is degraded by intrinsic phytase Plant seed utilize phytate as a source of inorganic phosphate during germination and thus tend to increase palatability and nutritional value.

Also, previous reports on the effect of germination on phytic acid in chickpea indicated that as a result of increased enzymes activity, more of phytic acid is hydrolyzed during germination (Reddy *et al.*, 1989 and Desphande 1983).Similarly, Shimelis and Rakshit (2007) also reported that a notable reduction in phytic acid of legumes of germination was obtained.

Table 6. Effect of processing treatments on phytic acid and tannin content of chickpea

Treatment	Phytate (mg/100g)	Tannin(mg/100g)
RC	98.06±0. 136[j]	191.80±0.325[i]
SO-6	79.12±0.309[h]	174.63±0.52[g]
SO-12	65.82±0.328[d]	130.74±0.39[e]
SO-18	58 .95±0.329[c]	129.14±0.28[e]
SP-24	77.98±0.190[g]	179.14±0.26[hg]
SP-36	65.80±0.340[e]	123.98..017[e]
SP-48	63.94±0.148[d]	112.37±0.174[d]
DE	60.49±0.683[c]	66.22±0.39[b]
UNS- cooked	89.25±0.377[i]	189.79±0.34[h]
SO- cooked	48.89±0.152[a]	99.18±0.440[c]
DE- cooked	54.75±0.379[a]	59.29±0.99[a]
AUT	74.04±0.489[f]	136.98±0.03[f]

Values are means of (± SD). Means not sharing a common superscript letter in a column are significantly different at $P<0.05$ (LSD)

RC- Raw chickpea
SO- soaked chickpea for 6, 12 and 18 hr
DE-dehulled chickpea
UNS- cooked- un-soaked cooked chickpea
SO- cooked- soaked cooked chickpea
DE- cooked- dehulled cooked chickpea
AUT - autoclaved chickpea
SP- Sproutedchickpeafor24, 36and48hr

4.2.2 Condensed Tannins

Tannin content of untreated seed was (191.80±0.325 mg /100gm). There are significant decreased in Tannin content were observed between processing techniques including cooking followed by dehulling, dehulling , sprauting for 48hr,36hr and soaking for 18hr significant reduced the tannin content to (59.29±0.99, 66.22±0.39, 112.37±0.174, 123.98..017and 129.14±0.28 mg /100gm) respectively.

A significant reduction ($p<0.05$) was observed in Tannin content of chickpea seeds during dehulling since condensed tannin are mainly localized in legumes seed hull of testa. Most tannin located in the testa its physical removal in tannin content Ahmed et al. (2005) and various studies have reported the reduction in tannin content of legumes during soaking.

However, in the present study, soaking was taken as the preparatory step of germination which have attributed to leaching in to soaking media (Vijayakumari *et al.* 2007).

Effect of germination on tannins content of chickpea is shown in Table 6. Decrease in tannin content was observed during germination. More reduction in tannin content was observed in sprouted chickpea tannin reduction during germination is usually attributed to enzymatic hydrolysis by polyphenol oxidaase (Reddy *et al* 1986 and Alonso *et al* 2005)

Mean values for the effect of cooking on tannin are presented in table 6. Highly significant differences ($P<0.05$) in tannin content of chickpeas have been observed on cooking. Among treatments without soaking no significant reduced were found in chickpea. However, Tannin content levels were significantly reduced by autoclave, cooking, dehulling and soaking followed by cooking. The effects of cooking and autoclaving treatments were effective in reducing condensed tannin (El-Adawy, 2002).

4.3. Effect of processing treatment on total mineral contents of chickpea

Mineral contents of raw, cooked and sprouted chickpea seeds are presented in Table 7. Chickpea seeds were found to contain calcium, phosphorus, zinc and iron 162.37±0.602, 30.66±0.476, 2.94±0.07 and 4.76±0.546 respectively. The mean values of selected mineral contents were different from raw samples in all the processing treatments. Total mineral contents showed significant differences ($p< 0.05$) among processing treatments.

Iron content of chickpea was significantly ($p<0.05$) reduce during soaking (table 7). After 18 hr soaking, a maximum loss occurred .The loss in iron on soaking may be attributed to leaching of the minerals. During sprouting, the iron content loss was low as compared to the raw Lee and Karunanithy (1990) found that the loss of iron was less during germination.

Table 7. Effect of processing treatment on total mineral contents of Chickpea (mg/100g)

Treatment	Total Fe	Total Zn	Total Ca	Total P
RC	4.76±0.546^{f}	2.94±0.070^{e}	162.37±0.602^{i}	30.66±0.476^{f}
SO-6	2.67±0.107^{cdb}	2.51±0.084^{bc}	129.27±0.384^{e}	22.76±0.337^{a}
SO-12	1.91±0.075^{a}	1.87±0.120^{ab}	126.32±0.346^{d}	20.75±0.356^{a}
SO-18	1.34±0.002^{a}	1.72±0.049^{a}	120.23±0.944^{c}	26.77±0.325^{d}
SP-24	3.18±0.061^{d}	2.58±0.410^{c}	140.89±0.153^{g}	28.79±0.351^{e}
SP-36	2.93±0.096^{e}	2.05±0.076^{a}	134.89±0.152^{f}	25.77±0.324^{b}
SP-48	2.58±0.012^{b}	2.09±0.016^{a}	128.35±0.539^{f}	24.86±0.331^{b}
DE	2.25±0.008^{a}	1.96±0.098^{a}	73.431±0.037^{b}	26.96±0.656^{e}
UNScooked	4.45±0.564^{g}	2.74±0.014^{ce}	149.94±0.122^{h}	29.06±0.414^{e}
SO- cooked	1.43±0.553^{a}	1.96±0.318^{a}	140.00±0.001^{g}	23.89±0.449^{c}
DE- cooked	2. 53±0.169^{a}	1.90±0.318^{a}	69.29±3.233^{a}	24.76±0.337^{c}
AUT	3.09±0.204^{cd}	2.72±0.042^{ce}	150.66±3.153^{h}	28.74±1.044^{e}

Values are means of (± SD). Means not sharing a common superscript letter in a column are significantly different at $P<0.05$ (LSD)

RC- Raw chickpea
DE-dehulled chickpea
SO- cooked- soaked cooked chickpea
DE- cooked- dehulled cooked chickpea
AUT - autoclaved chickpea
SP- Sproutedchickpeafor24, 36and48hr
SO- soaked chickpea for 6, 12 and 18 hr
UNS- cooked- un-soaked cooked chickpea

A significant ($p \leq 0.05$) variation in calcium was observed during soaking in chickpea. However, no significant ($p \leq 0.05$) difference was observed between the stages of germination. There was low in Calcium content of germinated chickpea when compared to raw (Stephens 2003). A significant ($p \leq 0.05$) reduction in zinc levels was observed during soaking and germination for 36 and 48hr. Urbano et al. (1995) found that soaking process prior to seed germination was responsible for the losses of zinc in small quantities.

reduction in the calcium, phosphorus, zinc and iron was noted during autoclaving and cooking without soaking Chickpea seeds, Trugo et al. (1990) reported no change in minerals content of legumes when subjected to heat treatments However, the loss was raised when soaked as well as soaked-dehulled seeds were cooked. Haytowitz & Matthews (1983) reported that soaking followed by cooking in boiling water caused great losses of minerals.

Dehulling of seeds promotes reducing the contents of these minerals. Minerals present in the hulls might have been lost during dehulling, therefore, contributing to the lower mineral contents in the dehulled chickpea seeds since the seed calcium is located in the seed coat. Chavan et al. (2001) and singh et al (1987) also reported a considerable reduction of calcium during dehulling of chickpea cotyledons.

Germination for 24, 36 and 48 h resulted in the losses of phosphorus. However, the losses did not vary significantly among the samples which is in agreement with those reported by Lee and Karunanithy (1990) who stated that the loss of divalent metals was low during germination due to their binding to protein and the formation of phytate and protein complexes.

4.3.1 The effect of processing treatment on molar ratios of minerals in chickpea

The molar ratio of Phy/Fe in untreated raw chickpea was 1.6±0.196 but it ranged after various processing from 1.75±0.206 to 2.93±0.173. The lowest ratio was obtained by un-soaked cooked and the highest in dehulled and cooked sampled in Table 8 . indicates that, there are significant differences with each processing treatments however, poor iron bioavailability was observed because of the high levels of phytic acid. Iron molar ratios > 1 is regarded as indicative of poor iron bioavailability (Melaku et al., 2005).

Cooked dehulled sample had the highest PA: Zn molar ratio of 3.34±0.049 followed by soaking for different times, sprouting and cooking methods. Phytates found in legumes are thought to be major contributors to reduced availability of zinc (Forbes et al. 1984). The PA:Zn molar ratios has been suggested to be an important determinant of zinc bioavailability from human diets (Oberleas and Harland 1981). Morris and Ellis (1980) reported that PA:Zn molar ratio of less than 10 provides adequate dietary zinc. Thus the results indicate positive zinc absorption.

Phytate: calcium molar ratio was below the critical molar ratio of 0.24 (Frontela et al., 2009), in all the processing techniques observed in the present study. Thus the results indicate positive Ca absorption. High calcium levels in foods can also promote the phytate-

induced decrease in zinc bioavailability when the [calcium x phytate]: / [zinc] millimolar ratio exceeds 0.5 (Melaku et al., 2005). However, in this study the values < 0.5 were observed in all the samples which indicate that the samples are high in calcium content and bioavailability

Table 8. The effect of processing methods on minerals- phytate molar ratios

Treatment	Phytate :Iron	Phytate: zinc	Phytate: calcium	[calcium x phytate]/[zinc] (mol/kg)
RC	1.60 ± 0.196^{a}	2.11 ± 0.101^{a}	0.036 ± 0.283^{f}	$0.\ 028 \pm 0.883^{k}$
SO-6	1.95 ± 0.152^{ab}	2.85 ± 0.033^{cd}	0.037 ± 0.297^{f}	0.015 ± 0.265^{j}
SO-12	2.35 ± 0.119^{cb}	3.22 ± 0.212^{ef}	0.031 ± 0.956^{c}	0.023 ± 0.595^{c}
SO-18	2.59 ± 0.001^{c}	3.19 ± 0.070^{ef}	0.032 ± 0.483^{c}	0.009 ± 0.003^{b}
SP-24	1.79 ± 0.008^{a}	2.68 ± 0.017^{c}	0.036 ± 0.171^{d}	0.002 ± 0.184^{g}
SP-36	2.35 ± 0.410^{dc}	3.11 ± 0.063^{e}	0.029 ± 0.452^{b}	0.010 ± 0.338^{d}
SP-48	2.52 ± 0.546^{d}	3.12 ± 0.113^{e}	0.030 ± 0.127^{c}	0.019 ± 0.027^{b}
DE	2.69 ± 0.021^{e}	2.79 ± 0.086^{d}	0.054 ± 0.169^{g}	0.012 ± 0.328^{h}
UNScooked	1.75 ± 0.206^{a}	2.49 ± 0.127^{b}	0.039 ± 0.190^{e}	0.017 ± 0.567^{i}
SO- cooked	2.47 ± 0.492^{d}	2.73 ± 0.008^{d}	0.027 ± 0.042^{a}	0.028 ± 0.020^{a}
DE- cooked	2.93 ± 0.173^{e}	3.34 ± 0.049^{f}	0.052 ± 0.229^{g}	$0.013 \pm \pm 0.554^{f}$
AUT	2.04 ± 0.146^{ba}	2.52 ± 0.102^{bcd}	0.029 ± 0.438^{b}	0.013 ± 0.460^{e}

Values are means of (± SD). Means not sharing a common superscript letter in a column are significantly different at *P*<0.05 (LSD)

RC- Raw chickpea
DE-dehulled chickpea
SO- cooked- soaked cooked chickpea
DE- cooked- dehulled cooked chickpea
AUT - autoclaved chickpea

SO- soaked chickpea for 6, 12 and 18 hr
UNS- cooked- un-soaked cooked chickpea

4.4.2 Effect of processing treatment on phytate phosphorous and non phytate phosphorous contents of Chickpea

Processing methods resulted in a significant (*p*<0.05) reduction phytic acid with a corresponding increase in non-phytate phosphorus. Soaking and sprouting of seeds significantly (p<0.05) reduced the phytin phosphorus after each stage of soaking being (22.26±0.132, 17.98±0.093 and 17.74±0.091 mg/100gm) and During germination

(21.97±0.053, 18.46±0.205 and 18.01±0.041) respectively. Indicating that longer soaking and germination periods were useful in reducing phytin phosphorus. Duhan et al. (2002) reported a significant decrease in the levels of phytic acid during germination and cooking the greater reduction of phytic acid in chickpea was attributed to the cooking method.

Table. 9. Effect of processing treatment on phytate phosphorous and non phytate phosphorous contents of Chickpea

Treatment	Total phosphorus (mg/100g)	Phytate phosphorus (mg/100g)	Non-phytate phosphorus (mg/100g)
RC	30.66±0.476^{f}	27.63±0. 037^{j}	3.03±0. 438^{a}
SO-6	22.76±0.337^{a}	22.26±0.132^{h}	5.49±0.468^{b}
SO-12	20.75±0.356^{a}	17.98±0.093^{cd}	9.76±0.449^{cd}
SO-18	26.77±0.325^{d}	17.74±0.091^{c}	9.03±0.417^{d}
SP-24	28.79±0.351^{e}	21.97±0.053^{g}	6.82±0.296^{c}
SP-36	25.77±0.324^{d}	18.46±0.205^{e}	6.84±0.372^{c}
SP-48	24.86±0.331^{b}	18.01±0.041^{d}	9.30±0.523^{e}
DE	26.96±0.656^{c}	18.45±0.192^{e}	8.51±0.463^{de}
UNS- cooked	29.06±0.414^{e}	25.14±0.099^{i}	3.92±0.313^{a}
SO- cooked	23.89±0.449^{c}	17.15±0.042^{b}	6.73±0.407^{c}
DE- cooked	24.76±0.337^{c}	16.55±0.106^{a}	8.20±0.230^{d}
AUT	28.74±1.044^{e}	20.86±0.138^{f}	9.82±0.984c^{d}

Values are means of (± SD). Means not sharing a common superscript letter in a column are significantly different at $P<0.05$ (LSD)

RC- Raw chickpea
SO- soaked chickpea for 6, 12 and 18 hr
DE-dehulled chickpea
UNS- cooked- un-soaked cooked chickpea
SO- cooked- soaked cooked chickpea
AUT - autoclaved chickpea
SP- Sproutedchickpeafor24, 36and48hr
DE- cooked- dehulled cooked chickpea

Phytase activity during germination resulted in hydrolysis of phytate phosphorus to inositol monophosphate. The liberated phosphorus is possibly transported to the embryo for further synthesis of organic phosphorus (Reddy *et al* 1989). Dehulling and autoclaving when compared to none soaked cooked and control chickpea significantly reduced phytate phosphorus

4.5. Effect of processing treatment on HCl -extractability of minerals in chickpea

Processing methods had a significantly ($p < 0.05$) positive effect on the HCl-extractability of dietary iron, zinc, calcium and phosphorus (Table 10). During 6 hr, soaking extractability of iron, zinc, calcium and phosphorus improved to 14.16±0.62, 17.43±0.09, 27.61±0.07 and 16.73±0.38 percent, respectively, as compared to the control. As the period of soaking was prolonged from 12 to 18 hr, an enhancement in calcium, phosphorus and iron extractability was observed.

Germination significantly altered HCl -extractability of minerals (Table 10) that may be an indication of minerals of their bioavailability to the human digestive system. HCl – extractability of phosphorus improved at all period of germination after 48 hr phosphorus extractability was the highest. Sprouting at all period of germination also enhanced the HCl - extractability of Ca, Fe and Zn.the enhancement was greater at longer period of germination.

HCl-extractability of divalent minerals has been attributed to the reduction of antinutrients like phytates Inositol hex phosphate and pent phosphates are the forms of phytate which exert negative impact on the HCl-extractability of divalent cations (Duhan *et al.*, 2002).

One phytate molecule possesses capacity to bind up to six divalent cations and one cation can possibly bridge at least two phytic acid molecules, depending upon the redox state (Graf and Easton, 1990). Breakdown of these phytate forms into lower phosphates have been found to reduce capability of binding divalent metal ions (Lonnerdal, 2000). Thus, phytase production during germination plays its role in the improvement of HCl-extractability of minerals especially Zn, Fe and Ca. As far as phosphorus extractability is concerned the phytate is the storage form of these minerals under the influence of processing treatments

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Table 10 Effect of processing treatments on HCl-extractability of minerals (%) in chickpea

Treatment	Extractable Fe	Extractable Zn	Extractable Ca	Extractable P
RC	11.21±1.099^{a}	14.29±0 .846^{a}	20.02±0 .456^{a}	11.78±0.311^{a}
SO-6	14.16±0.629^{b}	17.43±0.091^{b}	27.61±0.073^{c}	16.73±0.381^{b}
SO-12	18.44±0.395^{c}	20.78±0.059^{b}	21.56±0.883^{b}	19.50±0.084^{cd}
SO-18	20.99 1.025^{d}	22.79±0.916^{b}	28.52±0.171^{c}	21.60±0.231^{e}
SP-24	14.66±0.784^{b}	25.92±0.150^{d}	29.95±0.019^{d}	18.71±0.388^{c}
SP-36	22.93±1.725^{d}	30.62±0.984^{e}	37.84±0.581^{f}	25.99±1.873^{f}
SP-48	25.32±0.388^{d}	32.18±0.205^{e}	54.57±0.814^{g}	26.55±2.665^{f}
DE	14.49 ±2.128^{b}	28.18±0.375^{e}	24.66±1.599^{b}	25.04±0.091^{f}
UNScooked	11.94±0.240^{a}	23.34±0.181^{c}	23.88±0.885^{b}	18.90±0.707^{c}
SO- cooked	15.21±0.318^{b}	17.18±0.639^{b}	23.87±0.485^{b}	16.76±0.622^{b}
DE- cooked	12.78±0.000^{a}	18.27±0.348^{b}	26.26±0.008^{d}	15.22±1.088^{b}
AUT	16.76±0.325^{c}	24.12±0.209^{d}	20.92±0.108^{a}	16.32±1.244^{b}

Values are means of (± SD). Means not sharing a letter in a column are significantly different at $P<0.05$ (LSD)

RC- Raw chickpea
DE-dehulled chickpea
SO- cooked- soaked cooked chickpea
DE- cooked- dehulled cooked chickpea
AUT - autoclaved chickpea
SP- Sproutedchickpeafor24, 36and48hr
SO- soaked chickpea for 6, 12 and 18 hr
UNS- cooked- un-soaked cooked chickpea

During the germination processing of inositol hex phosphate hydrolyses, releasing free inorganic phosphorus and myoinositol (Hotz and Gibson, 2001). The liberated phosphorous becomes available for HCl-extractability. On cooking of unsoaked, soaked and soaked dehulled seeds, significant differences ($p < 0.05$) were noticed in the HCl-extractability of minerals in chickpea seeds. Autoclaving improved the extractability of chickpea seeds to a greater extent.

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

The processing methods employed in this study effectively reduced phytic acid and tannin content of chickpea, and improved HCl- extractablity of minerals. Also these processing methods improved nutritional qualities of chickpea. Specifically, germination resulted in increased protein, ash, fiber, carbohydrate content; whereas fat content was decreased.

Soaking processing methods caused increase of fat (6.04±0.126) carbohydrate (69.34±0.203) and fiber (3.87±0.31) except ash (1.01±0.040) and protein (14.21±0.02%) and cooking methods decreased protein (13.56±0.77) and ash (1.37±0.052) contents significantly. Dehulling process resulted increase in all nutritive values except ash.

The results also clearly indicated that total mineral content leached during soaking and cooking. Dehulling process also decreased the total mineral content, especially calcium value because of the removal of the hull. But, the HCl extractability of minerals was increased. This showed that HCl extractability and phytate has inverse relationship. Autoclaving methods of processing also resulted in total mineral retention.The bioavailability of zinc, calcium and millimolar ratio is greater positive bioavailability where as poor bioavailability of iron was observed.

5.2. RECOMMENDATION

5. 2 Recommendation

In the phase of the present findings, processing methods such as germination, soaking, cooking and dehulling, reduced the phytate content of chickpea and increased the availability of minerals contained in the food in question. Further, the proximate composition of the chickpea was enhanced by the processing method mentioned hitherto. Therefore, it is recommended that these methods should be utilized by processors. As well, industrial utilization of locally-grown chickpea varieties for manufacturers to produce bakery products is encouraged.

Future works are also recommended in order to extend the present findings to its level highest. Such include:

- In vitro and in vivo analysis should be undertaken to further prove bioavailability of minerals besides molar ratio of phytate to minerals and HCl-extractability.
- Mechanisms to improve the iron content of chickpea through fortifying with iron rich plants to increase its nutritional quality for feeding infants and young children will be desirable

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Declaration

I, the undersigned, declare that this thesis is my original work; it has not been presented in Other universities, colleges or institutions, seeking for similar degree or other purposes. All Sources of the materials used in the thesis have been only duly acknowledged.

Name	**Signature**
Yordanos Fikre	________________

The work has been done under our supervision.

Name	**Signature**
1. Mr. Kelbesa Urga	
2. Dr Melese Abdisa	________________