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

**Study on the Effect of Traditional Processing on Proximate  
Composition and Bioavailability of Minerals in Chickpea  
(*Cicer arietinum*) grown in Ethiopia**

**By**  
**Dejene Dida**

*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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Addis Ababa, Ethiopia

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## Acronyms

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine triphosphate
CHO	Carbohydrate
DMT	Divalent metal transporter
DZARC	Debre Zeit Agricultural Research Center
EHNRI	Ethiopian Health and Nutrition Research Institute
FAO	Food and Agriculture Organization
LDL	Low density lipoprotein
ICARDA	International Center for Agricultural Research in Dry Areas
ICRISAT	International Crops Research Institute for the Semi-Arid Tropic
PA	Phytic acid
SPSS	Statistical Product and Service Solution
TTA	Titrateable acidity
rpm	revolution per minute

## ABSTRACT

*The effect of various traditional processing methods (boiling, roasting, germination and fermentation) on proximate composition, antinutritional factors and HCl-extractability of minerals of chickpea (*Cicer arietinum*) grown in Ethiopia was studied. Moisture content, crude protein, crude fat, crude fiber and total ash ranges were 5.9 -9.4%, 13.8-16.7%, 4.3-6.1%, 3.4-5.9% and 2.3-2.7% respectively. The range of iron, zinc, calcium and phosphorous were 4.0-6.8, 2.7-3.9, 135.8-207 and 252.2-298.11mg/100g respectively. And also the ranges for phytate and condensed tannin content were 72.07-97.46 and 99.26-175.23 mg/100g respectively on wet weight basis. The HCl - extractability (as an index of bioavailability) of iron, zinc, calcium and phosphorous in each of the processing methods was also studied. The results indicated that fermentation and germination were most effective in reducing Phytate and condensed tannin content and enhance HCl -extractability of minerals.*

**Key Words:** Traditional processing, Phytate, tannin, HCl -extractability.

# 1. Introduction

## 1.1. Background

Legumes play an important role in the agriculture and diet of many developing countries and are a major source of dietary nutrients for many people. However, their role appears to be limited because of several factors including low protein and starch digestibility, poor mineral bioavailability and high antinutritional factors (Negi *et al.*, 2001; Kamchan *et al.*, 2004). The role of seed legumes in the diets of animal and man in developed countries is well documented. There is a lack of sufficient animal protein, hence it is necessary to search for alternative sources of protein from lesser-known legumes instead of expensive and scarce animal protein (Waterlow, 1994).

Chickpea is a member of the cool season *Fabaceae* (*Leguminosae*) family of legumes (Nwokolo and Smart, 1996). In addition to being an important source of protein, chickpea is also reported to be a good source of minerals. This legume supplies larger amounts of calcium and phosphorus than do other legumes and contains more calcium than whole cow's milk (120 mg/100 g) (Nestares *et al.*, 1997).

Chickpea is a less labor-intensive crop and its production demands low external inputs compared to cereals. In Ethiopia, chickpea is widely grown across the country and serves as a multi-purpose crop (Shiferaw *et al.*, 2007). First, it fixes atmospheric nitrogen in soils and thus improves soil fertility and saves fertilizer costs in subsequent crops. Second, 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. Third, it reduces malnutrition and improves human health especially for the poor who cannot afford livestock products. It is an excellent source of protein, fiber, complex carbohydrates, vitamins, and minerals. Fourth, the growing demand in both the domestic and export markets provides a source of cash for smallholder producers. Fifth, it increases livestock productivity as the residue is rich in digestible crude protein content compared to cereals (Menale *et al.*, 2009).

In spite of a good nutritional profile, as well as reported medicinal properties, chickpea has several nutritional and processing problems, such as the presence of antinutrients, prolonged cooking time, and poor digestibility. Its chemical composition is subject to fluctuations, depending on various factors, e.g. cultivar and maturity stage, environment (mostly weather conditions), and agrotechnics. Some reports have also underlined Variations in the physical as well as the chemical composition of these legumes. These variations can be either due to intrinsic factors (mainly genetics, which are partly responsible for differences between cultivars and varieties) or to extrinsic factors, such as storage, type of soil, agronomic practices, climatic factors and technological treatments (Paolini *et al.*, 2003).

The abundance of anti-nutritional factors and toxic correlates in plants used as human foods and animal feeds certainly calls for concern. Therefore, ways and means of eliminating or reducing their levels to the barest minimum should be looked for. It could be wrongly argued that since the African cultural method of preparing food involves cooking, there is no cause for alarm. This is not entirely correct because although the toxic effects of most anti-nutritional factors present in plant food and feedstuffs can generally be eliminated by proper heat treatment, it should be appreciated that conditions may prevail whereby complete destruction may not always be achieved (Aykroyd *et al.*, 1982).

The nutritional value of a diet can not be determined based on the concentration of individual nutrients, as interactions between nutrients and with antinutrients affect bioavailability, which is the degree to which a nutrient is absorbed from the diet. Interactions are recognized when the response of an individual to a nutrient is not constant but varies depending on the level of another nutrient or antinutrient in the diet. Because the issues of nutrient bioavailability in food are complex, an alliance with human nutritionists can be the only way for agricultural scientists to take on nutritional quality as a specific objective for a food-based system (Graham *et al.*, 2000).

The intention of this study is to investigate and report the effects of traditional processing techniques on the nutritional value, mineral bioavailability and antinutritional factors of chickpea grown in Ethiopia. This monograph consists of literature survey, materials and methods of analysis, results and discussion, conclusion and recommendation.

## **1.2 Statement of the problem**

Information on the effect of different processing techniques on the anti-nutritional factors in human foods will attract interests of human nutritionists, public health and food regulatory bodies/authorities on the exploitation of these techniques so that the nutritive values of foods could be efficiently maximized.

Much of the available data and information on the nutrient and anti-nutrient composition of the more commonly used local foods such as chickpeas do not cover all the foods where available, and requires updating. This is because of the possible effects of variety/genetic origin, climate, soil, processing methods, pesticides and fertilizers on the chemical composition of the chickpea.

Several considerations justify the continued surveillance, knowledge and research on antinutritional factors and toxic substances naturally present in plants used as foods like chickpeas and ways of reducing them to safe level of consumption. The determination of minerals and trace elements in foodstuffs is also an important part of nutritional and toxicological analyses.

## **1.3 Significance of the Study**

In Ethiopia chickpea grain is widely used in different forms as green vegetable (green immature seed), 'Kollo' (soaked and roasted) and 'nifro' (boiled seeds) and 'wot' (sauces) made up of 'shiro' (powdered seeds) etc. In all the forms, it may be consumed alone or mixed with cereals. The traditional processing practices used to convert chickpea into consumable forms included soaking, fermentation, boiling, roasting etc.

The results of this study can be used to promote the consumption of the legume seeds of chickpea by enhancing the bioavailability of minerals to prevent micronutrient malnutrition.

- The processing technique is designed to formulate traditional preparation.
- The processing technique must be and affordable for the poor.
- It can alleviate co-existing micronutrient deficiencies in the entire household;
- The finding may be used as source of information for the public, researchers etc.

#### **1.4. Objectives of the Study**

##### **1.4.1 General Objective**

The general objective of the study is to investigate the effect of traditional processing methods on nutritional quality, antinutritional factors and bioavailability of minerals of chickpea grown in Ethiopia.

##### **1.4.2. Specific Objectives**

The specific objectives of this study are to:-

- Determine the proximate analysis (moisture content, total ash, crude protein, crude fiber, and crude fat and utilizable carbohydrate) of traditionally processed chickpea.
- Investigate the effect of boiling, roasting, germination and fermentation of chickpea on some anti-nutritional factors (phytate and tannins) and the bioavailability of minerals (Fe, Zn, Ca and P).

## **2. Literature Review**

### **2.1 Over view of Chickpeas**

#### **2.1.1 World Production of Chickpea**

Chickpea is one of the earliest cultivated vegetables, as it is believed to have originated in the Middle East approximately 7450 years ago (Maiti and Wesche-Ebeling, 2001). Chickpea (*Cicer arietinum* L.) is a major food legume in Southern Europe, North Africa, India and Middle East countries (Viveros *et al.*, 2001; Iqbal *et al.*, 2006). It is cultivated mainly in Algeria, Ethiopia, Iran, India Mexico, Morocco, Myanmar, Pakistan, Spain, Syria, Tanzania, Tunisia and Turkey (Naghavi and Jahansouz, 2005).

Chickpea has since been grown in temperate and semi-arid regions of the world such as Asia, Europe, Australia and North America. In 2004, 45 countries were actively producing chickpea, and together produced a total of 8.6 million metric tones. India was the leading producer of chickpea accounting for approximately 66% of the world's production. Turkey was the second largest producer, producing approximately 7% of the world supply, followed by Pakistan and Iran at approximately 6% and 4%, respectively. In contrast, Canada and the United States contribute very little to the total quantity of chickpea produced worldwide, as they account for approximately 1% and less than 1% of the world production, respectively (Smith and Jimmerson, 2005).

Chickpeas (*Cicer arietinum* L.) are one of the oldest and most widely consumed legumes in the world; it is a staple food crop particularly in tropical and subtropical areas (Alijaji and El Adway, 2006). The crop is grown on small-scale farms as a food and cash crop. The immature pods, shoots and seeds may be used as vegetables. In 2005 world production was 9,171,930 Tones from 11 million hectares of which 98% was from developing countries. Yields have remained relatively stable over the past two decades, ranging from 0.7 to 0.8 t/ha. The largest chickpea producer is India which produces in 2005 about 6,000,000 Tones (Table 2.1)

Table 2.1 World chickpeas productions in Metric tones

<i>Chick-Peas Production (Mt)</i>	<b>Year</b>					
	<b>2000</b>	<b>2001</b>	<b>2002</b>	<b>2003</b>	<b>2004</b>	<b>2005</b>
<b>India</b>	5,120	3,855	5,473	4,130	5,770	6,000
<b>Pakistan</b>	565	397	362	675	611	868
<b>Turkey</b>	548	535	650	600	620	610
<b>Iran, Islamic Rep of</b>	242	269	300	310	310	310
<b>Mexico</b>	234	326	235	240	240	240
<b>Myanmar</b>	84	119	212	228	230	230
<b>Australia</b>	162	258	136	178	140	189
<b>Ethiopia</b>	165	176	187	114	136	135
<b>Canada</b>	388	455	157	68	51	98
<b>Syrian Arab Republic</b>	65	60	89	87	45	55
<b>Morocco</b>	15	32	51	43	42	42
<b>Yemen</b>	37	37	36	36	36	36
<b>Malawi</b>	35	35	35	35	35	35
<b>Other Countries</b>	288	339	362	366	358	324
<b>World</b>	7,947	6,894	8,284	7,110	8,625	9,172

Source: FAOSTAT, 2008

Chickpea is the third most important pulse crop commodity in the world based on total production (Yust *et al.*, 2003). In 2003, India was both the leading producer and importer of chickpea, with approximately 259 thousand metric tones imported (30% of the total amount imported worldwide). Pakistan and Bangladesh were the second and third largest importers of chickpea worldwide, with approximately 123 thousand (14%) and 84 thousand metric tones (10%), respectively. Similar to chickpea production, the United States and Canada only marginally contributed to the total quantity of chickpea imported worldwide. In 2003–2004, the United States imported approximately 17 thousand metric tones (1%) (Smith and Jimmerson, 2005).

There are two main commercially available types of chickpea grown in the world: the desi and the kabuli chickpea. Desi chickpea seed is small with a dark irregular-shaped seed coat and is grown on semi-arid land. Kabuli chickpea (Garbanzo beans) is larger than desi chickpea, has a thin light-colored seed coat and is normally grown in



temperate regions of the world. A variety of desi and kabuli chickpeas have been developed and the characteristics of these cultivars may vary depending on the producing region (Agriculture and Agri-Food Canada, 2008).

The kabuli chickpea have normally larger seeds, better water uptake properties, shorter cooking time, lower crude fiber and higher caloric value than desi type. In the Indo-Pak Subcontinent, the desi chickpeas are used whole, shelled and split to produce dhal, or ground into fine flour called besan. Besan is used in many ways for cooking, including mixing with wheat flour to make roti or chapatti (kinds of bread), and for making sweets and snacks. The kabuli types are used mainly in salad bars and vegetable mixes. They are also used in preparing a wide variety of snack foods, soups, sweets, and condiments. Smaller size kabuli chickpeas are also milled for obtaining chickpea flour (Singh, 1988).



Figure1. Desi and Kabuli types of chickpeas

There is a high demand for world production, exports and imports of chickpeas due to the crop's nutritional value. Chickpea is high in protein, low in fat and sodium, cholesterol free and is an excellent source of both soluble and insoluble fiber, complex carbohydrates, vitamins, folate, and minerals, especially calcium, phosphorous, iron, and magnesium (Nwokolo and Smartt, 1996). Chickpea is a good source of dietary protein due to its well-balanced amino acid composition and protein bioavailability. The nutritional benefits of chickpea have led to its use in various culinary applications

such as hummus. In addition, chickpea is used in stews, soups and salads, and can be processed into flour (Yust *et al.*, 2003).

### **2.1.2 Chickpea sub-sector in Ethiopia**

In Ethiopia, the earliest finding of chickpea is reported in 1520 BC (Joshi *et al.*, 2001). Ethiopia is the largest producer of chickpea in Africa accounting for about 46% of the continent's production during 1994-2006. It is also the seventh largest producer worldwide and contributes about 2% to the total world chickpea production (Table 2.1).

Several new varieties, both *desi* and *kabuli* types, have been introduced and developed by International Crops Research Institute for the Semi-Arid Tropic (ICRISAT) and International Center for Agricultural Research in Dry Areas (ICARDA) and released by the national program. Chickpea is grown in Ethiopia, mainly by subsistence farmers usually under rain fed conditions. It is one of the main annual crops in Ethiopia both in terms of its share of the total cropped pulse area and its role in direct human consumption. It is grown widely across the highlands and semi-arid regions of the country (Bejiga *et al.*, 1996).

Chickpea, locally known as *shimbra*, is one of the major pulse crops (including faba bean, field pea, haricot bean, lentil and grass pea) in Ethiopia and it is the second most important legume crop after faba beans. It contributed about 16% of the total pulse production during 1999-2008. The total annual average (1999-2008) chickpea production is estimated at about 173 thousand tones. During the same period, chickpea was third after faba beans and field peas in terms of area coverage (Menale *et al.*, 2009).

## **2.2 Consumption and importance of chickpeas**

In Egypt, chickpea seeds are usually consumed at the raw green and tender stage (unripe stage), called Malana, or in the form of mature dry seeds after parching as a popular snack food. The dry seeds can also be consumed as 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 biscuits (El-Adawy, 2002).

Chickpea is a good source of dietary protein, fertility restorer through symbiotic nitrogen fixation, drought tolerant and break crop. The main use of chickpea is for human consumption and the seed provides an excellent source of protein, especially for vegetarians or vegans (Taylor and Ford, 2007). The seeds may be eaten as whole; split into halves after removing the seed coat (dhal), processed into flour (besan) or the young shoots may be eaten as a vegetable. Based on the seed type, two different trade classes are recognised, viz., *desi* and *kabuli*. The *desi* chickpea are usually decorticated and processed into flour while the *kabuli* type is used as whole grains (Millan *et al.*, 2006).

*Desi* chickpea has traditionally been used in the Indian subcontinent as a dhal (milled seeds) or the flour is used to make a variety of snacks and sweets. Chickpea has one of the highest nutritional compositions of any dry edible grain legume. Chickpea seed contain approximately 20-30% protein, 40% carbohydrate and 3-6% oil and are a rich source of minerals (Ibrikci *et al.*, 2003).

The chickpea is considered to be a healthy vegetarian food and it is one of the most important human and domestic animal foods in south Asia. It is a cheap source of high quality protein in the diets of millions in developing countries, who cannot afford animal protein for balanced nutrition. In addition to proteins, it is a good source of carbohydrates, minerals and trace elements (Huisman and Van der Poel, 1994).

Chickpea, like other legumes, not only brings to the cereal staple a variety of taste and texture but adds nutrients (carbohydrates, minerals) to the staple dish which ensure a balanced diet, meeting all nutrient requirements. Its flour, called Besan, (in India) is used in many ways for cooking, e.g. mixed with wheat flour to make roti or chapatti. Young plants and green pods are eaten like spinach. A small proportion of canned chickpea is also used in Turkey and Latin America (Duhan *et al.*, 1999).

Chickpea (*Cicer arietinum* L.), an annual herbage plant, is the third most important grain legume in the world on the basis of total grain production (FAO, 2000). In 1980s, hundreds of chickpea species were imported from the International Center for Agricultural Research in the Dry Areas (ICARDA) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and have been planted in Gansu, Qinghai and Xinjiang of China (Zhang *et al.*, 2007).

Chickpeas (*Cicer arietinum* L.) are one of the most utilized legumes in the world because they are considered to be an excellent source of dietary protein (Frias *et al.*, 2000). Chickpea mainly consists of a seed coat (the outermost part) and cotyledons (the inner part). Starch granules are held within the protein matrix in the cotyledons (Sayar *et al.*, 2001). In simple terms, chickpea grain comprises starch granules embedded in a protein matrix covered by a seed coat (Sayar *et al.*, 2003).

Chickpea seeds are usually cooked to produce a texture that is acceptable to the consumer and to improve the nutritional quality of the seed (Clemente *et al.*, 1998). Soaking before cooking allows water to be distributed between the starch and protein fractions within the legume and reduces the time needed to achieve an acceptable texture (Gowen *et al.*, 2007).

Legumes have been considered as a rich source of protein throughout the world and contain approximately three times more proteins than cereals. Chickpea (*Cicer arietinum* L.) is one of the top five important legumes on the basis of whole grain production. It is a staple food crop in many tropical and subtropical countries of Asia. Chickpea has been used for the preparation of various traditional foods, for example, as an ingredient in bakery products, imitation milk, infant foods formulations and meat product (FAO, 2000).

Chickpea is primarily a food crop used in various forms. In India the crop is consumed primarily as dhal, a preparation produced by decorticating the seed and separating the cotyledons. The decorticated and split cotyledons are then used to produce a thick soup that is generally served with rice. Chickpea is also used as a whole pulse and is

soaked and boiled. "Chole" is a traditional dish made from whole chickpeas in India. A popular use in the Middle East is as hummus, a dish made from cooked and ground chickpea that is mixed with tahini, olive oil, and various spices and eaten with traditional flat bread. Dry roasted chickpea seasoned with various spices is a popular snack in most countries of the Middle East and North Africa. In India, the young leaves of chickpea are often harvested green and cooked to make a dish similar to spinach (Zhang *et al.*, 2007).

In Ethiopia, chickpea can be processed and used in the form of dehulled (split seed or kik), and soaked and roasted (kolo or snacks) and boiled seeds ( 'nifro').

### **2.3. Chickpeas and Health**

Chickpea (*Cicer arietinum L.*) is an important pulse crop due to its protein content and wide adaptability as a food grain. It is a source of dietary protein in general and particularly for vegetarian segments of the Indian-subcontinent population. It is also used as a protein supplement in the European countries (Viveros *et al.*, 2001). Green chickpea is commonly used as a vegetable while dry seed is consumed as such, in the form of dhal, and in the form of fried products from its flour. Apart from being a valuable source of protein, consumption of legumes has also been linked to reduced risk of diabetes and obesity, coronary heart disease, colon cancer and gastrointestinal disorders. Consumption of legumes may also have a protective effect against prostate cancer in humans. The phenolic compounds present in these legumes are known to exhibit strong antioxidant, anti-mutagenic, and anti-genotoxic activities (Kolonel *et al.*, 2000; Bazzano *et al.*, 2001; Bourdon *et al.*, 2001; Hangen and Bennink, 2002).

Besides, it has a traditional medicinal value with germinated chickpea reported as hypocholesteremic (Geervani, 1991). *Desi* chickpeas have a very low 'glycemic index' making them a healthy food source for people with diabetes (Walker *et al.*, 2007).

On the medicinal side, chickpea seed is used as a tonic; stimulant and aphrodisiac. The seed is used as an appetizer and also has anthelmintic properties. It also alleviates

thirst and burning sensation Seeds are mainly used for the treatment of bronchitis, leprosy, skin diseases, blood disorders and biliousness. Seeds are also used for the treatment of diseases of the liver and spleen; seeds enrich the blood and cure skin diseases and inflammation of the ear. Among food legumes, chickpea is the most hypocholesteremic agent, and germinated chickpea is reported to be effective in controlling cholesterol level in rats (Pandey and Enumeratio, 1993; Warner *et al.*, 1995).

It is relatively inexpensive, and has been associated with the prevention of cardiovascular disease, managing type-2 diabetes and lowering low density lipoprotein (LDL) cholesterol levels. Insoluble dietary fiber present in chickpea has been associated with reducing the incidence of colon cancer, whereas soluble fiber has been demonstrated to have a beneficial effect on weight loss and weight management (Agriculture and Agri-Food Canada, 2006).

## ***2.4 Anti-nutrients***

An antinutrient is a substance occurring in the diet which acts antagonistically towards one or multiple nutrients, reducing bioavailability. This is usually done through complex formation which reduces nutrient absorption (Graham *et al.*, 2000).

### ***2.4.1 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. Polyphenols are found to interact with proteins and cause either inactivation of enzyme such as trypsin and chymotrypsin or make protein insoluble. Polyphenols inhibit several enzymes including  $\alpha$ -amylase, lipases, pectin esterases, cellulases and  $\beta$ -galactosidase (Salunkhe *et al.*, 1985). In addition to this, tannins reduce the bioavailability of vitamins and minerals (Chavan *et al.*, 1986).

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 coat. There is a considerable variation in seed coat color among the various chickpeas cultivars. The polyphenols in cultivars, which have darker testa color, inhibit the digestive enzyme activity more than cultivars with lighter testa color. These components impart astringent flavors, which are not always desirable. Some processing treatments such as dehulling and cooking considerably reduce the level of tannins in legume. As such, chickpea seeds with light color are preferred for whole seed consumption (Chavan *et al.*, 1986).

#### **2.4.2 Phytate**

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 phytate which has high density of negatively charged phosphate groups which form very stable complexes with mineral ions causing non-availability for intestinal absorption (Walter *et al.*, 2002).

Phytates are generally found in food high in fiber especially in wheat bran, whole grains and legumes (Lori *et al.*, 2001). The major concern about the presence of phytate in the human diet is its negative effect on mineral uptake. Minerals of concern in this regard include zinc, iron, calcium, magnesium, manganese and copper (Lopez *et al.*, 2002). The formation of insoluble mineral-phytate complexes at physiological pH values is regarded as the major reason for the poor mineral bioavailability, because these complexes are essentially non absorbable from the human gastrointestinal tract. Furthermore, the human small intestine has only a very limited capability to hydrolyse phytate due to the lack of endogenous phytate degrading enzymes and the limited microbial population in the upper part of the digestive tract. Solubility and stability of *myo*-inositol phosphate--mineral complexes have been found to decrease as the number of phosphate residues on the *myo*-inositol ring decreases. Therefore, removal

of phosphate residues from phytate results in a reduced impairment of intestinal uptake of essential dietary minerals (Sandberg *et al.*, 1999).

In isolated form only *myo*-inositol pentakisphosphate suppressed absorption of iron, zinc and calcium in humans, while *myo*-inositol tetrakis- and trisphosphates had no effect in the concentrations under investigation. In the presence of higher phosphorylated *myo*-inositol phosphates, however, *myo*-inositol tetrakis- and trisphosphates were shown to contribute to the negative effect of phytate on iron absorption. Because a strong negative correlation was found between zinc absorption and the sum of *myo*-inositol tris- through hexakisphosphate from cereal and legume meals, such a contribution is probably also true for zinc absorption (Sandberg, 1991).

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 (Drewnowski and Gomez-Carneros, 2000).

Phytate is also known to form complexes with proteins at both acidic and alkaline pH. This interaction may affect changes in protein structure that can decrease enzymatic activity, protein solubility and proteolytic digestibility. However, the significance of protein-phytate complexes in nutrition is still under scrutiny. Strong evidence exists that phytate-protein interactions negatively affect protein digestibility *in vitro* and the extent of this effect depends on the protein source (Cheryan, 1980).

## **2.5. Effects of Methods of Domestic Processing and cooking**

Khokhar and Chauhan (1997) reported the anti-nutritional factors in Moth Bean (*Vigna aconitifolia*). The dry seeds were given different treatments including soaking, sprouting and cooking and the changes in the level of the anti-nutritional factors were estimated. Soaking the seeds in plain water and mineral salt solution for 12 hr



decreased phytic acid to the maximum (46–50%) whereas sprouting for 60 hr had the most pronounced saponin lowering effect (46%). The cooking method of processing was less effective in reducing the levels of these anti-nutritional factors.

In general, diet-related factors have a greater influence on the bioavailability of the micronutrients in plant foods, particularly Ca, Fe and Zn, than on the macronutrients. The absorption of Ca, Fe and Zn is particularly affected. The net effect on the nutrient bioavailability depends on the balance between factors that either inhibit or enhance nutrient absorption and/or utilization in the whole diet (Sandstrom, 2001).

The adverse effects of some of the organic component in plant foods on nutrient bioavailability can be reduced by household food processing and preparation practices; these practices are summarized in Table 2.2.

Table 2.2. Influence of household food processing and preparation methods on bioavailability of nutrients in plant foods

Processing method	Main technical influences	Nutritional consequences
Thermal processing	Releases some vitamins from poorly-digested complexes Inactivates heat-labile anti-nutritional factors (e.g. protease inhibitors, $\alpha$ -amylase inhibitors, lectins, thiaminases, goitrogens) May degrade phytate, depending on temperature Gelatinizes starch	Enhances bioavailability of vitamin B <sub>6</sub> , niacin, folate and certain carotenoids Enhances digestibility of proteins and starch Enhances bioavailability of thiamine and I  May enhance Zn, Fe and Ca bioavailability Enhances digestibility
Baking	Induces Maillard browning in foods containing reducing sugars	Destroys basic essential amino acids lysine, arginine and methionine Reduces protein quality and protein digestibility
Boiling	Reduces oxalate content	Enhances Ca absorption
Germination and malting	Increases phytase activity via <i>de novo</i> synthesis or activation of endogenous phytase Reduces polyphenol content of some legumes (e.g. <i>Vicia faba</i> ) Increases $\alpha$ -amylase content of cereals (e.g. sorghum and millet)	Induces hydrolysis of phytate and hence increase Zn, Fe, Ca, and Mg absorption Enhances non-haem-Fe absorption  Facilitates starch digestion; may increase non-haem-Fe absorption through a change in consistency
Village-based milling or home pounding	Reduces phytate content of cereals with phytate localized in outer aleurone layer (rice, wheat, sorghum) or in germ (maize)	Enhances bioavailability of Zn, Fe, and Ca, although mineral content simultaneously reduced
Microbial fermentation	Induces hydrolysis of phytate by microbial phytase Increases content of organic acids  Microbial enzymes may destroy protein inhibitors that interfere with N digestibility	Enhances bioavailability of Zn, Fe and Ca May form soluble ligands with non-haem-Fe and Zn, and enhance bioavailability May improve protein quality in maize, legumes, groundnuts and pumpkin and millet seeds

Source: Sandstorm, 2001

## **2.6 Methods to reduce antinutritional factors in chickpeas**

### **2.6.1. Enzymatic Phytate Dephosphorylation during Food Processing in Order to Improve Mineral Bioavailability**

Biological processing techniques, such as soaking, germination, malting, cooking, hydrothermal processing and fermentation, result in the phytate dephosphorylation of the food. Dephosphorylation of phytate in food occurs by increasing the activity of naturally present phytate-degrading enzyme in plants and microorganisms. It must be noted that, during food processing or preparation, phytate is, in general, not completely hydrolysed by the endogenous Phytases of plants and microorganisms. For the optimization of the food process for increased mineral bioavailability by phytate degradation, it is crucial to know optimal conditions of the phytases responsible for phytate degradation. Naturally, there are dissimilarities in the capacities of various plant and microbial species to dephosphorylate phytate, due to differences in their intrinsic phytate-degrading activities (Egli *et al.*, 2002) and the properties of the enzymes, such as protein stability and pH, as well as temperature optimum for phytate degradation (Konietzny and Greiner, 2006).

During food processing or preparation phytate is, in general, not fully hydrolysed by the phytases naturally occurring in plants and microorganisms. It was found, however, that phytate must be reduced to very low levels to strongly increase mineral bioavailability, especially of iron (Hurrell, 2003).

To optimise food processing and preparation in respect to phytate degradation, it is essential to know the properties of the natural occurring phytases. In recent years several phytases from cereals, legumes and microorganisms used for food fermentation have been purified and their enzymatic properties have been determined. The properties of a purified enzyme, however, are not necessarily identical to the properties of the same enzyme in a food matrix. Temperature optimum for phytate dephosphorylation by a phytase of black beans (*Phaseolus vulgaris* var. Preto), for example, was determined to be 50 °C for the isolated enzyme and 65 °C for the

enzyme in the bean matrix . In addition, the bean matrix had a stabilizing effect on the bean phytase at higher temperatures (Greiner and Konietzny, 1998).

The removal of undesirable components is essential to improve the nutritional quality of legumes. In this way, these could effectively be utilized to their full potential 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. Soaking, cooking, fermentation and germination may improve the quality of legumes due to the removal of some antinutritional factors.

### ***2.6.2 Soaking***

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 (Greiner and Konietzny, 2006).

Moreover, soaking removes some of the non-nutritional compounds, which can be partly or totally solubilised and eliminated with the discarded soaking solution (Frias *et al.*, 2000). Some previous studies reported in the literature have analyzed the effect of the soaking conditions on the main quality parameters in the final product (Frias *et al.*, 2000; Sayar *et al.*, 2001). Frias *et al.* (2000) concluded that the seed coat controls water absorption up to a certain level of moisture. Frias *et al.* (2000) also concluded that the differences in water absorption might be due to the differential solubilisation

of the starch during soaking which is caused mainly by differences in starch structure, seed size and membrane permeability.

### **2.6.3 Germination**

Germination is a process widely used in legumes and cereals to increase their palatability and nutritional value, particularly through the breakdown of certain anti-nutrients, such as phytate and protease inhibitors. In non-germinated 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 germination a marked increase in phytate-degrading activity with a concomitant decline in phytate content was observed (Graham *et al.*, 2000).

Phytate is hydrolyzed during germination 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. Phytate levels resulting in a strong increase in mineral uptake could be achieved after 6 to 10 days of germination. Because long time periods are needed to improve mineral bioavailability through germination, this approach is meant to be useful for household applications, but it does not appear to be an economical industrial method for food processing (Graham *et al.*, 2000).

### **2.6.4 Fermentation**

Food fermentation covers a wide range of microbial and enzymatic processing of food and ingredients to achieve desirable characteristics such as prolonged shelf life, improved safety, and attractive flavor, and nutritional enrichment, elimination of anti-nutrients and promotion of health. Many cereals, legumes and vegetables are extensively used in the preparation of a variety of fermented foods. Microorganisms used for food fermentation may be part of the natural micro flora found in the raw material that is fermented or specially cultivated cultures designed to bring about specific changes in the material that is being fermented. Today, defined starter cultures

and controlled conditions are generally used in food fermentation. The type of microorganism, the fermentation conditions used, and the starting amount of phytate present in the raw material significantly affect the extent of phytate removal during the fermentation process. Major fermentation microorganisms include lactic acid bacteria, moulds and yeast (Graham *et al.*, 2000).

### **2.6.5 Cooking**

Because phytate is heat-stable, significant heat destruction of phytate during cooking is not expected to occur. Therefore, 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 (Greiner and Konietzny, 1998).

### **2.7 Minerals**

Food legumes are good sources of minerals. The most important minerals contained in chickpeas are calcium, phosphorus, magnesium, iron, copper, zinc, sodium and potassium. From a nutritional point of view, the difference between dehulled seeds and whole seed in mineral composition is marginal except for calcium. 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 (Salunkhe *et al.*, 1985; Chavan *et al.*, 1986).

Minerals, classified as micronutrients which are needed by our body in small amounts. Deficiency in minerals, however, can have a major impact on health such as anemia and osteoporosis that commonly occur in both developed and developing countries. This study focused only on iron (Fe), zinc (Zn), calcium (Ca), and phosphorous (P).

### **2.7.1 Calcium**

Calcium (Ca) is an essential nutrient for humans, but is quite often limited in diets of low-income sectors and is of particular concern for pre-school children, adolescents, and pregnant and lactating women. Chickpea seeds contain  $103 \pm 259$  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 (Abbo *et al.*, 2000).

### **2.7.2 Phosphorous**

Phosphorous (or phosphate) is part of the phospholipids, an essential functional component of cellmembranes, 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).

### 2.7.3 Iron

Iron is involved in many vital functions in the human body. First, iron is important for oxygen transport. Further, iron is essential to brain function and development and severe iron deficiency can cause retarded mental development, which may be irreversible (Walker *et al.*, 2007). Recently, iron supplementation to children has been shown to increase morbidity and possibly mortality among non-iron deficient individuals in malaria endemic areas (Iannotti *et al.*, 2006; Sazawal *et al.*, 2006).

It is likely that the harmful effects of iron supplementation has to do with the formulation and higher amounts of iron, and it is conceivable that dietary sources of highly available iron are not harmful. Dietary iron is present in foods in two main forms; haem iron only in foods of animal origin (high amounts in liver and red meat) and non-haem iron in both animal and plant foods, mostly in the ferric state. Haem iron and non-haem iron are absorbed through different mechanisms. Haem iron is transported into the enterocyte by the haem receptor, while non-haem iron uses the divalent metal transporter (DMT), which means that dietary ferric iron ( $\text{Fe}^{3+}$ ) must be reduced to ferrous iron ( $\text{Fe}^{2+}$ ) before uptake (Mackenzie *et al.*, 2005).

Absorption of non-haem iron can be enhanced or inhibited by various dietary components and thus depends on the meal composition. The absorption of haem iron is much higher than the absorption of non-haem iron; about 25% for haem iron and less than 10 for non-haem iron. Iron absorption is also influenced by total iron content in the diet (lower iron content increases absorption efficiency), and by iron status and physiological state of the individual (low iron stores and pregnancy increases absorption efficiency). Cowan *et al.*, (1967) reported that chickpea is a good source of iron and its availability is highest as compared to other food legumes.

### 2.7.4 Zinc

Zinc (Zn) is an essential nutrient for all forms of life. Zinc deficiency is a global micronutrient deficiency in humans. Worldwide, about 3 billion people, especially in developing countries, are affected by micronutrient deficiencies including Zn

deficiency. Zinc deficiency in human results in a number of health problems, such as impairments in linear growth, sexual maturation, learning ability, immune functions and the central nervous system, susceptibility to infection, impaired wound healing, etc (Brown *et al.*, 2001).

Based on national food balance data, approximately 20.5% of the world's population is estimated to be at risk of inadequate Zn intake, with the percentage of individuals at risk highest in South East Asia (33.1%), Sub Saharan Africa (28.2%), South Asia (26.7%) and Latin America and the Caribbean (24.8%) (Wuehler *et al.*, 2005).

Existence of high concentrations of phytic acid (PA, myo-inositol hexa phosphate) and fiber in diets is a major cause for the occurrence of mineral, especially Zn, deficiency in human beings. Cereals, legumes and nuts store phosphorus (P) as PA, which, together with inositol penta phosphate, is one of the main inhibitors of Zn absorption. Phytic acid is the storage form of P and usually accounts for 60-80% in wheat, 66-70% in barley, 71-88% in corn 50-70% in soybeans, 27-87% in lentils and 40 - 95% in chickpeas of total P ( Erdal *et al.*, 2002; Mate and Radomir, 2002).

#### **2.7.5 Bioavailability of minerals in legumes**

Being a good source of minerals, legumes fulfill dietary requirements of human in adequate manner among different food groups. Legume 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) (Grusak and Dellapenna, 1999). 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 (Wood and Grusak, 2007).



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 (Saikia *et al.*, 1999; Duhan *et al.*, 2002).

The bioavailability of a nutrient can be subdivided in three constituent phases i.e. availability in the intestinal lumen for absorption, absorption and/or retention in the body and finally utilization by the body. The 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 (Watzke, 1998).

### 3 .Materials and Methods

#### 3.1 Apparatus, chemicals and glass wares

All chemicals used for analysis had analytical grade and obtained from Neway Plc Company. Similarly, the glass wares were cleaned free from any possible contamination prior to analysis.

The main equipments used during the analysis are listed below:

DHG- 9055A, Memmert Germany	drying oven
OAKTON pH-110 Meter kit model 55615-80	pH meter
ARZ 140, N315, SNR, 1203290469, USA	Analytical balance
Digest stove	Crude protein analyzer
Carbolite, Aston Lane, Hope Sheffield 30 ZRR, England	Muffle furnace
DYNAC II, Clay Adams, division of Becton and Dickinson Company, USA	Centrifuge
Varian spectra AA – zoplus, Varian Australia, pty, ltd, Australia	Atomic Absorption Spectrophotometer
Beckman, Du-64 Japan	Spectrophotometer
Crude fat extractor	Fat determinator

### **3.2 Sample collection**

Chickpea seeds (*Cicer arietinum L.*), the variety DZ 10-11 were obtained from Debre Zeit Agricultural Research Center. The Debre Zeit Agricultural Research Center (DZARC) is the premier institute for chickpea research in Ethiopia. It is about 50 km, East of Addis Ababa.

### **3.3 Sample Preparation**

The Seeds were hand-sorted to remove wrinkled, moldy seeds and foreign materials.

The raw chickpea seeds ( used as control) prepared with out removing the seed coat and made to flour with electric grinder until to pass 0.425 mm sieve, this size is suitable for mineral as well as antinutritional analysis.

#### **3.3.1 Boiling**

Chickpea seeds were added into a pot containing tap water (1:10, w/v) and cooked in a pot containing tap water (94°C -96 °C ) on a hot plate until they became soft when felt between the fingers (90 min).Then dried in drying oven at 55<sup>0</sup>c for 24 hr and made to flour using electric grinder until to pass 0.425 mm sieve .

#### **3.3.2 Roasting**

**3.3.2.1 Dry Roasting:** The cleaned chickpea seeds were roasted on a metal pan for 15 minutes together with precleaned sand. The sand was used to uniform heating temperature and was cooled under a room fan. Then made to flour using electric grinder until to pass 0.425 mm sieve.

**3.3.2 .2 Wet Roasting:** The cleaned chickpea seeds were soaked in tap water (1:3, w/v) for 12 hr and then were roasted at 97 °C -101°C. Then dried in drying oven at 55<sup>0</sup> C for 12 hr and made to flour using Electric grinder until to pass 0.425 mm sieve.

### **3.3.3 Germination**

- Seeds were rinsed, and then soaked in tap water (1:3, w/v) for 12 hr at ambient temperature (22– 23°C).
- Seeds were drained and placed on perforated aluminum pans then were placed in a dark, for germination.
- The seeds were germinated for 24 hr.
- Dried samples were milled to flour with a miller to pass 0.425 mm sieve

### **3.3.4 Natural fermentation**

Suspensions of chickpeas flour in tap water were prepared in plastic containers at a concentration of 1:3 dilutions (w/v). The flour slurry was allowed to ferment naturally with only the microorganisms borne on or inside the seeds (endogenous micro flora on the seeds) at room temperature (22 -23<sup>0</sup>C) for 0, 24, 48 and 72 hr in plastic containers. The fermentation Water was decanted and samples were withdrawn and transferred to aluminum dishes after each fermentation time and dried in a hot air oven-drier (Mettler, Germany) at 70 °C for 36 hr.

Dried samples were ground with a miller to pass a 1 mm sieve and stored for analysis.

#### **3.3.4.1 pH determination**

The pH of the fermented samples was determined according to the method of Pearson (1971). The pH of the slurry was determined by dipping the electrode of the pH meter ( OAKTON pH-110) in the mixture. The pH of the fermented samples was determined by dipping the electrode of the pH meter in the homogenate fermented mixture slurries after the end of each fermentation period. . The pH meter was calibrated using pH 4.0, 7.0 and 10.0 buffers.

### **3.3.4.2 Determination of total titratable acidity**

Total titratable acidity expressed as percentage of lactic acid, was determined by titrating 30 ml of the homogenate samples used for pH determination against 0.1 N NaOH. First the distilled Water (1L) used for titration was titrated with 0.1 N NaOH and the volume of 0.1 N NaOH Consumed by water titration was considered as a blank. The volume of 0.1 N NaOH used for titration of the sample was noted after correcting the blank and triplicate determination was made (Pearson, 1971).

Calculation:

$$\% \text{ Lactic Acid} = V \times 0.009008 \times 100/W$$

Where: V=Volume of 0.1 N NaOH used for sample titration; 0.009008=Factor equivalent in which 1ml of 0.1N NaOH =0.009008g C<sub>3</sub>H<sub>6</sub>O<sub>5</sub>; W=Weight in gram of sample in the mixture (Pearson, 1971).

### **3.4 Proximate Analysis**

Moisture content, total ash, crude protein, crude fiber, and crude fat of the seed flours were determined according to AOAC (2000) using the official methods 925.09, 923.03, 979.09, 962.09, and 4.5.01, respectively.

#### **3.4.1 Determination of moisture content (AOAC 925.09, 2000)**

Empty dishes and lids (made of porcelain) were dried using air drying oven (Memmert, Germany) for 1 hour at 100<sup>0</sup>C, 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 105<sup>0</sup>C, and then the dishes and their contents were cooled in a discator to room temperature and reweighed.

Calculation

$$\text{Moisture (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

M<sub>1</sub>=mass of the dish, M<sub>2</sub>=mass of the dish and the sample before drying, and

M<sub>3</sub>=mass of the dish and the sample after drying

### 3.4.2 Determination of Crude protein (AOAC 979.09, 2000)

Protein (N×6.25) was determined by the Kjeldahl method. 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 alkalization with sodium hydroxide is steam distilled into boric acid and titrated with hydrochloric acid.

**Digestion:** About 0.5000g of fresh sample s were taken in a Tecator tube and 6ml of acid mixture (5 parts of concentrated ortho phosphoric 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<sup>0</sup>C, 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 deionized 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 condenser

is 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.

$$\text{mg nitrogen in the sample} = V \times N \times 14$$

$$\text{g nitrogen/100 g ample} = \text{mg of nitrogen} \times 100 / \text{mg sample}$$

$$\text{Total nitrogen (\%)} = (V - V_b) \times N \times 14 / W$$

$$\text{Crude protein (\%)} = \text{total nitrogen (\%)} \times 6.25$$

Where: V = volume of hydrochloric acid consumed to neutralize the sample; V<sub>b</sub> = the volume of acid consumed to neutralize the blank; N = normality of the acid; 14 = Eq. wt of Nitrogen; 6.25 = conversion factor from total nitrogen to crude protein.

### **3.4.3 Determination of crude fat content (AOAC4.5.01, 2000)**

Crude fat was determined by exhaustively extracting a known weight of sample in diethyl ether (boiling point, 55 °C) in a soxhlet extractor. The ether was evaporated from the extraction flask. The amount of fat was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage.

The extraction flasks were cleaned, dried in drying oven (Memmert, Germany) at 70°C for 1 hour, cooled in desiccators (with granular silica gel) for 30 minutes, and then weighed. The bottom of the extraction thimble was covered with about 2cm layer of fat free cotton. About 2.00 gram of fresh samples were added into the extraction thimbles, and then covered with about 2cm layer of fat free cotton. The thimbles with the sample content were placed into soxhlet extraction chamber. The cooling water was switched on, and a 50 ml of diethyl ether was added to the extraction flask through the condenser. The extraction was conducted for about 3 hrs. The extraction flasks with their content were removed from the extraction chamber and placed in the drying oven at 70°C for about 1hr, cooled to room temperature in the desicator for about 30 minutes and re-weighed.

$$W=W_2-W_1$$

$$\text{Fat g/100 g fresh sample} = (W \times 100) / W_0$$

Where: W = weight of fat; W<sub>2</sub> = weight of extraction flask after extraction (wt. of flask and fat); W<sub>1</sub> = weight of extraction flask before extraction (wt. of flask);

W<sub>0</sub> = weight of fresh Sample.

#### **3.4.4 Determination of crude fiber content (AOAC 962.09, 2000)**

Crude fiber was determined after digesting a known weight of chickpea flour by refluxing 1.25% boiling sulfuric acid and 28% boiling potassium hydroxide.

**Digestion:** About 1.6000g of fresh sample was placed into a 600ml beaker, 200ml of 1.25% H<sub>2</sub>SO<sub>4</sub> 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.

**Filtration:** 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

**Washing:** The residue in the crucible was washed with hot distilled water and filtered (repeated twice). The residue was washed with 1% H<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>SO<sub>4</sub> and filtered. Finally the residue was washed with water- free acetone.



**Drying and combustion:** The crucible with its content was dried for 2 hours in an electric drying oven at 1300C 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<sup>0</sup>C. The crucible was cooled in the desiccator and weighed.

Then the fiber was calculated as a residue after subtraction of the ash.

$$\text{Crude fiber g /100 g} = (W_1 - W_2) \times 100 / W_3$$

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

#### **3.4.5 Determination of total ash content (AOAC 923.03, 2000)**

Ash was determined by incineration of known weights of the samples in a muffle furnace at 550<sup>0</sup>C (Gallenkamp, size 3) until a white ash was obtained. Organic matter was 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<sup>0</sup>C in a muffle furnace. The ashing dishes (made of porcelain) were placed into a muffle furnace for 30 min at 550<sup>0</sup>C. 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<sup>0</sup>C 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.

Calculation:

$$\text{Total ash (\%)} = \frac{W_2 - W}{W_1 - W} \times 100$$

Where w= weight in grams of empty dish

W<sub>1</sub> =weight in grams of the dish plus the dried test material

W<sub>2</sub>= weight in grams of the dish plus ash

#### **3.4.6 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.

#### **3.4.7 Determination of gross energy**

Gross energy was determined by calculation from fat, carbohydrate and protein contents using the Atwater's conversion factors; 16.7 kJ/g (4 kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrates and expressed in calories ( Guyot *et al.*,2007).

### **3.5 Minerals Analysis**

The mineral contents were determined by the procedure of AOAC (1984). Calcium, iron, and zinc were determined using an Atomic Absorption Spectrophotometer while phosphorous was determined 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 Spectrophotometer (Varian SpectraAA-20Plus, Varian Australia Pty., Ltd., and

Australia) 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 to concentrations that fall within the working range (0, 0.6, 1.0, 1.4, 1.8,  $\mu\text{g/ml}$  for zinc analysis; 0.0, 1.0, 1.5, 2.0, and 3.0  $\mu\text{g/ml}$  for calcium analysis and 0, 2.0, 6.0, 10.0 12.0  $\mu\text{g/ml}$  for iron analysis). The Atomic Absorption Spectrophotometer (AAS) used for mineral determination were calibrated using standard solutions and the reagent blank solution was run with the sample.

**3.5.1 Mineral determination:** 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 (Whatman 42, 125mm) 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

$$M \text{ content (mg/100g)} = [(a-b) \times V]/10W$$

$$M \text{ content (mg/kg)} = ((a-b) \times V)/W$$

Where: W= Weight (g) of samples; V= Volume (V) of extract; a = Concentration

( $\mu$  g/ml) of sample solution; b = Concentration ( $\mu$  g/ml) of blank solution.

### **Phosphorous determination**

Phosphorous was determined by the colorimetric method using ammonium molybdate (AOAC, 1984). It was converted to phosphomolybdate, which was reduced to a blue

molybdenum compound by aminonaphtholsulphonic acid to give a blue molybdenum compound. A sample solution was 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 deionized 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 deionized 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.

Calculation:

First B was subtracted from all other readings

$$P \text{ mg/100g} = (A-B) \times 50 \times 100 \times /\text{Slope} \times \text{WF} \times 10$$

Where: A=Reading of the sample solution; B= Reading of the blank solution;

WF=Weight of fresh sample.

**3.5.2 HCl-extractability of Minerals (Bioavailability):** Minerals in the samples were extracted by the method described by 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 placed in a muffle furnace at 550°C for 4 hr. Thereafter, the samples were cooled and about 5ml of 5N HCl was added and boiled gently for 10min and then cooled, diluted to 100ml with

de ionized water. Minerals were determined as described above. Extractability of each element was calculated as a percentage of the total amount of the element.

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in 0.03N HCl } \left( \frac{\text{mg}}{100\text{g}} \right)}{\text{Total minerals } \left( \frac{\text{mg}}{100\text{g}} \right)} \times 100$$

### **3.6 Determination of Antinutritional Factors**

#### **3.6.1 Determination of phytate content**

Phytate was determined by the method of Latta and Eskin (1980) and later modified 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 FeCl<sub>3</sub>.6H<sub>2</sub>O and 0.3% of sulfosalicylic 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 measured at 500 nm using UV-VIS spectrophotometer (Beckman DU-64- spectrophotometer, USA).

A series of standard solution were prepared 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 absorbances of the solutions (both the sample and standard) were measured at 500nm by using deionized water as a blank. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation. Phytate: mineral molar ratios were calculated using the molecular weight of PA=660.

Calculation:

$$\text{Phytic acid in mg/100g} = (\text{absorbance-intercept})^3 / (\text{slope} \times \rho \times \text{wt. of Sample} \times 10)$$

Where,  $\rho$  is density

### **3.6.1.1 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).

$$\text{Phytate phosphorous (mg/100g)} = (A \times 28.18) / 100$$

Where: A = phytate content (mg/100g)

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

### **3.6.2 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 D-catechin 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-15. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation.

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)

### **3.7 Statistical analysis**

The analysis was carried out in three triplicates for all determinations. The mean and standard deviation of means were calculated. The data were analyzed by one way analysis of variance (ANOVA) in SPSS 15.0 for windows evaluation version computer programme was used to analyze the results. A multiple comparison procedure of the treatment means was performed by Duncan's new multiple range test (Duncan, 1955). Significance of the differences was accepted at  $P < 0.05$

## 4. Results and Discussion

### 4.1. Effect of processing on proximate composition of chickpea

#### 4.1.1 Protein

Chickpea exhibited quite different protein content, there are significant differences ( $P < 0.05$ ) in crude protein content were observed between each processing techniques of chickpea Table 4.1 .The crude protein content during germination, fermentation at 0 hr and fermentation at 24 hr were  $15.39 \pm 0.55$ ,  $16.17 \pm 0.17$ , and  $15.71 \pm 0.085$  % respectively. However, boiling, roasting and fermentation for more than 24 hr decreased the crude protein content.

Germination caused a slight decrease in crude protein of chickpea while compared to raw chickpea, but the effect was non-significant ( $P > 0.05$ ). It was noted in the earlier study that; proteolysis occurred during sprouting which could be resulted in an increase in non-protein nitrogen and free amino acids (Sangronis *et al.*, 2004). However, the analytical method employed in this study relied on determination of total nitrogen for protein calculation (AOAC, 2000). Thus, the change in the form of nitrogen by breaking peptide bonds does not affect the crude protein level (Torres *et al.*, 2007).

During germination, however, protein content significantly increased compared to boiling, wet roasting, dry roasting and fermenting for more than 24 hr chickpea flours. This increment might be attributed to the utilization of carbohydrates as source of energy during germination process. The results obtained were in agreement with those obtained with kidney bean (Alonso *et al.*, 2000) and mungbean (Mubarek, 2005).

Natural fermentation reduced the protein content significantly ( $P < 0.05$ ). The reduction of protein level after natural fermentation was also noticed in bean and cowpea (Granito *et al.*, 2005). Reduction in protein content due to fermentation could be attributed to proteolysis that results in the production of volatile ammonia which is a characteristic of such process of protein rich foods (Beaumont, 2002).



**Table 4.1 The effect of processing on proximate composition of chickpea**

Values are means of triplicates ( $\pm$  SD). Means not sharing a common superscript letter in a column are significantly

Sample code	Crude Protein%	Crude Fat%	Crude Fiber%	Moisture Content %	Total Ash%	Utilizable CHO%	Energy Kcal/(100g)
RC	16.73 $\pm$ 2.140 <sup>a</sup>	5.877 $\pm$ 1.006 <sup>a,b</sup>	5.877 $\pm$ 256 <sup>a</sup>	7.600 $\pm$ 0.100 <sup>d</sup>	2.627 $\pm$ 0.0555 <sup>a</sup>	61.220 $\pm$ 1.057 <sup>e</sup>	364.690 $\pm$ 4.741 <sup>d,e</sup>
BC	13.877 $\pm$ 0.206 <sup>d</sup>	4.77 $\pm$ 0.160 <sup>d,e</sup>	4.570 $\pm$ 0.347 <sup>a,e</sup>	8.867 $\pm$ 0.049 <sup>b</sup>	2.417 $\pm$ 0.015 <sup>d</sup>	65.320 $\pm$ 0.275 <sup>b,c</sup>	359.717 $\pm$ 5.187 <sup>f</sup>
WRC	13.823 $\pm$ 0.110 <sup>d</sup>	5.203 $\pm$ 0.091 <sup>c,d</sup>	5.190 $\pm$ 0.036 <sup>c</sup>	9.367 $\pm$ 0.133 <sup>a</sup>	2.300 $\pm$ 0.040 <sup>e</sup>	64.117 $\pm$ 0.111 <sup>d</sup>	359.257 $\pm$ 0.374 <sup>f</sup>
DRC	14.083 $\pm$ 0.201 <sup>c,d</sup>	6.067 $\pm$ 0.165 <sup>a</sup>	3.357 $\pm$ 0.095 <sup>f</sup>	5.907 $\pm$ 0.21 <sup>g</sup>	2.590 $\pm$ 0.080 <sup>a,b</sup>	68.670 $\pm$ 1.093 <sup>a</sup>	382.947 $\pm$ 0.936 <sup>a</sup>
GC	15.393 $\pm$ 0.546 <sup>a</sup>	4.260 $\pm$ 0.150 <sup>e</sup>	5.600 $\pm$ 0.60 <sup>b</sup>	6.900 $\pm$ 0.070 <sup>e</sup>	2.310 $\pm$ 0.076 <sup>e</sup>	65.547 $\pm$ 0.735 <sup>b</sup>	362.097 $\pm$ 0.936 <sup>e,f</sup>
FC-0	16.170 $\pm$ 0.171 <sup>a</sup>	5.730 $\pm$ 0.030 <sup>a,b,c</sup>	4.590 $\pm$ 0.020 <sup>e</sup>	8.60 $\pm$ 0.060 <sup>c</sup>	2.670 $\pm$ 0.010 <sup>a</sup>	62.240 $\pm$ 0.233 <sup>e</sup>	365.210 $\pm$ 0.936 <sup>e,f</sup>
FC-24	15.713 $\pm$ 0.085 <sup>a</sup>	5.723 $\pm$ 0.050 <sup>a,b,c</sup>	4.87 $\pm$ 0.0045 <sup>d</sup>	6.82 $\pm$ 0.105 <sup>e</sup>	2.533 $\pm$ 0.035 <sup>b</sup>	64.337 $\pm$ 0.146 <sup>c,d</sup>	371.800 $\pm$ 0.293 <sup>b</sup>
FC-48	14.933 $\pm$ 0.249 <sup>b,c,d</sup>	5.303 $\pm$ 0.080 <sup>b,c,d</sup>	5.593 $\pm$ 0.165 <sup>b</sup>	6.587 $\pm$ 0.081 <sup>f</sup>	2.493 $\pm$ 0.061 <sup>c,d</sup>	65.09 $\pm$ 0.325 <sup>b,c,d</sup>	367.823 $\pm$ 0.293 <sup>c</sup>
FC-72	14.995 $\pm$ 1.203 <sup>c,d</sup>	5.220 $\pm$ 0.046 <sup>b,c,d</sup>	5.680 $\pm$ 0.106 <sup>a,b</sup>	6.40 $\pm$ 0.020 <sup>f</sup>	2.450 $\pm$ 0.020 <sup>c,d</sup>	66.020 $\pm$ 0.416 <sup>b</sup>	367.98 $\pm$ 0.173 <sup>c</sup>

different at ( $P < 0.05$ ) as assessed by Duncan's multiple range tests.

RC-Raw chickpea

DRC-dry roasted chickpea

FC-24-fermented chickpea for 24 hr

BC-Boiled chickpea

GC-germinated chickpea

FC-48- fermented chickpea for 48 hr

WRC-Wet Roasted chickpea

FC-0-fermented chickpea for 0 hr

FC-72-fermented chickpea for 72 hr

Other reason could be the metabolism of amino acids by fermenting microorganisms in to organic acids, alcohols and aldehydes (Feron and Wache, 2006).

In the present study, the results obtained for protein ranged from 13.88 to 16.73 % which is similar to previous study. Chickpea seed contains 14.5 to 30.6% crude protein (Chavan *et al.*, 1986).

#### **4.1.2 Crude Fat**

Chickpea contains a notably varied percentage of crude fat. In the present study, the crude fat obtained ranged between 4.26 - 6.07%. The analysis of variance showed in Table 4.1 indicated that, there were significant ( $P < 0.05$ ) differences among each methods. Maximum reduction was observed during germination. This reduction was probably caused by breaking down of fat by beta oxidation with fat being used for energy purposes in embryo development. This observation is in agreement with the previous studies of El-Adway (2002) and Mubarek (2005).

As far as fermentation effect on crude fat of chickpea samples is concerned, there is a significant reduction ( $P < 0.05$ ) of crude fat content of chickpea flours. The bacterial growth could be responsible for this effect because bacterial species contain lipase activity at variable levels. However, crude fat content ranged between 5.12 to 8.57 g/100g in some chickpea cultivars with the mean value  $6.0 \pm 0.9$  g/100g (Patane, 2006). The crude fat content in chickpea was somewhat lower than these values but are in agreement to the fat content determined in Indian chickpea varieties (4.18-4.92 g/100g) (Singhai and Shrivartava, 2006).

#### **4.1.3 Moisture Content**

Moisture content determination is an integral part of the proximate composition analysis of food. Moisture content in chickpea flour determines its stability. During processing treatment, there are significant ( $P < 0.05$ ) differences among each processing methods. The results indicated that, the maximum and minimum moisture

content was observed in wet roasting and dry roasting  $9.37 \pm 0.13$ ,  $5.9 \pm 0.08\%$  respectively, whereas the decrease was observed for fermented chickpea flours. However, the relative decrease of moisture content may be attributed due to a variation in the treatment during the drying processes of the samples.

All the treatments except raw sample were subjected to drying operation in order to prepare flour for analysis at low moisture content, the flours possessed low water activity hindering any microbial growth. Moreover, dried flours are devoid of moisture required for the spore growth and physiological activity. The moisture content studied in the present study of chickpea flours were similar to the studies undertaken by (Patane, (2006) and Singihai and Shrivastava, (2006).

#### **4.1.4 Crude Fiber**

The other important nutritional components of chickpea seed is the crude fiber. There were significant differences between each processing methods of the samples in Table 4.1 . It is obvious that crude fiber content depends on the thickness of seed coat, because it is present mainly in the outer seed testa (Grela and Gunter, 1995).

In the present study, the natural fermentation resulted in the decreases of the crude fiber content, similar to the results of Ramachandran *et al.* (2005). The expected decrease in fiber content during fermentation could be attributed to the partial solubilisation of cellulose and hemi cellulosic type of material by microbial enzymes. A previous study has reported a significant decrease of fat, ash, and fiber contents after four days of maize fermentation (Ejigui *et al.*, 2005).

#### **4.1.5 Total Ash**

The data as shown in Table4.1 indicates that the ash content of the germinated and wet roasted samples  $2.31 \pm 0.07$  and  $2.30 \pm 0.04 \%$  were the lowest among all treated chickpea samples. The leaching of minerals from the seeds during soaking could be the reason. It was observed that legumes contained a large portion of water soluble ash which has the tendency to leach out during hydro processing of seeds .The ash content

in the present study of chickpea samples as previously reported values of ash in chickpea cultivars by Singhair and Shirvostava,( 2006) and Patane,( 2006).

#### **4.1.6 Carbohydrates**

Utilizable carbohydrate content was determined by difference. That means, there was no analysis conducted for utilizable carbohydrate determination. There are significant differences among each treatments at ( $P<0.05$ ). The processing methods increased the content of utilizable carbohydrate as compared to the raw sample ( $61.22 \pm 1.06\%$ ).

Moreover, boiling and roasting treatments increased significantly the total carbohydrates. The increase in total carbohydrates content of chickpea after cooking and drying would be attributed to the retro gradation of starch after gelatinization. This result was in agreement with Wang *et al.* (2008), which indicated starch content of chickpeas increased on cooking.

#### **4.1.7 Gross Energy**

The caloric value was calculated by multiplying the mean values of crude proteins, crude fat and total carbohydrate by AtWater factors of 4, 9 and 4, respectively. There were significant differences ( $P<0.05$ ) among each processing treatments. The calorific value (Kcal/100g) was highest in dry roasted followed by fermented samples. These values were found to be relatively high as compared to those reported earlier for desi chickpea (Khan *et al.*, 1995). Treatments by dry roasting and fermentation for 24, 48 and 72 hr have increased the total energy contents by 18.35%, 7.11%, 3.13% and 3.29%, respectively.

Table 4.2 Changes in pH and titratable acidity (TTA) as % lactic acid

Fermentation period (hrs)	pH	TTA (as% lactic acid)
0	6.28±0.290 <sup>a</sup>	0.48± 0.001 <sup>d</sup>
24	4.90± 0.060 <sup>b</sup>	0.56±0.020 <sup>c</sup>
48	4.41 ±0.013 <sup>c</sup>	0.65±0.020 <sup>b</sup>
72	4.25 ± 0.002 <sup>c</sup>	0.69± 0.021 <sup>a</sup>

Values are means of triplicates (± SD). Means not sharing a common superscript letter in a column are significantly different at (P < 0.05) as assessed by Duncan's multiple range tests.

FC-0- fermented chickpea for 0hr    FC-24-fermented chickpea for 24 hr  
 FC-48-fermented chickpea for 48hr    FC-72-fermented chickpea for 72 hr

As shown in Table 4.2, as fermentation period increases the pH decreases with a concomitant increase in acidity. The pH drop was probably the result of microbial activity on chickpea flour converting some of the carbohydrates in to organic acids such as lactic acid, citric acid, acetic acids and other volatile short chain fatty acids. A decrease in pH with a corresponding increase in titratable acidity has been reported in fermentation of various food grains (Abdel haleem *et al.*, 2008; Shimelis and Rakshit, 2008). According to these authors, the production of lactic acid bacteria during fermentation has attributed to the decrease in pH.

Like many traditionally fermented products, the drop in pH and an increase in TTA were a means for protection from many food pathogens (Shimelis and Rakshit, 2008). Organic acids produced during fermentation also can potentially enhance Iron and zinc absorption via the formation of soluble ligands (Gibson *et al.*, 2006).

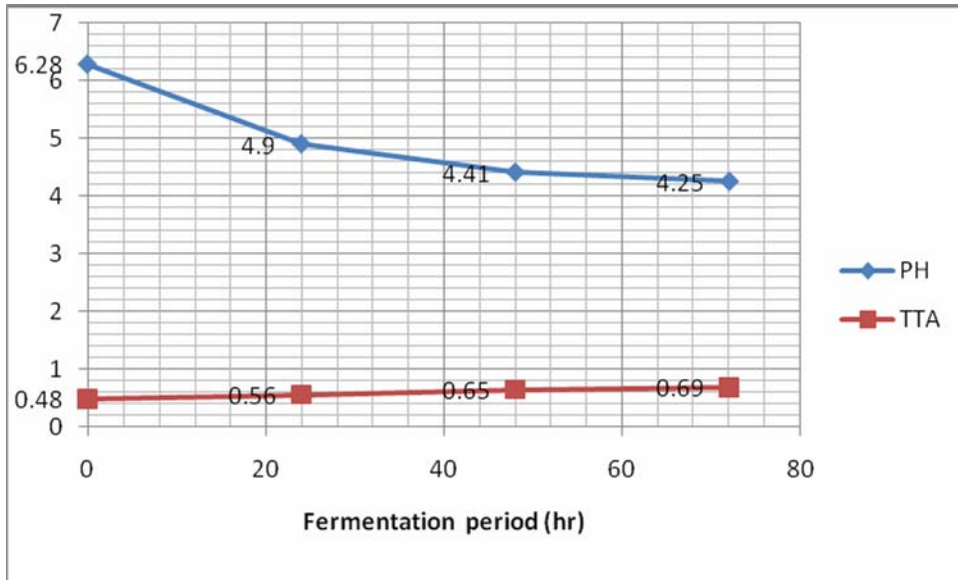


Figure 2. Change in pH and TTA (as % of lactic acid)

Table 4.3 The effect of processing treatment on molar ratios of phytate: Iron, Phytate: zinc, phytate= calcium and [calcium x phytate]/ [zinc]

Sample code	Phytate :Iron	Phytate: zinc	Phytate: calcium	[calcium x phytate]/[zinc] (mol/kg )
RC	1.233±0.057 <sup>e</sup>	2.440±0.100 <sup>d</sup>	0.020±0.00 <sup>c</sup>	0.127±0.110 <sup>e</sup>
BC	2.00±0.100 <sup>a</sup>	3.007±0.0306 <sup>b</sup>	0.030±0.00 <sup>b</sup>	0.145±0.1237 <sup>b</sup>
WRC	2.007±0.153 <sup>a</sup>	3.447±0.153 <sup>a</sup>	0.030±0.00 <sup>b</sup>	0.155±0.524 <sup>a</sup>
DRC	2.08±0.100 <sup>a</sup>	3.330±0.0173 <sup>a</sup>	0.040±0.00 <sup>a</sup>	0.135±0.015 <sup>d</sup>
GC	1.123±0.047 <sup>f</sup>	2.253±0.0493 <sup>e</sup>	0.020±0.00 <sup>c</sup>	0.139±0.246 <sup>c</sup>
FC-O	1.617±0.0252 <sup>b</sup>	2.587±0.023 <sup>c</sup>	0.040±0.00 <sup>a</sup>	0.106±0.049 <sup>f</sup>
FC-24	1.280±0.020 <sup>d</sup>	2.390±0.036 <sup>d</sup>	0.040±0.00 <sup>a</sup>	0.082±0.115 <sup>g</sup>
FC-48	1.310±0.040 <sup>d</sup>	2.233±0.012 <sup>f</sup>	0.040±0.00 <sup>a</sup>	0.076±0.069 <sup>h</sup>
FC-72	1.553±0.058 <sup>c</sup>	1.953±0.155 <sup>g</sup>	0.030±0.008 <sup>b</sup>	0.075±0.185 <sup>h</sup>

Values are means of triplicates ( $\pm$  SD). Means not sharing a common superscript letter in a column are significantly different at ( $p < 0.05$ ) as assessed by Duncan's multiple range tests.

RC-Raw chickpea

DRC- dry roasted chickpea

FC-24- fermented chickpea for 24 hr

BC-Boiled chickpea

GC- germinated chickpea

FC-48- fermented chickpea for 48 hr

WRC-Wet Roasted chickpea

FC-0-fermented chickpea for 0 hr

FC-72-fermented chickpea for 72 hr

Data in Table 4.3 indicates that, there are significant differences with each processing treatments. Phytate: Iron molar ratio indicates, however, poor iron bioavailability because of the high levels of phytic acid. The present study the results on phytate: iron molar ratios  $> 0.15$  which is regarded as indicative of poor iron bioavailability (Melaku *et al.*, 2005).

Phytate : Zinc molar ratio indicated in the Table 4.3 statistically significant at ( $P < 0.05$ ) among each techniques, however, in all cases the ratio is very small ( $< 5$ ) showing that high indicative of Zn bioavailability. On the other hand, Phytate:

zinc molar ratios  $>15$ , indicative of poor zinc bioavailability (Melaku *et al.*, 2005; Walingo, 2009).

Children in rural Ethiopia are especially very prone to deficiencies of minerals and trace elements, as they eat from the family dish and often cannot meet their specific nutrient needs. This is supported by Melaku *et al.* (2005), who showed that supplementation with zinc increased the linear growth of infants, particularly those who were stunted. Hence, phytate: Zinc molar ratio is considered a better indicator of zinc bioavailability than total dietary phytate levels alone (Kelbessa and Narasimha, 1998).

The phytate: calcium molar ratio was below the critical molar ratio of 0.24 (Frontela *et al.*, 2009), in all the processing treatment techniques observed in the present study. Thus the result indicates favorable 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 (Kelbessa and Narasimha, 1998; Melaku *et al.*, 2005). However, in this study the values  $< 0.5$  were observed in all the samples processing treatments which indicate that the samples are high in calcium content Table 4.3

#### **4.2 Effect of processing treatment on phytate phosphorous and non phytate phosphorous contents of Chickpea**

Analysis of variance showed as in Table 4.4, among each processing treatments there were significant differences ( $P < 0.05$ ) in total Phosphorous, phytate phosphorous and non phytate phosphorous. However, there were no significant differences ( $P > 0.05$ ) in case of phytate phosphorous during boiling, dry roasting and fermentation of chickpea at 0 hr. This could be attributed due to heat resistance of phytate during such cooking treatments and absence of phytase enzyme in fermentation at 0 hr. But during germination and fermentation there was a reduction of phytate phosphorous as a result of phytase activity. Non-phytate phosphorous is the difference between total phosphorous and phytate phosphorous. That means when there is high phytate



phosphorous, there will be low non-phytate phosphorous in the corresponding processing treatment.

Table 4.4 The effect of processing treatment on phytate phosphorous and non-phytate phosphorous contents of chickpea

Sample code	Total phosphorus (mg/100g)	Phytate phosphorus (mg/100g)	Non-phytate phosphorus (mg/100g)	Proportion of phosphorous as phytate (%)
RC	298.160±0.318 <sup>a</sup>	27.467±0.210 <sup>a</sup>	270.693±0.240 <sup>b</sup>	9.210±0.060 <sup>c</sup>
BC	265.363±0.717 <sup>f</sup>	27.383±0.240 <sup>a</sup>	237.98±0.479 <sup>g</sup>	10.317±0.065 <sup>b</sup>
WRC	260.620±0.77 <sup>g</sup>	25.997±0.136 <sup>b</sup>	234.623±0.656 <sup>h</sup>	9.973±0.032 <sup>d</sup>
DRC	252.187±0.441 <sup>h</sup>	27.057±0.174 <sup>a</sup>	225.130±0.515 <sup>i</sup>	10.723±0.032 <sup>d</sup>
GC	294.257±0.345 <sup>b</sup>	20.330±0.340 <sup>f</sup>	273.927±0.553 <sup>a</sup>	6.907±0.121 <sup>h</sup>
FC-O	270.083±0.604 <sup>c</sup>	27.357±0.206 <sup>a</sup>	242.723±0.755 <sup>f</sup>	10.13±0.095 <sup>c</sup>
FC-24	271.063±0.582 <sup>c</sup>	24.590±0.328 <sup>c</sup>	246.673±0.253 <sup>c</sup>	9.073±0.101 <sup>c</sup>
FC-48	272.317±0.500 <sup>d</sup>	22.740±0.210 <sup>d</sup>	249.673±0.412 <sup>d</sup>	8.347±0.070 <sup>f</sup>
FC-72	274.60±0.674 <sup>c</sup>	21.603±0.240 <sup>c</sup>	252.997±0.911 <sup>c</sup>	7.893±0.105 <sup>g</sup>

Values are means of triplicates (± SD). Means not sharing a common superscript letter in a column are significantly different at (p< 0.05) as assessed by Duncan's multiple range tests.

RC-Raw chickpea                      DRC-dry roasted chickpea                      FC-24-fermented chickpea for 24 hr  
 BC-Boiled chickpea                      GC- germinated chickpea                      FC-48- fermented chickpea for 48 hr  
 WRC-Wet Roasted chickpea                      FC-0- fermented chickpea for 0 hr                      FC-72- fermented chickpea for 72 hr

Fermentation and germination lowered the levels of phytate P in all the samples with a simultaneous increase in non phytate phosphorous significantly (P < 0.05) in Table 4.4. Thus, the hydrolytic reduction of phytic acid during fermentation and germination may have contributed the degradation of phytic acid. Hence, the lower the phytate

phosphorus, the more bioavailable was phosphorus in the fermented and germinated samples. Generally, diets with phosphorus as phytate (%)  $\leq$  60 % are regarded as being adequate in bioavailable phosphate (Melaku *et al.*, 2005). However, the high proportion of phosphate as phytate, decreased the bioavailability of minerals and trace elements (Melaku *et al.*, 2005).

Cleavage of phosphorus from phytic acid may explain the improved availability of phosphorus in fermented and germinated chickpea. Similarly, natural fermentation has been reported earlier to increase the HCl-extractability of phosphorus with a corresponding decrease in the phytic acid content of pearl millet flour (Khetarpaul and Sharma, 1997). A corresponding decrease in phytate phosphorus and enhancement in the non phytate phosphorus were noticed in the present study.

Abdel-Rhaman *et al.* (2005) concluded that, in various seeds, phytic acid positively correlates with total P, correlation coefficients being greater than 0.90. Factors that affect the total phosphorous content, such as soil, available phosphorous, variety, climatic condition and fertilizers, can influence the phytic acid concentration.

### 4.3 Antinutritional factors content

Table 4.5 The effect of processing treatment on phytate and Condensed tannin content

Sample code	Phytate (mg/100g)	Condensed tannin (mg /100g)
RC	97.46±0.750 <sup>a</sup>	175.23±0.31 <sup>a</sup>
BC	97.17±0.855 <sup>a</sup>	174.37±0.49 <sup>a</sup>
WRC	91.93±0.850 <sup>b</sup>	160.10±0.56 <sup>c</sup>
DRC	96.00±0.62 <sup>a</sup>	174.9±0.26 <sup>a</sup>
GC	72.067±1.33 <sup>f</sup>	99.26±2.99 <sup>f</sup>
FC-0	97.067±0.75 <sup>a</sup>	174.8±0.90 <sup>a</sup>
FC-24	84.26±1.16 <sup>c</sup>	169.9±0.82 <sup>b</sup>
FC-48	80.68±0.75 <sup>d</sup>	150.10±1.4 <sup>d</sup>
FC-72	76.67±0.85 <sup>e</sup>	142.77±0.83 <sup>e</sup>

Values are means of triplicates (± SD). Means not sharing a common superscript letters in a column are significantly different at (p< 0.05) as assessed by Duncan's multiple range tests.

RC-Raw chickpea

DRC- dry roasted chickpea

FC-24- fermented chickpea for 24 hr

BC-Boiled chickpea

GC-germinated chickpea

FC-48- fermented chickpea for 48 hr

WRC-Wet Roasted chickpea

FC-0- fermented chickpea for 0 hr

FC-72-fermented chickpea for 72 hr

#### 4.3.1 Condensed tannin content

The mean values for the effect of processing treatments on tannins are presented in Table 4.5. The analysis of variance of the data shows significant differences (p<0.05) among different processing methods. However, during boiling, dry roasting and fermentation at (0hr) has non-significant differences compared to the raw samples. During fermentation for 24, 48, 72 hr and germination of chickpea, the tannin content decreased by 3.1%, 14.4%, 18.5% and 43.4%, respectively. Germination was the first

best treatment, contributing in the reduction of tannin in chickpea. In the present study, losses of tannins after germination for 24 hr was in agreement with previous study of El-Adawy (2002), in which tannin loss after germination for 24 hr ranged from 43% to 59 % in chickpea samples.

Process variables and microorganisms play a vital role in the reduction of tannins. These results were in agreement with earlier findings of Moreno *et al.* (2004) who observed that optimum fermentation time and temperature are quite essential to obtain maximum tannin reduction in chickpea. Various studies have reported the reduction in tannin content of legumes during soaking (Vijayakumari *et al.*, 2007). However, in the present study, soaking was taken as the preparatory step of germination which have attributed to leaching in to soaking media.

Moreover, tannin reduction during germination is usually attributed to enzymatic hydrolysis by polyphenolase (Reddy *et al.*, 1985). Wet roasting of chickpea experienced a significant decrease ( $p < 0.05$ ) in tannin content. This reduction could be due to leaching of out tannin from the surface by soaking and wetting of seeds during germination and enzymatic hydrolysis.

#### **4.3.2 Phytic Acid (Phytate)**

Phytate (mg/100g) content of chickpea expressed as plus or minus of the standard deviation is shown in Table 4.5 as affected by different processing treatments. There are no significant differences among boiling, dry roasting and fermentation at initial (0hr). However, there is a significant difference among wet roasted, germinated and fermented at 24, 48 and 72 hr of chickpea in the content of phytate. The highest reduction of phytate was observed in the germination processing treatment than other cases. It reduced by 35.1% compared to raw samples.

Fermentation and germination of chickpea has been proven as the best strategy to reduce the phytate level. The microorganisms used in the chickpea processing reduced the phytate to a great extent by a virtue of their ability to produce phytase with simultaneous lowering down the pH of the substrate. The phytate reduction in legumes

such as chickpea is the best suitable process by enhancing phytase activity. However, there is no reduction in boiling and dry roasting of chick pea. This could be attributed due to heat stable nature of phytate in these processing treatments. Phytic acid is a source of phosphorous and cations for the seeds that begin to sprout. It is also a source phosphates and inositol, which can be generated by the hydrolysis mediated by phytase during germination. During initial period of fermentation phytase activity was low, thus is why no significant differences compared to raw sample. But, as the period of fermentation increased, a nominal decrease in phytate was observed in the present study.

Elk hail *et al.*, (2001) used both malting and natural fermentation process alternatively and achieved up to 83% reduction in phytate. Reduction in phytic acid contents of cereal and legume seeds with such processing treatments has been frequently reported (Ibrahim *et al.*, 2002). This has been attributed to an increase of phytase activities in fact; this enzyme makes the phytates soluble and released soluble protein and minerals. Phytates in chick pea seeds are more prone to hydrolysis during sprouting than other legumes (Chitra *et al.*, 1996).

#### **4.4 Effect of processing treatment on total mineral contents of Chickpea**

The mineral contents of chickpea were shown in Table 4.6. The values of mineral contents were different slightly from raw samples in all the processing treatments examined in the treatments. The total mineral contents were lowered as compared to the raw sample. The total iron content showed significantly difference ( $p < 0.05$ ) in each processing treatments. During boiling, wet roasting and dry roasting decreased by 38.29%, 40.50% and 48.09% respectively. This reduction could be attributed due to the minerals leached from the chickpea seeds in to the water at different rates during cooking treatments.

The total zinc content was significantly also different ( $p < 0.05$ ) among each treatments. Moreover, high reduction in wet roasting and dry roasting by 32.66% and 28.35% respectively. However, slight decrease in germination and fermentation treatments.

Similarly, the total calcium contents was significantly different ( $p < 0.05$ ) among each treatments. High reduction was observed, particularly for fermentation process. The results of the present study contradicts with the observation made by Ejigui *et al.* (2005) that fermentation does not have an overall effect on the contents of total minerals. On the other hand, the reduction of total minerals in some of the samples may be ascribed due to microorganisms could have utilized some of the hydrolyzed elements for their metabolic activities and lost through decantation and the minerals could have been lost in the fermentation medium and decant of fermentation water during the drying process.

There are several conflicting reports about mineral values in lactic acid fermentation. Abdel-Rahaman *et al.* (2008) observed an increase in both total and available amounts of calcium, iron, zinc and phosphorous after 14 hr of pearl millet lactic acid fermentation. According to Odumodu (2007), fermentation was found to enhance both the macro elements and the micronutrients of the fermented grains up to 72 hr, thereafter there were fluctuations in values which could be attributed to the metabolic activities of the microorganisms. However, all settled that fermentation ameliorates bioavailability of minerals in one way or another.

The total phosphorous content was significantly different ( $p < 0.05$ ) between each treatment. High retention was observed during germination and high lost was during dry roasting. The reduction in the phosphorous content with roasting treatments might be attributed to the loss of nutrients while treating at high temperature (Malik *et al.*, 2002). Leaching out of solid matter during pre germination, (soaking) could be the reason for significant reduction of mineral matter during wet roasting.

Table 4.6 The effect of processing treatment on total (mg/100g) and HCl-extractability of Fe, Zn, Ca and P

Sample code	Fe		Zn		Ca		P	
	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable
RC	6.790±0.020 <sub>a</sub>	30.260±0.610 <sup>h</sup>	3.95±0.03 <sup>a</sup>	45.68±0.25 <sub>g</sub>	207.4±0.81 <sup>a</sup>	28.04±0.63 <sub>h</sub>	298.14±0.30 <sub>a</sub>	32.76±0.67 <sub>g</sub>
BC	4.193±0.015 <sub>e</sub>	31.050±0.560 <sup>f</sup> <sub>g</sub>	3.21±0.04 <sup>d</sup>	47.1±0.66 <sup>f</sup>	191.43±0.58 <sub>b</sub>	30.23±0.32 <sub>g</sub>	265.37±0.74 <sup>f</sup>	38.87±0.36 <sup>f</sup>
WRC	4.043±0.015 <sup>f</sup>	37.196±0.398 <sup>f</sup>	2.66±0.07 <sup>e</sup>	50.26±0.79 <sub>e</sub>	178.52±1.79 <sub>c</sub>	32.9±0.50 <sup>f</sup>	260.63±0.74 <sub>g</sub>	36.93±0.56 <sub>e</sub>
DRC	4.003±0.025 <sup>f</sup>	32.003±0.405 <sup>f</sup>	2.83±0.03 <sup>e</sup>	47.58±0.8 <sup>f</sup>	162.6±0.79 <sup>e</sup>	29.22±1.14 <sub>g</sub>	252.21±0.48 <sub>h</sub>	34.69±0.91 <sup>f</sup>
GC	5.443±0.047 <sub>c</sub>	62.957±0.351 <sup>a</sup>	3.15±0.06 <sup>d</sup>	72.41±0.86 <sub>b</sub>	170.75±1.31 <sub>d</sub>	58.03±0.60 <sub>b</sub>	294.16±0.23 <sub>b</sub>	46.32±0.59 <sub>a</sub>
FC-O	5.117±0.095 <sub>d</sub>	40.56±0.76 <sup>e</sup>	3.7±0.1 <sup>b,c</sup>	61.44±1.25 <sub>e</sub>	163.76±0.47 <sub>e</sub>	41.10±0.57 <sub>e</sub>	269.98±0.44 <sub>e</sub>	39.90±0.71 <sup>d</sup>
FC-24	5.923±0.006 <sub>b</sub>	44.41±0.46 <sup>d</sup>	3.59±0.02 <sup>c</sup>	63.48±0.84 <sub>d</sub>	137.12±1.25 <sup>f</sup>	44.70±0.62 <sub>d</sub>	270.78±0.84 <sub>d</sub>	40.81±0.71 <sub>d</sub>
FC-48	5.213±0.130 <sub>d</sub>	50.54±0.75 <sup>c</sup>	3.55±0.03 <sup>c</sup>	66.35±0.42 <sub>c</sub>	135.81±1.06 <sup>f</sup>	48.74±0.47 <sub>c</sub>	272.34±0.53 <sub>d</sub>	42.09±0.58 <sub>c</sub>
FC-72	4.187±0.02 <sup>e</sup>	60.69±0.82 <sup>b</sup>	3.86±0.32 <sup>a</sup> <sub>b</sub>	74.79±0.56 <sub>a</sub>	163±0.22 <sup>e</sup>	56.97±0.35 <sub>a</sub>	274.63±0.69 <sub>c</sub>	44.35±0.57 <sub>b</sub>

Values are means of triplicates (± SD). Means not sharing a common superscript letters in a column are significantly different at (p< 0.05) as assessed by Duncan's multiple range tests

RC -Raw chickpea

DRC-dry roasted chickpea

FC-24-fermented chickpea for 24 hr

BC-Boiled chickpea

GC- germinated chickpea

FC-48- fermented chickpea for 48 hr

WRC-Wet Roasted chickpea

FC-0- fermented chickpea for 0 hr

FC-72- fermented chickpea for 72 hr

#### **4.5 Effect of processing on HCl-extractability of minerals in chickpea**

Analysis of variance technique which was applied to HCl-extractability of iron data showed that highly significant ( $p < 0.05$ ) differences in Table 4.6. A high level of Fe extractability was noted ( $62.69 \pm 0.86\%$ ) during germination. However, when the fermentation processing results were compared to the corresponding raw samples, iron achieved higher extractability at 72 hr ( $60.69 \pm 0.82\%$ ) almost twice as compared to raw samples.

In chickpea, the HCl-extractability of minerals was greatly affected owing to reduction in phytate by germination and fermentation by a concomitant improvement in the HCl extractabilities of minerals. It could be basically due to the fact that more phytase will be available to solublise phytate present in flour (Idris *et al.*, 2005). Similarly, on germination, phytase activated and causes the degradation of phytic acid (Abdelrahaman *et al.*, 2007).

The results for the effect of processing treatments on HCl-extractability of zinc in chickpea differed highly significant ( $P < 0.05$ ) the least HCl-extractability was attained by raw followed by dry roasting and boiling process ( $45.68 \pm 0.25$ ,  $45.8 \pm 0.80$ , and  $47.100 \pm 0.66\%$ ) respectively. On the other hand, the highest Zn HCl-extractability was observed during germination and fermentation which were increased almost by half compared to the raw sample.

The process of germination and fermentation are associated with a significant ( $P < 0.05$ ) enhancement in the bioavailability of calcium Table 4.6 Hussein and Ghanen (1999) also found an appreciable improvement in calcium bioavailability after germination and fermentation of legume samples could be contributed to simultaneous reduction of phytic acid, tannin and dietary fiber. Several reports also show the negative correlation of phytic acid and dietary contents of foods with percent of calcium bioavailability (Kamachan *et al.*, 2004).

The HCl-extractability of phosphorous was improved significantly ( $P < 0.05$ ) by the processing treatments. The high level of HCl-extractability of phosphorous was



achieved by germination ( $46.32 \pm 0.07\%$ ) and natural fermentation ( $44.35 \pm 0.57\%$ ) at 72 hr. This results in close proximity to each other. Chickpea flour with out treatment (raw) showed minimum HCl- extractability of phosphorous ( $32.76 \pm 0.67\%$ ) followed by boiling, dry roasting and wet roasting respectively.

As far as phosphorous extractability is concerned, the phytate is the storage of this mineral under the influence of processing treatment during germination and fermentation. Processing of phytate rich flours inositol hexaphosphate hydrolyses, releasing free inorganic phosphorous and myoinositol phosphates or inositol (Hotz and Gibson, 2001). Thus, liberated phosphorous becomes available for HCl-extractability.

According to Sandberg and Andlid, (2002), there are differences in optimal conditions for phytate degradation between plant species. pH Optimal of some legumes are neutral or alkaline. Hurrell *et al.* (2003) indicated 5-5.05 is an optimal pH range of phytase activity. Other researchers also reported that 4-6 as pH optima of phytase activity (Shimelis and Rakshit, 2008). In this study, it was found that after 48hrs fermentation, the percentage decreased in phytate content was 20.79%. In general, as far as individual treatments are concerned, high level of HCl-extractability was achieved by fermentation and germination.

## **5. Conclusion and Recommendation**

### **5.1 Conclusion**

This study has demonstrated that the processing of chickpea using traditional methods such as boiling, roasting, germination and fermentation resulted in a significant reduction of phytic acid and tannin contents. Germination and fermentation appear to be the best alternative for chickpea preparation. The contents of phytate and condensed tannin reduced greater than 30% during germination and fermentation than other processing methods. Therefore, traditional household practices such as fermentation and germination can decrease the phytate content significantly, and thus need to be encouraged to address the problem of zinc and iron deficiency particularly in rural areas of Ethiopia where most diets are based on cereals.

The results also clearly indicated that such processing methods may be useful for improving the nutritional quality of the chickpea with respect to crude protein, crude fat, crude fiber, total ash and utilizable carbohydrate as well as mineral bioavailability and can be used as supplement to the rural areas.

This study also provides data on the content of total zinc, iron, and calcium and phosphorous and their relative bioavailability. Although there are foods relatively rich in zinc and iron, high levels of inhibitors of absorption, especially phytic acid and tannins, impair the bioavailability of these minerals. Thus, the consumption of diets based on legumes, without animal products that are consumed only in limited amounts, could alleviate deficiencies, particularly of zinc and iron.

The mineral HCl-extractability (an index of mineral bioavailability) of chickpea samples increased through fermentation and germination up to 50%.

## 5.2 Recommendation

Diet-related factors in plant foods that affect bioavailability include: the chemical form of the nutrient in food and nature of the food matrix; interactions between nutrients and other organic components (e.g. phytate, dietary fibre, oxalic acid, protein, fat, ascorbic acid); pretreatment of food as a result of processing and/or preparation practices. Consequently, household strategies that reduce the content or counteract the inhibiting effects of these factors on micronutrient bioavailability are urgently needed in developing-country settings like Ethiopia. Examples of such strategies include: germination, microbial fermentation or soaking to reduce the phytate and polyphenol content of legumes and unrefined cereal porridges used for young child feeding; addition of ascorbic acid-containing fruits to enhance non-haem-Fe absorption.

Degradation of phytate can occur both during food processing and in the gastrointestinal tract. This degradation has nutritional importance because it has been demonstrated that such controlled degradation improves the uptake of essential dietary minerals, such as Fe and Zn.

An integrated approach that combines a variety of the strategies such as, including the addition of even a small amount of animal source foods is probably the best strategy to improve the nutrient bioavailability in diets based on plant foods. In the selection of cultivars to be released from agricultural research centers, equal attention should be given to nutritional and antinutritional compositions in addition to, consumer yield and production aspects.

The following recommendations are made based on a holistic view of the subject area:

- The use of indigenous food processing technologies such as fermentation and germination etc should be studied for different varieties of chickpeas grown in Ethiopia.
- The amino acid profile of various cultivars of chickpeas grown in Ethiopia should be studied.
- There is an urgent need to improve the nutritional quality of plant-based foods in Ethiopia, especially those used for feeding infants and young children.

- Studies should be undertaken for new product development like weaning food mixes, and cookies etc. using chickpea.
- Industrial utilization of locally-grown crops for manufacture of convenient local recipes including fortified products has to be studied.
- Moreover, other antinutritional factors of chickpea should be studied.

## 6. REFERENCES

- Abbo,S., Grusak,M.A., Tzuk,T., and R. Reifen,R.(2000). Genetic control of seed weight and calcium concentration in chickpea seed; Blackwell Wissenschafts-Verlag, Berlin ISSN 0179-9541. *Plant Breeding*, 119; 427-431.
- Abdelhaleem, W. H., El Tinay, A. H., Mustafa, A.I. and Babiker, E.E. (2008). Effect of fermentation, malt-pretreatment and cooking on antinutritional factors and protein digestibility of sorghum cultivars. *Pakistan Journal of Nutrition*, 7; 335- 341.
- Abdelrahman, S.M., H.B. Elmaki, W.H. Idris, A.B. Hassan, E.E. Babiker and A.H. El- Tinay. (2007). Antinutritional factor content and hydrochloric acid extractability of minerals in pearl millet cultivars as affected by germination. *Int. J. Food Sci. Nutr.* 58:16-17.
- Abdel-Rhaman, S.M., Babiker, E.E. and El Tinay, A.H. (2005).Effect of fermentation on antinutritional factors and Hcl extractability of minerals of pearl millet cultivars. *J. of Food Technology*, 3;516-522.
- Agriculture and Agri-Food Canada (2006). Chickpeas: Situation and outlook. Bi-weekly Bulletin, 19(13). [www.agr.gc.ca](http://www.agr.gc.ca) Retrieved 20.08.09.
- Agriculture and Agri-Food Canada (2008). Dry peas: Situation and outlook. Bi-weekly Bulletin,21(2). [www.agr.gc.ca](http://www.agr.gc.ca) Retrieved 20.08.09.
- Alajaji,S.A.and El-Adawy ,T.A .(2006).Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods . *Journal of Food Composition and Analysis*.
- Alonso,R., Aguirre,E.A. and Marzo,F., (2000).Effect of extrusion and traditional processing methods ,on antinutritional and invitro digestability of protein and starch in faba bean and kidney bean . *Food Chem.*, 68; 159-165.
- AOAC. (1984). Official Methods of Analysis Association of Official Analytical Chemists.4<sup>th</sup> Edition. Washington, DC: Association of Official Analytical Chemists (AOAC).
- AOAC.(2000). Official methods of analysis. (Vol.ii 17th ed.), of AOAC International Assn. of Official Analytical Chemists, Washington, DC, USA, OfficialMethods 925.09, 923.03, 979.09, 962.09, 4.5.01
- Aykroyd WR, Doughty J, Walker, A., (1982). Legumes in Human Nutrition. Food and

- Agricultural Organization of the United Nations (FAO), Food and Nutrition  
PapNo.20, FAO, Rome.
- Bazzano, L., He, J., Ogden, L. G., Loria, C., Vupputuri, S., Myers, L., (2001). Legume  
Consumption and risk of coronary heart disease in US men and women.  
*Archive International Medicine*, 161; 2573–2578.
- Beaumont, M. (2002). Flavouring composition prepared by fermentation with *Bacillus*  
spp. *Int.J. Food Microbial.* 75:189-196.
- Bejiga, G., Eshete, M. and Anbessa, Y. (1996) Improved cultivars and production  
technology of Chickpea in Ethiopia. Debre Zeit Agricultural Research Center  
(DZARC), Debre Zeit, Ethiopia, 60pp
- Bourdon, I., Olson, B., Backus, R., Richter, B. D., Davis, P. A., and Schneeman, B. O.  
(2001). Beans, as a source of dietary fiber, increase cholecystokinin and a  
polipoprotein B48 response to test meals in men. *Journal of Nutrition*, 131;  
1490–1585
- Brown KH, Wuehler SE, Peerson JM. (2001). The Importance of Zinc in Human  
Nutrition and Estimation of the Global Prevalence of Zinc Deficiency. *Food Nutr.*  
*Bull.* 22: 113-125.
- Burns, RR. (1971). Methods for estimation of tannin in grain Sorghum. *Agronomic*  
*Journal*; 63:511–512
- Chavan, J. K., Kadam S. S., and Salunkhe, D. K. (1986). Biotechnology and  
technology chickpea (*Cicer arietinum* L.) seeds. *CRC Critical Reviews in Food*  
*Science and Nutrition* 25: 107-158.
- Chauahan, M., And Mahjan, H. (1988) Effect of natural fermentation on the  
extractability of minerals from pearl millet flour. *J. FoodSci.*, 53:1576-1577
- Cheryan, M., (1980). Phytic acid interactions in food systems, *Crit. Rev. Food Sci.*  
*Nutr.* 13; 297–355
- Chitra, U., Singh, U., and Rao, P. V. (1996). Phytic acid, in vitro protein digestibility,  
dietary fiber and minerals of pulses as influenced by processing methods. *Plant*  
*Foods for Human Nutrition*, 49; 307–316
- Clemente, A., Sanchez-Vioque, R., Vioque, J., Bautista, J., Millan, F., (1998). Effect  
of processing on water absorption and softening kinetics in chickpea  
(*Cicer arietinum* L.) seeds. *J. Sci. Food Agri* 78 (2), 169–174.
- Cowan, J.W., M. Esfahani, J.P. Salji and Nahapetian, A. (1967). Nutritive value of

- Middle Eastern Foods. III. Physiological availability of iron in selected foods common to Middle East. *J. Sci. Food Agri.*, 18: 227-8.
- Drewnowski, A. and Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *The American Journal of Clinical Nutrition*, Vol. 72, pp. 1424
- Duhan, A., Khetarpaul, N., and Bishnoi, S. (1999). In starch digestibility (in vitro) of various pigeonpea cultivars through processing and cooking. *Ecology of Food and Nutrition*, 37;557–568
- Duhan A., Khetarpaul, N., and Bishnoi, S. (2002). Content of phytic acid and HCl extractability of calcium, phosphorus and iron as affected by various domestic processing and cooking methods. *Food Chem.* 78:9-14.
- Duncan, D.B., (1955). Multiple ranges and multiple F-tests. *Biometrics*, 11: 1-42
- Egli, I., Davidsson, L., Juillerat, M. A., Barclay, D., and Hurrell, R. F. (2002). The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feeding. *Journal of Food Science*, 67; 3484- 3488
- Ejigui, J., Savoie, L., Marin, J. and Desrosiers, T. (2005). Beneficial changes and drawbacks of a traditional fermentation process on chemical composition and antinutritional factors of yellow maize (*Zea mays*). *Journal of Biological Sciences*, 5; 590-596.
- El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. *Plant Foods for Human Nutrition*, 57; 83–97
- Elkhalil, E.A.I., A.H. El-Tinay, B.E. Mohamed and E.A.E. Elsheikh. (2000). Effect of malt pretreatment on phytic acid and in vitro protein digestibility of sorghum flour Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Shambat, Sudan .
- Erdal I, Yilmaz A, Taban S, Eker S, Torun B, Cakmak I .(2002). Phytic Acid and Phosphorus Concentrations in Seeds of Wheat Cultivars Grown with and without Zinc Fertilization. *J. Plant Nutr.* 25(1): 113-127
- FAO (2000). FAO yearbook production. Rome, Italy: FAO.
- FAO (2008). Food and Agricultural Organization Statistical Database. Rome: FAO (Food and Agricultural Organization at [www.faostat.org](http://www.faostat.org))

- Feron, G. and Y. Wache. (2006). Microbial biotechnology of food flavour production. Food Biotechnology. CRC Press, Boca Raton, FL. USA
- Frias, J., Vidal-Valverde, C., Sotomayor, S., Diaz-Pollan, C., Urbano, G., (2000). Influence of processing on available carbohydrate content and antinutritional factors of chickpeas. *European Food Research and Technology* 210 (5), 340–345.
- Frontela, C., Scarino, M.L., Ferruzza, S., Ros, G. and Martínez, C. (2009). Effect of dephytinization on bioavailability of iron, calcium and zinc from infant cereals assessed in the Caco-2 cell model. *World Journal of Gastroenterology*, 28; 1977-1984.
- Geervani, P. (1991) Utilization of chickpea in India and scope for novel and alternative uses. In *Uses of tropical grain legumes*. Patancheru, India: ICRISAT, pp. 47–54.
- Gibson, R, S., Perlas, L. and Hotz, C. (2006). Improving the bioavailability of nutrients in plant foods at the household level. *Proceedings of the Nutrition Society*, 65; 160–168.
- Golden, M. (2009). Nutrient requirements of moderately malnourished populations of children. *Food Nutr Bull*.
- Gowen, A., Abu-Ghannam, N., Frias, J., Oliveira, J., (2007). Modeling the water absorption process in chickpeas (*Cicer arietinum* L.). The effect of blanching pretreatment on water intake and texture kinetics. *Journal of Food Engineering* 78 (3), 810–819.
- Graham, RD, . Humphries, JM, . and Kitchen, JL, .( 2000). Nutritionally enhanced cereal. A sustainable foundation for a balanced diet; *Asia Pacific J Clin Nutr* ; 9:S91–S96
- Granito M., A. Torres, J. Frias, M. Guerra and C. Vidal-Valverde. (2005). Influence of fermentation on the nutritional value of two varieties of *Vigna sinensis*. *Eur. Food Res. Technol.* 220:176-181.
- Grela, E.R. and K.D. Gunter. 1995. Fatty acid composition and tocopherol content of some legume seeds. *Anim. Feed Sci. Technol.* 52:325-331.
- Greiner, R. and Konietzny, U. (1998) . Endogenous phytate-degrading enzymes are responsible for phytate reduction while preparing beans (*Phaseolus vulgaris*), *J. Food Process. Preserv.* 29; 321–331.
- Greiner, R., and Konietzny, U. (2006). Phytase for food application. *Food*



- Technology and Biotechnology, 44(2), 125–140
- Grusak, M.A. and Dellapenna, D. (1999). Improving the nutrient composition of plants to enhance human nutrition and health. *Ann. Rev. Plant Phys. Plant Mol. Biol.* 50:133-161.
- Guyot, J.P., Rochette, I. and Treche, S. (2007). Effect of fermentation by amyolytic lactic acid bacteria, in process combinations, on characteristics of rice/soybean slurries: A new method for preparing high energy density complementary foods for young children. *Food Chemistry*, 100;623–63.
- Hangen, L., and Bennink, M. R. (2002). Consumption of black beans and navy beans (*Phaseolus vulgaris*) reduced azoximethane-induced colon cancer in rats. *Nutrition Cancer*, 44; 60–65.
- Hotz, C. and R.S. Gibson. (2001). Assessment of home-based processing methods to reduce the phytate content and phytate/zinc molar ratio of white maize (*Zea mays*). *J. Agric. Food Chem.* 49:692-698.
- Huisman, J., and Van der Poel, A. F. B. (1994). Aspects of the nutritional quality and use of cool season food legumes in animal feed. In F. J. Muehlbauer and W. J. Kaiser seeds (Eds.), Expanding the production and use of cool season food legumes. Dordrecht: Kluwer Academic Publishers.
- Hussein, L., and Ghanem, K. Z. (1999). Calcium bioavailability from selected Egyptian foods with emphasis on the impact of germination and fermentation. *Int.J. Food Sci. Nutr.*, 50;351–356.
- Hurrell, R.F. (2003) .Influence of vegetable protein sources on trace element and mineral bioavailability, *J. Nutr.* 133 (Suppl.) 2973–2977.
- Iannotti LL, Tielsch JM, Black MM, Black RE(2006 ).Iron supplementation in early childhood:health benefits and risks. *Am J Clin Nutr*; 84:1261-76
- Ibrahim, S. S., Habiba, R. A., Shatta, A. A., and Embaby, H. E. (2002). Effect of soaking, germination, cooking and fermentation on antinutritional factors in Cowpeas. *Nahrung*, 46; 92–95.
- Ibrikci, H., Knewton, S.J.B. and Grusak, M.A. (2003) Chickpea leaves as a vegetable green for humans: evaluation of mineral composition. *Journal of the Science of Food and Agriculture*, 83; 945-950.
- Idris, W.H., A.B. Hassan, E.E. Babiker and A.H. El Tinay. 2005. Effect of malt pretreatment on antinutritional factors and HCl extractability of minerals of

- sorghum cultivars. *Pak. J.Nutr.* 4 (6):396-401
- Iqbal A, Ateeq N, Khalil IA, Perveen S, Saleemullah S (2006). Physicochemical characteristics and amino acid profile of chickpea cultivars grown in Pakistan. *J. Foodservice.* 17: 94-101
- Joshi, P. K., Parthasarathy Rao, P, Gowda, C. L. L., Jones, R. B., Silim, S. N., Saxena, K.B and Jagdish Kumar. (2001) .The world chickpea and pigeonpea Economies: Facts, Trends, and Outlook. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 68 pp. ISBN 92- 9066-443-6. Order code BOE 030.
- Kamchan, A., Puwastien, P., Sirichakwal, P. P., and Kongkachuichai, R. (2004). *In vitro* calcium bioavailability of vegetables, legumes and seeds. *Journal of Food Composition and Analysis*, 17; 311–320.
- Kelbessa, U. and Narasimha, H.V. (1998). Phytate: zinc and phytate x calcium: zinc molar ratios in selected diets of Ethiopians. *Bulletin of the Chemical Society of Ethiopia*, 12.
- Khan, M.A., N. Akhtar, I. Ullah and S. Jaffery( 1995). Nutritional evaluation of Desi and Kabuli chickpeas and their products commonly consumed in Pakistan. *Int. J. Food Sci. Nutr.* 46(3):215-23
- Khetarpaul, N. and Sharma, A. (1997).Effect of fermentation with whey on the HCl extractability of minerals from rice-dehulled black gram blends. *Journal of Agriculture and Food Chemistry*,45;2258–2261.
- Khokhar S, Chauhan BM, (1997). Antinutritional factors in Moth Bean (*Vigna aconitifolia*):Varietal Differences and Effects of Methods of Domestic Processing and Cooking. *Food Chem.* 59: 367-371
- Kolonel, L. N., Hankin, J. H., Whittemore, A. S., Gallagher, R. P.,Wilken, L. R., John, E. M., (2000). Vegetables, fruits, legumes and prostate cancer: a multiethnic case-control study. *Cancer Epidemiol Biomark review*, 9; 793–804.
- Latta, M. and Eskin, M. (1980). A simple and rapid colorimetric method for phytate determination. *Journal of Agricultural and Food Chemistry*, 28; 1315–1317.
- Lopez,H.W., Leenhardt,F., Coudray, C.,and Remesy,C., (2002). Minerals and phytic acid interactions: Is it a real problem for human nutrition?, *Int. J. Food Sci. Technol.* 37; 727– 739.
- Lori O, Thava V and James HH (2001). Phytic acid. *Food Rev Int* 17: 419-431.

- Lou, H.X., Y. Yamazaki, T. Sasaki, M. Uchida, H. Tanaka and S. Oka. (1999). A-type procyanidins from peanut skin. *Phytochemistry*. 51:297-308.
- Mackenzie B, Garrick MD(2005). Iron Imports. II. Iron uptake at the apical membrane in the intestine. *Am J Physiol Gastrointest Liver Physiol* 289:G981-G986.
- Malik, M., U. Singh and S. Dahiya, (2002). Nutrient composition of pearl millet as influenced by genotypes and cooking methods. *J. Food Sci. and Techno.* 39: 463-468.
- Manary MJ, Hart CA, Whyte MP(1998 ). Severe hypophosphatemia in children with Kwashiorkor is associated with increased mortality. *J Pediatr*;133:789-91.
- Mate H, Radomir L (2002). Phytic Acid Content of Cereals and Legumes and Interaction with Proteins. *Periodica Polytechnica Ser.Chem. Eng.* 46 (1-2): 59-64
- Maiti, R., and Wesche-Ebeling, P. (2001). In R. Maiti and P. Wesche-Ebeling (Eds.), *Advances In chickpea science* (pp. 1). Science Publishers Inc.
- Melaku, U., West, C.E., and Habtamu, F. (2005). Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *Journal of Food Composition and Analysis*, 18; 803–817.
- Menale ,K., Bekele S., Solomon A., Tsedeke A., Geoffrey M., SetotawF., Million E., and Kebebew A. (2009). Current Situation and Future Outlooks of the Chickpea sub-sector in Ethiopia; Ethiopian Institute of Agricultural Research (EIAR ), Debre Zeit Agricultural Research Center ,Debre Zeit , Ethiopia .
- Millan, T., Clarke, H.J., Siddique, K.H.M., Buhariwalla, H.K., Gaur, P.M., Kumar, J., Gil, J., Kahl, G.and Winter, P. (2006). Chickpea molecular breeding: New tools and concepts. *Euphytica*, 147;81-103.
- Moreno, C.R., E.O.C. Rodríguez, J.M. Carrillo, O.G.C. Valenzuela and J.B. Hoyos. (2004).Solid state fermentation process for producing chickpea(*Cicer arietinum* L) tempeh flour.Physicochemical and nutritional characteristics of the product. *J. Sci. Food Agric.* 84(3):271-278.
- Mubarek,A..E..( 2005).Nutritional Composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. *Food Chem.*, 89; 489-495.
- Naghavi MR, Jahansouz MR (2005). Variation in the agronomic and morphological traits of Iranian chickpea accessions. *J. Integr. Plant. Biol.* 47(3): 375-379

- Negi, A., Boora, P., and Khetarpaul, N. (2001). Starch and protein digestibility of newly released moth bean cultivars: Effect of soaking, germination and pressure-cooking. *Nahrung*, 45(4), 251–254.
- Nestares, T., Urbano G., Lopez-Frias, M., Barrionuevo, M. (1997). Nutritional assessment of magnesium from raw and processed chickpea (*Cicer arietinum* L.) in growing rats. *J. Agric. Food Chem.*, 45; 3138-3142.
- Nwokolo, E., and Smartt, J. (1996). In E. Nwokolo (Ed.), Food and feed from legumes and oilseeds . Chapman and Hall publishing. 82 and 84.
- Odumodu, C.U. (2007). Optimum fermentation period for micronutrients content of cereal based complementary food. *Pakistan Journal of Nutrition*, 6;518-523.
- Pandey, G., and Enumeratio, G. (1993). *Planta Medica Gyanendra Ausadhiya Padapavali*. Delhi, India: Spring, p. 116.
- Paolini, R., Colla, G., Saccardo, F., and Campiglia, E. (2003). The influence of plant crop density on the efficacy of mechanical and reduced chemical weed in lentil (*Lens culinaris* Medik). *Italian Journal of Agronomy*, 7; 85–94.
- Patane, C. (2006). Variation and relationship among some nutritional traits in Sicilian genotypes of chickpea (*Cicer arietinum* L.). *J. Food Qual.* 29:282-293.
- Pearson, D. (1971). *The chemical Analysis of Foods*. 6th Edition. Chemical publishing company, Inc. New York .
- Ramachandran, S., A. Bairagi and A.K. Ray ( 2005). Improvement of nutritive value of grasspea (*Lathyrus sativus*) seed meal in the formulated diet for rohu, *Labeo rohita* (Hamilton) fingerlings after fermentation with a fish gut bacterium. *Biores. Tech.* 96:1465-1472.
- Rao, P.U. and Y.G. Deosthale. (2006). Polyphenoloxidase activity in germinated legume seeds. *J. Food Sci.* 52(6):1549-1551.
- Reddy, N.R., M.D. Pierson, S.K. Sathe and D.K. Salunkhe. (1985). Dry bean tannins: A review of nutritional implications. *J. Am. Oil Chem. Soc.* 62(3):541-549.
- Saikia, P., Sarkar, C.R. and Borua, I. (1999). Chemical composition, antinutritional factors and effect of cooking on nutritional quality of rice bean [*Vigna umbellata* (Thunb; Ohwi and Ohashi)]. *Food Chem.* 67(4):347-352.
- Salunkhe, D. K., S. S. Kadam and J. K. Chavan. (1985). *Postharvest Biotechnology of Food Legumes*. Florida: CRC Press.
- Sandberg, A.S. and Andlid, T. (2002). Phytogetic and microbial phytases in human

- nutrition. *International Journal of Food Science and Technology*, 37;823-833.
- Sandberg,A.S. (1991). The effect of food processing on phytate hydrolysis and availability of iron and zinc, *Adv. Exp. Med. Biol.* 289; 499–508.
- Sandberg,A.S., Brune,M., Carlsson,N.G.,Hallberg,L., and Skoglund, L.(1999). Rossander- Hulthen, Inositol phosphates with different numbers of phosphate groups influence iron Absorption in humans, *Am. J. Clin. Nutr.* 70; 240–246.
- Sangronis, E., M. Rodríguez, R. Cava and A. Torres. (2006). *Eur. Food Res. Tech.* 222(1-2): 144-148.
- Sandstrom B (2001) Micronutrient interactions: effects on absorption and bioavailability.*British Journal of Nutrition* 85;S181–S185.
- Sayar, S., Turhan, M., Gunasekaran, S., (2001) Analysis of chickpea soaking by simultaneous water transfer and water–starch reaction. *J.Food Eng.* 50 (2), 91–98.
- Sayar, S., Turhan, M., Korsel, H., (2003). Application of unreacted-core model to in situ gelatinization of chickpea starch. *J. Food Eng.*60 (4), 349–356.
- Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A et al. (2006). Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community based, randomised, placebo-controlled trial. *Lancet* 367:133-43.
- Shiferaw, B, Jones R, Silim S, Tekelewold H and Gwata E. (2007) Analysis of Production costs, market opportunities and competitiveness of desi and kabuli chickpea in Ethiopia. IMPS(Improving productivity and market Success) of Ethiopian Farmers Project Working Paper 3. ILRI (International Livestock Research Institute), Nairobi, Kenya. 48pp
- Shimelis, E.A. and Rakshit, S.K. (2008). Influence of natural and controlled fermentations on  $\alpha$ -galactosides, antinutrients and protein digestibility of beans (*Phaseolus vulgaris* L.). *International Journal of Food Science and Technology*, 43; 658–665.
- Singhai, B. and S.K. Shrivastava. (2006).Nutritive value of new chickpea (*Cicer arietinum* ) varieties. *J. Food Agric. Environ.* 4(1):48-53.
- Singh, U. (1988). Antinutritional factors of chickpea and pigeonpea and their removal by processing. *Plant Foods for Human Nutrition*, 38;251–256.
- Smith, V., and Jimmerson, J. (2005). Chickpeas (Garbanzo beans). Agricultural

- Marketing Policy Center Montana State University, Briefing No. 55. Taylor, P.W.J. and Ford, R. (2007). Chickpea. In Pulses, Sugar and Tuber Crops: Genome Mapping and Molecular Breeding in Plants, Vol. 3 (Kole, C., ed. Berlin Heidelberg: *Springer-Verlag*, pp. 109-121.
- Torres, A., J. Frias, M. Granito and C. Vidal-Valverde. (2007). Germinated *Cajanus cajan* seeds as ingredient in pasta products: chemical, biological and sensory evaluation. *Food Chem.* 101:202-211.
- Vaintraub, I.A. and Lapteva, N.A.(1988). Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Analytical Biochemistry*, 175: 227.
- Vijayakumari, K., M. Pugalenti and V. Vadivel. (2007). Effect of soaking and hydrothermal processing methods on the levels of antinutrients and in vitro protein digestibility of *Bauhinia purpurea* L. seeds. *Food Chem.* 103:968-975.
- Viveros, A., Brenes, A., Elices, R., Arija, I., and Canales, R. (2001). Nutritional value of raw and autoclaved kabuli and desi chickpea (*Cicer arietinum* L.). *British Plant Science*, 42; 242– 251.
- Walingo, M.K. (2009). Indigenous food processing methods that improve zinc absorption and bioavailability of plant diets consumed by the kenyan population. *African Journal of Food Agriculture Nutrition and Development*, 9; 523-535
- Walker SP, Wachs TD, Gardner JM, Lozoff B, Wasserman GA, Pollitt E. (2007). Child development: risk factors for adverse outcomes in developing countries. *Lancet* 369:145-57.
- Walter HL, Fanny L, Charles C and Christian R. (2002). Minerals and phytic acid interaction: is it a real problem for human nutrition. *Int J Food Sc Tech* 37:727-739.
- Warner, P. K. W., Nambiar, V. P. K., and Remankutty, C. (1995). Indian medicinal plants. Chennai, India: Orient Longman, p. 773.
- Waterlow, J. C. (1994). Childhood malnutrition in developing nations: Looking back and looking forward. *Annual Review of Nutrition*, 14; 1–19.
- Wang, N., Hatcher, D. W., and Gawalko, E. J. (2008). Effect of variety and processing on nutrients and certain antinutrients in field peas (*Pisum sativum*). *Food Chemistry*, 111; 132–138.
- Watzke, H.J. (1998). Impact of processing on bioavailability examples of minerals in foods. *Trends Food Sci. Tech.* 9:320-327.

- Wood, J.A. and M.A. Grusak.( 2007). Nutritional value of chickpea. p. 101-142. In Redden, W. Chen, B. Sharma and Y. Yadav (eds.) Chickpea breeding and management. Oxford University Press, New York, USA.
- Wuehler SE, Peerson JM, Brown KH. (2005). Use of National Food Balance Data to Estimate the Adequacy of Zinc in National Food Supplies: Methodology and Regional Estimates.*Pub. Health Nutr.* 8:812-819
- Yust, M. M., Pedroche, J., Giron-Calle, J., Alaiz, M., Millan, F., and Vioque, J. (2003).Production of ace inhibitory peptides by digestion of chickpea legumin with alcalase. *Journal of Food Chemistry*, 81; 363–369.
- Zhang, T., Jiang, B., and Wang, Z. (2007). Gelation properties of chickpea protein isolates.*Food Hydrocolloids*, 21; 280–286.

## **DECLARATION**

I, the under signed, declare that this thesis is my original work and that all the sources of materials used for the thesis have been correctly acknowledged.

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The thesis has been submitted with my approval as a supervisor.

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