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EFFECTS OF DIFFERENT PROCESSING METHODS ON CHEMICAL COMPOSITION AND PHYTOCHEMICALS PRESENT IN CHICKPEA (*Cicerareitinum L.*)

Basanti Thapa[1] and Alok Shrestha[1]*
[1]Department of Food Technology, GoldenGate International College, Kathmandu, Nepal.
[*]Correspondance: ashrestha8@hotmail.com

Abstract

This study is focused on the estimation of proximate composition, phenol, flavonoid, tannin and phytate content of raw, germinated and cooked samples of chickpea. The total carbohydrates, crude protein, fat, crude fiber, ash, and moisture content of raw sample was found to be 59.53±1.84, 24.39±0.89, 5.56±0.26, 1.84±0.05, 3.31±0.03 and 7.24±0.08 g/100g, respectively. The crude protein, ash and crude fiber content of germinated sample increased up to 3.29, 2.35 and 38.25%, respectively. Whereas, the total carbohydrate and fat were reduced up to 2.46 and 1.21%, respectively. In cooked sample total carbohydrate and crude fiber increased by 0.81 and 34.02%, respectively. Similarly, crude protein, fat and ash decreased up to 5.20, 9.35 and 6.04%, respectively. This decrease might be attributed to their diffusion into cooking water. The phenolic content of raw, germinated and cooked samples were found to be 76.80±0.98, 68.71±0.39, 41.87±2.08mg GAE[1]/100g as gallic acid equivalent, respectively. The flavonoid content was determined to be 57.46±1.54, 48.16±0.49, 28.75±1.05 mg RE[2]/100 g as rutin equivalent, respectively. Tannin content was assessed as 64.08±1.13, 41.06±0.56, 33.05±1.27 mg/100 g as tannic acid, respectively. Likewise, phytate content was found to be 119.14±3.38, 44.17±1.10, 51.26 ±3.62 mg/100g, respectively. In case of processed samples all of the parameters reduced significantly. These significant losses can be attributed to those water-soluble phenolics that were leached during soaking and cooking as well as due to breakdown of phenolics during different processing conditions.

Introduction

Chickpea is the common name for an annual plant, *Cicer arietinum*, of the Fabaceae (or Leguminosae) family that is widely cultivated for its typically yellow-brown, pea-like seeds. The name also is used for these edible seeds, which form in short pods and are popular in various cuisines. Other common names for this seed are garbanzo bean, Indian pea, cecibean, bengal gram, kadale kaalu, sanaga pappu, and shimbra. Chickpea is considered the third most important pulse in the world, being widely grown in many subtropical and warm-temperate regions. Commercially, there are two main kinds of chickpeas. First, Desi, has small, darker seeds and a rough coat and is cultivated mostly in the Indian subcontinent, Ethiopia, Mexico, and Iran. And second, Kabuli has lighter colored, larger seeds and a smoother coat which is mainly grown in Southern Europe, Northern Africa, Afghanistan, and Chile, and also was introduced during the eighteenth century to the Indian subcontinent. Kabuli (meaning "from Kabul" in Hindi, since they were thought to have come from Afghanistan when first seen in India) is the type widely grown throughout the Mediterranean (Mansfeld, 2008).

Chickpeas are amazing in their nutrient content. They are good source in micro-nutrient supplement in our diet. Chickpeas are good source of protein, dietary fibers, minerals and bioactive polyphenols and they also contribute to polyphenol intake from other foods (Scalbert *et al*., 2005). This work will help in promotion and potential commercialization of the legume.

The aim of this research work is to study the effect of different processing conditions on chemical composition and phytochemicals present in a chickpea.

Materials and methods

Raw material collection and preparation

The "desi" variety of chickpea were collected from local market of Battisputali, Kathmandu, Nepal on December, 2016. The sample thus collected were subject to winnowing and hand sorting to remove stones, dust materials, stalks, broken, immature and undersized grains. Then it was soaked in 1000ml distilled water for 12 hours prior to cooking. The raw ones were ground with the help of mortar and pestle into the powder and sieved through 1mm mesh size. The grinded sample was packed into the air tight zip line bags and stored for further analysis.

Cooking

Cooking was carried out separately in boiling distilled water in a standard laboratory hotplate to maintain uniform and constant temperature. Boiling was continued and samples (2 seeds)

were withdrawn using a spatula at 5 minute intervals up to 30 minutes and thereafter, after every 2 minutes and tested for softness by pressing between finger and thumb. After the desirable softness was obtained the beans were allowed to drain over a wire screen and were surface-dried with a filter paper. Then, they were wrapped around in an aluminium foil and were placed in an air tight zip line bag which was further placed in an air tight bag and was stored for further analysis.

Germination

At first, the seeds were hand-sorted to remove wrinkled, moldy seeds and foreign materials. The sample was then washed and cleaned with tap water. Then, it was soaked for a whole night at room temperature. After that the sample was placed under wet muslin cloth and left for germination for 48 hr at room temperature (28°C) without direct contact with sun light.

Physicochemical properties

Hundred (100) kernels weight

One hundred bean seeds were counted randomly in triplicate and weighed separately. Mean of three replications was reported (Singh *et al*., 2005).

Bulk density

Seeds were poured into a known volume from a fixed height (30 centimetres) and mass of samples occupying the volume was determined. The ratio was calculated as g/mL (Singh *et al*., 2005).

Hydration capacity

Seeds, weighing 100 g in triplicate, were counted and transferred to a measuring cylinder, along with 100 mL of water. The cylinder was covered with aluminium foil and left overnight at room temperature (27±2 °C). After 24 hours (h), the seeds were drained; superfluous water was removed with filter paper and swollen seeds separated and reweighed (Adebowale *et al*., 2005; Singh *et al*., 2005).

Swelling capacity

Seeds, weighing 100 g in triplicate were counted, their volume noted and soaked overnight. The volume of soaked seeds was noted in a graduated cylinder. Swelling capacity per seed was determined as Adebowale *et al*., (2005).

Moisture content

Moisture content was determined by placing sample in hot air oven at 105±5 °C to dry until constant weight was obtained (AOAC, 2005).

Proximate composition

The sample was analyzed to obtain the chemical composition such as total carbohydrate, crude protein, crude fat, total ash and crude fiber. The minerals content of the sample was also determined as Calcium, Iron and Magnesium. The official methods of analysis were carried out to analyze the sample for different characteristics (AOAC, 2005).

Cooking properties

Cooking time

Two grams of soaked (overnight) chickpea was taken separately and cooked in 20 mL distilled water in a boiling water bath. The cooking time was determined on removing seeds at different time intervals during cooking and pressing between index finger and thumb until no uncooked core was left (Seena and Sridhar. 2005; Thomas *et al.,* 2013; Singh *et al.,* 2005).

Water uptake ratio

Two grams of beans were cooked in distilled water for a minimum cooking time in a boiling water bath, drained and blotted to remove superficial water. The cooked samples were weighed and water uptake ratio was calculated (determined as increase in weight of bean samples after cooking) (Seena and Sridhar. 2005; Thomas *et al*., 2013; Singh *et al*., 2005).

Elongation ratio

Mean length of ten cooked bean seeds were divided by length of 10 raw bean seeds to determine elongation ratio (Seena and Sridhar. 2005; Thomas *et al*., 2013; Singh *et al*., 2005).

Gruel solid loss

Two grams of sample was cooked in distilled water for a minimum cooking time in a boiling water bath, the gruel was transferred to 50 mL standard flasks after many washings and made up to volume with distilled water and mixed. Aliquots gruel was evaporated to determine the percent gruel loss (Seena and Sridhar. 2005; Singh *et al.,* 2005).

Chemical analysis

Total phenolics and flavonoids

The method described by Sadasivam and Manickam (2008) with slight modification was used for determining total phenolics. 0.5 g powdered sample was taken and extracted using 10 mL 80% ethanol which was left at 50 °C for 3 hours then centrifuged for 15 minutes. The extract then was filtered and collected in a separate test tube while 10 mL ethanol was added to the residue and allowed to leave overnight followed by centrifugation for 15 minutes. The extracts then were combined and volume was made up to 25 mL by 80% ethanol. Evaporation was carried out until sample dried and small portion of alcoholic extract (10 mL) on a water bath was extracted; the residue obtained was dissolved with 10 mL of distilled water and used for the determination for both phenolics and flavonoids determination. 0.5 mL of an aqueous solution was pipetted into a test tube where 2.5 mL distilled water and 0.5 mL of Folin-Ciocalteau reagent was added and mixed. 3 ml of 20 % sodium carbonate (Na_2CO_3) solution was added after 3 minutes which was then mixed and left for 2 hours at room temperature. The absorbance of the resulting solution was recorded at 760nm with spectrophotometer and phenolic content was calculated as gallic acid.

Total flavonoid determination was done as per. 0.2 mL 5% $NaNO_2$ solution was added into 2 mL aliquot, mixed and left for six minutes. Then, 0.2 mL 10% $AlCl_3$ was added, mixed and left for six minutes. Next, 1 mL NaOH was added, mixed and left for 15 minutes. Finally, the absorbance was recorded at 500 nm in spectrophotometer and results were expressed as rutin equivalent (RE). Rutin is the glycoside bond between the flavonol quercetin and the disaccharide rutinose.

Tannin content

Tannin was determined by Folin-Denis method as described by Sadasivam and Manickam, (2008). 1g sample with 50 mL distilled water was boiled for 30 minutes, the solution was cooled and centrifuged and the final volume was made up to 100 mL. Then the solution prepared was filtered through filter paper (Whatman no.1). One ml of filtrate was taken in which 0.5mL Folin-Denis reagent was added, then 1ml of sodium carbonate was added and volume made up to 10 mL. Then the prepared solution was left at room temperature for 30 minutes and then the absorbance was taken at 760 nm against a reagent blank. The tannin content was calculated as tannic acid equivalent.

1 O.D = 0.1931 mg; O.D = optical density

Phytate content

The phytate content was determined as described in Sadasivam and Manickam (2008). The phytic acid was extracted with trichloroacetic acid and precipitated as ferric salt. The iron content of the precipitate was determined colorimetrically and the phytate: phosphorous content calculated from this value assuming a constant 4 Fe: 6P molecular ratios in the precipitate. The mg iron present in $Fe(NO_3)_3$ was calibrated from the standard curve. And the phytate content determined on the basis of following relation:

1 mg iron = 0.8321 mg phytate.

Data analysis

All experiments were carried out in triplicate and average values were reported. The data obtained in this experiment were statistically analysed by using Genstat 5 Release 12.1 software program developed by The Null Corporation, 2009. Experimental data obtained were statistically analysed using one way Analysis of variance (no blocking) at 5% level of significance. The means were compared by using least significant method.

Results and Discussion

The chick pea sample was collected from local market of Kathmandu. Chickpea (*Cicer arietinum L.*) is nutritious as well as rich in phytochemicals and not much research has been carried out in raw, germinated and as well as in the cooked ones. The chickpea sample was cooked and analyzed for their phytochemical composition along with their cooking properties. Another sample was left for germination and its proximate composition and phytochemical composition was analyzed.

Proximate composition of chickpea

The proximate analysis gives information about the nutritional and biochemical composition of the food. The proximate composition was determined and was statistically analyzed. The result obtained from the proximate analysis of chickpea is shown below.

Proximate composition of chickpea sample (g/100g)

Parameters	Amount (g/100g)
Total Carbohydrate	59.53 ± 0.84
Crude Protein	24.39 ± 0.89
Crude Fat	5.56 ± 0.26
Total Ash	3.31 ± 0.03
Crude Fiber	1.84 ± 0.05

*Values expressed are mean ± standard deviation

Physicochemical properties of Chickpea

Physical parameters are important in determining the quality of legume and also their acceptability by the consumers. The data obtained through the analysis relating to physical properties of seeds are given below.

Physicochemical properties of Chickpea

Parameters	Amount
100 kernel weight (g)	218
Bulk Density (kg/m^3)	740
Hydration capacity	0.11 ± 0.0
Swelling capacity	0.14 ±0.01
Moisture content (%)	7.24 ± 0.28

*Values expressed are mean ± standard deviation.

The physical properties of the chickpea sample were carried out. 100 kernels weight of chickpea was 218 g and bulk density of the sample was 740 kg/m^3. Similarly, the hydration capacity and swelling capacity of the samples was found to be 0.11 and 0.14, respectively. Hydration capacity determines the extent to which seeds absorb water on soaking. Rakshit and Shimelis (2005) concluded that the legumes with higher hydration capacity, hydration index, swelling capacity and swelling index would require less cooking time, which is useful for saving fuel energy.

The moisture content of the sample was found to be 7.24%. The physico-chemical properties, as mentioned above are considered important parameters, as they basically play an important role in cooking. The results of the present study are similar with those mentioned by previous workers (FRD, 2015; Rakshit and Shimelis, 2005; Wang *et al*., 2003) for other legumes. They reported that the legumes having the higher hydration and swelling capacity as well as index require less cooking time. Hardness after cooking increases with a decrease of hydration capacity (g/seed) in the different varieties, but decreases with cooking time. A large hydration capacity leads to better cooking quality (less cooking time and texture) and quicker sprouting.

Cooking Properties of Chickpea

Cooking time is considered as one of the main factors used for evaluating pulse cooking quality. Longer cooking times result in a loss of nutrients, so, the cooking time is of paramount importance for consideration. The results obtained for cooking properties of chickpea are given below.

Cooking properties of chickpea

Parameters	Amount
Cooking time (min)	32.70 ± 0.35
Water uptake ratio	1.65 ± 0.0
Elongation ratio	1.1 ± 0.0
Gruel solid loss (%)	8.25 ± 0.15

*Values expressed are mean ± standard deviation

The cooking time of the sample was found to be 32.70 ± 0.35 min. The water uptake ratio and elongation ratio of the sample was obtained as 1.65 and 1.1 respectively. Similarly, the gruel solid loss of the cooked sample was found to be 8.25 ± 0.15 %. As the samples having higher swelling and hydration capacities, they required less cooking time. Cooking time of beans has been found to significantly decrease in beans that are soaked prior to cooking (Berrios *et al*., 1999; Rakshit and Shimelis, 2005). Similar findings relating to cooking properties of legumes have been reported in the FRD (2015).

Effect of processing on proximate composition of chickpea

Some proximate composition in processed chickpea were analysed and compared.

Effect of cooking and germination on proximate composition of chickpea (g/100g db)

Parameters	Raw	Cooked	Germinated
Total Carbohydrate	59.53^a± 1.84	60.02^a± 0.64	58.09^a± 0.87
Moisture	7.24^a± 0.08	8.71^b± 0.11	7.83^c± 0.08
Crude Protein	24.39^a± 0.89	23.12 ^{a}± 1.17	25.22^a± 0.64
Crude Fat	5.56 ^{a}± 0.26	5.04^b± 0.04	5.47^b± 0.32
Total Ash	3.31 ^{a}± 0.03	3.11^a± 0.02	3.39^a± 0.12
Crude Fiber	1.84 ^{a}± 0.05	2.67 ^{b}± 0.04	2.98^c ± 0.21

Means in the same column with different letters are significantly ($p < 0.05$) different.

Means ± standard deviation of three determinations.

*

By difference

Means in the same column with different letters are significantly ($p < 0.05$) different.

Means ± standard deviation of three determinations.

Proximate composition of raw and processed chickpeas are presented above. The moisture contents of chickpea were in the range of 7.24-8.71%. But all the raw, cooked and germinated samples were significantly different from eachother. Cooking of chickpea showed higher value (8.71%) but the germinated chickpea showed slightly lower value (7.83%) than cooked chickpeas. Similarly, the amount of moisture content increase from 9.75 to 10.50% in seeds. The protein content of raw chickpea had highest value than both cooked and germinated chickpea. The carbohydrate content was in a range of 58.09-60.02%. Hence, cooking treatments significantly decrease protein, ash and fat contents, these decreases might be attributed to their diffusion into cooking water. The decrease ash content of cooked sample was related to that fact that mineral or ash content of vegetative tissues are positively related (Tsialtas *et al.,* 2002). Whereas, crude fiber was increased by cooking treatments; this increase could have been due to protein-fiber complexes formed after possible chemical modification induced by the soaking and cooking of dry seeds. These results are in agreement with those obtained by Guo *et al*., (2008) and Guo *et al*., (2010).

Germination of chickpea resulted in significant ($p<0.05$) increase in crude protein and crude fiber compared to the raw seeds. This increase may be due to the use of seed components and degradation of protein to simple peptides during the germination process. However, germination caused decrease in fat and total carbohydrate contents. These decreases could be attributed to their use as an energy source to start germination process. Ash was not significantly ($p >0.05$) affected by germination.

Effect of processing on minerals composition

Mineral content in various conditions (mg/100g)

Parameters	Raw	Germinated	Cooked
Ca	155	140	109
Mg	152	150	145
Fe	6.12	5.72	4.98

The Ca, Mg and Fe content of raw chickpea are 155, 152 and 6.12 mg/100g, respectively. Minerals were more reduced in cooked chickpeas. The Ca and Mg contents were reduced in very small amount during treatment. Here, Ca reduced up to 29% in cooked sample and 9.6% in germinated sample. Likewise, Mg reduced up to 1.88% and 1.31% in cooked and germinated samples, respectively. This decrease is mainly due to the minerals leached from the chickpea seeds into the water during cooking treatments. Whereas, germination resulted in the greatest retention of minerals. It showed noticeable decreases in the content of Ca and Mg. Similar finding were observed by Gupta *et al*., (2006) and Wang *et al*., (2010). They reported that, cooking beans and chickpeas in water significantly reduced the mineral contents. A decrease in the minerals was also noticed by Aletor and Ojo (1989) after cooking cowpea, which attributed mainly by the enhanced permeability of seed coat of legumes. However, germination slightly decrease Fe content. This result is somewhat in agreement with hose reported by Lee & Karunanithy (2002), who stated that the loss of divalent metals (Ca, Fe and Zn) was low during germination due to their binding to protein and the formation of a phytate-cation protein complexes.

Effect of processing on phenols, flavonoids, tannin and phyate.

Effect of cooking and germination on phenols, flavonoids, tannins and phytate

Parameters	Raw	Germinated	Cooked
Total Phenols (mg GAE[1]/100 g)	$76.80^{a}\pm 0.98$	$68.71^{b}\pm 0.39$	$41.87^{c}\pm 2.08$
Total Flavonoids (mg RE[2]/100 g)	$57.46^{a}\pm 1.54$	$48.16^{b}\pm 0.49$	$28.75^{c}\pm 1.05$
Tannin (mg TA[3]/100 g)	$64.08^{a}\pm 1.13$	$41.06^{b}\pm 0.56$	$33.05^{c}\pm 1.27$
Phytate (mg/100g)	$119.14^{a}\pm 3.38$	$44.17^{b}\pm 1.10$	$51.26^{b}\pm 3.62$

The values in the table are the means of triplicate ± standard deviation. All parameters are expressed on dry weight basis. One way ANOVA was carried out for raw, germinated and cooked samples each separately. Means ±standard deviation bearing similar superscripts in column are not significantly different ($p>0.05$).

1GAE = Gallic acid equivalent
2RE = Rutin equivalent
3TA = Tannic acid

Total phenol, flavonoid and tanin content varied significantly ($P<0.05$) in raw, cooked and germinated seeds. In raw chickpea total

phenolics was observed higher than cooked and germinated sample. However, the total phenolics was found lowest in cooked sample. There was about 46% loss in total phenolics after cooking. Whereas, after germination there was about 10.53% loss. The decrease in total phenol content during germination may be attributed to the polyphenol oxidase based enzymatic hydrolysis. On the other hand, for the flavonoids the total flavonoid content was decreased in both cooked and germinated chickpea. The highest % of loss in total flavonoid was seen almost 50% in cooked chickpea. And the lowest 16% in germinated chickpea. The phenomenon of loss of total phenolics might be due to longer soaking time, during which some polyphenols (condensed tanin) in the seed coat were hydrolysed and diffused into the soaking water. The substantial loss of phenolic components could also be due to breakdown of phenolics during processing. Although there are hundreds of varieties of dry edible beans in the world, data on phenolics in cooked legumes are very limited Bressani and Elias (1980) observed that about 30-40% of phenolics could be removed from common beans by cooking and discarding the cooking water. Xu and Chang, (2008) reported that about 75-79% of phenolics were leached into soaking and cooking water. Ismail *et al*., (2004) also reported that thermal treatment decreased the total phenolic content in all vegetables. The result obtained above was in accordance to the one as reported by Bressani and Elias (1980).

Tannin content in raw sample was found to be 64.08 mg TA^3/100 g. The highest reduction of about 48.42% was noted in cooked chickpea. Whereas, the reduction is less in germinated chickpea. The reason for loss of tannins during germination process is binding of polyphenols with other organic substances such as carbohydrate or protein. Apart from that, during the period of soaking prior to germination, the enzyme polyphenol oxidase may be activated, resulting in degradation and consequent losses of polyphenols. The value in cooked sample decreased significantly in comparison to uncooked raw ones. This might be due to complex change in chemical composition. Decrease in tannin might be attributed to leaching into soaking medium. Thermal processing might cause degradation of polyphenols and release bound phenolic compositions. These significant loss might be attributed to those water-soluble phenolics that were leached into soaking and cooking water before and during cooking as well as breakdown of phenolics during processing. The loss in tannin content in chickpea after being thermally processed were ranged from 48.94-55.10% by other researchers and the result obtained above is also in the same range. Similar results were obtained by Vijayakumari *et al.* (1998) for cooked seeds of *Vigna aconitifolia* and *Vigna sinensis*.

Phytic acid content was significantly decreased (63%) after germination. Whereas, in cooked chickpea it was decreased about 57.14%. Similar reduction pattern in phytic acid content during soaking, cooking or germination has been reported by many investigators, (Alonso *et al.*, 2000, Desphande and Sheryan, 1983; Vdal-Valaerde *et al.,* 1994, Sievwright and Shipe, 1986) for Chinas legumes, pea, faba pea, dry bean, lentil and black bean. The reduction could be due to increase in endogenous phytase activity. It could also be due to diffusion into the soaking medium also known as leeching out. As soaking of legumes in distilled water was an effective way of removing phytic acid from legumes. Phytates play an important role in mineral availability. Phytic acid reduces the availability of zinc, manganese, copper, molybdenum, calcium, magnesium, iron as well as protein. When bound to protein, phytic acid induces a decrease of solubility and functionality of the protein.

Conclusions

The findings of the study showed that desi variety of chickpea is rich in nutrients. Cooking and germination of chickpeas affected the phytochemical composition leading to reduction in phenols, flavonoids and tannins. Higher percentage of phytic acid was reduced in germinated chickpea. Germination of chickpea resulted in the greater retention of minerals than cooked chickpea. Cooking treatments significantly decrease protein, ash and fat contents whereas increases crude fiber. Germination resulted in significant increase in crude protein and crude fiber and decrease fat and total carbohydrate contents.

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