



DETERMINATION OF PROTEIN VALUE AND WATER ABSORPTION IN CHICKPEA (*CICER ARIETINUM L.*) SEEDS DURING GERMINATION

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ABSTRACT

Protein and water absorption values were investigated in Cagatay and Gokhoyuk varieties of chickpea (*Cicer arietinum*) seeds after germination. The crude protein content were determined as 29.14% by dry weight in Cagatay and 26.13% in Gokhoyuk varieties respectively. Following imbibition, the amount of water absorption increased up to approximately 50% to 60% in both varieties. Crude protein content increased by 10.4% and 14.2% and carbohydrate content increased by 0.45% and 0.41% respectively for Cagatay and Gokhoyuk varieties of chickpea seeds. In addition, total viability of microorganisms, yeasts and molds were also determined after germination. Inactivation of microorganisms (including yeast and molds) to acceptable safe limits were noted, after soaking in hot water at 95^o C for five minutes.

1. Introduction

Chickpea (*Cicer arietinum L.*) is one of the major source of plant dietary protein and particularly important for vegetarian segments of the world population. It is also used as a protein supplement in Europe and Australia and has been suggested to have significant effect on dietary quality, satiety and bowel health (Glemente and Olias, 2017; Murty *et al.*, 2010). Chickpea is an ancient crop and has been grown and consumed in tropical, sub-tropical and temperate regions for centuries. Chickpea is valued for its nutritive properties, economic source of protein and potentially health-beneficial bio-active compounds (Sofi *et al.*, 2020).

Chickpea is used exclusively as a food item in many countries (Gupta *et al.*, 2017; Jukanti *et al.*, 2012) and its traditional uses include boiling, roasting, canning or processing into humus, salad, soup and

stew. It is also known for its use in cosmetic and herbal medicine (Mahjour *et al.*, 2018; Ahmadi *et al.*, 2020). Apart from bioactive compounds, chickpea also contains high quantities of insoluble and soluble fibers which are associated with lowering of blood cholesterol levels, thereby lessening the risk of heart disease, stroke, type 2 diabetes and regular bowel movements (Hall *et al.*, 2016; Jukanti *et al.*, 2012).

Several studies have been conducted regarding amino acid content (Khan *et al.*, 1995; Sanjeewa *et al.*, 2010; Gupta *et al.*, 2018) chemical composition (Viveros *et al.*, 2001; El-Adawy 2002; Suhasini and Malleshi 2003), carbohydrate content (Hawkins and Johnson., 2005) and mineral content (Nestares *et al.*, 1999) in non-germinated chickpea seeds.

The aim of this research was to investigate the water absorption and changes in crude protein and carbohydrate contents in chickpeas

during germination at 5° C, total viability of microorganisms in the samples and extent of their inactivation following hot water treatment at 95° C for five minutes.

2. Materials and methods

2.1. Chickpea samples used in the study

Gokhoyuk (Vd.1.104) and Cagatay (Vd.1.101) varieties of chickpea seeds (*Cicer*

arietinum L.) harvested in October 2020, were obtained from Blacksea Agricultural Research Institute of Turkey based in Samsun. The samples were obtained in dry form (vacuum packed) in polyethylene bags. Nutrient composition of the chickpea varieties are shown in Table 1. The collected samples were stored in refrigerator for physical and chemical analysis.

Table 1. Nutrient composition of Cagatay and Gokhoyuk chick pea varieties

Genotype	Protein (%)	Ash (%)	Ca (ppm)*	Cu (ppm)	Fe (ppm)	K (ppm)	Mg (ppm)	Mn (ppm)	P (ppm)	Zn (ppm)
Vd.1.101 (Cagatay)	21.82	2.96	1434.50	11.68	35.48	12536.71	1712.69	27.77	3172.46	46.82
Vd.1.104 (Gokhoyuk)	24.05	2.80	1719.43	10.36	41.17	12751.74	1743.84	27.30	3502.05	39.49

2.2. Preparation of the samples for germination procedure

150 g of samples were weighed from each chickpea sample and placed in separate conical flasks. 600 ml of double distilled water was added to each and the flasks and covered with stretch film. The flasks were stored in the refrigerator at 5°C for 6 days. On the 7th day, the flasks were taken out, 50 g of moist samples were weighed out from each variety of chickpeas and placed in covered petri dishes and stored in the dark at 21°C temperature for 48 hours for germination (Ferreira et al., 2019). Duplicate samples were prepared, from each chickpea variety, for germination. During germination, the samples were sprayed with distilled water twice in a day. Protein contents were determined on 1, 2, 3, 4, 5 and 6th day of soaking in water (Kajihaua et al., 2014).

2.3. Determination of crude protein

The protein content, of chickpea samples, was measured by the Dumas Method, an official AOAC method (993.13) using a LECO FP 828 nitrogen analyzer (Jung et al., 2003).

The Jones conversion factor of 6.25 was used to convert the nitrogen (N) content as percentage (%) of protein in the sample $N \% \times 6, 25 = \text{protein } \%$ (Chang and Zhang., 2017; Jones., 1931)

The amount of crude protein in the samples was calculated as follows:

Protein content on a wet weight basis / dry solids (Chang and Zhang., 2017)

Table 2 shows the crude protein contents of the two varieties of chickpeas.

Table 2. Effects of soaking and germination on crude protein content of chickpea samples

Chickpea Variety	Before Germination	After Germination	After Germination (water soluble ash)	After Germination (water insoluble ash)
Cagatay Chickpea Vd.1.101	3.14	2.87	77.81	22.19
Gokhoyuk Chickpea VD.1.104	3.13	2.72	81.02	18.97

2.4. Determination of moisture content

The moisture content, of the chickpea samples, was determined from the weight loss,

following the evaporation process (Pearson., 1973). Figure 1 indicates the water absorption by the chickpea samples on 1, 2, 3, 4, 5 and 6th day of soaking in water.

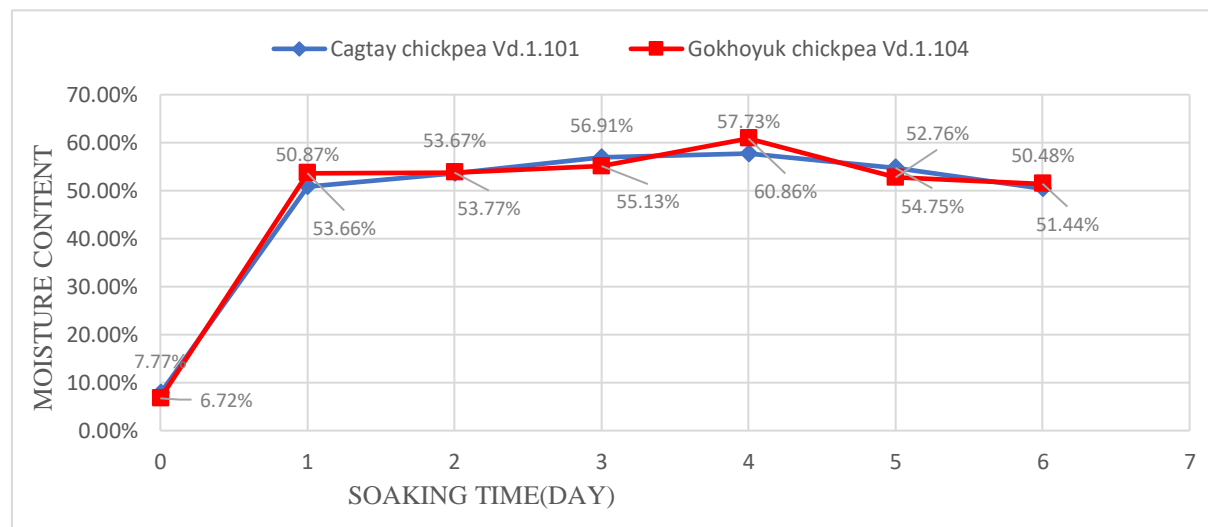


Figure 1. Changes in moisture content in chickpea during soaking

2.5. Determination of the ash content

The ash content, of chickpea samples, was determined by the standart AOAC method 923.03. which is based on the principle of calculating the amount of inorganic matter remaining as a residue after the burning of organic materials (FSSAI,2016).

The formulation for calculating the ash content is as follows:

$$\text{Total ash on dry basis (\% by weight)} = \frac{W_2 - W}{W_1 - W} \times 100$$

Where,

w₂- weight of the dish with ash (in g)

w- weight of the empty dish (in g)

w₁- weight of the dish with the dry sample (in g)

2.7. Determination of alkalinity of water-soluble ash in chickpeas after germination

The alkalinity of water-soluble ash, in germinated chickpea samples, was determined using the AOAC method 900.02. 10 ml of HCl was added to the water-soluble ash sample. It was titrated with 0.1N NaOH until a color change occurred using methyl orange indicator.

2.6. Determination of water-soluble and insoluble ash in chickpeas after germination

After germination process, water-soluble (organic) and insoluble (inorganic) ash amount in chickpeas was determined according to AOAC method 900.02. The chickpea samples were incinerated in the ash furnace. After combustion or complete acid-facilitated oxidation of organic matter, the organic ash was dissolved in boiling water and filtered to obtain the insoluble fraction (Harris and Marshall., 2017).

The water soluble and insoluble ash contents of chickpea samples before and after germination are given in Table 3.

The NaOH volume required for titration was used to determine the alkalinity of water soluble ash in the sample (Harris and Marshall., 2017).

2.8. Determination of total bacteria, yeast and mold

Fresh germinated (sprouted) and blanched (vacuum packed in a vacuum device, kept in a 95°C water bath for 5 minutes and cooled)

chickpea samples from both varieties were used for analyses. 10 g of each sample (fresh and blanched) was blended and homogenized (Stomacher AES Smasher) with 90 ml sterile peptone solution (0.1%) and serially diluted using sterile peptone water. Plate Count Agar (PCA) was used for total plate count after incubation at 35°C for 24 hours (Livingstone *et al.*, 1992). Yeast Extract Glucose Chloramphenicol (YGC) agar was used for yeast and mold count after incubation at 25°C for 5 days (AOAC 997.02., 2000).

3. Results and discussion

3.1. Effect of germination on chemical composition of chickpea

Germination may be characterized as a complicated bio-process consisting of three separate stages, depending on the seed's microstructure and water absorption capacity. The first stage is the imbibition stage, characterized by rapid water uptake. In the second stage, water intake decreases; however, with the sprouting of roots out of the seed coat in the third stage, water intake increases again (Ohanenye *et al.*, 2020). Germination, may be also identified as an effective and inexpensive method for improving the nutritional quality of grains and legumes (Khattak *et al.*, 2007).

Following 48 hours of germination, protein content of chick pea was found to increase substantially (from 18.48% to 24.46%). The close result was observed for Gamma Aminobutyric Acid (GABA) to increase from 6.42 mg/100 g to 24.576 g/100g (Ferreira *et al.*, 2019).

Germination process carried out under optimal conditions increased antioxidant activity and total phenolic compounds content of desi chickpea seeds significantly by (5707-14,361mmol TE /100g sample) and (97 to 201 mg GAE /100g sample) respectively. In addition protein content was also found to increase by 16.4% as compared to un-germinated samples ($p < 0.05$). Germination process was suggested to be an effective alternative method for increasing the nutritive value of chickpeas (Domínguez-Arispuro *et al.*, 2018).

In this study, the crude protein content of non-germinated Cagatay chickpea variety was found to be 25%, ash content 3.14% and moisture content 7.77%. For the Gokhoyuk chickpea variety, crude protein was 23.41%, ash 3.13% and moisture content 6.72%. Similar results were reported in a study conducted by Alajaji *et al.*, (2006) on non-germinated chickpea having crude protein content of 23.64%, ash 3.72% and moisture content of 10.35% (Alajaji and El-Adawy., 2006).

In addition to that, samples from two chick pea varieties (Cagatay and Gokhoyuk) were kept soaked in water for 6 days followed by a germination period of 2 days (48 hours). Significant increases in moisture and protein content was noted in both varieties. The highest crude protein content (28.83%) was recorded on the 5th day of soaking for the Cagatay chickpea variety. On the other hand, the highest crude protein content (30.53%) was detected on the fourth day for Gokhoyuk chickpea samples as given in Table 2 and Figure 1. As likewise, after 2 days (48 hours) of germination, an increase of 14.20% in the amount of crude protein for the Cagatay chickpea variety and an increase of 10.40% for the Gokhoyuk chickpea variety were observed.

Similar results were reported by Ferreira *et al.*, (2019); In their study, the amount of protein content of the chickpea sample initially was 18.4% but increased to 24.6% after 48 hours of germination (Ferreira *et al.*, 2019). In another study conducted by Xu *et al.*, (2019), the crude protein content of chickpea samples increased by 3.39g /100g, after 6 days of germination (Xu *et al.*, 2019).

The increased amount of crude protein during germination, was explained by the synthesis of enzymes by the seed during germination and the compositional change resulting from the degradation of other constituents (Xu *et al.*, 2019). In a study conducted by Dipnaik and Bathere., (2017), the protein content of chickpeas increased from 32% to 48% and a significant increase in alanine transaminase activity was observed after 12 hours of soaking and germination, compared to

raw chickpea samples (Dipnaik and Bathere., 2017).

The highest moisture rate was found to be 57.73% for Cagatay and 60.86% for Gokhoyuk chickpea varieties on the fourth day. As of the fifth day, a decrease in the moisture content was observed for both chickpea varieties as given in Figure 1. At the end of 48 hours of germination, moisture content of Cagatay chickpea type was determined as 56.60% and for Gokhoyuk chickpea it was determined as 52.36%.

Similar results were observed in the study conducted by Kajihaua et al., (2014), moisture and protein content of flour obtained from sprouted sesame seeds increased with soaking and sprouting. The seeds were immersed in water for 16 hours and germinated for a period of 36 hours. Moisture content increased from 3.97% to 4.99% and protein content increased from 26.09% to 47.64% in the 10th hour in the soaked samples. It was found that protein content increased from 26.09% to 45.64% (at 8th hour) and 48.70% (at 12th hour) holding time. However, with increasing the soaking time further, protein content decreased to 48.27% (14th hour) and 47.81% (at 16 hour) holding time, respectively (Kajihaua et al., 2014).

In a study conducted by Fouad and Rehab., (2015) on lentils, germination was found to

increase moisture content (from 25.42% to 39.25%), crude protein content (7.33% to 12.60%), and ash content (2.77 to 3.35%). On the other hand a decrease in total carbohydrate content (from 41.69% to 48.70%) and fat content (2.2g/100g to 0.90g/100g) was noted. It was stated that the increase in the amount of moisture was related to the increase in the number of hydrated cells, as the germination period extended. On the other hand, the decrease in the amount of fat it was associated with the increased lipolytic enzyme activity during germination. Finally, the authors reported that the decrease in the amount of ash was associated with the increase in phytase enzyme activity. Fat and total carbohydrate ratios that decreased during germination were attributed to the possibility of being used as an energy source during the germination phase (Fouad and Rehab., 2015).

In this study, ash content in non-germinated chickpeas was determined as 3.14% and 3.13% for Cagatay and Gokhoyuk varieties, respectively. The germination process resulted in a reduction in the ash content of both chickpea varieties this may be due to the amount of carbohydrate increased during germination (Table 3).

Table 3. Ash content of chickpea before and after germination (%)

Chickpea Varieties	Crude Protein Content (%) During Soaking							Crude Protein Content (%) After Germination (2 days)	
	Days	0	1	2	3	4	5		6
Cagatay Vd.1.101		25.0	22.79	25.14	25.52	28.74	28.83	23.92	29.14
Gokhoyuk Vd.1.104		23.41	24.06	23.40	26.52	30.53	25.08	25.43	26.13

Similar results were found by Ferreira et al., (2019) in their study with chickpeas. After 48 hours of germination, it was determined that the amount of ash, which was 3.3% in non-germinated form, decreased to 3.0% at the end of germination (Ferreira et al., 2019b).

In a study conducted by Xu et al., (2019) a small increase in ash content (3.13g/100g to 3.26g/100g) was observed on chickpea flour obtained after 6 days of germination (Xu et al., 2019). In another study performed by the same authors, an increase in the amount of ash content

(4.72g/100g to 5.07g/100g) was observed in protein isolates obtained from germinated chickpeas (Xu et al., 2020). The results were not consistent with our research this may be related with varieties of chickpeas.

On the other hand, Cornejo et al., (2015) reported a slight decrease in the amount of ash content (2.85g/100g to 2.35g/100g) in brown rice, at the end of the 48-hour germination process (Cornejo et al., 2015).

For those living in tropical and subtropical regions, legumes are included in diets as an indispensable protein source, as animal foods are consumed in limited quantities. In addition, chickpeas are considered a good source of carbohydrates, minerals and vitamins for vegans and vegetarians who meet most of their protein needs from legumes. In another study conducted by Oghbaei and Prakash., (2020) chickpeas were examined for nutritional quality after germination in distilled water supplemented with iron and zinc. A control sample was maintained consisting of chickpeas germinated in plain water. Ash content of control sample was 2.71g/100g whereas ash content of chickpeas germinated in water supplemented with 100 mg and 200 mg Fe was 3.11 g/100g and 4.11g/100g, respectively. Similarly, a significant increase in ash content (3.63g/100g) was also observed in chickpeas germinated in water supplemented with 100mg zinc (Oghbaei and Prakash., 2020).

3.2. Microbial load of germinated chickpea and effect of blanching

Following the *Escherichia coli* epidemic outbreaks in Germany and France in the spring and summer of 2011, the European Food Safety Authority (EFSA) assessed the public health risks of *Escherichia coli* producing Shiga toxin and other pathogenic bacteria that could contaminate sprouted seeds that could pose microbial food safety concerns, and therefore recommended that the general EU food safety hygiene guidelines be followed throughout the food chain, up to the final product (EFSA., 2011).

Bergspica et al. (2020) investigated Shiga toxin producing *Escherichia coli* (STEC), *Salmonella* spp. and *Listeria* spp. in 45 samples of microgreens, sprouted and un-sprouted seeds from retail markets. *Listeria monocytogenes* was not detected in any of the samples tested. In addition, *Listeria innocua* was detected in two (4.4%) of the samples. Three (6.7%) dried sprouted samples were found positive for STEC virulence genes. *Salmonella* spp. were detected in one sunflower seed sample (2.2%). According to the results of the study, it was reported that microgreens and seeds were generally safe, but since *E.coli* virulence genes were identified in 3 samples of dried sprouts, there could be some concerns regarding consumption of dried sprouts (Bergspica et al., 2020).

In this study, microflora changes that occurred during germination were examined and colonies were counted for yeast, mold and total bacteria which are important for health and were responsible for food spoilage. Microbiological analyses indicated that there were no increase in the numbers of yeast and molds during germination, however number of total bacterial colonies were too high to be counted by naked eyes. Total bacterial (plate) count were found to decrease to safe levels ($<10^3$ cfu/g) after blanching.

4. Conclusions

It may be concluded that consumption of germinated chickpeas is of significant importance from health perspectives due to its increased protein content, as compared to its non-germinated form. On the other hand, boiled or cooked forms of chickpeas must be preferred instead of non-cooked ones in terms of microbial contamination.

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