Pak. J. Agri. Sci., Vol. 56(X), xxx-xxx; 2019
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/19.7844
http://www.pakjas.com.pk

EFFECT OF CHICKPEAS (*Cicer arietinum*) GERMINATION UNDER MINERALS STRESS ON THE CONTENT OF ISOFLAVONES AND FUNCTIONAL PROPERTIES

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Germination of chickpeas was carried out in the light using a closed germination chamber at 25 ± 2°C and 80% R.H. Calcium, zinc and selenium were sprayed throughout the germination period in concentrations of 25, 50, 75, and 100mg/L. HPLC-UV was used at 260nm to analyze the contents of isoflavones from the samples collected on 1st, 3rd, 5th, 7th and 9th day of germination. Profound increase in the content of biochanin A and fermonenetin was found on 7th day of germination in WM sample with 3.08 and 5.50 mg/g, respectively. Whereas, biochanin glucoside and genistein were acquired in maximum content of 4.34 and 2.41 mg/g in 5D25 and 9D25. The results proved the use of minerals at 25 and 50mg/L during germination to be ideal for increased isoflavone as well as for increased mineral contents. Significant increase of 0.98 g/mL, 1.27 g/g and 2.29 mL/g was observed for bulk density (BD), oil holding capacity (OHC) and water holding capacity (WHC), in a samples germinated for 5 days. The results of emulsifying activity (EA), foaming capacity (FC) and foaming stability (FS) significantly decreased from 44.22-36.48%, 27.82-12.63% and 16.34-8.00%, respectively. Current results of isoflavones, minerals and functional properties encourage the utilization of germinated chickpeas at industrial level for as a functional and nutritional ingredient for the germination period of 5 days at mineral supplementation of 25-50mg/L
Keywords: Chickpeas, germination, isoflavones, HPLC-UV, minerals, functional properties

INTRODUCTION

Chickpea (*Cicer arietinum*) ranked 2nd among the cultivated legumes in the world Bar-El Dadon *et al.* (2014). India, Australia, Turkey and Pakistan are its top producers along with 37 other countries (Jiménez-Díaz *et al.*, 2015). For over 2500 years, China is using chickpeas not only for food but also for making natural Uygur traditional medicine (Xiao *et al.*, 2014). Recently, the diet based on the important bioactive components is getting attention in contrast to the high nutriton diet based on protein, fat and carbohydrates. (Torres-Fuentes *et al.*, 2015).

Due to anti-oxidative, anti microbial, insecticidal, anti-fungal, oestrogenical and contraceptive properties, isoflavones have gotten tremendous importance in the class of bioactive compounds. Furthermore, many epidemiological and clinical studies have proven role of isoflavones for prevention of cancer, obesity, cardiovascular disease and diabetes (Gao *et al.*, 2015). Biochanin A and biochanin B (fermonenetin) are abundantly found in chickpeas, as these names were originated from the word "chana", name of chickpea in Hindi/Urdu. Above mentioned isoflavones are quite similar in their chemical structure to daidzein and genistein with an exception of methylated 4' hydroxyl group (Aguilera *et al.*, 2011a; Megías et *al.*, 2016).

Recently, the demand of natural or minimal processed food with better nutrition is increasing (Palframan and Myers, 2016). The enhancement of nutritional value in beans and grains after germination is proved from many studies (Dueñas *et al.*, 2016). According to Zhang *et al.* (2012), germination not only improve the nutrition but also the mineral's bio-availability and trace elements. However, the bioactive components and end product value is dependent on the length of germination process, seed type, and other processing conditions (Bains *et al.*, 2014).

Minerals are important parts of our body and the role of zinc supplementation for bone development in women with postmenopausal is proved from different studies whereas calcium and vitamin D are well known for bone development (Khadilkar *et al.*, 2012). Some forms of cancers, hypertension, coronary artery disease, inflammatory problems are recovered by the use of selenium (Klein *et al.*, 2003; Nawrot *et al.*, 2007; Schnabel *et al.*, 2008; Asemi *et al.*, 2015; Duntas, 2009). Being a cheaper source of food and its

abundant availability in India, and Pakistan encourages the utilization of chickpeas with simple germination treatment to get a better nutrition with increased bioactive components. Keeping in view the importance of these minerals and isoflavones; the study was conducted by germinating the chickpeas using calcium, zinc and selenium in different concentrations to analyze isoflavones, minerals and functional properties as no previous was available to understand the effect of these minerals at specific concentrations on isoflavones after germination for a specific period of time.

MATERIALS AND METHODS

Chickpeas were procured from an industry in Xinjiang, China. Six different standards for isoflavones (biochanin A, rutin, fermonentin, genistein, daidzein and biochanin glucoside) were purchased from Sigma Aldrich, Shanghai, China. HPLC grade ethanol, methanol, acetic acid and acetonitrile were used. All other chemicals used were AR grade and ultrapure water provided by laboratory purification system was used throughout the experiments.

***Instruments*:** Biochemical incubator under controlled temperature and moisture was used for germination of chickpeas. All petri plates and mineral solutions were sterilized using Shenan DSX-280B autoclave. The eluents used in HPLC were sonicated using KG-500DE sonicator to remove bubbles. Waters apparatus equipped with UV detector and Atlantis T3 C_{18} column was used to analyze the isoflavones. Atomic absorption spectrophotometer equipped with graphite tube atomized (GTA 120) was used for detection of Ca and Zn, while selenium was detected at 196nm using atomic fluorescence spectrophotometer (AFS-930) with Se fluorescence lamp.

***Germination of Chickpeas*:** 1% $NaCLO_4$ solution was used to surface sterilize the seeds of chickpeas for almost 30 minutes followed by 3 times washing with distilled water. Four different concentrations 25, 50, 75 and 100 mg/L for three different minerals ($Cacl_2$, $ZnSO4.7H_2O$ and Na_2SeO_3) were prepared. Chickpeas were soaked for 14 h at 37 °C using tap water and different mineral solutions. Sterilized petri dishes with autoclaved cotton were prepared and soaked chickpeas were poured on the plates. Germination of chickpeas was carried out at 25 ± 2 °C and 80% relative humidity. The germinated seeds of chickpeas were procured on different days and were sprayed with 1mL of tap water and different minerals solutions each throughout the germination period with spraying gap of every 8hours.

***Sample preparation and analysis of isoflavones through HPLC-UV*:** Isoflavones were isolated by modifying the method of Gao et *al.* (2015). Germinated samples were oven dried at 50 °C. The moisture content of the germinated flours after drying was 10-11%. Grinding of dried chickpeas was done with laboratory mill and then passed through a 60 mesh sieve. Isoflavones were twice extracted by stirring 1 g of chickpea flour in 5 mL of 70% ethanol solution at 70 °C for 3 h. Furthermore, the supernatant was separated and centrifuged at 3000g for almost 10 min followed by filtration using Whatman filter paper. The isolated samples were stored at 4 °C before analyzing through HPLC.

High pressure liquid choromatography (HPLC) coupled with UV detector and 5C18 MS-II packed column was used to identify the isoflavones following the method of Franke and Custer (1994) with slight modifications. Water acidified by 10% acetic acid (v/v) was used as solvent and solvent B was HPLC grade acetonitrile used at a flow rate of 1mL/min. Column temperature and detection wavelength were 350 °C and 260nm, respectively. The elution of solvent was: A–B (21:79, v/v) linearly to A–B (71:29) in 7min and then held at A–B (21:79) for 13min. Calibration curve of standards was used to determine the concentrations of different isoflavones detected through HPLC-UV (ng/g; 1000000-3906.25; R^2 =0.99).

***Sample preparation and analysis of Ca and Zn through atomic absorption spectrophotometer (AAS)*:** Mineral analysis was carried out using the method of Tüzen (2003) with slight alterations. All glasswares were washed using 40% HNO_3 for a day before in a big container. 1g sample was used for dry ashing at 550 °C. The residue obtained after dry ashing was mixed with nitric acid for analysis of minerals. Ca and Zn were estimated by atomic absorption spectrophotometer (AAS) using hollow cathode lamp. The results of mineral estimation were expressed as mg/100g.

***Preparation and analysis of Se using Atomic Fluorescence Spectrophotometer (AFS)*:** Ventilated chamber was used to prepare a solution of HNO_3 and $HCLO_4$ in the ratio of 4:1 using a beaker with glass balls to avoid the acid reaction. Overnight cold digestion of the sample (1 g) in 10mL of above solution was done. The digested sample was then poured into a flask and heated at 100-150 °C using infrared oven until the yellow fumes changed in to white and then the temperature was increased to 200-220 °C until the fumes disappeared and transparent solution left back. The transparent solution obtained through digestion was taken off the oven and stored in the dark place for cooling down. The solution after cooling was further mixed with 10 mL ultrapure water and heated until evaporation. It was finally mixed with water in a volumetric flask to make final volume of 25mL. The analysis of selenium was done using atomic fluorescence spectrophotometer (AFS).

FUNCTIONAL PROPERTIES ANALSYSIS

***Bulk density (BD)*:** The chickpea flour samples were filled gently into a 10mL previously tared graduated cylinder. The bottom of the cylinder was gently tapped to avoid diminution of the sample level after filling to the 10mL mark. Bulk Density was the weight of sample per unit volume of sample calculated as g/mL (Du *et al.*, 2014).

***Water holding capacity (WHC)*:** 1 gram of germinated chickpea flour mixed with 10mL of distilled water was stirred for 24 hours in a centrifuge tube at 25 °C followed by centrifugation at 3000g for almost 25 minutes. Volume was measured by pouring the supernatant after centrifugation a 10mL graduated cylinder. Water holding capacity was determined as mL of the water held by 1g of the dried chickpea flour sample (Chau and Cheung, 1998).

***Oil holding capacity (OHC*):** 1 gram of different chickpea flours germinated at different intervals of time was mixed with vegetable oil the ratio of 1:10 and stirred for 30 minutes at room temperature. The mixture was then centrifuged at 3000g for 25 min and supernatant was transferred to the 10mL graduated cylinder. Milliliter of oil held by 1g of different chickpea flours was measured as oil holding capacity (Chau and Cheung, 1998).

***Emulsifying activity (EA)*:** Emulsifying activity (EA) was measured by following the method of Seena and Sridhar (2005) with slight modifications. 1 gram sample dissolved in 20mL distilled water was stirred for almost 25 minutes; 5mL were added to make the final volume of 25mL. Then corn oil (25mL) was added and homogenized for 3 minutes. The emulsion obtained was then centrifuged at 2100g for 5 minutes and volume was noted. The emulsifying activity was measured as the percentage of emulsified volume layer in the centrifuge tube as shown in eq. (1)

(1) Emulsifying activity (%) = (Height the emulsified layer in the tube ×100) / (Height of the total sample content)

***Foaming capacity (FC) and foaming stability (FS)*:** 1 gram sample was mixed and shaken vigorously with 50mL of distilled water for 5 minutes in a test tube. It was then quickly poured into a 250mL graduated cylinder and volume to foam was measured to know the foaming capacity. For foaming stability the volume of foam was recorded again after 30 and 60 minutes. The percentage of foaming capacity of and stability were measured using the following equations (Cheng and Bhat, 2016).

(2) FC (%) = (Volume after whipping - Volume before whipping) / (Volume before whipping) ×100

(3) FC (%) = (Foam volume after 10min-Foam volume before whipping) / (Initial foam volume) ×100

***Statistical Analysis*:** The results of all data were expressed with mean and standard deviation values; one-way analysis of variance (ANOVA) with post-hoc Dunnett's t test was used to analyze the data using the SPSS Statistics v. 19 software (IBM SPSS, New York, USA). Differences of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of germination on isoflavones content in Chickpeas

***Table 1*:** shows the results of different isoflavones obtained

Table 1. Effect of germination conditions on isoflavones content (mg/ g) of chickpeas.

Treatment	Biochanin A	Daidzein	Fermonenetin	Biochanin glucoside	Genistein	Rutin
NG	ND	ND	0.01 ± 0.000^{a}	0.03 ± 0.000^{a}	ND	0.11 ± 0.006^{d}
1D WM	0.01 ± 0.001^{b}	ND	0.22 ± 0.003^{h}	0.72 ± 0.003^{fgh}	0.29 ± 0010^{abc}	0.11 ± 0.003^{de}
1D 25	ND	ND	0.16 ± 0.003^{e}	0.48 ± 0.002^{cde}	0.11 ± 0.004^{ab}	0.30 ± 0.00^{n}
1D 50	ND	ND	0.09 ± 0.004^{c}	0.26 ± 0.003^{abc}	0.04 ± 0.000^{a}	0.03 ± 0.00^{a}
1D 75	ND	ND	0.21 ± 0.001^{h}	0.14 ± 0.001^{ab}	ND	0.03 ± 0.001^{b}
1D 100	0.06 ± 0.000^{c}	0.08 ± 0.000^{c}	0.20 ± 0.000^{g}	0.42 ± 0.000^{bcde}	0.12 ± 0.003^{ab}	0.22 ± 0.001^{l}
3D WM	0.97 ± 0.003^{l}	0.46 ± 0.003^{l}	1.36 ± 0.002^{o}	4.34 ± 0.002^{n}	2.15 ± 0.002^{i}	0.20 ± 0.001^{k}
3D 25	0.32 ± 0.003^{f}	0.22 ± 0.002^{i}	0.88 ± 0.003^{l}	2.11 ± 0.002^{j}	0.94 ± 0.061^{fg}	0.13 ± 0.002^{h}
3D 50	0.26 ± 0.002^{e}	0.17 ± 0.001^{f}	0.60 ± 0.002^{j}	1.95 ± 0.003^{j}	0.95 ± 0.003^{fg}	0.58 ± 0.003^{s}
3D 75	0.01 ± 0.002^{a}	0.13 ± 0.003^{e}	0.21 ± 0.001^{h}	0.97 ± 0.003^{h}	0.28 ± 0.001^{abc}	0.53 ± 0.001^{r}
3D 100	ND	0.04 ± 0.002^{a}	0.11 ± 0.002^{d}	0.33 ± 0.003^{abcd}	ND	0.46 ± 0.003^{q}
5D WM	0.59 ± 0.003^{h}	0.21 ± 0.001^{h}	1.09 ± 0.000^{m}	2.70 ± 0.706^{k}	1.35 ± 0.003^{h}	0.12 ± 0.001^{f}
5D 25	0.97 ± 0.003^{l}	0.46 ± 0.003^{l}	1.36 ± 0.002^{o}	4.34 ± 0.002^{n}	2.15 ± 0.002^{i}	0.20 ± 0.002^{k}
5D 50	0.32 ± 0.003^{f}	0.22 ± 0.002^{i}	0.88 ± 0.004^{k}	2.11 ± 0.002^{j}	0.49 ± 0.571^{cd}	0.13 ± 0.003^{h}
5D 75	0.09 ± 0.003^{d}	0.12 ± 0.003^{d}	0.35 ± 0.002^{i}	0.83 ± 0.005^{gh}	0.31 ± 0.002^{bc}	0.11 ± 0.000^{r}
5D 100	ND	0.08 ± 0.002^{c}	0.17 ± 0.004^{f}	0.59 ± 0.002^{def}	0.10 ± 0.001^{ab}	0.11 ± 0.000^{r}
7D WM	3.09 ± 0.003^{p}	0.23 ± 0.003^{i}	5.50 ± 0.000^{t}	2.93 ± 0.002^{k}	2.38 ± 0.002^{i}	0.12 ± 0.001^{g}
7D 25	2.62 ± 0.001^{n}	0.17 ± 0.001^{f}	3.94 ± 0.003^{s}	3.50 ± 0.002^{l}	$2.34 \pm 0..001^{i}$	0.24 ± 0.003^{m}
7D 50	1.55 ± 0.00^{m}	0.29 ± 0.003^{k}	2.79 ± 0.001^{q}	2.16 ± 0.002^{j}	1.07 ± 0.002^{g}	0.16 ± 0.002^{i}
7D 75	0.55 ± 0.003^{g}	0.07 ± 0.003^{b}	1.03 ± 0.002^{m}	1.47 ± 0.001^{i}	0.81 ± 0.004^{rf}	0.17 ± 0.003^{i}
7D 100	0.72 ± 0.003^{oi}	0.18 ± 0.002^{g}	1.24 ± 0.002^{n}	1.27 ± 0.001^{i}	0.65 ± 0.003^{de}	0.17 ± 0.001^{j}
9D WM	ND	ND	0.03 ± 0.003^{b}	0.06 ± 0.002^{a}	ND	0.05 ± 0.002^{c}
9D 25	2.80 ± 0.002^{o}	0.50 ± 0.003^{m}	3.58 ± 0.004^{r}	3.83 ± 0.004^{m}	2.41 ± 0.003^{i}	0.32 ± 0.003^{p}
9D 50	0.85 ± 0.003^{k}	0.28 ± 0.003^{j}	1.77 ± 0.002^{p}	1.92 ± 0.004^{j}	0.59 ± 0.003^{de}	0.23 ± 0.002^{m}
9D 75	0.72 ± 0.003^{i}	0.18 ± 0.001^{g}	1.23 ± 0.001^{n}	1.27 ± 0.001^{i}	0.65 ± 0.003^{de}	0.17 ± 0.002^{j}
9D 100	ND	ND	0.02 ± 0.000^{b}	0.06 ± 0.002^{a}	ND	0.05 ± 0.002^{c}

Data is expressed as mean ± standard deviation. Values with different letters in the same column are significantly different ($p < 0.05$). NG, Non-germinated; ND, Not detected; D, day/days; WM, without minerals; 25, 50, 75 and 100 shows the quantity of minerals added in mg L^{-1} of mineral solution.

after germination of chickpeas for different intervals and under stress of three different minerals. The HPLC spectra of different isoflavones discovered in chickpea after sprouting is shown in Fig.1, while standards peaks of isoflavones (biochanin A, fermonenetin, biochanin glucoside, genistein, daidzein and rutin) are shown in Fig.2. There was a significant increase in isoflavones after germination and a pronounced effect of germination period was found in the chickpea samples germinated under different conditions. Maximum contents of Biochanin A and fermonenetin were found at 7th day of germination with 3.09 mg/g and 5.50 mg/g, respectively. Dramatical increase in the content of biochanin A and fermonenetin could be seen from Table 1 as compared to their content in non-germinated sample.

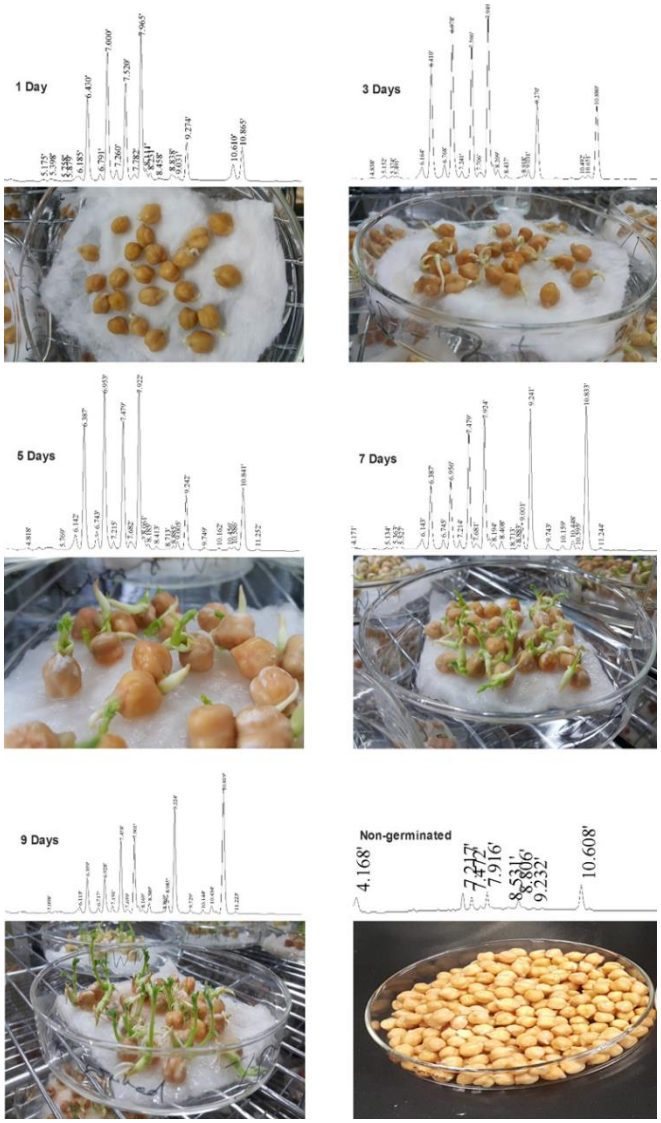

Figure 1. HPLC-UV peaks of isoflavones during different germination days.

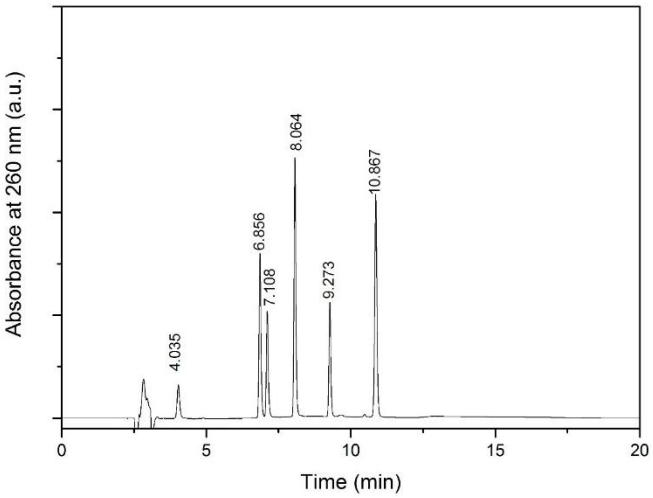

Figure 2. HPLC-UV peaks of isoflavones at 260nm. Peaks: 4.036, Rutin; 6.866, Daidzein; 7.108, Biochanin Glucoside; 8.064, Genistein; 9.273, Fermonenetin; 10.867, Biochanin A.

Gradual increase in biochanin glucoside and genistein was found as 0.03-3.83 mg/g and 0.00-2.41 mg/g, respectively, with biochanin glucoside was found maximum in 5D25 after 5 days of germination at 25 mg/L concentration of minerals. The content of rutin was maximum (0.58 mg/g) in 3D50 and significant decrease was observed with only exception in 9D25 where it was 0.32 mg/g. Highest amount of daidzein was discovered in 9D25 compared showing the effect germination period which significantly enhanced the isoflavones content. Further, 3DWM and 5D25 were resulted to have same concentration of daidzein. According to findings of previous studies, biochanin A, formononetin, genistein, and their glycoside conjugates are the major isoflavones found in chickpeas (Wu *et al.*, 2012). Literature have proved the presence of about 31 different isoflavones in soybean and chickpeas discovered from chromatographic techniques based on analyzing the retention time of their chromatogram, spectra obtained through UV, and their mass fragments. According to the study of Wu *et al.* (2012) chickpeas have almost four times more isoflavones than soybean.

The isoflavones are normally identified using UV absorption spectra as it has a specific UV absorption band II with wavelength (240−280nm) to absorb A-ring benzoyl system. Series of chemical groups and their formations are responsible for absorption of isoflavones by UV spectra including aromatic acyle group, the number and position of aglycones hydroxyle, and the patterns of glycocidic substitution. Rise in content of isoflavones could be the reason of different metabolic pathways as they are found as precursor of isoflovones in different legumes.

Sprouting in the light has a pronounced effect on the content of isoflavones. According to Phommalth *et al.* (2008), the germination of soybean in the light were found to synthesize

1.53 times more isoflavones as compared the soybean sprouted in the dark. Photosynthesis during the process of germination is another reason for the production of isoflavones as well as enhancement of malonyl-CoA and coumaroyl-CoA which are precursors for isoflavones production during germination. Together with the process of photosynthesis, the presence of light during the process of sprouting tends to increase the effect of initial enzymes like pheylalanine ammonia-lyase for production of isoflavones. Further, the little decline in the content of isflavones could be the result of transformation of isoflanoids to other isoflavones after the prolonged germination period of 9 days (Liu *et al.*, 2002; Graham, 1991).

***Effect of germination and minerals addition on the final mineral content of chickpeas*:** Supplementation of minerals was found to have a significant ($p \leq 0.05$) effect in the content of calcium with germination period. 1st day of sprouting had a non-significant increase in the content of calcium (Table 2); while a significant rise was noticed in calcium content on 5th day in 5D50 with 275.75 g/100g. The results of Li *et al.* (2014) while working on the germination of peanut seeds had a similar trend for the concentration of calcium. Some other studies on mungbean germination were shown to have a significant effect on calcium (Embrey, 1921; Tso, 1926). The absorption of minerals content by chickpea seeds during the process of germination was another reason of increased content of calcium.

A pronounced increase in zinc levels was observed (Table 2) after 5 days of germination. There was a significant decrease in the levels of zinc after 5th day of germination, where maximum zinc content was 4.12 mg/100g in 5D100. The rise in the levels of minerals content of a sample without minerals supplementation could be the reason of tap water used during the germination period. Even the simple process of germination resulted in the enhancement of minerals contents for ideally a period of 5 to 7 days. A little loss in the levels of the minerals in the earlier samples could be the result of leaching of minerals during the process of soaking done prior to sprouting (Bains *et al.*, 2014)

The result of selenium content obtained through atomic fluorescence spectrophotometer are shown in Table 2. It could be seen from the results that there was a profound increase in the levels of selenium after germination, especially after 7 days in 7D50 with a content of 2309 ng/g. Similar results have

Table 2. Effect of germination conditioins on mineral content of chickpeas.

Treatment	Ash %	Ca mg 100^{-1} g	Zn mg100^{-1} g	Se ng g^{-1}
NG	1.22 ± 0.007^{a}	145.64 ± 0.438^{a}	2.51 ± 0.014^{d}	310.92 ± 0.057^{b}
1D WM	1.39 ± 0.014^{b}	141.02 ± 0.346^{a}	2.42 ± 0.021^{c}	280.02 ± 2.333^{a}
1D 25	2.21 ± 0.014^{g}	179.34 ± 0.362^{b}	2.03 ± 0.021^{a}	402.60 ± 1.831^{c}
1D 50	2.22 ± 0.007^{g}	187.51 ± 1.068^{bc}	2.43 ± 0.007^{d}	480.32 ± 5.508^{e}
1D 75	2.19 ± 0.007^{ef}	175.01 ± 0.226^{b}	2.33 ± 0.028^{c}	494.88 ± 3.076^{e}
1D 100	2.73 ± 0.014^{j}	188.44 ± 0.304^{bc}	2.29 ± 0.035^{b}	451.78 ± 1.110^{d}
3D WM	2.12 ± 0.007^{d}	176.53 ± 1.888^{b}	2.64 ± 0.021^{e}	618.34 ± 1.838^{f}
3D 25	2.29 ± 0.021^{h}	203.19 ± 0.573^{bcde}	2.70 ± 0.028^{f}	780.18 ± 5.671^{g}
3D 50	2.22 ± 0.014^{g}	195.46 ± 0.643^{bcd}	2.88 ± 0.028^{g}	850.96 ± 2.871^{h}
3D 75	2.17 ± 0.014^{d}	191.91 ± 1.287^{bc}	2.53 ± 0.007^{e}	1107.06 ± 3.514^{i}
3D 100	2.11 ± 0.021^{d}	185.98 ± 0.170^{b}	2.30 ± 0.007^{bc}	1149.40 ± 0.799^{j}
5D WM	2.21 ± 0.014^{g}	195.98 ± 0.043^{bcd}	2.87 ± 0.014^{h}	1116.58 ± 1.881^{i}
5D 25	3.09 ± 0.014^{m}	245.35 ± 0.633^{fgh}	3.02 ± 0.014^{o}	1868.88 ± 2.977^{v}
5D 50	3.00 ± 0.007^{l}	275.75 ± 0.230^{def}	3.68 ± 0.014^{n}	1707.10 ± 2.899^{t}
5D 75	2.50 ± 0.021^{i}	243.07 ± 0.820^{fgh}	3.29 ± 0.028^{l}	1319.54 ± 0.863^{l}
5D 100	2.98 ± 0.014^{l}	260.02 ± 0.831^{gh}	4.12 ± 0.021^{k}	1559.12 ± 0.764^{p}
7D WM	2.01 ± 0.014^{c}	220.34 ± 0.997^{cdef}	3.02 ± 0.043^{i}	1456.26 ± 0.863^{o}
7D 25	2.13 ± 0.014^{d}	234.49 ± 0.361^{efg}	3.20 ± 0.028^{l}	1768.94 ± 3.599^{u}
7D 50	2.04 ± 0.007^{c}	273.88 ± 1.011^{h}	3.45 ± 0.014^{n}	2309.00 ± 0.778^{w}
7D 75	2.20 ± 0.007^{fg}	201.89 ± 1.648^{bcde}	3.13 ± 0.028^{j}	1531.96 ± 0.969^{p}
7D 100	2.96 ± 0.014^{k}	188.00 ± 0.686^{bc}	2.95 ± 0.035^{h}	1583.36 ± 2.107^{q}
9D WM	2.20 ± 0.014^{fg}	219.54 ± 0.636^{cdef}	3.07 ± 0.021^{j}	1641.42 ± 2.667^{s}
9D 25	2.06 ± 0.014^{c}	233.33 ± 1.584^{efg}	3.43 ± 0.021^{m}	1613.42 ± 1.287^{r}
9D 50	2.19 ± 0.014^{fg}	202.94 ± 0.035^{bcde}	3.75 ± 0.014^{o}	1396.40 ± 2.737^{n}
9D 75	2.12 ± 0.014^{d}	192.34 ± 0.544^{bc}	3.20 ± 0.021^{k}	1364.32 ± 0.042^{m}
9D 100	2.28 ± 0.028^{h}	179.33 ± 0.856^{b}	2.90 ± 0.014^{h}	1205.34 ± 2.114^{k}

Data is expressed as mean ± standard deviation. Values with different letters in the same column are significantly different ($p < 0.05$). NG, Non-germinated; ND, Not detected; D, day/days; WM, without minerals; 25, 50, 75 and 100 shows the quantity of minerals added in mg L^{-1} of mineral solution

been observed to check the effect of sodium selenite on germinated chickpeas (Zhang *et al.,* 2012).

The results of germination were found to increase the content of not only isoflavones but also a tremendous increase in the content of minerals was also observed. It could also be seen from the Table 2 that the sample without minerals addition had increased content of selenium proving the ability of tap water to enhance minerals during sprouting. The decrease in the content of selenium after 7 days of germination could be the activation of plant defense system towards external stress (Mishra *et al.,* 2012).

***Effect of germination and mineral fortification on functional properties of chickpeas*:** The results for the effect of germination on functional properties of different chickpeas samples are shown in Table 3. The range of results for the bulk density (BD) of different chickpea samples was 0.84 to 0.98 g/mL. Significant increase for bulk density was found in 5D50 and NG sample. Further, the period of germination and the increased content of minerals had an inverse effect on BD as it was proved from previous studies too (Benítez *et al.,* 2013). However, this low bulk density could be used as a functional tool to produce several formula foods due to high volume and porous structure.

The results of water holding capacity (WHC) were in the range of 1.93-2.29 mL/g for different samples (Table 3). The levels of WHC for chickpeas in this study were little lower than those observed by Aguilera *et al.* (2011b) for beans and almost same to the results observed for germinated mucuna beans. Water holding capacity is responsible for the production of many products including puddings, meat products, and some doughs, where texture is an important parameter to keep the water and let it not dissolve in the protein. Our results of WHC for chickpeas germinated under different conditions are good enough to use it for the production of several textural products.

The values of oil holding capacity obtained after germination of chickpeas in different conditions could been seen in Table 3. The results of OHC showed a significant differences for different samples and values ranged between 0.79 and 1.33 g/g. Germination for 5 days found to give the maximum value of OHC in 5D50. Further, the decrease in OHC was observed with the increasing concentration of mineral supplementation. The deformation in the structure occurred during the process of sprouting could be the reason of rise in OHC in different chickpea samples as previously studied by Elkhalifa and Bernhardt (2010) and Aguilera *et al.* (2011b).

Table 3. Effect of germination under mineral stress on functional properties of Chickpeas.

Treatment	Bulk Density g/mL	Oil holding capacity g/g	Water holding capacity mL/g	Emulsifying activity %	Foaming Capacity %	Foaming Stability 30 %	Foaming Stability 60 %
NG	0.84±0.014L^{m}	0.79±0.007^{o}	1.92±0.007^{o}	44.22±0.014^{a}	27.82±0.700^{a}	16.34±0.000^{a}	9.93±0.071a
1D WM	0.88±0.014^{jk}	0.86±0.014^{n}	2.11±0.014^{i-m}	43.30±0.099^{ab}	25.48±0.354^{c}	15.33±0.148^{c}	9.76±0.014^{bc}
1D 25	0.90±0.007^{ghij}	0.95±0.028^{lm}	2.09±0.028^{lmn}	42.94±0.071^{bc}	25.11±0.148^{cd}	15.59±0.212^{b}	9.70±0.057^{cd}
1D 50	0.92±0.007^{defg}	0.99±0.007^{jkl}	2.16±0.014^{fg}	41.56±0.474^{de}	26.27±0.346^{b}	15.01±0.028^{d}	9.54±0.057^{f}
1D 75	0.88±0.007^{ijk}	0.90±0.007^{mn}	2.10±0.007^{j-m}	39.58±0.615^{ghi}	24.92±0.092^{d}	14.75±0.276^{e}	9.43±0.021^{g}
1D 100	0.86±0.007^{kl}	0.88±0.007^{n}	2.07±0.007^{mn}	39.72±0.382^{gh}	24.11±0.148^{e}	14.27±0.078^{f}	9.40±0.007^{gh}
3D WM	0.96±0.014^{abc}	1.03±0.057^{ij}	2.20±0.021^{de}	41.99±0.184^{d}	23.23±0.304^{g}	12.47±0.028^{g}	9.63±0.021^{de}
3D 25	0.91±0.007^{efgh}	1.06±0.021^{hi}	2.20±0.007^{de}	42.07±0.057^{cd}	23.81±0.240^{ef}	11.94±0.057^{hi}	9.65±0.014^{de}
3D 50	0.95±0.007^{bcd}	1.13±0.021^{fg}	2.24±0.021^{bcd}	40.89±0.311^{ef}	24.19±0.219^{e}	11.85±0.170^{hij}	9.59±0.014^{ef}
3D 75	0.91±0.007^{ghij}	1.01±0.014^{jk}	2.13±0.014^{g-j}	38.79±0.318^{i-l}	23.39±0.085^{fg}	11.40±0.240^{lm}	9.33±0.014^{hi}
3D 100	0.91±0.021^{ghij}	0.98±0.014^{kl}	2.10±0.007^{klm}	38.24±0.304^{k-o}	23.07±0.078^{g}	11.06±0.042^{nop}	9.12±0.057^{k}
5D WM	0.98±0.007^{a}	1.21±0.045^{cd}	2.24±0.021^{bcd}	40.00±0.156^{fg}	19.02±0.035ij	12.07±0.057^{h}	9.33±0.014^{hi}
5D 25	0.97±0.021^{ab}	1.27±0.028^{b}	2.27±0.021^{ab}	39.34±0.163^{ghij}	19.50±0.071^{l}	11.81±0.212^{ij}	9.25±0.042^{ij}
5D 50	0.98±0.007^{a}	1.33±0.007^{a}	2.29±0.014^{a}	38.93±0.255^{h-k}	20.15±0.255^{h}	11.65±0.007^{jk}	9.12±0.007^{k}
5D 75	0.94±0.007^{cdef}	1.11±0.021^{fgh}	2.17±0.035^{fg}	38.00±0.021^{l-o}	18.50±0.219^{j}	11.23±0.156^{mn}	9.02±0.007^{l}
5D 100	0.93±0.014^{defg}	1.02±0.014^{ijk}	2.01±0.021^{klm}	37.55±0.297^{nop}	17.90±0.163^{k}	10.94±0.021^{p}	8.92±0.085^{m}
7D WM	0.94±0.014^{bcde}	1.21±0.021^{cd}	2.20±0.021^{ef}	38.51±0.544^{jklm}	16.38±0.495m	11.56±0.007^{kl}	9.02±0.007^{l}
7D 25	0.93±0.007^{defg}	1.24±0.007^{bc}	2.22±0.007^{cde}	37.90±0.170^{l-o}	17.05±0.028L	11.18±0.057^{mno}	9.83±0.071^{B}
7D 50	0.92±0.014^{defgh}	1.28±0.007^{b}	2.25±0.007^{bc}	37.83±0.219^{mno}	17.23±0.148^{l}	11.05±0.049^{nop}	9.60±0.085^{ef}
7D 75	0.89±0.007^{ijk}	1.20±0.007^{cde}	2.15±0.007^{ghi}	36.90±0.163^{pq}	16.13±0.141^{m}	10.89±0.127^{pqr}	9.20±0.014^{jk}
7D 100	0.86±0.014^{klm}	1.12±0.007^{fg}	2.11±0.007^{jklm}	36.73±0.417^{pq}	15.55±0.290^{n}	10.69±0.028^{qrs}	8.97±0.028^{lm}
9D WM	0.91±0.014^{fghi}	1.15±0.0.042^{ef}	2.14±0.007^{ghij}	38.04±0.064^{k-o}	13.24±0.304^{op}	10.96±0.042^{op}	8.74±0.042^{n}
9D 25	0.90±0.007^{hij}	1.20±0.014^{cd}	2.08±0.042^{mn}	37.47±0.049^{op}	13.38±0.219^{op}	10.91±0.028^{pq}	8.70±0.049^{n}
9D 50	0.85±0.007^{lm}	1.19±0.049^{de}	2.16±0.021^{gh}	37.120±0.007^{k-n}	13.50±0.085^{o}	10.83±0.071^{p-s}	8.46±0.028^{o}
9D 75	0.84±0.007^{m}	1.10±0.021^{gh}	2.12±0.014^{h-l}	36.90±0.163^{pq}	12.92±0.049^{pq}	10.67±0.007^{rs}	8.32±0.035^{p}
9D 100	0.85±0.028^{lm}	1.02±0.007^{jk}	2.06±0.007^{n}	36.48±0.078^{q}	12.63±0.177^{q}	10.60±0.071^{s}	8.00±0.021^{q}

Data is expressed as mean ± standard deviation. Values with different letters in the same column are significantly different ($p < 0.05$). NG, Non-germinated; ND, Not detected; D, day/days; WM, without minerals; 25, 50, 75 and 100 shows the quantity of minerals added in mg L^{-1} of mineral solution.

Further, the different hydrophobic interactions of starch, dietary fibers and amino acids could also be a reason of different alterations in OHC. The process of sprouting exposes the amino acid sites and enhance the content of dietary fiber which ultimately improve the OHC, providing the food with better mouth-feel and strong flavor retention.

Emulsifying activity (EA) was significantly changed, and profound decrease in EA was found with increasing period of germination. The range of decrease in EA was 44.22%-36.48%. The interactions of amino acids and starch under severe mineral contents and the long sprouting period could be the reason of the decrease in the EA. Benítez *et al.* (2013) while working on germination of different legumes found similar results of EA.

The result of foaming capacity was 27.83% in non-germinated chickpea sample while the significant decrease (12.63%) was observed with the increasing period of germination as the value of FC in 9D100. The decrease in foaming capacity in our results is also proved from many other studies where different processing treatments of legumes tend to decrease FC (Jogihalli *et al.,* 2017a; Wani *et al.,* 2015). Maximum foaming stability noted after the time spam of 30 minutes was 16.32% in non-germinated chickpea sample. Non-significant difference in Fc was observed after 60 minutes in the FC which ranged from 9.93-8.00%. The decrease in FC after germination could also be seen from the results of Jogihalli *et al.* (2017b).

CONCLUSION

The study provides the usefulness of germination under mineral stress for a specific period of time to not only increase the content of isoflavones and minerals but also the functional properties of chickpeas. Based on present results, the germination period of 5 to 7 days and the minerals concentration of 25-50mg/L is found ideal to increase the content of isoflavones and minerals while improving the functional properties. The results of our study encourage the food industry to utilize chickpeas by applying simple process of germination to develop nutritious and functional food products.

***Acknowledgements*:** This study was supported by the grants from the State Key Research and Development Plan (No. 2017YFD0400200), the Key Research and Development Program of Jiangsu Province (BE2017374) and Jiangsu province "Collaborative Innovation Center for Food Safety and Quality Control" industry development program.

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