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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Study on the effect of traditional processing methods on nutritional composition and anti nutritional factors in chickpea (*Cicer arietinum*)

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Abstract: The effect of various traditional processing methods (boiling, wet roasting, dry roasting, germination and fermentation) on nutritional composition: moisture content, crude protein, total ash, crude fiber and crude fat was performed using standard AOAC methods. Utilizable Carbohydrate content was calculated by difference and the gross energy content (Caloric value) was determined by calculation from fat, carbohydrate and protein contents using the Atwater's conversion factors and also anti nutritional factors (Phytate, condensed tannin) in chickpea (*Cicer arietinum*) were studied. The ranges for Moisture content, crude protein, crude fat, crude fiber, total ash, utilized carbohydrate and gross energy were 5.9–9.4%, 13.8–16.7%, 4.3–6.1%, 3.4–5.9%, 2.3–2.7%, 61.22–68.67% and 359.26–382.95% respectively. As fermentation period increases from 0hr (pH = 6.28) to 72hr (pH = 4.25), the pH decreases with a concomitant increase in acidity. And also the ranges for phytate and condensed tannin content were 72.07–97.46 and 99.26–175.23 mg/100 g respectively. The results indicated that fermentation and germination were most effective in reducing Phytate and condensed tannin content.

Subjects: Food Chemistry; Substitutes - Food Chemistry; Food Analysis

Keywords: traditional processing; nutritional composition; anti nutritional factors

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PUBLIC INTEREST STATEMENT

Globally, chickpea is mostly consumed as a seed food in several different forms and preparations are determined by ethnic and regional factors. Chickpea, locally known as' shimbra' in Amharic, is one of the major pulse crops in Ethiopia and it is the second most important legume crop after faba beans. In Ethiopia chickpea grain is widely used in different forms as green vegetable (green immature seed), dehulled (split seed or "kik"), and "Kollo" (soaked and roasted) and "nifro" (boiled seeds) and "wot" (sauces) made up of "shiro" (roasted and powdered seeds). Chickpea is a cheap source of protein in the diets of millions in developing countries, who cannot afford animal protein for balanced nutrition. The results of this study can be used to promote the consumption of chickpea for various traditional preparation at home level as well as would add to scientific data base for users to develop and design in food formulation for manufacturers in food industries.

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1. Introduction

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 (Alajaji & El-Adawy, 2006). 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, Vidal-Valverde, Sotomayor, Diaz-Pollan, & Urbano, 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, Turhan, & Gunasekaran, 2001). In simple terms, chickpea grain comprises starch granules embedded in a protein matrix covered by a seed coat (Sayar, Turhan, & Köksel, 2003).

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 & Agri-Food Canada, 2008). 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, Jones, Silim, Tekelewold, & Gwata, 2007).

Chickpea seed is processed and cooked in a variety of forms depending upon traditional practices and taste preferences. Different domestic processing methods (decortications, soaking, sprouting, fermentation, boiling, roasting, parching frying, and steaming) was used to obtain a suitable texture for the consumer, improvement in the nutritional factors and increase the protein digestibility (Attia, 1994; Clemente, Sanchez-Vioque, Vioque, Bautista, & Millan, 1998). In spite of a high nutritional value, 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. Cooked chickpea seeds had a decrease of methionine, cysteine, tyrosine and leucine. The highest reductions being in cysteine (15%) and lysine (13.20%) (Clemente et al., 1998).

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, Colla, Saccardo, & Campiglia, 2003). The chickpea is considered to be a healthy vegetarian food and it is one of the most important human and domestic herbal 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 & Van Der Poel, 1994).

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. 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 (Menale et al., 2009). The objective of this study was to determine the effects of traditional processing methods on nutritional composition, and anti-nutritional factors of Chickpea (*Cicer arietinum*) flours.

2. Materials and methods

2.1. Sample collection and preparation

Chickpea seeds (*Cicer arietinum* L.)*,* the variety DZ 10–11 (desi type) were obtained from Debire 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. The Seeds were hand-sorted to remove wrinkled, moldy seeds and foreign materials. The raw chickpea seeds (used as control) prepared without removing the seed coat and made to flour with electric grinder (NIMA-8300 Burman, Germany) until to pass through 0.425 mm sieve mesh size, and finally packed into airtight polyethylene plastic bags.

2.2. Experimental design

Completely randomized design was used in this experiment and the effect of traditional processing methods (boiling, wet roasting, dry roasting, germination and fermentation) on nutritional composition and anti-nutritional factors (phytate and condensed tannin) of chickpea flours were studied.

2.3. Boiling

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

2.4. Dry roasting

The cleaned chickpea seeds were roasted on a metal pan for 15 min together with pre cleaned 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 0.42 mm sieve mesh size.

2.5. Wet roasting

The cleaned chickpea seeds were soaked in tap water (1:3, w/v) for 12 h and then were roasted at 97–101°C. Then dried in drying oven at 55°C or 12 h and made to flour using electric grinder until to pass 0.425 mm sieve mesh size.

2.6. Germination

Seeds were rinsed, and then soaked in tap water (1:3, w/v) for 12 h at ambient temperature (22–23°C) and were drained and placed on perforated aluminum pans then were placed in a dark, for 24 h germination and made to flour using electric grinder until to pass 0.425 mm sieve mesh size.

2.7. 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°C) for 0, 24, 48 and 72 h 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 (Memmert, Germany) at 70°C for 36 h. Dried samples made to flour using electric grinder to pass 0.425 mm sieve mesh size and stored for analysis.

2.8. pH determination

The pH of the fermented samples was determined according to the method of Pearson (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.

2.9. 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 according to (Pearson, 1971).

3. Nutritional content analysis

3.1. Determination of moisture content

The moisture content of the chickpea samples was determined according to Association of Official Analytical Chemists (AOAC, 2000) sub component 925.09 by oven drying method. A clean, empty aluminum dishes, and its lids (made of porcelain) were dried in drying oven (Memment, Germany) at 100°C for 1 h, and cooled in a desiccator (with granular silica gel) for about 30 min, and weighed. The samples prepared for each traditional processing method in triplicates were mixed thoroughly, and about 3.0 g of chickpea flou was weighed in triplicate. The dishes and the samples were placed in the drying oven, and dried for 3 h at 105°C. After drying, the samples were cooled in a desiccator for 30 min, and re-weighed until constant weight obtained.

3.2. Determination of crude protein

The Protein content of the Chickpea flour samples was determined according to AOAC (2000) sub component 979.09 by the Kjeldahl method in which digestion, distillation and titration were involved. All nitrogen is converted to ammonia by digestion with a mixture of concentrated sulfuric acid and concentrated orthophosphoric acid containing copper sulfate and potassium sulfate as a catalyst. The ammonia released after alkalinization with sodium hydroxide is steam distilled into boric acid and titrated with hydrochloric acid.

3.2.1. Digestion

About 0.5000 g of chickpea flour samples were taken in a Tecator tube and 6 ml of acid mixture (5 parts of concentratedortho phosphoric acid and 100 parts of concentrated sulfuric acid) was added, mixed, thoroughly and 3.5 ml 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 3.0000 g of the catalyst mixture (ground 0.5000 g 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°C, the tubes were lowered into the digester. The digestion was continued until a clear solution was obtained, about 1 h. The tubes in the rack was transferred into the fume hood for cooling, a 15 ml of deionized water was added, and shaken to avoid precipitation of sulfate in the solution.

3.2.2. Distillation

A 250 ml conical flask containing 25 ml 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 5 ml deionized water and the rinses were added into the solution. A 25 ml 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 100 ml 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 150 ml was collected. The tip was rinsed with a few milli-liter of water before the receiver was removed.

3.3. Determination of crude fat content

The crude fat content of chickpea flour samples was determined according to AOAC (2000) Official using sub component 920.39. The extraction flasks with boiling chips were cleaned, dried in drying

oven (Memmert, Germany) at 90°C for 1 hour, cooled in desiccators (with granular silica gel) for 30 min, and then weighed. The bottom of the extraction thimble was covered with about 2 cm layer of fat free cotton. About 3.00 g of chickpea flour sample was added into the extraction thimbles, and then covered with about 2 cm 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 (boiling point, 55°C) was added to the extraction flask through the condenser. The extraction was conducted for about 3 h. The ether was evaporated from the extraction flask. The extraction flasks with their content were removed from the extraction chamber and placed in the drying oven at 90°C for about 1 hr, cooled to room temperature in the desiccator for about 30 min and re-weighed. The amount of fat was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage.

3.4. Determination of crude fiber content

Crude fiber content of Chickpea flour samples was determined according to AOAC (2000) using sub component 962.09 in which the steps of digestion, filtration, washing, drying and combustion was involved. Crude fiber was determined after digesting known weight of chickpea flour by refluxing 1.25% boiling sulfuric acid and 28% boiling potassium hydroxide.

3.4.1. Digestion

About 1.6000 g of Chickpea flour was placed into a 600 ml beaker, 200 ml of 1.25% H_2SO_4 was added, and boiled gently exactly for 30 min 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 min boiling, 20 ml of 28% KOH was added and boiled gently for a further 30 min, with occasional stirring.

3.4.2. 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_2SO_4 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_2SO_4 and filtered. Finally the residue was washed with water-free acetone.

3.4.3. Drying and combustion

The crucible with its content was dried for 2 h in an electric drying oven at 130°C and cooled for 30 min in the desiccator (with granular silica gel), and then Weighed. The crucible was transferred to a muffle furnace (Gallen kamp, size 3) and incinerated for 30 min at 550°C. The crucible was cooled in the desiccator and weighed. Then the fiber was calculated as a residue after subtraction of the ash.

3.5. Determination of total ash content

Total ash content of chickpea flour samples was determined according to AOAC (2000) sub component 923.03. The crucible and lid placed in the muffle furnace (Carbolite CSF 1200) at 550°C overnight to ensure that impurities on the surface of crucible are burned off and the crucible cooled in the desiccator for 30 min. Then the crucible and lid weighed. About 3.0 g of chickpea flour of each traditional processing method in triplicates was added into each crucible and heated over low Bunsen flame with lid half covered. When fumes are no longer produced, crucible and lid placed in the muffle furnace and then heated at 550°C overnight. During heating, the crucible was not covered with the lid. But the lid placed after complete heating to prevent loss of fluffy ash. Finally taken out of the Muffle Furnace placing immediately in a desiccator till cooled to room temperature, and each crucible with lid plus ash was re-weighed. Weight of total ash was calculated by difference, and expressed as percentage of samples.

Table 1. The effect of traditional processing methods on crude protein, crude fat, crude fiber, moisture content, total ash, utilizable carbohydrate and caloric value/energy of chickpea

Sample code	Crude protein%	Crude fat%	Crude fiber%	Moisture content %	Total ash%	Utilizable CHO%	Energy kcal/(100 g)
RC	16.73 ± 2.140^a	$5.88 + 1.006^{a,b}$	5.88 ± 2.56^a	7.60 ± 0.100^d	2.63 ± 0.0555^a	61.22 ± 1.057^e	$364.69 \pm 4.741^{d,e}$
BC	13.88 ± 0.206^d	$4.77 \pm 0.160^{d,e}$	$4.57 \pm 0.347^{d,e}$	8.87 ± 0.049^b	2.42 ± 0.015^d	$65.32 \pm 0.275^{b,c}$	359.72 ± 5.187^f
WRC	13.82 ± 0.110^d	$5.20 \pm 0.091^{c,d}$	5.19 ± 0.036^c	9.37 ± 0.133^a	2.30 ± 0.040^e	64.12 ± 0.111^d	359.26 ± 0.374^f
DRC	$14.08 \pm 0.201^{c,d}$	6.07 ± 0.165^a	3.36 ± 0.095^f	5.91 ± 0.21^g	$2.59 \pm 0.080^{a,b}$	68.67 ± 1.093^a	382.95 ± 0.936^a
GC	15.39 ± 0.546^a	4.26 ± 0.150^e	5.60 ± 0.60^b	6.90 ± 0.070^e	2.31 ± 0.076^e	65.55 ± 0.735^b	$362.09 \pm 0.936^{e,f}$
FC-0	16.17 ± 0.171^a	$5.73 \pm 0.030^{a,b,c}$	4.59 ± 0.020^e	8.60 ± 0.060^c	2.67 ± 0.010^a	62.24 ± 0.233^e	$365.21 \pm 0.936^{e,f}$
FC-24	15.71 ± 0.085^a	$5.72 \pm 0.050^{a,b,c}$	4.87 ± 0.0045^d	6.82 ± 0.105^e	2.53 ± 0.035^b	$64.34 \pm 0.146^{c,d}$	371.80 ± 0.293^b
FC-48	$14.93 \pm 0.249^{b,c,d}$	$5.30 \pm 0.080^{b,c,d}$	5.59 ± 0.165^b	6.59 ± 0.081^f	$2.49 \pm 0.061^{c,d}$	$65.09 \pm 0.325^{b,c,d}$	367.82 ± 0.293^c
FC-72	$14.99 \pm 1.203^{c,d}$	$5.22 \pm 0.046^{b,c,d}$	$5.68 \pm 0.106^{a,b}$	6.40 ± 0.020^f	$2.45 \pm 0.020^{c,d}$	66.02 ± 0.416^b	367.98 ± 0.173^c

Notes: RC: raw chickpea; BC: boiled chickpea; WRC: wet roasted chickpea; DRC: dry roasted chickpea; GC: germinated chickpea; FC-0: fermented chickpea for 0 h; FC-24: fermented chickpea for 24 h; FC-48: fermented chickpea for 48 h; and FC-72: fermented chickpea for 72 h.

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.

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3.6. Determination of Utilizable carbohydrate

The Utilizable Carbohydrate content was calculated by difference. The mathematical expression is as follows:

$$100 - (\%\text{Moisture} + \%\text{Crude protein} + \%\text{Crude fiber} + \%\text{Total ash} + \%\text{Crude fat}).$$

3.7. Determination of gross energy

The gross energy content was determined by calculation from Crude fat, carbohydrate and Crude protein contents using the Atwater's conversion factors; The mathematical expression is as follows:

$$\text{Gross energy (kCal/g)} = (4\ \text{kCal/g} \times \text{Crude protein}) + (9\ \text{kCal/g} \times \text{Crude fat}) + (4\ \text{kCal/g} \times \text{Carbohydrate}).$$

4. Antinutritional factors

4.1. Determination of phytate content

Phytate was determined by the method of Latta and Eskin (Latta & Eskin, 1980) and later modified by Vantraub and Lapteva (Vaintraub & Lapteva, 1988). About 0.1000 g of fresh samples were extracted with 10 ml 2.4% HCl in a mechanical shaker (Eberbach) for 1hour at an ambient temperature and centrifuged at 3000 rpm for 30 min. The clear supernatant was used for phytate estimation. A 2 ml of Wade reagent (containing 0.03% solution of $FeCl_3.6H_2O$ and 0.3% of sulfosalicilic acid in water) was added to 3 ml of the sample solution (supernatant) and the mixture was mixed on a Vortex (Maxi Maxi II) for 5 s. 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 0.2 N HCl. A 3 ml of standard was added into 15 ml of centrifuge tubes with 3 ml of water which were used as a blank. A 1 ml of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 5 s. The mixtures were centrifuged for 10 min and the absorbances of the solutions (both the sample and standard) were measured at 500 nm by using deionized water as a blank. A standard curve was made from absorbance vs. concentration.

4.2. Determination of Condensed tannin

Tannin content was determined by the method of Burns (Burns, 1971). About 2.0000 g of chickpea flour was weighed in a screw cap test tube. The chickpea flour was extracted with 10 ml of 1% HCl in methanol for 24 h at room temperature with mechanical shaking. After 24 h shaking, the solution was centrifuged at 1,000 rpm for 5 min. A 1 ml 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 40 mg of D-catechin was weighed and dissolved in 1,000 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 1 ml with 1% HCl in methanol. A 5 ml of vanillin-HCl reagent was added into each test tube. After 20 min, the absorbance of sample solutions and the standard solution were measured at 500 nm by using water to zero the spectrophotometer.

4.3. Statistical analysis

All measurements were carried out in triplicate for each of the Samples (three times). The mean and standard deviation (SD) of means were calculated. The data were analyzed by one way analysis of variance (ANOVA) using SPSS version 15.0 software for windows. All values were presented as means of triplicates ± SD and compared using Duncan's multiple range tests (Duncan, 1955). A level of $p < 0.05$ was used to indicate significant differences among the samples.

5. Results and discussion

5.1. Effect of Traditional processing methods on nutritional composition of chickpea

There are significant differences ($p < 0.05$) in crude protein content were observed between each processing methods of chickpea (Table 1). The crude protein content during germination,

fermentation at 0 h and fermentation at 24 h were 15.39 ± 0.55, 16.17 ± 0.17, and 15.71 ± 0.085% respectively. However, boiling, roasting and fermentation for more than 24 h 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, Rodríguez, Cava, & Torres, 2006). 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 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, Aguirre, & Marzo, 2000; Mubarak, 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, Torres, Frias, Guerra, & Vidal-Valverde, 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). 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, Kadam, & Salunkhe, 1986).

In the present study, the crude fat obtained ranged between 4.26 and 6.07%. The analysis of variance showed in Table 1 indicated that, there were significant ($p < 0.05$) differences among each processing 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-Adawy, 2002; Mubarak, 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 and 8.57/100 g in some chickpea cultivars with the mean value 6.0 ± 0.9 g/100 g (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 (4.18–4.92/100 g) (Singhai & Shrivastava, 2006).

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 ± 0.13, 5.9 ± 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 process 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 was similar to the studies undertaken by (Patane, 2006; Singhai & Shrivastava, 2006).

There were significant differences between each processing methods on crude fiber content of the samples (Table 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 & Günter, 1995). In the present study, the natural fermentation resulted in the decreases of the crude fiber content, similar to the results of (Ramachandran, Bairagi, & Ray, 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, Savoie, Marin, & Desrosiers, 2005).

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Table 2. Changes in pH and titratable acidity (TTA) as % lactic acid in fermented Chickpea flour		
Fermentation period (hrs)	**pH**	**TTA (as% lactic acid)**
0	6.28 ± 0.290[a]	0.48 ± 0.001[d]
24	4.90 ± 0.060[b]	0.56 ± 0.020[c]
48	4.41 ± 0.013[c]	0.65 ± 0.020[b]
72	4.25 ± 0.002[c]	0.69 ± 0.021[a]

Notes: FC-0: fermented chickpea for 0hr; FC-24: fermented chickpea for 24 h; FC-48: fermented chickpea for 48hr; FC-72: fermented chickpea for 72 h.

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.

The data as shown in Table 1 indicates that, the total ash content of the germinated and wet roasted samples 2.31 ± 0.07 and 2.30 ± 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 (Patane, 2006; Singhai & Shrivastava, 2006).

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 ± 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 retrogradation of starch after gelatinization. This result was in agreement with (Wang, Hatcher, & Gawalko, 2008), which indicated starch content of chickpeas increased on cooking.

The caloric value (gross Energy) was calculated by multiplying the mean valves 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 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, Akhtar, Ullah, & Jaffery, 1995). Treatments by dry roasting and fermentation for 24, 48 and 72 h have increased the total energy contents by 18.35, 7.11, 3.13 and 3.29%, respectively.

As shown in Table 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 (Abdelhaleem, El Tinay, Mustafa, & Babiker, 2008; Shimelis & Rakshit, 2008).

The mean values for the effect of traditional processing methods on tannins are presented in Table 3. The analysis of variance of the data showed significant differences ($p < 0.05$) among different processing methods. However, during` boiling, dry roasting and fermentation at (0 hr) has non-significant differences compared to the raw samples. During fermentation for 24, 48, 72 h and germination of chickpea, the tannin content decreased by 3.1, 14.4, 18.5 and 43.4%, respectively. In the present study, losses of tannins after germination for 24 h was in agreement with previous study of (El-Adawy, 2002), in which tannin loss after germination for 24 h ranged from 43% to 59% in chickpea samples These results were in agreement with earlier findings of (Moreno, Rodríguez, Carrillo, Valenzuela, & Hoyos, 2004) who observed that optimum fermentation time and temperature are quite essential to obtain maximum tannin reduction in chickpea. Various studies have

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Table 3. The effect of traditional processing methods on phytate and Condensed tannin content in Chickpea flour		
Sample code	Phytate (mg/100 g)	Condensed tannin (mg/100 g)
RC	97.46 ± 0.750[a]	175.23 ± 0.31[a]
BC	97.17 ± 0.855[a]	174.37 ± 0.49[a]
WRC	91.93 ± 0.850[b]	160.10 ± 0.56[c]
DRC	96.00 ± 0.62[a]	174.9 ± 0.26[a]
GC	72.07 ± 1.33[f]	99.26 ± 2.99[f]
FC-O	97.07 ± 0.75[a]	174.80 ± 0.90[a]
FC-24	84.26 ± 1.16[c]	169.90 ± 0.82[b]
FC-48	80.68 ± 0.75[d]	150.10 ± 1.4[d]
FC-72	76.67 ± 0.85[e]	142.77 ± 0.83[e]

Notes: RC: raw chickpea; BC: boiled chickpea; WRC: wet roasted chickpea; DRC: dry roasted chickpea; GC: germinated chickpea; FC-0: fermented chickpea for 0 h; FC-24: fermented chickpea for 24 h; FC-48: fermented chickpea for 48 h; and FC-72: fermented chickpea for 72 h.

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.

reported the reduction in tannin content of legumes during soaking (Vijayakumari, Pugalenthi, & Vadivel, 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, Pierson, Sathe, & Salunkhe, 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.

There are no significant differences ($p > 0.05$) among boiling; dry roasting and fermentation at initial (0 hr) on phytate as presented in Table 3. However, there is a significant difference among wet roasted, germinated and fermented at 24, 48 and 72 h 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. The microorganisms produced 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 chickpea. 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.

Both malting and natural fermentation process alternatively and achieved up to 83% reduction in phytate (Elkhalil, El-Tinay, Mohamed, & Elsheikh, 2001). Reduction in phytic acid contents of cereal and legume seeds with such processing treatments has been frequently reported (Ibrahim, Habiba, Shatta, & Embaby, 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 chickpea seeds are more prone to hydrolysis during sprouting than other legumes (Chitra, Singh, & Venkateswara Rao, 1996).

6. Conclusion
The results of this study showed that the traditional processing methods (boiling, wet roasting, dry roasting, germination and fermentation) of chickpea were very important because, that increased

utilizable carbohydrate as well as gross energy. The results also clearly indicated that such traditional processing methods may be useful for improving the nutritional quality of the chickpea with respect to crude protein, crude fat, crude fiber, moisture content and total ash. In particular, 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 traditional processing methods.

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